CHAPTER 9

Laboratory Evaluation of Kidney Disease

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Diseases of the kidney are often "silent" until late in the course of disease, when clinical signs and symptoms of uremia mark the onset of kidney failure. In contrast, laboratory evaluation for kidney disease reveals earlier manifestations and is an essential part of the clinical assessment of health and disease. In this chapter, we begin with a general approach to the laboratory evaluation of acute and chronic kidney disease (CKD). We then focus on glomerular filtration rate (GFR) as an index of overall kidney function, and proteinuria and other abnormalities in the urine sediment as markers of structural damage. In addition, we review all aspects of the routine urinalysis. Tubular functions, including concentration and dilution of the urine, urinary acidification, and reabsorption and secretion of electrolytes and other solutes are described in other chapters, as are production of hormones and metabolism by the kidney and novel biomarkers for specific diseases.

GENERAL APPROACH

Recent guidelines define kidney diseases according to alterations in kidney structure and function and their duration (Fig. 9.1 and Table 9.1).[1,2] Kidney diseases are further classified by severity of reduction in GFR and magnitude of albuminuria and by cause, reflecting the pathogenesis and pathologic abnormalities. The level of GFR is generally accepted as the best overall index of kidney function, and other kidney functions often decline in parallel to GFR in acute and chronic kidney diseases. Albuminuria generally reflects structural damage to the glomerular filtration barrier. Both measures appear to reflect kidney involvement in systemic vascular diseases as well as primary kidney diseases, and recent studies show that the severity of reduced GFR and magnitude of albuminuria are associated with a graded increase in risk for adverse outcomes across a wide variety of settings, including patients with acute and chronic kidney diseases, patients with increased risk from cardiovascular disease, and the general population (Figs. 9.2 and 9.3).[3] Abnormalities in the urine sediment, such as renal tubular cells and cellular casts, signify kidney damage and may provide a clue to the cause of kidney disease, but quantification is not well studied. Abnormalities on imaging studies and pathologic abnormalities are sufficient for diagnosis of acute or chronic kidney disease. A history of kidney transplantation is sufficient for a diagnosis of chronic kidney disease.

Recent guidelines also suggest simplification of initial diagnostic testing for detection and evaluation of acute and chronic kidney diseases. Although the importance of timed urine collections is acknowledged for gold standard measures of GFR and albumin excretion rate, they are impractical for routine general clinical practice. In this chapter, we emphasize initial testing using estimation of GFR from serum levels of endogenous filtration markers, estimation of albumin excretion rate from untimed "spot" urine albumin-to-creatinine ratio, and interpretation of reagent pads on the urine dipstick. Timed urine collections can be considered for more accurate assessment of GFR or albuminuria or further evaluation of abnormalities observed on the urine dipstick.

GLOMERULAR FILTRATION RATE

Glomerular Filtration: Determinants and Measurement

Normal Glomerular Filtration

The human kidney contains approximately 1 million glomeruli.[4,5] This number is determined at birth but is quite variable and a lower nephron number may be associated with development of hypertension and kidney disease in later life.[6,7] Each glomerulus attains an adult size of approximately 150 to 200 μm in diameter, providing a total surface area provided for filtration that approximates 1 square meter.[8] Approximately 180 L per day (or 125 mL per minute) of tubular fluid are produced from the rich renal plasma flow by the process of ultrafiltration. Glomerular filtration, driven by the high hydrostatic pressure across the glomerular capillaries, is facilitated by a hydraulic permeability of the glomerular capillary wall that is one to two orders of magnitude greater than other capillaries.[9]

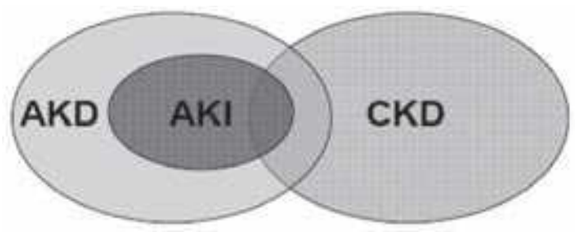


FIGURE 9.1 Conceptual model for integration of acute kidney injury (*AKI*), chronic kidney disease (*CKD*), and acute kidney diseases and disorders (*AKD*). Overlapping ovals show the relationships among AKI, AKD, and CKD. AKI is a subset of AKD. Both AKI and AKD without AKI can be superimposed upon CKD. Individuals without AKI, AKD, or CKD have no known kidney disease or disorder (NKD), not shown here. (Reproduced from Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Int Suppl.* 2012;2(1):1–126.)

The glomerular filtration barrier is both size- and charge-dependent. Substances with molecular weights lower than 10,000 daltons cross the glomerular capillary wall as easily as water and electrolytes.[10–12] Micropuncture sampling of glomerular filtrate in amphibians and mammals shows the filtrate to be identical in nonprotein composition to plasma, with electrolyte concentrations conforming to the Gibbs-Donnan relationship.[11,13] As discussed later, plasma proteins are excluded from the filtrate as a consequence of the unique structure of the glomerular capillary wall.

Determinants of the Glomerular Filtration Rate

In principle, the GFR is dependent on the number of nephrons (N) and the single-nephron glomerular filtration rate (SNGFR), as described below:

$$GFR = N \times SNGFR \quad (1)$$

In normal individuals, regulation of GFR occurs via regulation of SNGFR. In patients with kidney disease, in whom the nephron number may be reduced, regulation of SNGFR remains important in modulating GFR. SNGFR is determined by two major factors. The first factor is the net ultrafiltration pressure (P_{UF}), determined by the difference between the net transcapillary hydraulic pressure (ΔP) favoring filtration and the net oncotic pressure ($\Delta\pi$)

9.1 Definitions of Kidney Disease

	Functional Criteria	Structural Criteria
Acute kidney injury (AKI)	Increase in serum creatinine by 50% within 7 days, OR Increase in serum creatinine by 0.3 mg/dl within 2 days, OR Oliguria	No criteria
Chronic kidney disease (CKD)	GFR $<$60 mL/min/1.73 m^2 for $\geq$3 months	Kidney damage for $\geq$3 months, including Albumin excretion rate $>$30 mg/d, OR, Urine sediment abnormalities, OR, Imaging abnormalities, OR Pathologic abnormalities, OR History of kidney transplantation
Acute kidney diseases and disorders (AKD)	AKI, OR GFR $<$60 mL/min/1.73 m^2 $<$3 months, OR Decrease in GFR by $>$35% or increase in serum creatinine by $>$50% for $<$3 months	Kidney damage for $<$3 months, as defined by above
No kidney disease or disorder (NKD)	GFR $>$60 mL/min/1.73 m^2, AND Stable serum creatinine	No kidney damage

Note: AKI and CKD have formal consensus definitions. The definition for AKD is proposed as an operational definition to classify individuals with alterations in kidney function and structure and function who do not meet the definitions for AKI and CKD. NKD indicates no functional or structural alterations that meet the definition for AKI, CKD, or AKD. Clinical judgement is required or individual decision-making regarding the extent of evaluation that is necessary to assess kidney function and structure. Glomerular filtration rate (GFR) may be assessed from estimated or measured GFR. Estimated GFR does not reflect measured GFR in AKI as accurately as in CKD. Albuminuria may be assessed from timed urine collections or "spot" urine albumin-to-creatinine ratio. Novel markers of kidney damage have been proposed, but none have been validated for inclusion in the definitions of AKI or CKD. A history of kidney transplantation is considered a marker of kidney damage for CKD but not AKD.

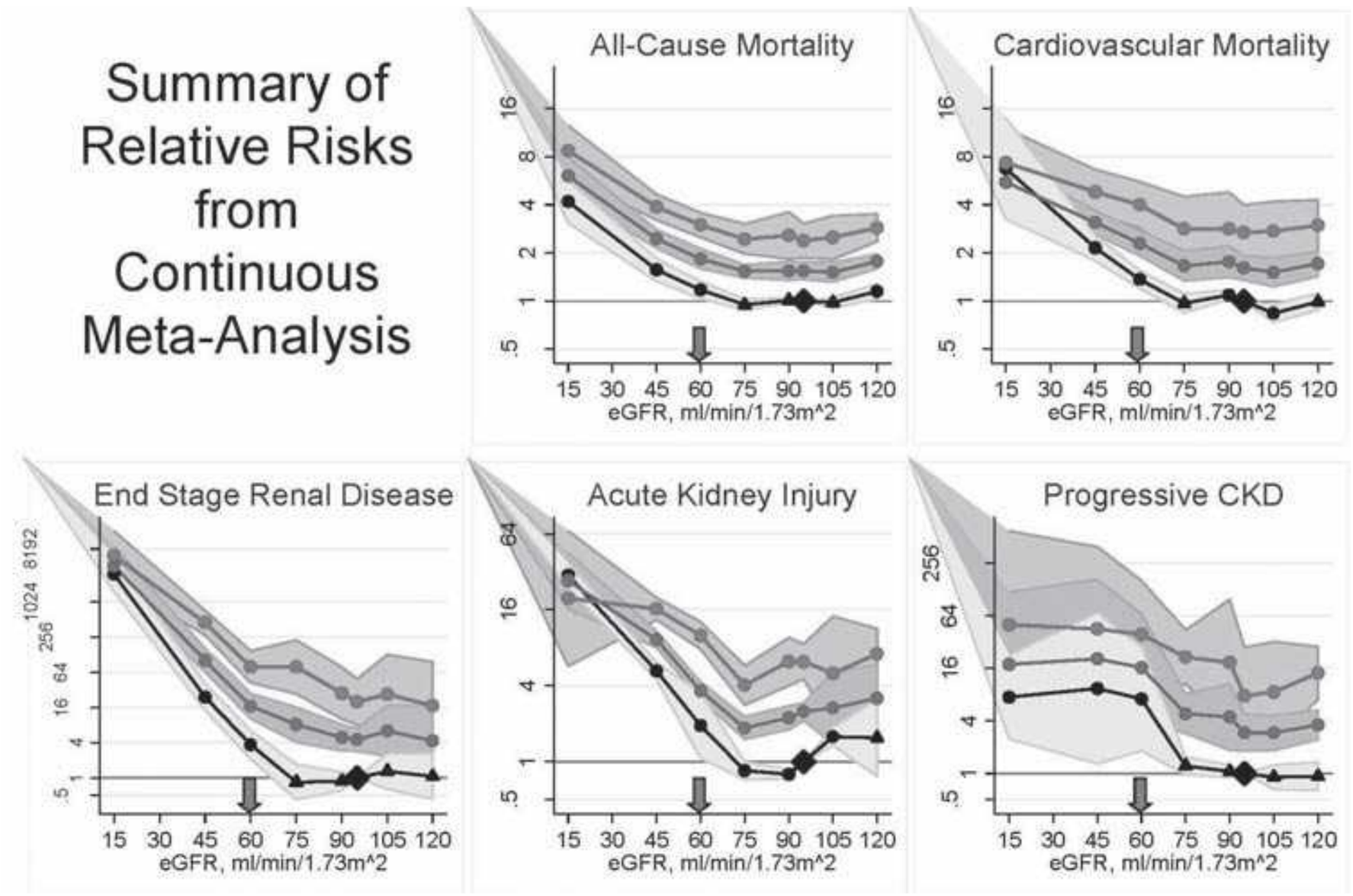


FIGURE 9.2 Summary of KDIGO Controversy Conference continuous meta-analysis (adjusted relative risk [RR]) for general population cohorts with albumin-to-creatinine ratio (ACR). Mortality is reported for general population cohorts assessing albuminuria as urine ACR. Kidney outcomes are reported for general population cohorts assessing albuminuria as either urine ACR or dipstick. Estimated glomerular filtration rate (eGFR) is expressed as a continuous variable. The three lines represent urine ACR of <30 mg per g or dipstick negative and trace (*blue*), urine ACR 30 to 299 mg per g or dipstick 1+ positive (*green*), and urine ACR >300 mg per g or dipstick ≥2+ positive (*red*). All results are adjusted for covariates and compared with reference point of eGFR of 95 mL/min/1.73m^2 and ACR of <30 mg per g or dipstick negative (*diamond*). Each point represents the pooled RR from a meta-analysis. *Solid circles* indicate statistical significance compared with the reference point ($P < 0.05$); *triangles* indicate nonsignificance. *Red arrows* indicate eGFR of 60 mL/min/1.73m^2, threshold value of eGFR for the current definition of chronic kidney disease (CKD). HR, hazards ratio; OR, odds ratio. (Reproduced from Levey AS, de Jong PE, Coresh J, et al. The definition, classification and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int*. 2011;80:17–28.) (See Color Plate.)

opposing filtration. ΔP is determined by the difference between the glomerular capillary hydraulic pressure (P_{GC}) and that in the earliest proximal tubule (P_T). $\Delta\pi$ is determined by the glomerular oncotic pressure alone as the ultrafiltrate is virtually protein free. The second factor, K_f, describes the surface area and permeability characteristics of the glomerular ultrafiltration barrier. This relationship can be expressed by the equation:

$$\text{SNGFR} = K_f(\Delta P - \Delta\pi) \quad (2)$$

Absent from this equation is the renal plasma flow rate. Alterations in renal plasma flow affect SNGFR largely by affecting $\Delta\pi$. Changes in determinants of SNGFR as plasma traverses the glomerular capillary are demonstrated in Figure 9.4. For a detailed analysis of these determinants and the multiple factors that result in the regulation of glomerular filtration, the reader is directed to Chapter 2.

In acute and chronic kidney disease, decreased GFR can be due either to a decrease in nephron number or SNGFR. Interestingly, in a number of experimental chronic kidney diseases characterized by decreased nephron number, SNGFR is elevated, perhaps reflecting compensation in processes to maintain whole kidney GFR. Moreover, in some diseases, increased SNGFR precedes the decline in nephron number, thereby raising the hypothesis that hyperfiltration in single nephrons may give rise to the development or progression of chronic kidney disease.[14]

Normal Range and Variability of Glomerular Filtration Rate

The GFR cannot be measured directly. Instead, as discussed later, it is assessed from the urinary clearance of an ideal filtration marker, such as inulin. When measured repeatedly in a single individual, under constant conditions and according to a standard protocol, the GFR appears relatively constant. Homer Smith measured the inulin clearance in one "hospitalized but otherwise normal subject" 15 times during 1 year; the range was 113 to 137 mL per minute with a mean of 122 mL per minute.[15] However, variation among individuals is quite large, and normal values show considerable spread. As discussed later, the major causes of variability in healthy individuals are age, gender, and body size. Hence,

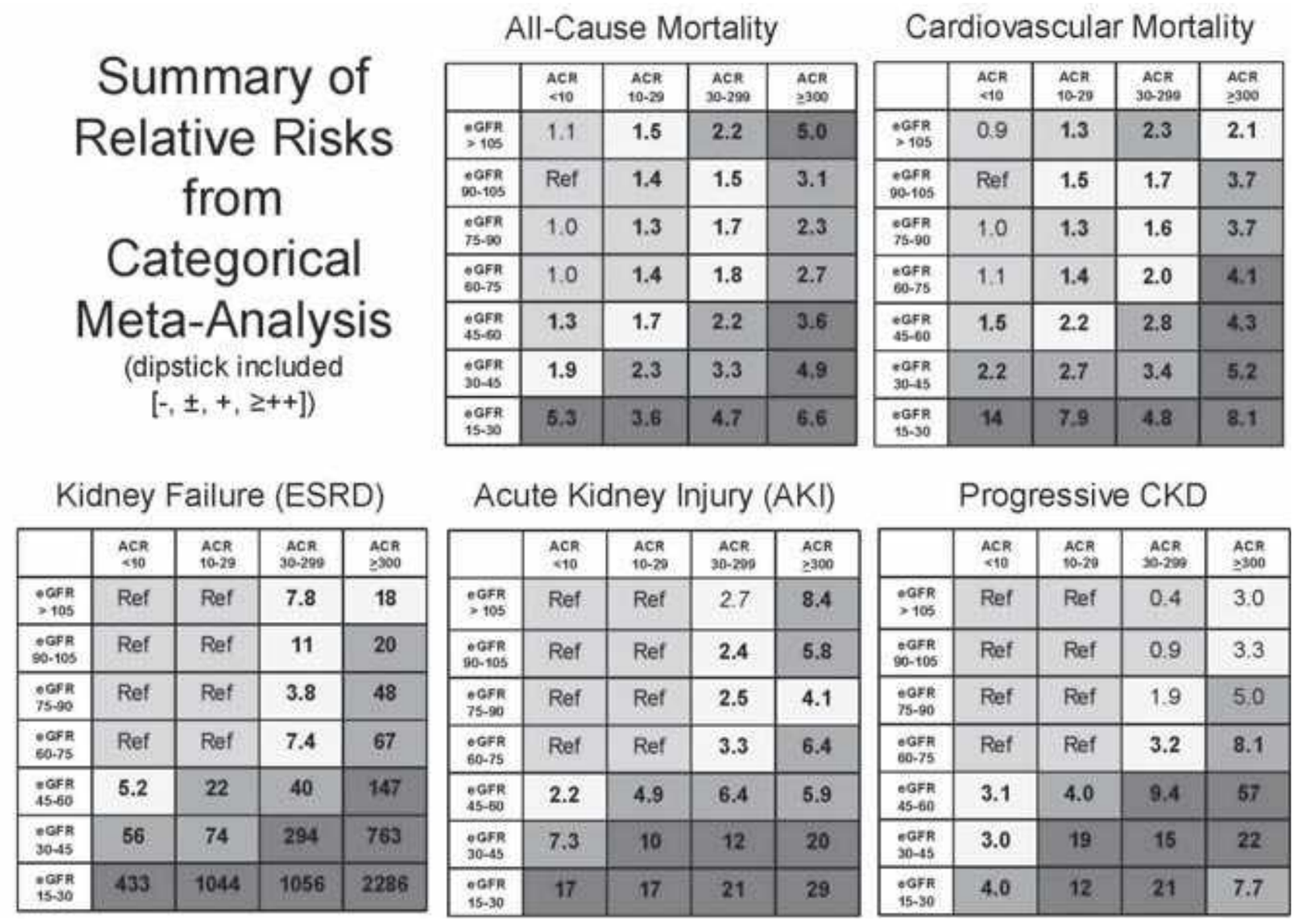


FIGURE 9.3 Summary of KDIGO Controversy Conference categorical meta-analysis (adjusted relative risk [RR]) for general population cohorts with albumin-to-creatinine ratio (ACR). Mortality is reported for general population cohorts assessing albuminuria as urine ACR. Kidney outcomes are reported for general population cohorts assessing albuminuria as either urine ACR or dipstick. Estimated glomerular filtration rate (eGFR) and albuminuria are expressed as categorical variables. All results are adjusted for covariates and compared with the reference cell (*Ref*). Each cell represents a pooled relative risk from a meta-analysis; *bold numbers* indicate statistical significance at $P < .05$. Incidence rates per 1,000 person-years for the reference cells are 7.0 for all-cause mortality, 4.5 for cardiovascular disease mortality, 0.04 for kidney failure, 0.98 for acute kidney injury (AKI), and 2.02 for kidney disease progression. Absolute risk can be computed by multiplying the RRs in each cell by the incidence rate in the reference cell. Colors reflect the ranking of adjusted relative risk. The point estimates for each cell were ranked from 1 to 28 (the lowest RR having rank number 1, and the highest number 28). The categories with rank numbers 1 to 8 are *green*, rank numbers 9 to 14 are *yellow*, the rank numbers 15 to 21 are *orange*, and the rank numbers 22 to 28 are colored *red*. (For the outcome of kidney disease progression, two cells with RR 1.0 are also green, leaving fewer cells as orange.) (Reproduced with permission from Levey AS, de Jong PE, Coresh J, et al. The definition, classification and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int*. 2011;80:17–28.) (See Color Plate.)

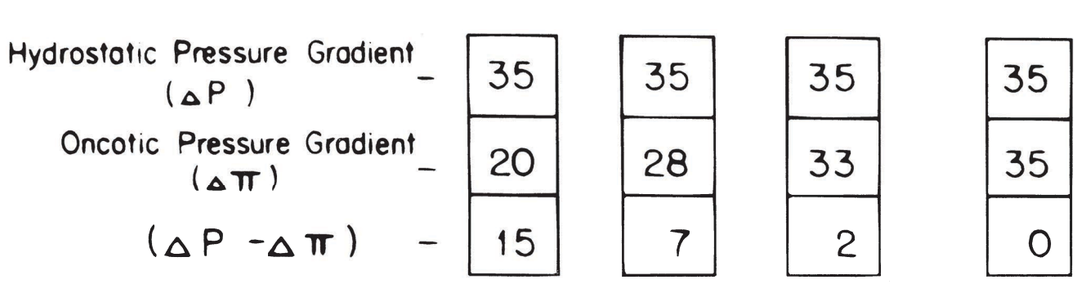


FIGURE 9.4 The changes in hydrostatic and oncotic pressures that occur as plasma traverses the glomerular capillary. As water is filtered without protein, the oncotic pressure gradually rises, thereby decreasing the net pressure favoring filtration. The pressure favoring filtration falls toward zero and filtration stops in this model before the plasma reaches the efferent arteriole. (From Deen WM, Robertson CR, Brenner BM. Glomerular ultrafiltration. *Fed Proc*. 1974;33:14, with permission.)

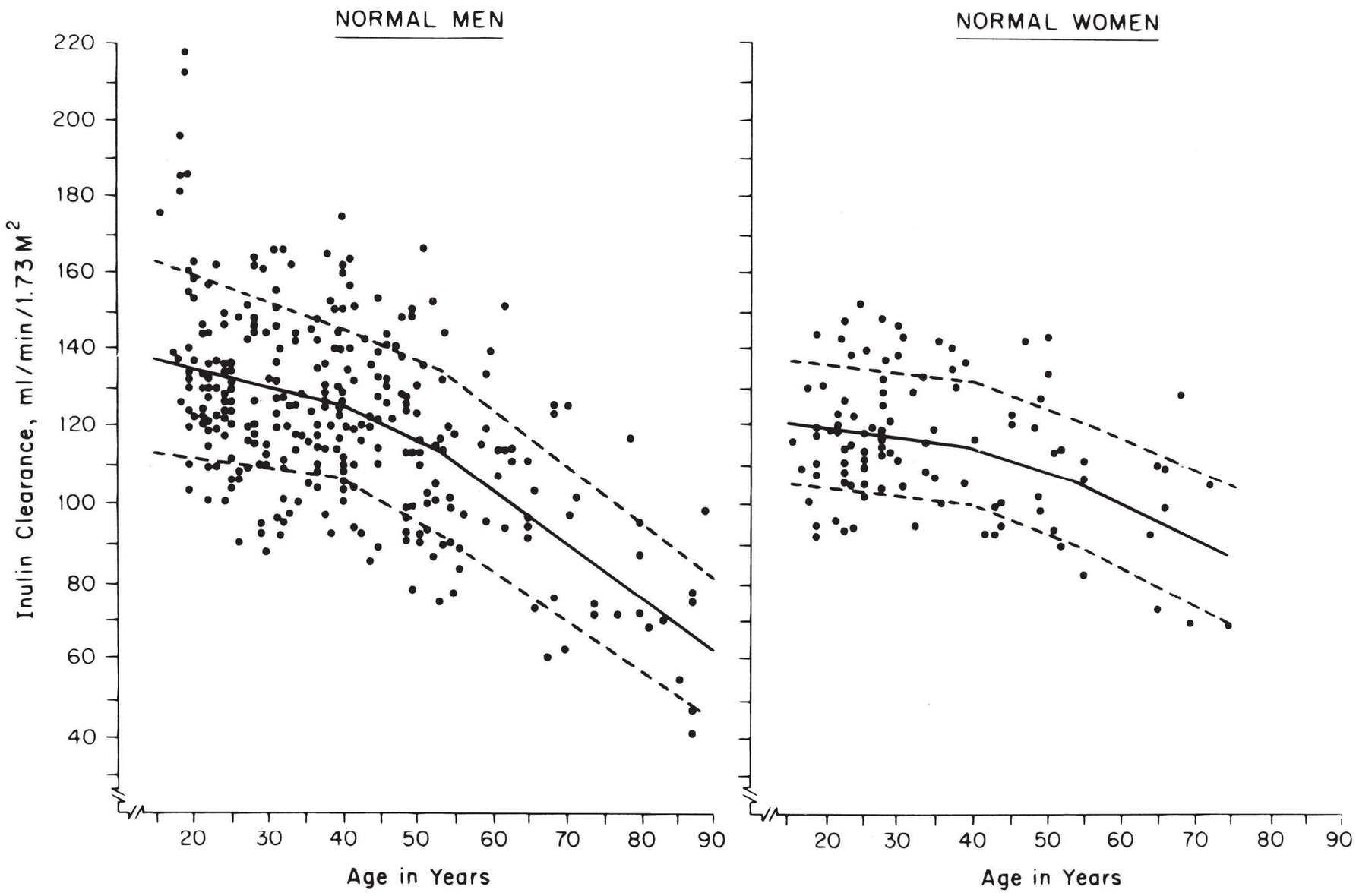


FIGURE 9.5 Normal values for glomerular filtration rate, adjusted for body surface area, in men and women of various ages. (From Wesson LG, ed. *Physiology of the Human Kidney*. New York: Grune & Stratton; 1969, with permission.)

measured values of GFR are typically adjusted for body size (surface area) and are traditionally compared to normative values for age and gender (Fig. 9.5).[16] Even after elimination of these sources of variation, important variability remains. A compilation of inulin clearance measurements in hydrated young adults (adjusted to a standard body surface area of 1.73 m^2) shows the mean value in men to be 131 mL per minute with a coefficient of variation (CV) (defined as the standard deviation divided by the mean) of 18%, and the mean value in women to be 120 mL per minute, with a CV of 14%.[15,16] The following sections discuss causes of normal variation. These same factors also contribute to variation in GFR in patients with kidney disease.

Age, Sex, Body Size, and Ethnicity. The surface area adjustment was first introduced to minimize variability in urea clearance results among normal adults and children.[17–19] Based on the relationship of GFR to glomerular surface area, it is not surprising that the level of GFR is related to kidney size, which in turn, is related to body surface area and metabolic activity.[20] Measured values for GFR are conventionally factored by 1.73 m^2, the mean surface area of men and women 25 years of age. Nonetheless, as described earlier, surface-area adjusted values for GFR are approximately 8% higher in young men than in women of the same age.

Glomerular tuft volume, renal size, and GFR increase during growth and development. The surface area adjustment is not appropriate for newborns, whose adjusted GFR is less than 50% of the value achieved at approximately 1 year of age.[21,22] More recent studies strongly suggest that in newborns, GFR should be expressed in mL/min/kg, with the normal value being 0.6 to 1.6 mL/min/kg. Such an approach reduces the apparent variation in measured GFR more than 10-fold.[23] Beyond age 1 to 2 years, however, GFR values in normal children, adjusted to 1.73 m^2, are the same as those for young adults.

The appropriateness of the surface area correction in obesity remains controversial.[24] Because adipose tissue is less metabolically active than lean body mass, the physiologic matching of GFR to body surface area may not be the same in obese as in lean individuals. There are few data to relate measured GFR to body size, metabolic activity, and risks for development of kidney disease in obesity, leaving many important questions unanswered.[25] Are estimates of body surface area from height and weight as accurate in obese as in lean individuals? Does GFR increase with weight gain in proportion to body surface area? If so, is the resulting hyperfiltration associated with increased risk for development of kidney disease, as hypothesized in other conditions with hyperfiltration, such as diabetes? If so, indexing GFR to body surface area in obesity may obscure detection of an important marker of disease. Cross-sectional and longitudinal studies of measured GFR in obesity, in association with measures of body size and metabolic activity, and markers of kidney damage are necessary to answer these questions.[24]

Most studies of measured GFR in populations without kidney disease have been conducted in North America or

Europe, so data on nonwhite races and other ethnicities is limited. Reports on small to moderate numbers of subjects have suggested a lower average value,[26,27] but these studies are somewhat limited by differences in GFR measurement methods and by incomplete ascertainment of protein intake (see below). A more recent report from a representative population in Pakistan suggests mean values of GFR in young adults only slightly below those in whites, with similar age-related decline.[28]

In older studies, both cross-sectional and longitudinal studies in normal men demonstrate an age-related decline in GFR of approximately 10 mL/min/1.73 m^2 per decade after the age of 30 years.[16,29–31] Recent studies in the general population have not been performed, but studies in kidney donors demonstrated a 4 mL/min/1.73m^2 lower measured GFR per decade up to the age of 45 years and a 7.5 mL/min/1.73m^2 lower measured GFR per decade thereafter.[32] Thus, using the data from the general population, during the 50 years from age 30 to 80, GFR declines by almost 40% from approximately 130 to 80 mL/min/1.73 m^2. Cross-sectional studies in normal women indicate roughly similar results, but comparable longitudinal studies have not been performed and there may be subtle differences related to effects of hormones, pregnancy, and propensity toward illnesses that impact the kidney. This age-related decline in GFR is consistent with the anatomic observation that the number of glomeruli in the normal human kidney declines with age; in the sixth and seventh decades, the number of glomeruli is less than one-half the number present in young adults.[4,33] The cause of age-related decline in GFR is not completely understood, but progressive glomerular sclerosis, independent of traditional kidney disease risk factors, likely contributes to the loss of glomeruli.[34,35] Recent epidemiologic studies demonstrate that this decline in GFR is associated with increased risk for all-cause and cardiovascular disease mortality as well as kidney disease, casting doubt on the traditional interpretation that it is normal.[36]

Pregnancy. Marked increases in GFR occur during pregnancy; elevations to an average as much as 50% occur during the first trimester, and these high levels persist until shortly after term.[37–40] These increments in GFR are associated with an increase in renal plasma flow and relatively constant filtration fraction throughout most of pregnancy, reflecting hemodynamic consequences of widespread vasodilatation. Late in pregnancy, it appears that hyperfiltration becomes dependent on reduced plasma oncotic pressure. This change persists in the very early postpartum period, but the GFR returns to normal in the first 4 to 8 weeks following the end of pregnancy.[40,41]

Interestingly, pregnancy-induced hyperfiltration also occurs in women with preexisting chronic kidney disease.[42] This observation suggests that the physiologic vasodilatation of pregnancy can further augment the single-nephron hyperperfusion and hyperfiltration associated with chronic kidney disease. However, this phenomenon may be restricted to women with only very mild reductions in GFR. Improvement of GFR was not observed in one study of 23 women with chronic kidney disease and pre-pregnancy serum creatinine levels greater than 1.4 mg/dL.[43]

Protein Intake. The effect of protein intake to modulate GFR in experimental animals was recognized 70 to 80 years ago.[44,45] It is now clear that these effects occur in humans, although the magnitude of the effect varies widely among studies.[46] Important causes of variation include the duration of protein feeding (habitual protein intake vs. meat meals or amino acid infusions), the type of protein (animal vs. vegetable or soya protein sources; essential vs. nonessential amino acids), and the filtration marker used to measure GFR (inulin vs. creatinine).

In a classic study, Pullman et al.[47] placed healthy humans on low (0.1 to 0.4 g/kg/day), medium (1.0 to 1.4 g/kg/day), and high (2.6 g/kg/day) protein diets for 2 weeks. Compared to the low protein diet, inulin clearance increased after ingestion of the medium and high protein diets by 9% and 22%, respectively. These changes were accompanied by parallel changes in renal plasma flow, indicating a hemodynamic basis for the changes in GFR. A longer period of habituation may have greater effects on GFR. Similarly, in patients with chronic malnutrition, inulin clearance was 27% to 64% lower than after repletion of nutritional status,[48–51] and returned to near normal values only after 1 month of refeeding. In addition, malnourished patients had smaller kidneys, suggesting that differences in kidney function were due to structural as well as hemodynamic alterations.[48] Increases in GFR and kidney size in association with increased protein intake have been noted in diverse clinical circumstances, such as in patients receiving total parenteral nutrition and in insulin-dependent diabetic patients with poor metabolic control.[52] Some studies suggest a greater response to animal than vegetable protein in habitual diets as well as in response to protein loads.[53–55] A recent study assessing the impact of sustained high protein feeding demonstrated an increase in GFR in young subjects (24 ± 1 years old), but actually a small decrease in GFR in older subjects (70 ± 2 years old).[56]

After a meat meal, GFR, renal plasma flow, and splanchnic blood flow rise within an hour and remain elevated for several hours.[57] In humans, the increment in inulin clearance is about 10%,[58,59] and appears to be less than the increment in creatinine clearance.[46] Nonessential amino acids are more potent than essential amino acids in inducing the postprandial rise in GFR, and branched-chain amino acids appear to have little or no effect.

It had been proposed that protein-induced hyperfiltration represents "renal reserve capacity," which is lost prior to the reduction in baseline GFR associated with kidney disease.[60] However, it has now been shown conclusively that changes in GFR occur in response to changes in habitual protein intake or meat meals in patients with kidney disease and reduced GFR.[59–63] This is consistent

with studies in animals with experimental kidney diseases, which show that changes in protein intake further modulate the determinants of single-nephron GFR. In particular, a high protein diet raises the already increased glomerular plasma flow and transcapillary hydrostatic pressure gradient.[64,65] Thus, protein-induced hyperfiltration augments the hyperperfusion and hyperfiltration of chronic kidney disease.

Diurnal Variation. A normal diurnal variation in filtration rate occurs, with 10% higher values occurring in the afternoon than in the middle of the night.[66] In large part, the diurnal variation is thought to be related to variation in protein intake during the day.[16,60] Possibly, diurnal variation may also be related to transient reductions in GFR associated with exercise. Indeed, a decrease of 40% or more is seen with severe exertion.[16,67,68] However, diurnal variation is also observed in quadriplegics,[69] arguing against physical activity as the sole cause of diurnal variation. Possibly, diurnal variation may also reflect variation in hydration. GFR increases with overhydration and decreases with water restriction. However, the changes are small except when gross disturbances in fluid balance occur.

Antihypertensive Therapy. As a result of powerful mechanisms for autoregulation of renal hemodynamics (Chapter 3), the level of GFR remains relatively constant throughout a wide range of blood pressure. Nonetheless, antihypertensive therapy can be associated with reductions in GFR, due, in part, to the effect of lowering blood pressure and, in part, to specific effects of classes of antihypertensive agents. Indeed, marked reduction in GFR can complicate treatment in patients with severe hypertension and acute or chronic kidney disease,[70] which is an effect thought to be due to the loss or reset of autoregulation due to sclerosis of the renal vasculature from hypertensive injury.[71] In normal individuals and in patients with kidney disease, GFR is transiently reduced by a variety of antihypertensive agents, including diuretics, beta-blockers, central alpha-2 agonists, and peripheral alpha blockers.[72] In contrast, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), calcium channel blockers, and directly acting vasodilators do not regularly lower GFR in healthy subjects. A large study in patients with chronic kidney disease and well-controlled hypertension showed persistent small (less than 5 mL per minute), but significant, reductions in GFR associated with the use of ACE inhibitors as well as diuretics and beta-blockers.[73] In addition, after controlling for the effect of these classes of antihypertensive agents, a small effect of lowering blood pressure remained. Because the effects of the various classes of medications and of lowering blood pressure appear to be independent, a clinically significant reduction in GFR could occur in patients with chronic kidney disease undergoing treatment with multiple antihypertensive agents.

Measurement of the Glomerular Filtration Rate

Clearance. As mentioned earlier, the GFR is assessed from the clearance of filtration markers, substances excreted by glomerular filtration that can be used to assess the GFR. The "gold standard" for the measurement of GFR is the urinary clearance of inulin. The term clearance was introduced into kidney physiology by Van Slyke and his colleagues in reference to studies of the excretion of urea in 1929.[18] Two years later, Jollife and Smith extended the use of the term to the excretion of creatinine and later to the excretion of many other substances.[74] In the many decades since these pioneering studies, the concept of clearance has maintained its primacy as the cornerstone of our understanding of the measurement of glomerular filtration.

The clearance of a substance is defined as the rate at which it is cleared from the plasma per unit concentration. The clearance of substance "x" (C_x) is given in the following equation:

$$C_x = A_x / P_x \tag{3}$$

where A_x is the amount of x eliminated from the plasma and P_x is the average plasma concentration. Hence, C_x is expressed in units of volume per time. The value for clearance does not represent an actual volume, but a virtual volume of plasma that is completely cleared of the substance per unit of time, without reference to the route of elimination. The value for clearance is related to the efficiency of elimination: the greater the rate of elimination, the higher the clearance.

Relationship of Glomerular Filtration Rate to Urinary Clearance. For a substance that is cleared by urinary excretion, the clearance formula may be rewritten as follows:

$$C_x = U_x \times V / P_x \tag{4}$$

where U_x is the urinary concentration of x and V is the urine flow rate. The term $U_x \times V$ is defined as the urinary excretion rate of x. If substance x is filtered freely across the glomerular capillary walls and excreted only by glomerular filtration, then the rate of filtration is equal to the rate of urinary excretion:

$$GFR \times P_x = U_x \times V \tag{5}$$

where the term $GFR \times P_x$ is defined as the filtered load of x. By substitution into Equation 9.2:

$$C_x = GFR \tag{6}$$

Hence, substance x would be defined as an "ideal filtration marker" whose urinary clearance could be used to measure GFR.

However, if substance x is also reabsorbed or secreted by the renal tubules, then the following equations apply:

$$U_x \times V = GFR \times P_x - TR_x + TS_x \quad (7)$$

$$GFR = (U_x \times V - TR_x + TS_x) / P_x \quad (8)$$

$$GFR = C_x - TR_x / P_x + TS_x / P_x \quad (9)$$

where TR_x and TS_x are the rates of tubular reabsorption and secretion of x, respectively, and TR_x/P_x and TS_x/P_x are the clearances of substance x due to reabsorption (C_{TRx}) and secretion (C_{TSx}), respectively. In this case, the rate of urinary excretion ($U_x \times V$) does not equal the filtered load ($GFR \times P_x$), and clearance does not equal GFR. Therefore, the value for urinary clearance of x (C_x) is determined not only by the rate of glomerular filtration, but also by the mechanism of excretion by the kidney. For substances that are filtered and secreted, clearance exceeds GFR, and for substances that are filtered and reabsorbed, clearance is less than GFR.

Inulin Clearance. The requirements for an ideal filtration marker, as outlined by Smith,[15] include the following:

1. It is freely filtered at the glomerulus. It passes from the glomerular capillary blood into Bowman's space unhindered by its size, charge, or binding to plasma proteins.
2. It is not altered during its passage through the nephron. It is not reabsorbed, secreted, synthesized, or metabolized by the tubules.
3. It is physiologically inert and does not alter the function of the kidney.

Inulin, a 5,200-dalton, inert, uncharged polymer of fructose, meets these criteria, and it remains the standard for experimental and clinical measurement of GFR.[15,75,76] The conclusion that inulin is freely filtered and is neither secreted nor reabsorbed in the normal kidney was originally based on indirect evidence, but a large body of direct micropuncture observations have verified this assumption.[11,77–80] Similar evidence is not available, however, in all experimental kidney diseases. For example, in several models of acute kidney failure with extensive tubular basement membrane damage, leakage of inulin across the tubules is readily demonstrated.[81,82] In such situations, of course, the urinary excretion of inulin is less than the filtered load, and inulin clearance is less than GFR.

Although the measurement of inulin clearance is a highly accurate and reproducible means of estimating GFR, there are several disadvantages that make it impractical for clinical use. First, the classical method includes measurement under fasting conditions in the morning, a continuous intravenous infusion, multiple clearance periods requiring repetitive blood and urine collections over 3 hours, oral water loading to stimulate diuresis, bladder catheterization to assure complete urine collection, and careful timing of blood sampling at the midpoint of the urine collection. Period-to-period variability in GFR (intratest variation; expressed as CV) is approximately 10%. Intratest variation may reflect incomplete bladder emptying and is often used to judge the quality of a urinary clearance study.[15] However, one recent study has shown that the precision of GFR determinations is only weakly affected by intratest variability,[83] probably because averaging over several clearance periods minimizes error due to incomplete bladder emptying. In a study in normal individuals using the classical method of inulin clearance, the CV for repeated measurements within an individual (intertest CV) was 7.5%.[84] These estimates of measurement error are probably lower than would be observed in most clinical settings. Second, inulin is difficult to dissolve in aqueous solutions, difficult to measure, and is in short supply. Because of these disadvantages, clinical assessment of GFR generally utilizes other filtration markers and clearance methods.

Urinary Clearance of Endogenous Filtration Markers. In principle, the simplest alternative to inulin clearance would be the urinary clearance of an endogenous filtration marker. The advantage of this method is that clearance can be computed from urine collections and blood sampling under usual clinical conditions without the need for administration of an exogenous marker. Indeed, this method is widely used for measuring creatinine clearance, as discussed later. The most common method is to collect a 24-hour urine collection and a single serum measurement, assuming a steady state. The urine collection is performed at home. At the onset of the collection period, the patient is instructed to empty the bladder and discard the urine. During the collection period, all subsequent urine is saved. At the end of the period, the patient is asked to void completely and to add this last specimen to the urine collection. Shortly thereafter, the blood sample is obtained.

Unfortunately, the accuracy of this method is limited because neither creatinine nor any other known endogenous filtration marker meets all the criteria for an ideal filtration marker and because timed urine collections under usual clinical conditions are notoriously inaccurate. Errors in timing or completeness can result from misunderstanding by the patient or personnel of the instructions, such as omitting urine specimens during the interval or incompletely emptying the bladder at the start or end of the collection period. At first glance, it might appear that the use of short urine collection intervals, such as 1-hour, carried out under close supervision by trained personnel might overcome these difficulties. However, using a shorter collection period, the small errors due to incomplete bladder emptying would have a greater impact on the estimate of the urine volume and hence the urine flow rate. Indeed, the 1-hour technique has been largely abandoned because the extra effort and personnel required do not significantly improve the accuracy as compared to the 24-hour

clearance.[85] However, averaging the results of three to four 30-minute collection periods does significantly improve the accuracy, probably due to cancellation of errors from incomplete bladder emptying.[86]

A similar method can be used to compute clearance for patients who are not in a steady state balance by obtaining additional blood samples during the urine collection to estimate the average serum concentration. The most common strategies are to collect blood at the mid-point of the urine collection, or at the beginning and end of the urine collection, and to average the serum concentrations.

Alternative Clearance Methods and Exogenous Filtration Markers. All alternative clearance methods have been designed to facilitate GFR measurement; however, all have limitations that should be understood for proper interpretation. Table 9.2 summarizes the strengths and limitations of these alternative clearance methods and markers, as well as the gold standard method.[75,87–89]

Changes to the clearance method include substitution of bolus intravenous or subcutaneous injection for a constant intravenous infusion and use of plasma clearance techniques to eliminate the need for urine collection. With a bolus injection, the pattern of decline in serum levels is more accurately modeled as an exponential rather than linear of decline.[83] In the bolus subcutaneous technique, the marker substance (e.g., ^{125}I-iothalamate, ^{51}Cr-EDTA) can be given with a small dose of aqueous epinephrine to slow its release into the circulation, providing fairly constant plasma levels.[90,91] More recently subcutaneous continuous infusions have been used.[92]

Plasma clearance is computed from Equation 9.3 using either the entire area or a one-compartment or two-compartment model of the plasma disappearance plot.[93–95] There are several caveats. First, a relatively long time (3 to 5 hours) is required to accurately determine the declining plasma concentration of the marker, with longer times for people with reduced GFR. Second, filtration markers utilized for this method must meet an additional criterion of rapid equilibration with the extracellular volume, and inulin is therefore not appropriate for use.[96] Third, for some markers, simultaneous assessment of plasma and urinary clearance of a filtration marker typically yields a higher level for plasma clearance, presumably due to extrarenal excretion of the marker.[97,98] This underestimation is more apparent at a lower GFR. Fourth, plasma clearance overestimates GFR in patients with moderate to severe edema probably because of the larger than expected volume of distribution and lower than expected plasma levels of the marker.[99]

Alternative exogenous markers include radioisotope-linked markers ^{125}I-iothalamate, ^{51}Cr-ethylene diamine tetraacetic acid (EDTA), and its analogue, ^{99m}Tc-diethylene triamine pentaacetic acid (DTPA), that can be readily and inexpensively measured using radioactive counters; and nonradioactive markers iohexol and iothalamate that can be measured by X-ray fluorescence and high performance liquid chromatography (HPLC) methods. The advantage of the latter two is the avoidance of radiation exposure; however, the assay methods are more expensive and generally performed in specialized laboratories. All other filtration markers deviate from ideal behavior. Overall, there is suggestion by some but not all studies that iothalamate clearance results in a higher GFR than inulin clearance, presumably due to secretion of iothalamate by the tubules. Other studies suggest that iohexol clearance may underestimate inulin clearance. DTPA readily dissociates from its radioactive tracer, allowing binding of the tracer to plasma proteins leading to retention of the tracer and underestimation of GFR.

GFR can also by measured by counting of a radioactive exogenous filtration marker over the kidneys and bladder. This technique can be combined with renal imaging, usually using ^{99m}Tc-DTPA, and is useful for determination of split kidney function.[88,100] Several studies indicate poor correlation of ^{99m}Tc-DTPA dynamic renal imaging with simultaneous urinary or plasma clearance, reflecting both bias and imprecision, and lesser accuracy than estimated GFR.[101–103] Currently, magnetic resonance imaging (MRI) is being investigated for measurement of GFR. Many protocols are in use which will require consolidation before introduction into clinical practice.[104,105]

Because of these limitations, all values for measured GFR contain an element of error, which differentiates them from true GFR. As such there is variability in the literature as to how each of these markers and methods compare to the gold standard method.

Estimation of the Glomerular Filtration Rate

Relationship of Glomerular Filtration Rate to the Plasma Solute Concentration

The plasma level of a solute (P_x) is determined by its generation (G_x) from cells and diet, extrarenal elimination (E_x) by gut and liver, and urinary excretion ($U_x \times V$) by the kidney (Fig. 9.6).[106] Physiologic processes other than GFR that affect the plasma level of a solute (P_x) are termed "non-GFR determinants." The following discussion relates concepts of plasma levels of filtration markers, their non-GFR determinants, and the physiologic basis for GFR estimating equations.

An important concept for this discussion is the steady state of solute balance. A steady state with regard to substance x is achieved when the rate of generation in body fluids (either from endogenous production or exogenous intake) is constant and equal to its rate of elimination from body fluids (either from excretion or metabolism). Therefore, in the steady state, the plasma concentration of substance x is constant:

$$G_x - E_x = U_x \times V \quad (10)$$

where G_x and E_x are the rates of generation and extra-renal elimination of x. If the substance is excreted only in the

9.2 Strengths and Limitations of Glomerular Filtration Rate Measurement Methods and Markers

Approach	Strengths	Limitations
Methods		
Urinary Clearance		
Bladder catheter and continuous intravenous infusion of marker	■ Gold standard method	■ Invasive
Spontaneous bladder emptying	■ Patient comfort ■ Less invasive	■ Possibility of incomplete bladder emptying ■ Low flow rates in people with low levels of GFR
Bolus administration of marker	■ Shorter duration	■ Rapidly declining plasma levels at high levels of GFR ■ Longer equilibration time in extracellular volume expansion
24-hour urinary collection		■ Cumbersome ■ Prone to error
Plasma clearance	■ No urine collection required ■ Potential for increased precision	■ Overestimation of GFR in extracellular volume expansion ■ Inaccurate values with one-sample technique, particularly at lower GFR levels ■ Longer duration of plasma sampling required for low GFR
Nuclear imaging	■ No urine collection or repeated blood samples required ■ Relatively short duration	■ Less accurate
Markers*		
Inulin	■ Gold standard ■ No side effects	■ Expensive ■ Difficult to dissolve and maintain into solution ■ Short supply
Creatinine	■ Endogenous marker, no need for administration ■ Assay available in all clinical laboratories	■ Secretion which can vary among and within individuals
Iothalamate	■ Inexpensive ■ Long half-life	■ Probable tubular secretion ■ Requirement for storage, administration, and disposal of radioactive substances when iothalamate-125 used as tracer ■ Use of nonradioactive iothalamate requires expensive assay ■ Cannot be used in patients with allergies to iodine
Iohexol	■ Not radioactive ■ Inexpensive ■ Sensitive assay allows for low dose	■ Possible tubular reabsorption or protein binding ■ Use of low doses requires expensive assay ■ Cannot be used in patients with allergies to iodine ■ Nephrotoxicity and risk for allergic reactions at high doses

(continued)

9.2 Strengths and Limitations of Glomerular Filtration Rate Measurement Methods and Markers (continued)

Approach	Strengths	Limitations
EDTA	■ Widely available in Europe	■ Probable tubular reabsorption ■ Requirement for storage, administration, and disposal of radioactive substances when ^{51}Cr is used as tracer
DTPA	■ Widely available in the United States ■ New sensitive and easy to use assay for gadolinium	■ Requirement for storage, administration, and disposal of radioactive substances when ^{99m}Tc used as tracer ■ Requires standardization for ^{99m}Tc ■ Dissociation and protein binding of ^{99m}Tc ■ Concern for NSF when gadolinium is used as the tracer

^{51}Cr, chromium-51; ^{99m}Tc, technetium-99m; DTPA, diethylene triamine pentaacetic acid; EDTA, Ethylenediaminetetraacetic acid; GFR, glomerular filtration rate; NSF, nephrogenic systemic fibrosis.

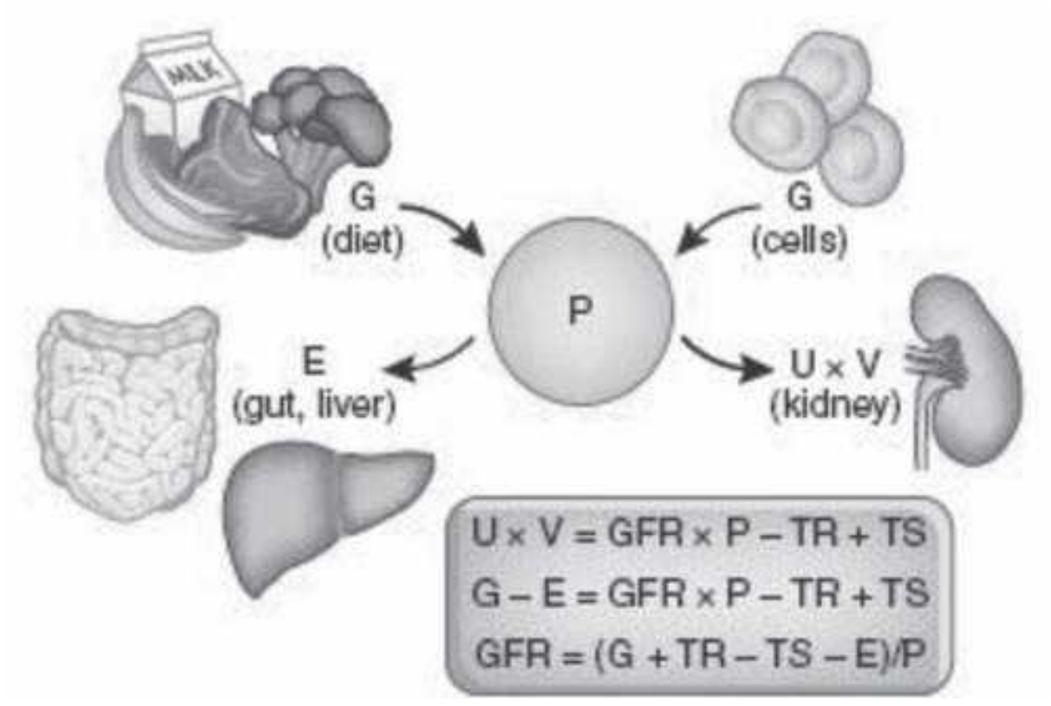


FIGURE 9.6 Determinants of the serum level of endogenous filtration markers. The plasma level (P) of an endogenous filtration marker is determined by its generation (G) from cells and diet, extrarenal elimination (E) by gut and liver, and urinary excretion (UV) by the kidney. Urinary excretion is the sum of filtered load (GFR × P), tubular secretion (TS), and reabsorption (TR). In the steady state, urinary excretion equals generation and extrarenal elimination. By substitution and rearrangement, GFR can be expressed as the ratio of the non-GFR determinants (G, TS, TR, and E) to the plasma level. (Reproduced from Stevens LA, Levey AS. Measured GFR as a confirmatory test for estimated GFR. *J Am Soc Nephrol.* 2009;20(11):2305–2313.)

urine, in the steady state, the rate of generation can be assessed from the urinary excretion rate.

$$G_x = U_x \times V \quad (11)$$

By rearrangement of Equations 9.7 and 9.10 and solving for P_x, we obtain the following:

$$P_x = (G_x - TR_x + TS_x - E_x) / GFR \quad (12)$$

Hence, P_x is inversely related to GFR, and directly related to its non-GFR determinants.

$$GFR = (G_x - TR_x + TS_x - E_x) / P_x \quad (13)$$

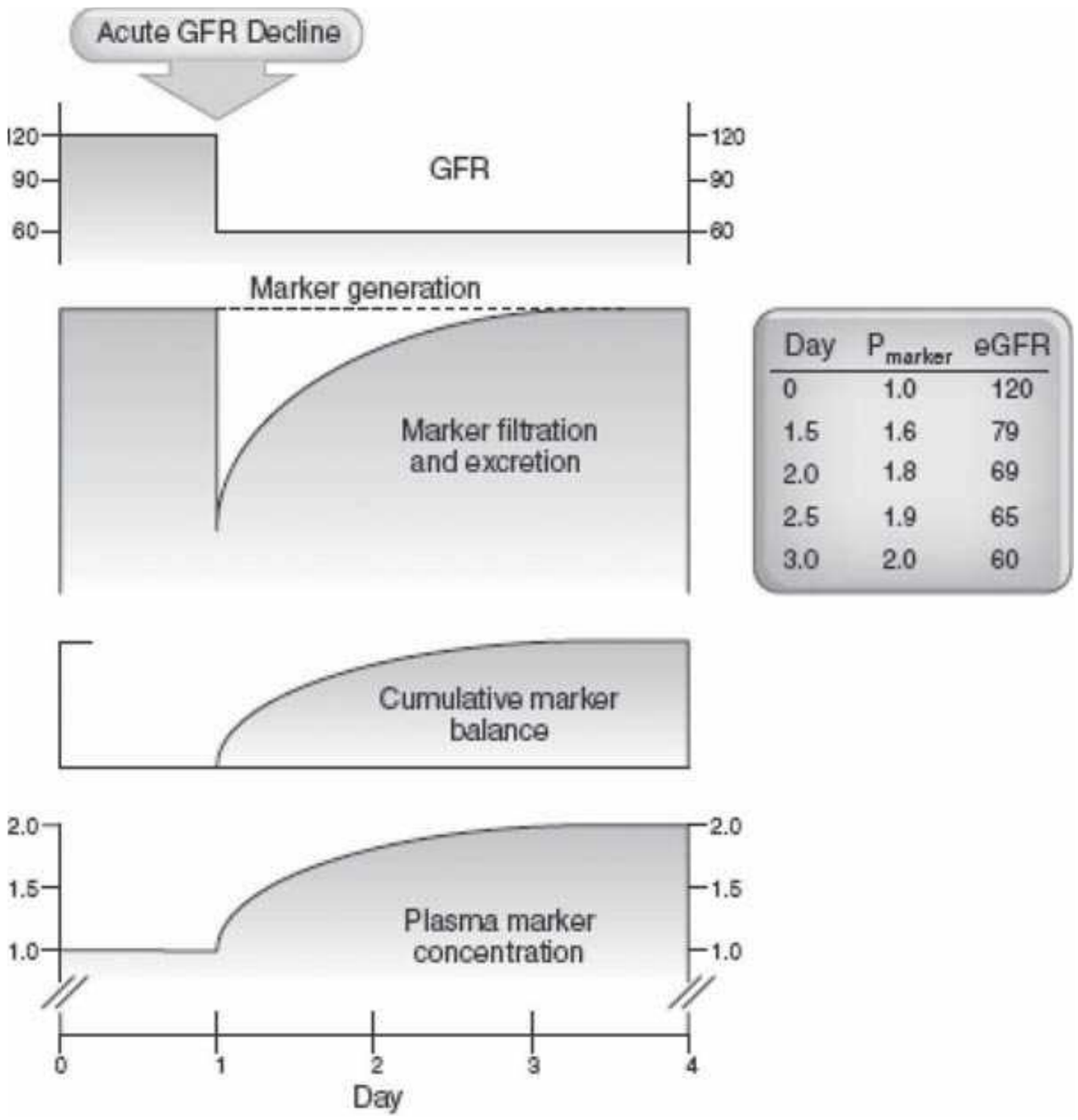
For a substance that is eliminated entirely by glomerular filtration, this relationship simplifies to the following.

$$GFR = G_x / P_x \quad (14)$$

If the rate of generation is constant across individuals and over time, the level of GFR can be estimated by the plasma level and proportionality constant.

$$GFR = k / P_x \quad (15)$$

Figure 9.7 shows the hypothetical change in levels of a filtration marker GFR after an acute change in GFR.[106,107] After an acute GFR decline, generation of the marker is unchanged, but filtration and excretion are reduced, resulting in retention of the marker (a rising positive balance) and a rising plasma level (non–steady state). Although GFR remains reduced, the rise in plasma level leads to an increase



Day	P_{marker}	eGFR
0	1.0	120
1.5	1.6	79
2.0	1.8	69
2.5	1.9	65
3.0	2.0	60

FIGURE 9.7 Effect of an acute glomerular filtration rate (GFR) decline on generation, filtration, excretion, balance, and serum level of endogenous filtration markers. GFR is expressed in units of milliliter per minute per 1.73 m^2. Tubular secretion and reabsorption and extrarenal elimination are assumed to be zero. (Reproduced with permission from Stevens LA, Levey AS. Measured GFR as a confirmatory test for estimated GFR. *J Am Soc Nephrol.* 2009;20(11):2305–2313. Modified from Kassirer JP. Clinical evaluation of kidney function—glomerular function. *N Engl J Med.* 1971;285:385–389.)

in filtered load ($GFR \times P_x$) until it equals generation (G_x). At that time, cumulative balance and the plasma level plateau at a new steady state. In this example, a halving of GFR is associated with a doubling of the plasma concentration of the marker.

Physiologic Basis of Glomerular Filtration Rate Estimating Equations

This section discusses general principles of GFR estimating equations. Specific estimating equations for GFR are discussed in more detail later in the chapter. Estimating GFR from the plasma level of endogenous filtration markers has the advantages of eliminating the need for infusion of an exogenous filtration marker and urine collections. Unfortunately, the plasma levels of all endogenous filtration markers are influenced by physiologic processes other than GFR, these processes are generally not measured in clinical practice, and clinical conditions affecting these physiologic processes are not known for all filtration markers.

Estimating equations for GFR are regression equations that estimate measured GFR from plasma levels of endogenous filtration markers and demographic and clinical variables as observed surrogates for the unmeasured physiologic processes (non-GFR determinants).[108] By definition, an estimating equation provides a more accurate estimate of measured GFR than the plasma concentration alone. For example, the equation below shows the hypothetical relationship of numerical values for demographic and clinical variables X, Y, and Z to generation of the filtration marker (G_x).

$$G_x \sim X + Y + Z \tag{16}$$

Therefore, by substitution into Equation 9.14

$$eGFR = (bX + cY + dZ) / aP_x + \varepsilon \tag{17}$$

where eGFR is estimated GFR and a, b, c, and d are regression coefficients relating P_x and other variables to measured GFR, and ε is the error based on uncertainty due to measurement, biologic variability, and statistical techniques used to derive the coefficients. Estimating equations for GFR are often expressed on the logarithmic scale and, therefore, have the appearance of

$$\log eGFR = aP_x + bX + cY + dZ + \varepsilon \tag{18}$$

$$eGFR = [P_x]^a \times X^b \times Y^c \times Z^d \times \varepsilon \tag{19}$$

where a is a negative coefficient to account for the inverse relationship between GFR and the plasma level of the filtration marker.

GFR estimating equations are derived in the steady state; hence, GFR estimates are more accurate in the steady state than in the non–steady state. In the non–steady state (Fig. 9.7), the rate and direction of change in the level of the

filtration marker and eGFR are affected by the magnitude of change in GFR, but also by the non-GFR determinants and the volume of distribution of the filtration marker.[109] Hence, the plasma level of the filtration marker reflects the magnitude and direction of the change in GFR but does not accurately reflect the level of GFR. After a fall in GFR, the decline in eGFR is less than the decline in GFR, and eGFR thus exceeds GFR. Conversely, after a rise in GFR, the rise in eGFR is less than the rise in GFR, and eGFR is thus less than GFR. As the plasma level approaches the new steady state, eGFR approaches GFR and the level of the filtration marker varies inversely with GFR.

Development and Validation of Glomerular Filtration Rate Estimating Equations

Development and validation of GFR estimating equations should be undertaken with appropriate attention to epidemiologic and statistical techniques. In general, a large sample size (n > 500 subjects) with a wide range of GFR is required for developing a GFR estimating equation. It is important to include both men and women across a wide age range and from a variety of racial and ethnic groups for international use. Validation should be undertaken in a separate population, selected according to similar criteria and with similar clinical and demographic characteristics to the development population. GFR should be measured in both populations using either inulin or an exogenous filtration marker and clearance method validated against inulin clearance. Plasma or serum concentrations of the endogenous filtration markers should be measured using assays calibrated to reference standard. The development process should proceed according to a protocol for introduction and selection of important covariates that are hypothesized to reflect non-GFR determinants of the filtration markers. The validation process should systematically evaluate bias, precision, and accuracy in the overall validation population and in clinically relevant subgroups (Table 9.3).[110] Bias reflects a systematic difference in performance, generally due to differences between the development and validation population in measurement methods for GFR, assays for filtration markers, or selection of study subjects. Imprecision reflects random error, and is generally greater at higher GFR values, due to greater GFR measurement error and greater variation in non-GFR determinants, than at lower GFR. In principle, the use of multiple filtration markers can improve precision by cancelling errors due to variation in non-GFR determinants.

Creatinine as a Filtration Marker

Creatinine is the most frequently measured endogenous filtration marker in routine clinical practice. It has been estimated that serum creatinine is measured more than 280 million times per year in the United States.[111] The classical assay was first introduced more than 125 years ago by Jaffé.[112] The normal level of GFR is sufficient to maintain a low concentration of creatinine in serum, approximately 0.7 to 0.9 mg per dl in healthy young people. Reference ranges cited by clinical laboratories vary because of variation in serum creatinine assays. More importantly, reference ranges are difficult to interpret because of variation among individuals in non-GFR determinants (Table 9.4)[113]; serum creatinine may not rise above the upper limit of the reference range unless GFR is less than 60 mL/min/1.73 m^2. Recent interest in more accurate GFR estimation has led to worldwide standardization of serum creatinine assays and reporting of estimated GFR when serum creatinine is measured.[114] Using eGFR overcomes some of these limitations, but imprecision remains, especially at higher GFR.

9.3 Metrics for Evaluation of Glomerular Filtration Rate Estimating Equations

Criteria	Metric	Definition
Bias	Median difference	mGFR − eGFR
	Median percent difference	(mGFR − eGFR)/mGFR * 100
Precision	IQR difference	Interquartile range of (mGFR − eGFR)
	IQR % difference	Interquartile range of [(mGFR − eGFR)/m GFR] * 100
Accuracy	Median absolute difference	Median of the absolute value of eGFR − mGFR
	P_{30}	Percent of estimates within 30% of measured GFR
	RMSE	Square root of mean $(\log mGFR - \log eGFR)^2$

*Measures of accuracy assess precision when bias is 0 (development datasets).
IQR, interquartile range; eGFR, estimated glomerular filtration rate; mGFR, measured glomerular filtration rate; RMSE, root-mean-square deviation.
From Stevens LA, Zhang Y, Schmid CH. Evaluating the performance of equations for estimating glomerular filtration rate. J Nephrol. 2008;21(6):797–807.

9.4 Clinical Conditions that Cause Errors in the Estimation of GFR from Measurement of Creatinine Clearance or Serum Creatinine

Condition	Effect on C_{cr}	Effect on P_{cr}	Comment
Plasma Ketosis	None	Increase	Interference with the picric acid assay for creatinine
Medications			
Certain cephalosporins or flucytosine	None	Increase	Interference with the picric acid and iminohydrolase assays for creatinine, respectively
Cimetidine or trimethoprim	Decrease	Increase	Inhibition of tubular secretion of creatinine
Dietary Protein			
Ingesting cooked meat	Increase	Increase	Transient increase in GFR and creatinine generation
Restriction of dietary protein	Decrease	Decrease	Sustained decrease in GFR and creatinine generation
Muscle Change			
Vigorous prolonged exercise	Decrease	Increase	Transient decrease in GFR and increase in muscle creatinine generation
Muscle wasting	None	Decrease	Decrease in muscle creatinine generation
Muscle growth	None	Decrease	Increase in muscle creatinine generation
Kidney Disease[a]	Increase	Decrease	Decrease in GFR, but stimulation of tubular secretion of creatinine, and possible decrease in creatinine generation

[a]Effects on C_{cr} and P_{cr} relative to effects on GFR (i.e., C_{cr} is higher than expected and P_{cr} is lower than expected for the reduction in GFR; see text).
C_{cr}, creatinine clearance; P_{cr}, serum creatinine; GFR, glomerular filtration rate.
From Levey AS. Clinical evaluation of renal function. In: Greenberg A, ed. Primer of Kidney Diseases. San Diego: Academic Press; 1998:23.

Kidney Handling of Creatinine

Creatinine is small (molecular weight 113 daltons, molecular radius 0.3 nm) and not bound to plasma proteins; hence, it passes freely through the glomerular capillary wall into the Bowman's space. However, it is also secreted by the tubules, probably by the same pathway used for other organic cations.[115] Therefore, creatinine is excreted not only by glomerular filtration, but also by tubular secretion.

$$U_{cr} \times V = GFR \times S_{cr} + TS_{cr} \quad (20)$$

where Scr is serum creatinine concentration (virtually identical to plasma concentration) and TScr is the rate of tubular secretion. Consequently, it is not an ideal filtration marker. The true relationship between creatinine clearance and GFR is as follows

$$C_{cr} = GFR + TS_{cr} / S_{cr} \quad (21)$$

where TS_{cr} / P_{cr} is the clearance of creatinine due to tubular secretion (C_{TScr}). Thus, at all levels of GFR, creatinine clearance exceeds GFR by an amount equal to the clearance of creatinine due to tubular secretion.

Tubular Secretion of Creatinine. Creatinine secretion was recognized long ago,[116] and has been reemphasized in the modern era.[117] It was not initially recognized as a limitation to the estimation of GFR from creatinine clearance; the major reason was related to the method of measurement of serum creatinine used in the past. As discussed later, the classical method, the Jaffé reaction, used a colorimetric reaction that detects both creatinine and a number of noncreatinine chromogens in serum, but not in urine. Thus, the serum "chromogen creatinine" exceeded the true serum creatinine measured by more accurate methods, and using the "chromogen creatinine" to calculate creatinine clearance led to a systematic underestimation of the true value. On the other hand, because of tubular secretion, the true creatinine clearance exceeded GFR. The net result was that estimated creatinine clearance deviated little from GFR in normal individuals. With the introduction of more accurate methods to measure serum creatinine, the discrepancy between creatinine clearance and GFR became more apparent.

Using older assays, the level of serum creatinine in the low range is overestimated, and average creatinine secretion in normal individuals accounted for 5% to 10% of the excreted creatinine. Hence, creatinine clearance exceeded GFR by approximately 10 mL/min/1.73 m^2. However, with the newer assays, normal serum levels are lower, so creatinine secretion can exceed GFR by much larger amounts. The magnitude of this overestimation has not been well quantified. Most studies find proportionately greater creatinine secretion in patients with reduced GFR, which leads to a clear disparity between creatinine clearance and GFR.[118] Moreover, the magnitude of creatinine secretion is variable among individuals and over time. Only some of the factors responsible for this variability are known. The level of GFR appears to be a major determinant.[117] The mean difference between C_{cr} and GFR (the clearance due to tubular secretion) within the range of GFR from 40 to 80 mL/min/1.73 m^2 is approximately 35 mL/min/1.73 m^2 and lower at lower GFR.

Other factors determining the magnitude of creatinine secretion are the type of kidney disease and the quantity of dietary protein intake. Patients with polycystic kidney disease and tubulointerstitial diseases have lower mean values for creatinine clearance due to secretion than patients with glomerular diseases and other diseases,[61] perhaps reflecting more serious tubular injury and limitation of tubular secretion. On the other hand, higher protein intake is associated with higher mean values for creatinine clearance due to secretion,[61] perhaps due to stimulation of secretion due to protein ingestion. This finding may account for the greater effect of protein loads on creatinine clearance compared to GFR.[46]

Several commonly used medications, including cimetidine and trimethoprim,[119] competitively inhibit creatinine secretion, thereby reducing creatinine clearance and raising the serum creatinine concentration, despite no effect on GFR. Clinically, it can be difficult to distinguish a rise in serum creatinine due to drug-induced inhibition of creatinine secretion from a decline in GFR. A clue to inhibition of creatinine secretion is that urea clearance and blood urea nitrogen concentration are unchanged.

Some investigators have proposed using cimetidine to inhibit creatinine secretion during creatinine clearance measurements, thereby permitting a more accurate assessment of GFR.[120,121] However, complete inhibition of creatinine secretion may require prolonged high dose cimetidine therapy.[122] Variable inhibition of tubular secretion by cimetidine makes interpretation of the test difficult.

Tubular Reabsorption of Creatinine. To a limited extent, creatinine may also be reabsorbed by the tubules. Studies in normal animals and humans with very low urine flow rates,[123–125] and in patients with decompensated congestive heart failure or uncontrolled diabetes mellitus[126–130] have demonstrated a ratio of clearances of creatinine and inulin <1.0. Reabsorption of creatinine may be due to its passive back-diffusion from the lumen to blood because of the high tubular creatinine concentration that occurs during low urine flow. Based on the clearance ratios observed in these studies, the maximum effect of creatinine reabsorption probably would be a 5% to 10% decrease in creatinine clearance.

Creatinine Metabolism

Generation. Creatinine is distributed throughout total body water. It is generated in muscle from the nonenzymatic conversion of creatine and phosphocreatine (Fig. 9.8).[131] Approximately 98% of the total creatine pool is contained in muscle and about 1.6% to 1.7% per day is converted to creatinine.[131] For example, in an individual with a total creatine pool of 100 g, creatinine generation would be 1.6 to 1.7 g per day. Thus, creatinine generation is proportional to muscle mass, which can be estimated from age, gender, and body size (Fig. 9.9).[132] Based on five reports containing data on 1,100 healthy individuals and patients without renal or hepatic disease, Walser derived the following equations to estimate urine creatinine excretion[133]:

$$eU_{cr} \times V = 28.2 - 0.172 \times \text{age (men)} \quad (22)$$

$$eU_{cr} \times V = 21.9 - 0.115 \times \text{age (women)} \quad (23)$$

where creatinine excretion (given in mg/kg/day) is assumed to equal creatinine generation and age is given in years. These equations do not take into account racial and ethnic differences in muscle mass. African American men and women have higher muscle mass and, consequently, higher creatinine excretion than their European American counterparts.[134–138]

Recently, Ix and colleagues derived equations in a pooled dataset of six studies of 2,466 black and white subjects with and without kidney disease and diabetes.[139] These equations were more accurate than those proposed by Walser and may be more generalizable.

$$\begin{aligned} eU_{cr} \times V = {} & 879.89 + 12.51 \times \text{weight (kg)} \\ & - 6.19 \times \text{age} + 34.51 \text{ (if black)} \\ & - 379.42 \text{ (if female)} \end{aligned} \quad (24)$$

$$\begin{aligned} eU_{cr} \times V = {} & 1115.89 + 11.97 \times \text{weight (kg)} \\ & - 5.83 \times \text{age} - 60.18 \\ & \times \text{phosphorus (mg/dl)} + 52.82 \\ & \text{(if black)} - 368.75 \text{ (if female)} \end{aligned} \quad (25)$$

The relationship of creatinine generation to age, gender, and body weight is affected by muscle mass and diet. Muscle wasting is associated with a decreased creatine pool, which leads to decreased creatinine generation and excretion.[140–143] However, some muscle diseases are associated with increased creatine turnover,[141] which in principle could transiently

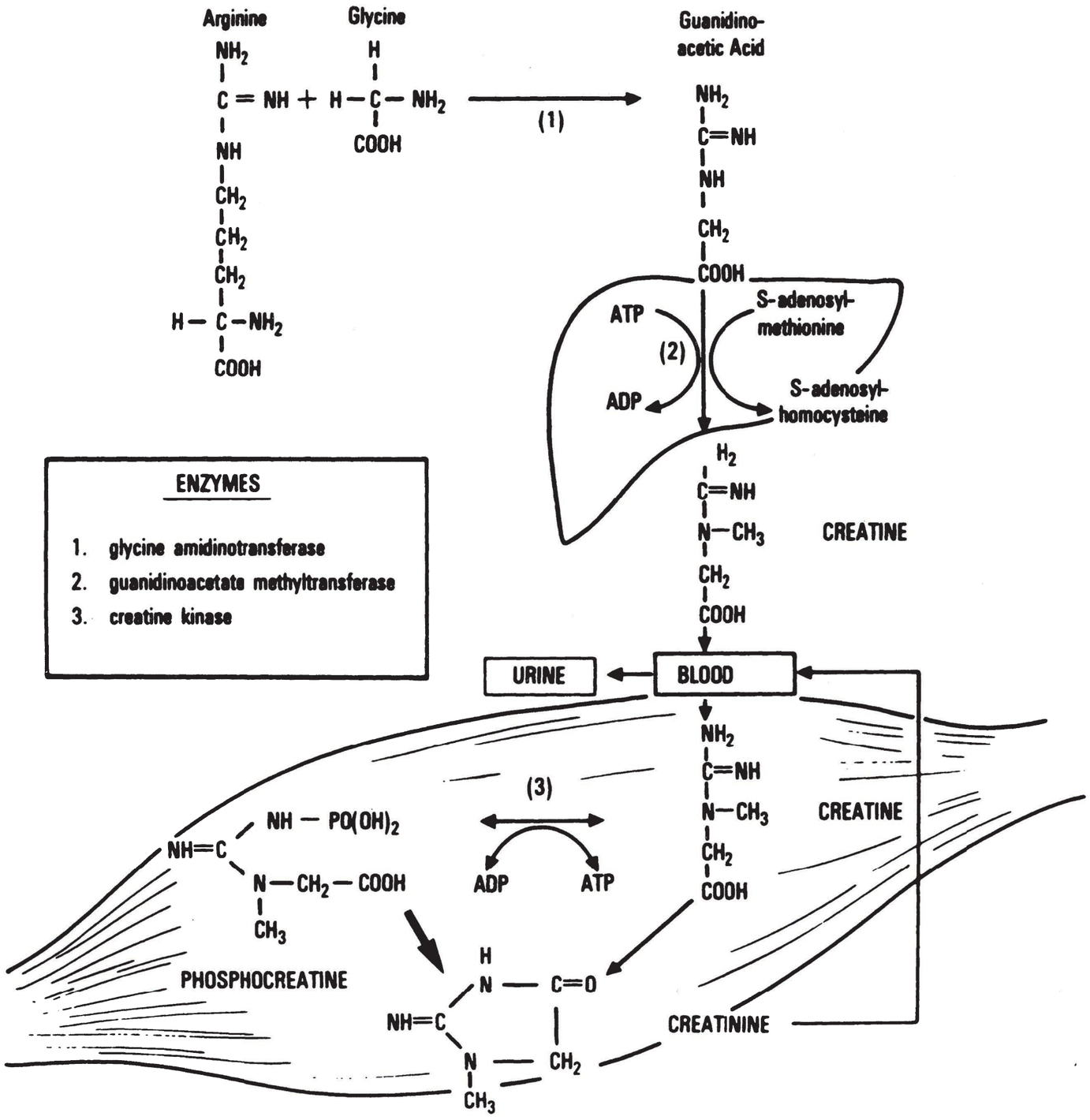


FIGURE 9.8 Pathways of creatinine metabolism. (From Heymsfield SB, Arteaga C, McManus C, et al. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr.* 1983;37:478, with permission.)

increase creatinine generation and excretion. Reduction in dietary protein causes a decrease in the creatine pool by 5% to 15%, which is probably due to the reduction of the availability of creatine precursors, arginine, and glycine.[131,144] Of greater importance is the effect of creatine in the diet. Creatine is contained largely in meat; uncooked lean beef contains about 3.5 to 5 mg of creatine per g.[145,146] Elimination of creatine from the diet decreases urinary creatinine excretion by as much as 30%.[144,147,148] Conversely, ingesting a creatine supplement increases the size of the creatine pool and increases creatinine excretion.[144,149–151] Meat intake also affects creatinine generation and excretion independent of its effect on the creatine pool. During cooking, a variable amount (18% to 65%) of the creatine in meat is converted to creatinine, which is absorbed from the gastrointestinal tract. Therefore, following ingestion of cooked meat, there is a sudden transient increase in the serum creatinine concentration and urinary creatinine excretion. These findings are not observed when a similar quantity of uncooked meat is ingested.[152,153]

Extrarenal Elimination. Extrarenal loss of creatinine is not detectable in normal individuals, but may account for up to 68% of daily creatinine generation in patients with severe decrease in GFR. One likely, but still not established, mechanism is degradation of creatinine within the intestinal lumen by microorganisms due to induction of the enzyme creatininase.[154–158]

Thus, in patients with kidney disease, creatinine excretion underestimates creatinine generation:

$$U_{cr} \times V = G_{cr} - E_{cr} \quad (26)$$

where E_{cr} is the rate of elimination of creatinine by extrarenal routes.

Measurement of Creatinine

Creatinine can be measured easily in serum, plasma, and urine and a variety of methods are used by clinical laboratories. The National Kidney Disease Education Program (NKDEP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have recently completed standardization of serum creatinine assays to minimize differences in clinical laboratories and facilitate more accurate reporting of estimated GFR.[114,159] The reference standard for creatinine assay is isotope dilution mass spectrometry (IDMS) using either gas or liquid chromatography.[114,160,161] All instruments can now be calibrated to standardized serum creatinine using secondary reference materials and proficiency testing programs.[162] Calibration does not eliminate the problem of interference by specific substances in serum with specific assays.[161]

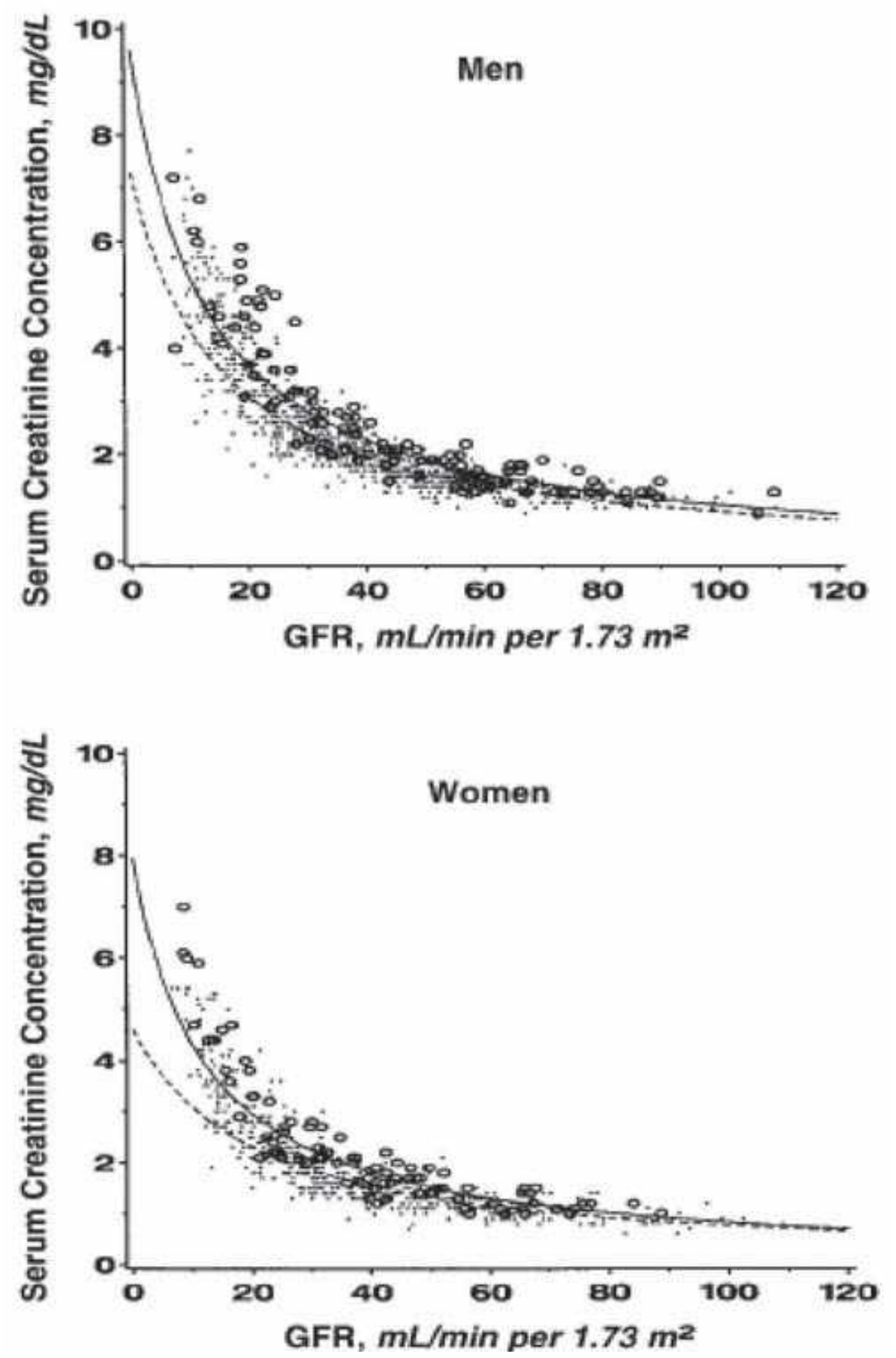


FIGURE 9.9 Relationship of serum creatinine concentration to measured glomerular filtration rate (GFR) in the Modification of Diet in Renal Disease Study. GFR was measured as the urinary clearance of ^{125}I-iothalamate. Serum creatinine concentration was measured using a Beckman Astra CX3 analyzer and a kinetic alkaline picrate assay.[33,47] Regression lines were computed from the relationship of reciprocal of serum creatinine versus GFR. When GFR is 60 mL/min/1.73 m^2, the 95% confidence interval for the serum creatinine concentration is 1.4 to 1.8 mg per dl for white men (n = 802) and 1.3 to 1.5 for African American men (n = 113) (*left panel*), and 1.1 to 1.4 mg per dl (97.2 and 123.8 μmol per L) for white women (n = 502) and 1.0 to 1.2 mg per dl (88.4 and 106.1 μmol per L) for African American women (n = 84) (*right panel*). These levels are close to the upper limit of the reference range. Confidence intervals for serum creatinine levels are wider at lower levels of GFR. (Reproduced with permission from Stevens LA, Coresh J, Greene T, et al. Assessing kidney function—measured and estimated glomerular filtration rate. *N Engl J Med*. 2006;354(23):2473–2483.)

The classic method uses the Jaffé reaction in which creatinine reacts directly with picrate ion under alkaline conditions to form a red-orange complex that is easily detected and quantified.[163] However, in normal subjects, up to 20% of the color reaction in serum or plasma is due to substances other than creatinine. Two classes of positive interferences have been described: substances such as glucose, ascorbate, and uric acid, which slowly reduce the alkaline picrate, and substances such as acetoacetate, pyruvate, other ketoacids, fluorescein, furosemide, hemoglobin, paraquat and diquat, and serum proteins which react with alkaline picrate to form colored complexes. The error in measurement can be greater, however, in diabetic ketoacidosis due to the increased concentration of acetoacetate, and in patients taking certain cephalosporins which can contribute to the colorimetric reaction. Very high serum bilirubin levels can cause falsely lower creatinine levels. In patients with kidney disease, noncreatinine chromogens are not retained to the same degree as creatinine. Consequently, the overestimation of serum creatinine and the corresponding underestimation of creatinine clearance are reduced. In general, noncreatinine chromogens are not present in sufficient concentration in urine to interfere with creatinine measurement. Hence, measurement of creatinine clearance in normal individuals using the Jaffé reaction results in values that are approximately 20% lower than the true value.

The kinetic alkaline picrate method takes advantage of the differential rate of color development for noncreatinine chromogens compared to creatinine. It significantly reduces, but does not eliminate, both types of positive interferences described earlier. A survey by the College of American Pathologists (CAP) in 2004 found that assays based on the alkaline picrate method were the most widely used in clinical laboratories in the United States.[162]

To circumvent interferences in the alkaline picrate reaction, other methods have been developed which are increasingly used by clinical laboratories. Enzymatic methods include the creatinine iminohydrolase and creatininase-creatinase-sarcosine oxidase methods. The antifungal agent, flucytosine, interferes with the creatinine iminohydrolase method, whereas bilirubin, dopamine, dobutamine, ascorbic acid, and sarcosine may interfere with the creatinase-creatininase methods. HPLC is a fairly sensitive and analytically specific method for measuring serum creatinine, but technically more difficult than enzymatic methods. Enzymatic and HPLC methods usually provide values that are 10% to 20% lower than kinetic alkaline picrate methods and are closer to the reference standard.

Serum Creatinine as an Index of Kidney Function

Based on substitutions and rearrangements of Equations 9.20 and 9.24, the relationship between GFR and serum creatinine is as follows:

$$GFR = (G_{cr} - E_{cr} - TS_{cr}) / S_{cr} \quad (27)$$

Estimating equations have been developed to estimate creatinine clearance[164–170] and GFR.[171–174] Most use age, sex, and body size as surrogates for creatinine generation. According to the June 2008 Chemistry Survey of the College of American Pathologists (CAP), 77% of clinical laboratories report eGFR when serum creatinine is measured.[114]

Due to its relative ease of use, one of the first estimating equations to be widely used is the Cockcroft and Gault formula.[164]

$$eC_{cr} = [140 - \text{age} \times \text{body weight}] \times 0.85 \text{ (if female)} / [S_{cr} \times 72] \quad (28)$$

where C_{cr} is expressed in mL per minute, age is expressed in years, body weight is expressed in kg, and S_{cr} is expressed in mg per dl. The formula was derived in 236 men (mean measured creatinine clearance of 73 mL per minute) in 1973. The formula for women was based on the assumption that creatinine generation is 15% less in women than in men. The Cockcroft and Gault formula was extensively validated before standardization of creatinine assays, but cannot be re-expressed for use with standardized creatinine assays. Use of standardized serum creatinine values in the Cockcroft and Gault equation leads to overestimates of creatinine clearance. Because measured creatinine clearance exceeds measured GFR, these overestimations may be particularly misleading.

Recent studies have developed equations to estimate GFR rather than creatinine clearance. The most commonly used equation is the Modification of Diet in Renal Disease (MDRD) Study.[132,175]

$$\text{eGFR} = 186 \times S_{cr} \text{ (mg per dl)}^{-1.154} \times \text{age (years)}^{-0.203} \times 0.742 \text{ (if female)} \times 1.210 \text{ (if black)} \quad (29)$$

where eGFR is expressed in mL/min/1.73 m^2, S_{cr} is expressed in mg per dl, and age in years. The MDRD Study equation has now been re-expressed for standardized serum creatinine as

$$\text{eGFR} = 175 \times \text{standardized } S_{cr} \text{ (mg per dl)}^{-1.154} \times \text{age (years)}^{-0.203} \times 0.742 \text{ (if female)} \times 1.210 \text{ (if black)} \quad (30)$$

The MDRD Study equation was developed in 1,628 patients with chronic kidney disease (mean GFR of 40 mL/min/1.73 m^2) who were predominantly white and had predominantly nondiabetic kidney disease. The equation was reported in 1999 and has been validated in African Americans with hypertensive nephrosclerosis, diabetic kidney disease, and kidney transplant recipients.[176] Inclusion of the race term significantly improved the prediction, which is likely because of the larger muscle mass in African Americans compared to whites. The MDRD Study equation is more accurate than the Cockcroft-Gault equation as well as measured urinary creatinine clearance. Its main disadvantage is a systemic underestimation of measured GFR and imprecision at higher values. Because of this, NKDEP recommends that eGFR >60 mL/min/1.73 m^2 using this equation not be reported as a numeric value.

In 2009, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) reported a more accurate equation using a large diverse dataset pooled from multiple studies. The development dataset included 8,254 individuals from 10 studies with a mean measured GFR of 68 mL/min/1.73 m^2. The validation dataset included 3,859 individuals from 16 additional studies with measured GFR.[177]

$$\text{eGFR} = 141 \times \min(\text{standardized } S_{cr}/\kappa, 1)^{\alpha} \times \max(\text{standardized } S_{cr}/\kappa, 1)^{1.209} \times 0.993^{\text{age}} \times 1.1018 \text{ (if female)} \times 1.159 \text{ (if black)} \quad (31)$$

where eGFR is expressed in mL/min/1.73 m^2, standardized serum creatinine is expressed as mg per dl, age is expressed in years, κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates the minimum of S_{cr}/κ or 1, and max indicates maximum of S_{cr}/κ or 1. The CKD-EPI equation uses the same variables as the MDRD Study equation, but includes a nonlinear term for serum creatinine that substantially reduces bias at higher GFR, enabling numeric eGFR reports throughout the range (Fig. 9.10). The main disadvantage is imprecision in the high range for eGFR. Most but not all studies confirm the greater accuracy of the CKD-EPI equation compared to the MDRD Study equation.[178–184] In addition, because of lesser bias, use of the CKD-EPI equation leads to lower prevalence estimates of decreased GFR in cross-sectional studies and more steep risk relationships of eGFR to adverse outcomes in longitudinal studies.[111]

Modifications to the MDRD Study and CKD-EPI equations have been proposed to account for racial, ethnic, and regional differences in diet and muscle mass.[185–187] Where these modifications lead to more accurate GFR estimations, it may be reasonable to substitute them for the MDRD Study and CKD-EPI equations, but it is not clear from the current literature whether these modifications truly reflect population differences in non-GFR determinants or methodologic differences, such as GFR measurement, serum creatinine assay, or subject selection.

Currently, most clinical laboratories report eGFR using the MDRD Study. In April 2011, large commercial clinical laboratories in the United States began to use the CKD-EPI equation and it is likely that it will be used more widely in the future. Only a small number of clinical laboratories in the United States report estimated creatinine clearance using the Cockcroft and Gault equation. However, since 1979, the U.S. Food and Drug Administration (FDA) has recommended the Cockcroft and Gault equation for pharmacokinetic studies used for drug development and labeling. For these reasons, drug dosing recommendations by pharmacists are generally based on estimated creatinine clearance computed using the Cockcroft and Gault rather than the MDRD Study or CKD-EPI

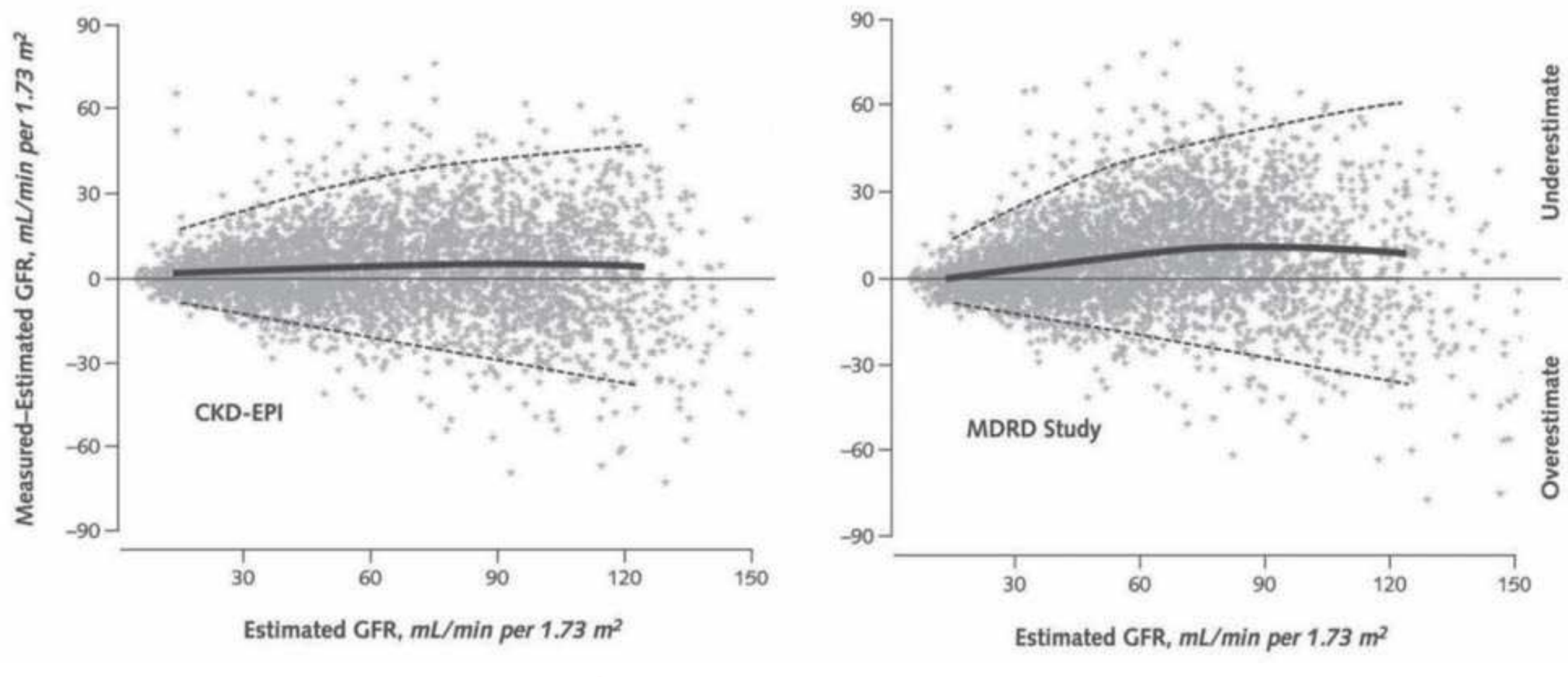


FIGURE 9.10 Comparison of performance of Modification of Diet in Renal Disease (MDRD) Study and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations by estimated glomerular filtration rate (GFR) in the external validation dataset. *Left*. Measured versus estimated GFR. *Right*. Difference between measured and estimated versus estimated GFR. Shown are smoothed regression line and 95% confidence interval (computed using the lowest smoothing function in R), using quantile regression, excluding lowest and highest 2.5% of estimated GFR values. To convert GFR from mL/min/1.73 m^2 to mL/s/m^2, multiply by 0.0167. (Reproduced with permission from Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604–612.)

equations.[188] One study shows a relatively high concordance in drug dosing recommendation using all three of the equations compared to measured GFR, and the NKDEP suggests using GFR estimates reported by clinical laboratories for drug dosing.[189,190] Further guidance by the FDA is needed.

In summary, there are limitations to the use of estimating equations based on the physiologic, analytical, and statistical principles described earlier.[106,108] Tables 9.4 and 9.5 list clinical situations in which estimating equations may not be accurate and clearance measurements may be indicated as a confirmatory test.[106]

9.5 Clinical Situations in Which Estimating Equations for Creatinine Clearance or Glomerular Filtration Rate Measurements May Not be Accurate and Clearance Measurements May be Recommended

- Extremes of age and body size
- Severe malnutrition or obesity
- Diseases of skeletal muscle
- Paraplegia or quadriplegia
- Vegetarian diet
- Rapidly changing kidney function
- Pregnancy
- Prior to dosing drugs with significant toxicity that are excreted by the kidneys

Urea as a Filtration Marker

A relationship between serum urea and kidney function was recognized long before the development of the concept of clearance of or techniques to assess GFR.[191] The factors influencing both the production of urea and its renal excretion, however, are considerably more complex and variable than those for creatinine (Table 9.6).[113] In the United States, urea is traditionally assayed as urea nitrogen. The usual concentration of serum urea nitrogen (for historical reasons, often referred to as the blood urea nitrogen, or BUN) in healthy young people is in the range of 8 to 12 mg per dl, but the reference ranges in clinical laboratories are wider to take into account variation among individuals. The urea clearance is rarely used today as a measure of kidney function, and the serum urea nitrogen concentration has been replaced largely by the serum creatinine concentration as an index of GFR in routine clinical practice. Nonetheless, measurement of the BUN remains useful both as a diagnostic aid in distinguishing among the various causes of acute decline in GFR and as a rough correlate of uremic symptoms in kidney failure. To understand the utility and shortcomings of BUN measurements, a brief summary of the kidney handling and metabolism of urea is presented subsequently.

Kidney Handling of Urea

Urea (molecular weight 60 daltons) is filtered freely by the glomerulus and reabsorbed in both the proximal and distal

9.6 Clinical Conditions that Cause Errors in the Estimation of Glomerular Filtration Rate from Measurement of Urea Clearance or Blood Urea Nitrogen

Condition	Effect on C_{urea}	Effect on BUN	Comment
Extracellular Volume			
Dehydration	Decrease	Increase	Increased urea reabsorption
Reduced renal perfusion (volume depletion, congestive heart failure)	Decrease	Increase	Reduced GFR, increased urea reabsorption, increased urea generation
Overhydration	Increase	Decrease	Reduced urea reabsorption
Increased renal perfusion (volume expansion, pregnancy, syndrome of inappropriate ADH secretion)	Increase	Decrease	Increased GFR, reduced urea reabsorption
Dietary Protein or Catabolism			
Restriction of dietary protein	Decrease	Decrease	Sustained decrease in GFR and reduced urea generation
Increased dietary protein	Increase	Increase	Sustained increase in GFR and increased urea generation
Accelerated catabolism (fever, trauma, GI bleeding, cell lysis, therapy with tetracycline or corticosteroids)	None	Increase	Increased urea generation
Liver Disease	Decrease[a]	Decrease[a]	Decreased GFR, decreased urea reabsorption, decreased urea generation
Kidney Disease	None[a]	Decrease[a]	Decreased GFR, no change in urea reabsorption, decreased urea generation (if dietary protein is restricted)

[a]Effects on C_{urea} and BUN relative to effects on GFR (i.e., C_{urea} is lower than expected for the reduction in GFR).
ADH, antidiuretic hormone; BUN, blood (serum) urea nitrogen; C_{urea}, urea clearance; GFR, glomerular filtration rate; GI, gastrointestinal.
From Levey AS. Clinical evaluation of renal function. In: Greenberg A, ed. Primer of Kidney Diseases. San Diego: Academic Press; 1998.

nephron. Hence, urea excretion ($U_{UN} \times V$) is determined by both the filtered load and tubular reabsorption (TR_{UN})

$$U_{UN} \times V = GFR \times BUN - TR_{UN} \quad (32)$$

Consequently, clearance of urea (or urea nitrogen, C_{UN}) is less than GFR

$$C_{UN} = GFR - TR_{UN} / BUN \quad (33)$$

A large fraction of the filtered load of urea is reabsorbed in the proximal convoluted tubule. In the medullary collecting duct, urea reabsorption is linked closely to water reabsorption. In the absence of antidiuretic hormone (diuresis), the medullary collecting duct is relatively impermeable to urea; thus, urea reabsorption is minimal. Conversely, in the presence of antidiuretic hormone (antidiuresis), permeability rises and urea reabsorption increases. In normal individuals, the ratio of urea clearance to GFR varies from as high as 0.65 during diuresis to as low as 0.35 during antidiuresis.

In patients with GFR less than 20 mL/min/1.73 m^2, the ratio of urea clearance to GFR is higher (0.7 to 0.9) and is not influenced greatly by the state of diuresis. Thus, urea clearance is approximately 5 mL per minute less than GFR. By coincidence, at this level of GFR, the difference between the values of GFR and urea clearance is similar to the difference between the values of creatinine clearance and GFR. Hence, the average of the clearances of urea and creatinine approximates the level of GFR.[172,173] This coincidence provides a relatively simple method to assess GFR in advanced renal disease. A single blood sample and 24-hour urine collection may be analyzed for creatinine and urea nitrogen

and the values for clearance may be averaged. However, the kidney handling of urea and creatinine is influenced by different physiologic and pathologic processes and may vary independently, causing deviations from this approximation.

Urea Metabolism

The metabolism of urea, its relationship to dietary protein intake, and the effect of renal insufficiency on protein metabolism are discussed in detail in Chapter 72. Briefly, urea is the end product of protein catabolism and is synthesized primarily by the liver. Approximately one quarter of synthesized urea is metabolized in the intestine to carbon dioxide and ammonia; thus, the ammonia that is generated returns to the liver and is reconverted to urea.

Dietary protein intake is the principal determinant of urea generation and may be estimated as follows:

$$EPI = 6.25 \times G_{UN} \quad (34)$$

where EPI is estimated protein intake, G_{UN} is urea generation, and both are measured in g per day.[192] Usual protein intake in the United States is approximately 100 g per day,[193–195] corresponding to a usual value for urea nitrogen generation of approximately 15 g per day.

In the steady state, urea generation can be estimated from the measurements of urea excretion, as shown below:

$$G_{UN} = U_{UN} \times V + 0.031 \times \text{weight} \quad (35)$$

where G_{UN} and $U_{UN} \times V$ are measured in g per day, weight is measured in kg, and 0.031 g/kg/day is a predicted value for nitrogen losses other than urine urea nitrogen.[196] For a 70-kg individual with a dietary protein intake of 100 g per day, urea excretion and other nitrogen losses would be approximately 13 and 2 g per day, respectively.

Measurement of Urea

The urease method assays the release of ammonia in serum or urine after reaction with the enzyme urease.[197] The presence of ammonium in reagents or use of ammonium heparin as an anticoagulant may falsely elevate the BUN, as can the drugs chloral hydrate, chlorbutanol, and guanethidine.[198] Urea is also subject to degradation by bacterial urease. Bacterial growth in urine samples can be inhibited by refrigerating the sample until measurement or by adding an acid to the collection container to maintain urine pH <4.0.

Blood Urea Nitrogen as an Index of Kidney Function and Protein Intake

In the steady state, the BUN level reflects the levels of urea clearance and generation.

$$BUN = G_{UN} / C_{UN} = U_{UN} \times V / C_{UN} \quad (36)$$

Consequently, many factors influence the level of BUN (Table 9.6). Nonetheless, the BUN can be a useful tool in some clinical circumstances.

As mentioned earlier, the state of diuresis has a large effect on urea reabsorption and a small effect on GFR, but does not affect creatinine secretion. Hence, the state of diuresis affects urea clearance more than creatinine clearance, and is reflected in the ratio of BUN to serum creatinine. The normal ratio of BUN to serum creatinine is approximately 10:1. In principle, a reduction in GFR without a change in the state of diuresis would not alter the ratio. However, conditions causing antidiuresis (dehydration or decreased kidney perfusion) would decrease GFR and increase urea reabsorption, thus raising the BUN-to-creatinine ratio. Consequently, the BUN-to-creatinine ratio is a useful aid in the differential diagnosis of acute GFR decline. Conversely, overhydration or increased renal perfusion would raise GFR and decrease urea reabsorption, thus lowering the serum creatinine and the BUN-to-creatinine ratio.

Also important is the well-recognized relationship of the level of renal function, the BUN level, and clinical features of uremia. A useful "rule" is that a BUN level greater than 100 mg per dl is associated with a higher risk of complications in both acute and chronic kidney failure and may indicate the need to initiate dialysis.[199,200] In both acute and chronic kidney disease, restriction of dietary protein intake to 40 to 50 g per day would reduce urea nitrogen excretion to approximately 4.5 g per day. Consequently, the BUN level might rise to only 40 to 60 mg per dl, despite severe reduction in GFR. Although protein restriction may temporarily ameliorate some of the uremic symptoms, severe reduction in GFR is associated with development of uremic symptoms despite only moderate elevation in BUN.

Urea generation and the BUN are also influenced by factors other than protein intake.[192] An increase is observed after the administration of corticosteroids, diuretics, or tetracyclines; after the absorption of blood from the gut; and in infection, renal failure, trauma, congestive heart failure, and sodium depletion. Decreases in urea generation and BUN may occur in severe malnutrition and liver disease. These conditions may also affect the BUN and the BUN-to-creatinine ratio.

Cystatin C as a Filtration Marker

Cystatin C has been proposed as an endogenous filtration marker. Assays for cystatin C are available in some countries in Europe but are not yet available in the United States. Research studies show that serum levels in healthy young adults are approximately 0.8 mg per L.[201] Studies in human subjects demonstrate a good correlation of serum cystatin C levels with GFR; typically better than that of serum creatinine levels alone, but equivalent or worse than serum creatinine adjusted for age, sex, and race.[202,203] A summary of issues related to its kidney handling, metabolism, measurement, and use as an index of GFR is presented subsequently. Table 9.7 lists the factors that influence the level of cystatin C.

9.7 Clinical Conditions that Cause Errors in the Estimation of Glomerular Filteation Rate from Measurement of Cystatin C

Condition	Effect on Cystatin C	Comment
Demographics		
Age	Decrease	
Male sex	Increase	
Race	No change	When tested in blacks vs. whites; finding has not been validated and requires testing in other racial groups
Cell turnover		
Inflammation	Increase	Seen in inflammatory conditions as indicated by WBC, CRP
Corticosteroids	Increase	
Hyperthyroid	Increase	
Hypothyroid	Decrease	
Diabetes	Increase	
Fat Mass	Increase	
Kidney Disease	Increase	Decreased GFR, suspect may be decrease in extrarenal elimination of cystatin C at low levels of GFR

CRP, C-reactive protein; GFR, glomerular filtration rate; WBC, white blood cell.

Kidney Handling of Cystatin C

Based on its small size (13 kD) and limited direct measurements in the rat, it appears that cystatin C is freely filtered.[204–211] It is then reabsorbed and catabolized by the renal tubules.[204–206] Urinary cystatin C is a marker of kidney damage; it is found in the urine of patients with tubulointerstitial kidney disease,[212] in particular in patients with acute kidney disease, and some glomerular diseases,[213] presumably due to impaired catabolism.[207,208] There is no evidence for tubular secretion,[210] whereas there is indirect evidence for extra-renal elimination of cystatin C in animal studies.[204,211] Direct evaluation of kidney handling in humans has not yet been performed.

Cystatin C Metabolism

Cystatin C is a nonglycosylated basic protein—its mRNA is found in every human tissue.[214] Molecular analysis of its promoter suggests that cystatin C is encoded on a "housekeeping" gene.[214,215] Indirect evidence suggests that there is variability in the generation rate, in particular with states associated with higher or lower cell turnover, such as hyperthyroid or hypothyroid states,[216] or steroid use.[217–220] Epidemiologic studies have suggested that non-GFR determinants of cystatin C—age, sex, body mass index, diabetes, white blood cell count, albumin, and C-reactive protein—were significantly related to higher levels of cystatin C,[202,221] whereas other studies have not shown a relationship to inflammation[222] or diet.[223]

Measurement of Cystatin C

There are several commercially available autoanalyzers to assay cystatin C. At present, methods use nephelometric, turbidimetric, or enzyme-linked immunosorbent assay (ELISA) methods. Despite high precision and reproducibility of the assays, there are large differences among them.[224–226] One study has compared two turbidimetric and one nephelometric cystatin C assay and showed large variation when the assays are used with patient samples, but not when control samples were used, suggesting interference of the assays with substances found in patient samples.[225] Other studies have shown large within and between laboratory variations even for the same assay.[225,227] Variations in the assay would lead to inaccurate GFR estimates. Recently, the International Federation of Clinical Chemists (IFCC) made available a reference material for cystatin C that will allow for standardization of the assays across platforms.[228] At present, the reference materials are not yet FDA approved, and so likely standardization of the commercial platforms will not be uniform in the United States until at least 2015.

Cystatin C as an Index of Kidney Function

Based on these considerations the relationship of GFR to serum levels of cystatin would be as follows:

$$GFR = G_{cys} / S_{cys} \quad (37)$$

Several factors could influence the level of cystatin C independent from the GFR, leading to errors in estimation of GFR (Table 9.7).

Multiple studies have compared serum cystatin C and creatinine as filtration markers in the general population,[182] in those with CKD,[202,229,230] and in special populations with reduced muscle mass[203,231–234] where cystatin C is hypothesized to have a particular advantage and results are mixed. In general, when the two analytes are compared alone, cystatin C appears to be a better filtration marker. When compared

to GFR estimates based on serum creatinine adjusted for age, sex, and race, there is no clear advantage of cystatin C. These results do not suggest that cystatin C should replace creatinine or that there are specific populations in which cystatin C should be used. In combination, creatinine and cystatin C result in a more precise estimate of GFR than either marker alone.[202,235–240] In acute GFR decline, studies in animals and in humans demonstrate that cystatin C increases prior to serum creatinine, and has been interpreted as a more sensitive marker; however, few studies have compared changes in cystatin C to changes in measured GFR.[241]

In contrast to the data on cystatin C as a marker of GFR, the data on cystatin C as a prognostic marker show that it provides consistently better information than creatinine or creatinine-based estimating equations.[241] It is not known whether this improvement is because cystatin C is indeed a better marker of kidney function in these study populations or because non-GFR determinants of cystatin C are also associated with adverse outcomes, as described previously.

PROTEINURIA

The plasma filtered by the kidneys each day contains approximately 11,000 to 14,000 g of protein, yet the final urine is virtually protein-free due to selectivity of glomerular filtration. This conservation of essential proteins is necessary for oncotic regulation, for immune protection, for normal coagulation, and for a host of other vital processes.

An increased protein excretion rate (proteinuria) is usually due to kidney disease, and most kidney diseases are associated with some degree of proteinuria. Proteinuria does not generally cause clinical signs or symptoms. An exception is the nephrotic syndrome, characterized by loss of proteinuria sufficient to cause hypoalbuminemia, edema, and hypercholesterolemia (usually >3.5 g per day). The detection and evaluation of lesser quantities of proteinuria has gained additional significance years as multiple studies have demonstrated its diagnostic and prognostic importance. It has long been known that the degree of proteinuria is a risk factor for kidney disease progression. It has now been shown that the presence of even mildly increased amounts of protein in the urine serves as an independent risk marker for cardiovascular disease and death, independent of other risk factors such as diabetes, hypertension, or advancing age. Recent experimental and clinical studies also suggest an important role for proteinuria in the pathogenesis of the progression of kidney disease.[242] The physiology of protein handling by the kidney and the pathophysiology of proteinuria are extensively covered elsewhere in this book.

This section considers (1) mechanisms by which the kidney handles proteins, (2) methods to measure urine protein, (3) patterns of proteinuria, and (4) clinical interpretation of proteinuria. For several reasons, clinical terminology is slowly changing to focus on albuminuria rather than proteinuria. Albumin is the principal component of urinary protein in most kidney diseases. Recent recommendations for measurement of urine proteins emphasize quantification of albuminuria rather than total protein[1,243,244]; recent epidemiologic data demonstrate a strong graded relationship of the quantity of urine albumin with both kidney and cardiovascular disease risk[36,245–247]; and a recent international conference suggested classification of kidney disease by albuminuria in addition to GFR.[3] In this chapter we will refer to proteinuria when discussing general concepts and will refer either to total protein, albumin, or other specific proteins when discussing measurements, patterns, and interpretation of proteinuria.

Protein Handling by the Kidney

In healthy individuals, the daily urinary protein excretion averages 40 to 80 mg, and the upper limit of normal ranges from 75 to 150 mg. Urine protein is a mixture of plasma proteins that cross the filtration barrier and other proteins that originate in the tubules and lower urinary tract. Of the total, albumin constitutes 30% to 40%, immunoglobulin G (IgG) 5% to 10%, light chains 5%, and IgA 3%. Tamm-Horsfall protein (THP), also known as uromodulin, is a glycoprotein not found in plasma[248,249] and is the most abundant protein in normal human urine and constitutes the remainder.[250] Large molecules, such as IgD and IgM, normally are not detected in the urine.[248,251]

The handling of plasma proteins by the kidney is complex, but consists of two major components: the permeability of the glomerular filter to plasma proteins and the tubular metabolism of filtered proteins. For a detailed review of these mechanisms, the reader is referred to Chapters 72 and 73.

Urine Proteins of Plasma Origin

Low Molecular Weight Proteins. Low molecular weight proteins (less than 25,000 daltons or radius less than 2.3 nm) are extensively filtered by the glomeruli, taken up by the tubules, and subsequently handled by proximal tubular degradation.[252] Biologically important low molecular weight proteins handled by the kidney include enzymes (lysozyme and ribonuclease), immunoglobulins (light chains and beta-2 microglobulin), fibrin-fibrinogen degradation products, and hormones (insulin, growth hormone, and parathyroid hormone). The tubular concentration of these proteins ranges from 50% to 90% of their plasma concentrations (Table 9.8). Low molecular weight proteins are small enough that their charge plays only a minor role in their filtration.

Despite the significant amount of low molecular weight protein that is filtered, only minimal quantities appear in the urine. The proteins are taken up in the proximal tubule and hydrolyzed into amino acids by the vacuolar-lysosomal system. Small amounts of these proteins are actually reabsorbed intact.[253–255] The tubular capacity for some of these low molecular weight proteins is significantly greater than the filtered load. For example, when purified lysozyme was given in an isolated perfused rat kidney, lysozyme did not appear in the urine until the filtered load was increased

9.8 Handling of Plasma Proteins by the Kidney

Protein	Molecular weight (daltons)	Approximate Stokes-Einstein radius (nm)	Approximate ratio of glomerular filtrate to plasma concentrations
Inulin (for reference)	5,200	1.4	1.0
Insulin	6,000	1.6	0.9
Lysozyme	14,600	1.9	0.75
Myoglobulin	16,900	1.9	0.75
Parathyroid hormone (cow)	9,000	2.1	0.65
Growth hormone (rat)	20,000	2.1	0.6–0.7
Light chains	44,000	2.8	0.09[a]
Amylase	48,000	2.9	0.02
Albumin	69,000	3.6	0.02
Gamma globulin	160,000	5.5	0.0
Ferritin	480,000	6.1	0.02

[a]Can be as high as 0.45 if light-chain monomers predominate over dimers in plasma.
From Kanwar YS. Biology of disease: biophysiology of glomerular filtration and proteinuria. Lab Invest. 1984;51:7. Maack T, Johnson B, Kau ST, et al. Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. Kidney Int. 1979;16:251.

nearly threefold.[253] Through the process of filtration, tubular absorption, and excretion, the kidney accounts for between 30% and 80% of the metabolic clearance of low molecular weight proteins.[253]

Immunoglobulin light chains are handled in a similar manner. The monomer (molecular weight 22,000 daltons) is filtered freely and then degraded by the tubules with small amounts appearing in the urine. In contrast, the dimer (molecular weight 44,000 daltons, radius 2.8 nm) is restricted with only approximately 10% filtered. Horseradish peroxidase, a neutral tracer molecule of similar weight and size to light chains, is handled in a similar manner.[256,257]

Albumin is the principal plasma protein and has a molecular weight of 69,000 and radius of 3.6 nm. Under normal situations, any significant amount of albumin is prevented from entering the urine space by the glomerular permselectivity barrier. However, under certain conditions such as reduced glomerular plasma flow, albumin passes into the urine, demonstrating that size selectivity alone is not sufficient to restrict the filtration of albumin. Rather, it appears that the negative charge on the various structures of the glomerular barrier contributes significantly to restricting the filtration of albumin. Of these structures, the negative charge on the basement membrane has been considered the major obstacle to albumin crossing the glomerular capillary wall.[257–263]

Changes in filtered albumin sufficient to account for heavy proteinuria have been documented by micropuncture studies in experimental nephrotic syndrome and by indirect studies in humans. In rats with aminonucleoside nephrosis, the increase in filtered albumin accounts entirely for the increase in protein excretion.[264,265] In fact, in animals with either aminonucleoside nephritis or nephrotoxic nephritis, the proximal tubular albumin concentration is increased 8- to 12-fold.[265,266] These animals may excrete 100 to 400 times as much albumin as controls.[265,266] In humans, clearance studies have provided indirect evidence of increased filtration of protein; in patients with nephrotic syndrome, the minimal protein concentration in the glomerular filtrate (calculated by correcting the urine protein concentration by the fraction of water reabsorbed) far exceeds the concentration of filtered protein observed in the proximal tubular fluid in normal animals.[267] Furthermore, urine albumin excretion is linearly related to plasma albumin concentration when the latter is increased by infusion.[267,268] Such a relationship is characteristic of substances excreted mainly by glomerular filtration.[15]

Although increased filtration of protein appears to be the major factor leading to proteinuria, the specific defects in the capillary wall that are responsible for the protein loss are not completely defined. New information regarding the

major role of the podocyte and its slit diaphragm in many proteinuric diseases has become available from multiple studies.[269–272]

Filtered albumin is also absorbed and catabolized by the proximal tubule with little or no reabsorption of intact albumin. As in the case of low molecular weight proteins, there is excess capacity above normal, but this pathway is saturable.[273] This explanation of albumin handling by the kidney has been challenged lately, with the suggestion that albumin is, in fact, highly filterable and not impeded by charge, but handled largely by tubular reabsorption.[274] Rebuttals against this theory have been effective, including new insights regarding the importance of the podocyte slit diaphragm in genetic causes of proteinuria and the molecular mechanisms of albumin transport in the proximal tubule. Presently glomerular size and charge selectivity are still considered the main barriers to albuminuria.[275,276] For a detailed discussion regarding the pathophysiology of glomerular proteinuria, the reader is directed to Chapter 45.

Large Plasma Proteins. Large molecular weight plasma proteins are restrained from crossing the glomerular barrier. Proteins such as globulins (molecular weight 160,000 daltons, radius, 5.5 nm) are undoubtedly restricted by the basement membrane, but the contribution of the endothelial fenestrae is uncertain. A tiny fractional clearance of large plasma weight proteins has been established and animal studies suggest that this is due to the presence of rare, very large pores in the glomerular ultrafiltration barrier.[277] Changes in glomerular plasma flow do not alter the restriction of these molecules from the urine space.

Proteins in Urine Not of Plasma Origin

The major protein in normal human urine that has no counterpart in plasma is THP, a glycoprotein with a molecular weight of 7 million.[250,278,279] It is excreted in amounts of 20 to 100 mg per day.[280,281] Immunofluorescent staining techniques in human kidneys have demonstrated that THP is confined to the cells lining the thick ascending limb of Henle's loop and the most proximal part of the distal convoluted tubule, which strongly suggests that these cells are the source of the THP in the urine.[282,283] THP is the major protein component of urinary casts.[282,284] Excretion of this protein increases only slightly in patients with nephrotic syndrome, and its excretion rate does not appear to be related quantitatively either to the number of casts or to the degree of proteinuria.[284] In vitro studies indicate that the addition of albumin to THP-containing solutions leads to precipitation of THP,[284] which suggests that increased albumin excretion may lead to precipitation of THP in the tubules causing cast formation. The structure and function of this unusual glycoprotein has been extensively reviewed.[250] Mutations in the gene coding for THP are associated with rare hereditary tubulointerstitial kidney diseases, and in large populations, genetic variation is associated with CKD.[285,286]

In addition to THP, many other discrete proteins unrelated to plasma proteins have been identified in trace amounts in urine.[278] These proteins presumably originate in the lower urinary tract and prostate gland. Endothelin, the potent endogenous vasoconstrictor peptide, is produced by renal epithelial cell lines in vitro, appears in human urine, and may serve as a nonspecific marker of kidney damage.[287] In fact, intense efforts are underway to identify urinary proteins that may signal acute or chronic kidney damage.[288]

Measurement of Urine Protein

Urine protein excretion is routinely measured to detect, evaluate, and manage kidney disease. Historically, total urine protein was considered the preferred measure of proteinuria because of the simplicity of the assays. However, due to lack of a gold standard for total urine protein, and due to the evidence that albumin excretion rises substantially before total urine protein becomes abnormal, there has been a shift of emphasis to measurement of albuminuria. Nonetheless, total urine protein is still widely measured using a variety of methods (Table 9.9).[289]

The simplest and most widely used methods are semiquantitative tests done on random urine samples. Although these tests are extremely useful in screening for proteinuria, they detect an abnormal concentration of total urine protein, not an abnormal excretion rate. Therefore, they might be positive in patients with low urine volume even if the excretion rate is normal, and they may be negative in patients with high urine volume even if the excretion rate is elevated. For more definitive evaluation and management of patients with proteinuria, quantitative protein analysis must be undertaken in timed urine collections. A number of different methods are available for assay of specific proteins.[290] Semiautomated, two-dimensional, electrophoretic systems, which employ ultrathin gels, combined with silver staining, allow the detection of a host of specific urinary proteins on a routine basis.[291,292] These techniques also improve the characterization of urinary proteins with molecular weights less than 70,000.[293] Specific immunoassays are available for detection of individual proteins within the urine, as described subsequently for albumin. Additionally, broad descriptions of patterns of urinary protein excretion have become possible by proteomic techniques.[294]

Semiquantitative Tests for Total Urine Protein

Semiquantitative tests for urinary protein involve either precipitation of protein or protein-induced color changes of an indicator dye on a dipstick. The precipitation tests may be performed by adding either 5% sulfosalicylic acid or concentrated nitric acid to an aliquot of urine or by heating the urine and adding glacial acetic acid.[289] With these methods, the quantity of precipitate is graded from 0 (no precipitate) to 4+ (heavy gelatinous precipitate). Urine samples with a protein concentration as low as 5 to 10 mg per dl will give a positive reaction with the acetic acid precipitation test,

9.9 Definitions of Proteinuria and Albuminuria

	Urine Collection Method	Name (units)	Interpretation of Results: Normal to High Normal	High	Very High
Albumin	24-hour collection	AER (mg/day)	<30	30–300	>300
	Spot urine albumin-to-creatinine ratio	ACR (mg/g)	<30	30–300	>300
Total Protein	24-hour collection	PER (mg/day)	<150	150–499	>500
	Spot urine	PCR (mg/mmol, mg/g)	<150	150–499	>500
	Spot urine	Protein dipstick	negative to trace	trace to 1+	>1+

To convert from mg/g creatinine to mg/mmol of creatinine multiply by 0.113.
ACR; albumin/creatinine ratio; AER, albumin excretion rate; PCR, protein/creatinine ratio; PER, protein excretion rate.

but radiopaque contrast materials, tolbutamide, or large amounts of penicillin, nafcillin, or oxacillin may produce a false-positive reaction.[289,295,296]

The dipstick test for protein (also see Urinalysis section) utilizes a paper strip impregnated with a pH indicator dye (tetrabromophenol blue) buffered to maintain the pH in the paper at 3.0. The test is based on the capacity of proteins to change the color of tetrabromophenol, and is more sensitive to albumin than other proteins.[289] The degree of color change is roughly proportional to the amount of protein present, with the color varying from yellow, with low protein concentrations, to blue, with high protein concentrations. A color comparison chart is provided with the dipstick that contains a scale of protein concentrations as well as a 0 to 3 or 4+ rating. It should be noted that the correlation between color change and actual protein concentration is only approximate. In one study, for example, comparison with quantitative methods indicated agreement only 60% to 70% of the time.[297] The use of the dipstick test is further restricted by the finding of substantial interobserver variation between technicians in interpretation of the results,[298,299] which can be improved by semiautomated and automated reading devices.[300] Additionally, different brands of dipsticks may have different performance characteristics.

The dipstick method has the advantage that it is not affected by urine turbidity, radiopaque material, or drugs.[289,296] It can give a false-positive value in highly buffered alkaline urine, but such samples are encountered rarely. The major fault of the dipstick test is its insensitivity. Although dipstick tests can detect protein concentrations as low as 6 to 15 mg per dl,[298] it is only protein concentrations of 30 mg per dl and above that are detected with certainty. Below this level, the test is negative or trace positive in over half the samples tested.[297,301] In a patient excreting 300 mg of protein per day in a total volume of 1,500 mL, the protein concentration is only 20 mg per dl, and this concentration may not be detected using the dipstick method. Also, the test is insensitive to light chains and can give a negative reaction even when the excretion of this protein is moderately high.[302–304] In selected populations, dipstick screening for proteinuria carries a high risk for false-positive and -negative results with a sensitivity of less than 67% and specificity of 74%.[305]

Quantitative Tests for Total Urine Protein

As discussed previously, the major limitation to tests for total urine protein is the absence of an absolute gold standard due to the presence of many different types of protein in the urine in health and disease. Thus, it is not possible to entirely standardize measurements across laboratories or to determine precise cut-off values for the definition of normal or various diseases. Despite this limitation, a large number of tests are available.

Excretion in Timed Collection. Quantitative methods for measuring protein excretion have been traditionally based on precipitation of protein, usually accomplished using trichloroacetic acid or sulfosalicylic acid. Presently, these methods have been largely replaced by precipitation with other agents, such as benzethonium chloride or benzalkonium chloride, or by colorimetric methods employing automated dye binding assays, using pyrogallol red or pyrocatechol violet dyes.[306,307]

In the precipitation methods, the denaturing substance is added to an aliquot of urine, and the turbidity, measured with a photometer or nephelometer, is compared to standards prepared by the addition of known amounts of

protein to urine. The dye binding assays use a photometer to measure absorbance at a given wavelength of color.[308] These methods remain only roughly quantitative, however, because they have a CV as large as 20%.[289,309] Light chains (Bence Jones protein), however, are effectively measured by these methods, although the precision of measurement is poor compared to pheresis and ELISA. Relative insensitivity to globulins has been reduced by the use of TCA or other precipitating agents, but still is an issue.[309] Iodinated contrast material can falsely elevate the turbidity regardless of agent, and it is best to wait 24 hours after contrast to determine protein excretion rates.[310] With all of these tests, the protein concentration is multiplied by the total volume of the sample and result reported in milligrams or grams per unit of time (usually 24 hours).

Excretion in an Untimed Collection ("Spot Urine Sample"). Twenty-four-hour protein excretion can be easily approximated by measurement of both protein and creatinine in a random urine specimen. Because the excretion of both creatinine and protein is fairly stable throughout the day, if the daily creatinine excretion is known, the ratio of the concentrations of protein and creatinine in a random urine specimen provides an estimate of the daily protein excretion (Fig. 9.11).[311–313] In most circumstances, however, urine creatinine excretion is not known, but is assumed to be 1.0 g per day. However, as discussed before, in the steady state, creatinine excretion is a reflection of creatinine generation, which is affected by age, sex, race, and body size and changes in GFR. Thus, the protein-to-creatinine ratio may differ substantially from protein excretion rate, especially in the non–steady state. Nonetheless, within populations, correlations of protein-creatinine ratio with protein excretion rate are moderate to high and associations of protein-creatinine ratio with disease outcomes are strong. Consequently, this test is now widely used as a first quantitative test, with confirmation using a timed urine collection if necessary.

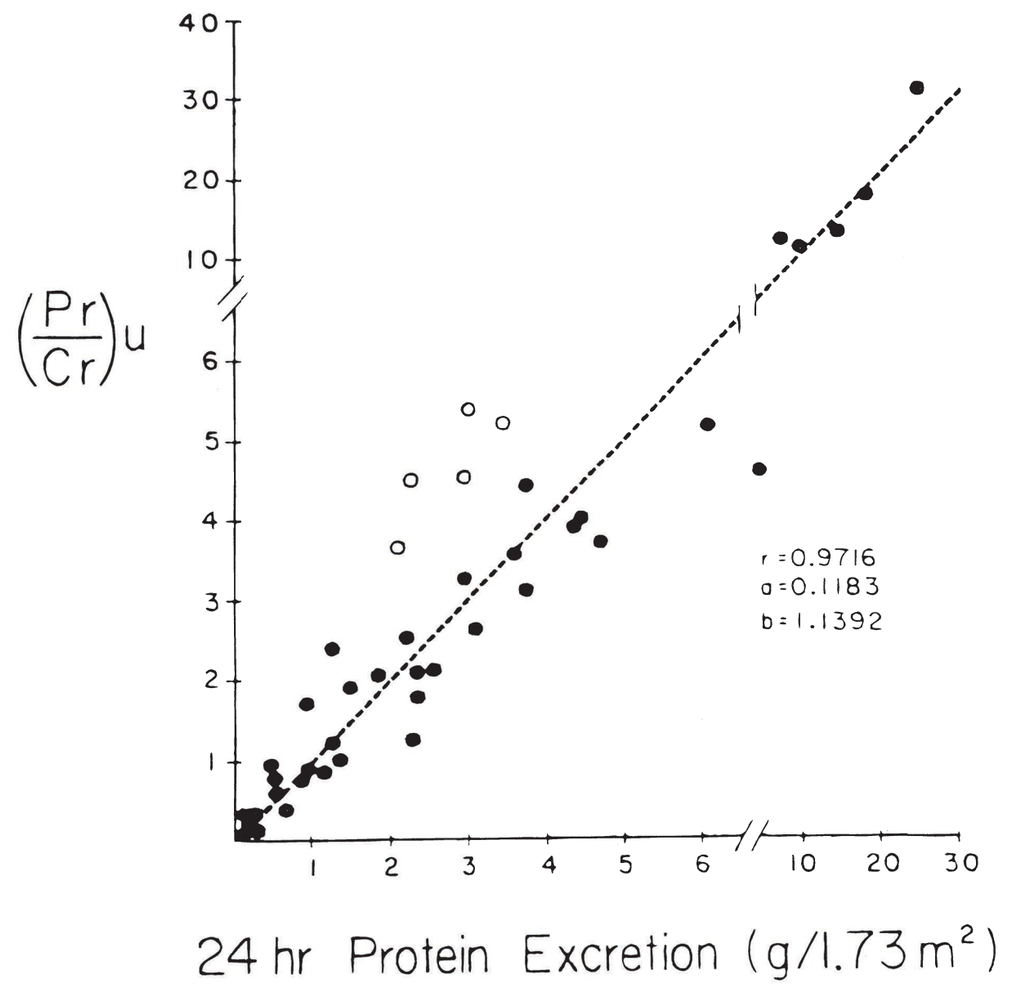


FIGURE 9.11 Ratio of urinary protein to creatinine concentration (Pr/Cr) of random single voided urine samples expressed as a function of protein excretion per 24 hours per 1.73 m^2. (From Ginsberg JM, Chang BS, Matarese RA, et al. Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med.* 1983;309:1543, with permission.)

Recently, dipsticks have become more widely available as an alternative method to measure the urine protein-to-creatinine ratio with initial results suggesting excellent reasonable correlation with standard measures for screening.[314,315]

It has also been demonstrated that the protein-osmolality ratio in a random urine sample may reliably predict protein excretion rates. This ratio was successful in screening for abnormal proteinuria in normal and proteinuric populations with a sensitivity of 96% and a specificity of 93%, superior to routine dipstick performance and equal to the protein-creatinine ratio.[316] Adequate validation studies have not been performed to date, and this test cannot yet be recommended in place of the protein-creatinine ratio.

Clinical practice guidelines by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) recommend measurement of total urine protein in adults using spot urine protein-to-creatinine ratios and expressing the results as total protein in milligrams per creatinine in gram. The normal value varies with the laboratory, but is approximately <200 mg per g (Table 9.9).

Tests for Specific Proteins

Albumin. Specific radioimmunoassay is the standard test for detecting and quantitating albumin concentrations, although turbidimetric assays can be used with similar precision.[317] HPLC techniques allow for even more accurate and early detection of abnormal albumin excretion rates but are not widely available and also have limitations in reproducibility.[318] Many different screening tests are also available as qualitative screens with varying ability to detect albuminuria in the normal and above normal range.[319–322]

Albumin excretion in timed urine samples is considered the gold standard for classification of albuminuria. Given the wide variability in urinary albumin excretion at the low range, several urine samples should be tested to classify albumin as high.[323] The albumin-to-creatinine ratio in a spot urine sample is widely used as a first test for albuminuria, with high correlation with albumin excretion rate,[324,325] but is subject to the same limitations as the protein-to-creatinine ratio. Moreover, spot samples for albumin concentration or for albumin-to-creatinine ratio are associated with a strong graded relationship with adverse outcomes in the general population and populations at increased risk for cardiovascular and kidney diseases, with increased risk detectable at levels greater than 10 mg per g.[3,247,326] Some but not all

recent studies suggest optimal prediction of outcome with albumin-to-creatinine ratio (tested in first morning samples) rather than other measures of proteinuria.[327,328] NKF KDOQI, Kidney Disease: Improving Global Outcomes (KDIGO), and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) National Kidney Disease Education Program (NKDEP) all recommend spot urine albumin-to-creatinine ratio for detecting and following chronic kidney disease in adults (Table 9.9).[1,243,329–331]

Dipsticks are also available to measure the albumin-to-creatinine ratio[332] but validation studies remain mixed in terms of their sensitivity and specificity,[333] although performance of these dipsticks are improved by adjustment for specific gravity.[334] Presently, they may be most useful for screening high-risk populations with quantification better served by laboratory testing.

Immunoglobulin Light Chains. Identification of immunoglobulin fragments in the urine is useful in establishing the diagnosis of multiple myeloma and other monoclonal gammopathies. Light chains are often sought by the traditional Bence Jones test, a method that depends on the unusual solubility characteristics of these proteins. When the urine is heated to 45°C to 55°C, light chains precipitate, particularly when the pH is brought to 4.9 by the addition of an acetate buffer.[335] When the urine is then brought to a boil, the precipitated light chains redissolve partially or completely. This test is difficult to carry out properly and can be rather insensitive. It is positive only when the concentration of light chains exceeds 800 to 1,600 mg per L,[336,337] and even in the presence of such concentrations, it may still be falsely negative.[304,336,338] As noted earlier, the semiquantitative dipstick test also may be negative when light chains are present in the urine due to the insensitivity of the indicator dye to globulins.[302,303] By far the most sensitive tests for detection of light chains and other immunoglobulin fragments are routine electrophoresis and immunoelectrophoresis. In the presence of light chains, routine electrophoresis discloses a monoclonal peak, and immunoelectrophoresis (of concentrated urine) makes it possible to accurately identify the specific protein, even at low concentrations.

Patterns of Proteinuria

Proteinuria can be classified according to its pathophysiology into three major groups: glomerular proteinuria, tubular proteinuria, and overproduction proteinuria.

Glomerular Proteinuria

Glomerular proteinuria is defined as proteinuria due to increased permselectivity of the glomerular filtration barrier to plasma proteins. Therefore, the hallmark of glomerular proteinuria is albuminuria (Fig. 9.12). Albuminuria may be a transient phenomenon in normal individuals without kidney disease. However, persistent albuminuria (albumin-creatinine ratio >30 mg per g for 3 or more months)

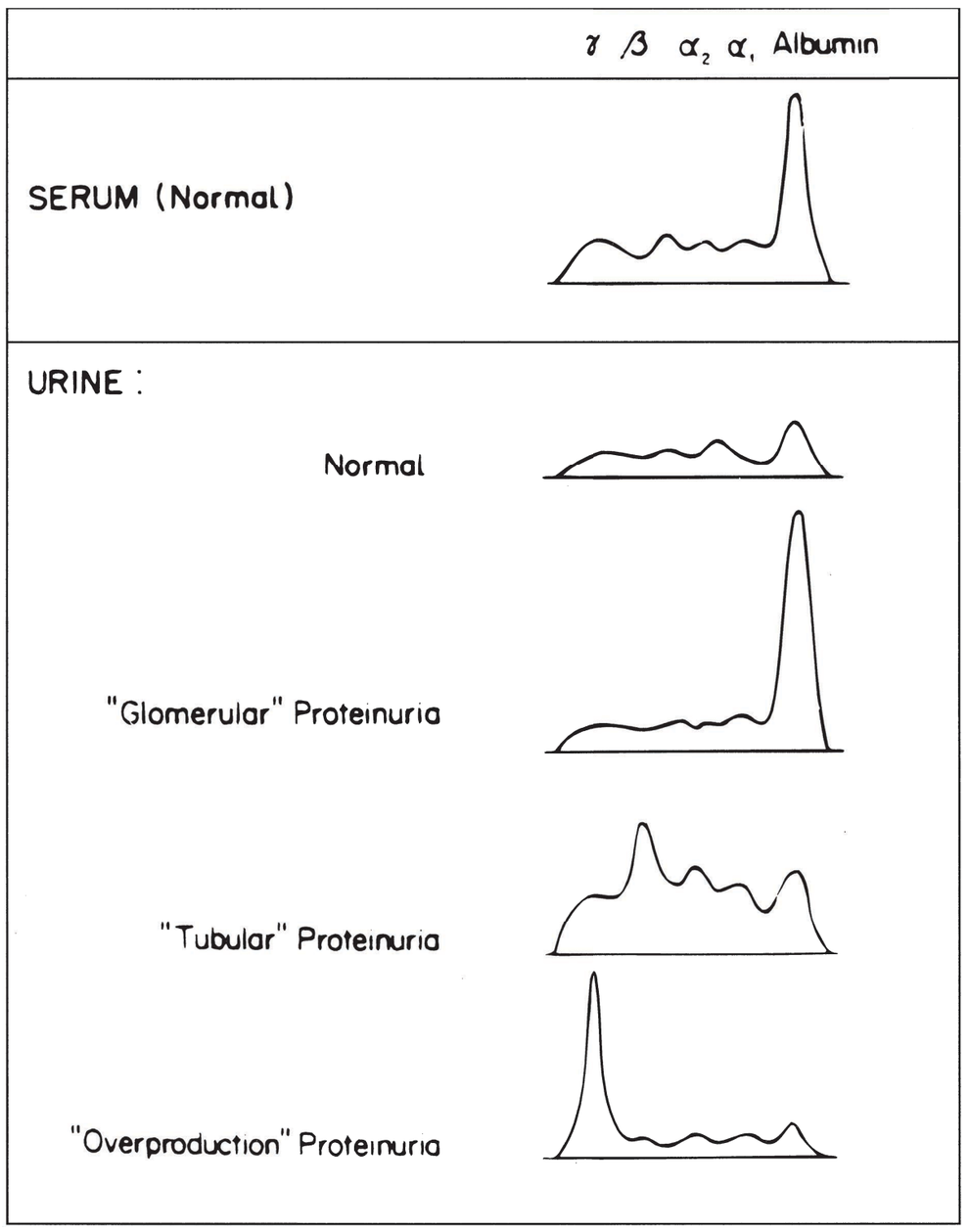


FIGURE 9.12 Electrophoretic patterns of normal serum, normal urine, and urine with three types of abnormal protein excretion. The electrophoresis pattern shown for overproduction proteinuria is taken from a patient with multiple myeloma.

indicates the presence of kidney disease, even when kidney function is normal, when the urine sediment contains no abnormalities, and when the patient has no signs or symptoms of kidney disease.[339–341] Indeed, albuminuria occurs in the great majority of kidney diseases. Whether the primary site of injury is the glomerulus or the tubulointerstitial compartment, albumin makes up 60% to 90% of the urinary protein (Fig. 9.12). The excretion of low molecular weight proteins usually remains minimal.[342,343]

The persistent excretion of small amounts of albumin has been termed microalbuminuria. The term has been defined variably, but usually refers to an albumin excretion rate of 30 to 300 mg per day, which is above the normal values of 5 to 20 mg per day, but less than that detected by tests for total urine protein (i.e., 200–300 mg per day). Albumin excretion greater than 300 mg per day is sometimes referred to as macroalbuminuria or clinical proteinuria, because it can sometimes be detected by the usual dipstick. Despite their widespread usage, these terms should probably be abandoned, because they are technically incorrect or imprecise. Neither microalbuminuria nor macroalbuminuria refer to the size of urinary albumin fragments, and clinical proteinuria does not convey a specific meaning. It has now been proposed to classify albuminuria using the albumin-to-creatinine ratio as normal (<10 mg per g), high normal (10–29 mg per g),

high (30–299 mg per g), very high (300–1,999 mg per g), or nephrotic (>2,200 mg per g).[344]

An albumin excretion rate between 300 and 2200 mg per day, corresponding to a total protein excretion rate between 500 mg and 3,500 mg per day can be seen in many types of CKD, whereas excretion rates greater than this amount are almost invariably the result of glomerular disease. Persistent excretion of ≥2.2 g per day of albumin or ≥3.5 g per day of total protein usually leads to the nephrotic syndrome.[345] Clinical and laboratory evaluation can identify the cause of CKD in many patients with the nephrotic syndrome, but the various histologic subtypes can be definitively identified only by kidney biopsy.

Functional Albuminuria. A transient increase in albumin excretion occurs in a variety of physiologic and experimental settings in the absence of kidney disease. Protein excretion is increased twofold to threefold during and immediately following heavy exercise,[346–348] and the increase is accounted for largely by plasma protein components.[249,349,350] Minor abnormalities in the urine sediment can accompany the proteinuria, but both the proteinuria and the sediment abnormalities usually disappear within hours after the completion of exercise. Similar increases in protein excretion can be induced by fever,[351] severe emotional stress,[352] infusions of norepinephrine or angiotensin,[353,354] and prolonged assumption of the lordotic position.[355] In addition, mild to moderate proteinuria often is observed in patients with congestive heart failure.[356]

Orthostatic Albuminuria. In patients with CKD, proteinuria typically increases in the upright position to levels above those present in the recumbent position. This orthostatic change in excretion appears to have no special diagnostic or prognostic importance. The finding of proteinuria (mainly albumin) only in the upright position is known as orthostatic or postural proteinuria.[357,358] In this condition, total daily excretion usually does not exceed 1 g. Postural proteinuria occurs in the healing phase of many glomerular diseases and also in the absence of kidney disease. In the latter group, minor histologic abnormalities are found on kidney biopsy in approximately one half of the patients.[359] Kidney biopsy in patients with postural proteinuria typically discloses few morphologic abnormalities either on light or electron microscopy,[359–361] although in two reports both immunoglobulins and complement were identified in a substantial fraction of such patients on immunofluorescence microscopy.[362,363] The significance of this finding is uncertain.

To test for postural proteinuria, the patient is instructed to collect a urine sample in the upright position, while carrying out his other usual daily activities. A 16-hour collection can begin in the morning and end just before the patient goes to bed. On retiring, the patient begins an 8-hour recumbent urine collection, including voiding at the time of arising. The amount of protein in both samples is extrapolated to 24 hours. Patients with postural proteinuria have an increased excretion in the specimen collected in the upright position and a normal excretion in the specimen collected when recumbent. If protein excretion is increased in both specimens, the patient has persistent rather than postural proteinuria.

Long-term follow-up studies strongly suggest that postural proteinuria is a benign condition.[358,364] After 10 years of follow-up in one study, it was found that over half of the patients no longer had proteinuria, somewhat less than half continued to have postural proteinuria, and only a small minority developed persistent proteinuria.[365] Decreased GFR was not observed, and hypertension was a rare occurrence. After 20 years of follow-up of many of the same patients, all those examined had normal kidney function, the prevalence of hypertension was no different from that in the general population, and only one third had proteinuria. In half of the proteinuric group, the pattern of protein excretion was still the postural variety.[366] Thus, the prognosis of patients with postural proteinuria appears to be excellent, and patients with this condition should be reassured about the benign nature of the disorder.

Protein Selectivity. Proteinuria can be classified into either a selective or a nonselective pattern, based on a comparison of the clearance of larger molecular weight proteins, such as globulins, with the clearance of albumin.[290,291,367] Investigators have sought to gauge the severity of the glomerular leak by measuring the relative clearance rates of proteins of various sizes.[268,368,369] In patients with proteinuria secondary to a wide variety of CKDs, the clearance rates of large molecules, such as IgG, range from less than 10% to greater than 60% of the clearance rate of albumin or transferrin, a protein similar in size to albumin. Patients with a clearance ratio of IgG/albumin (or transferrin) of less than 0.10 are considered to have only a modest increase in glomerular permeability and are defined as having a "highly selective" pattern of protein excretion. Conversely, patients in whom the clearance ratio is 0.5 or greater are considered to have a relatively porous filter and are defined as having a poorly selective pattern.

Studies of the pattern of protein excretion have shown that the majority of patients with proteinuria have a nonselective pattern.[368,369] Among patients with the idiopathic nephrotic syndrome, however, two populations emerge. One group has selective proteinuria and, in most cases, has minimal change in the disease. The second group has nonselective proteinuria and usually has one of the more severe histologic varieties, such as membranous nephropathy or membranoproliferative glomerulonephritis. Because of this correlation and the frequency of the minimal change lesion among patients with the idiopathic nephrotic syndrome, selectivity studies could have some value in predicting the presence of the minimal change lesion. However, the technical difficulties involved in carrying out the protein analyses, the failure of the test to distinguish among the many subgroups of nephrotic syndrome, and the low risk of kidney biopsy relative to its diagnostic yield have made measurements of selectivity superfluous in the study of the nephrotic patient.

Tubular Proteinuria

A pattern of abnormal protein excretion in which low molecular weight proteins predominate is found in patients with a diverse group of kidney diseases characterized by primary tubular injury. This includes hereditary tubular disorders, such as Fanconi syndrome and Wilson disease,[370] chronic potassium depletion, acute renal failure due to acute tubular necrosis, Balkan nephropathy,[371] and cadmium poisoning.[372,373] The low molecular weight proteins excreted by these patients are the plasma constituents described earlier that are present in only minute amounts in the urine of normal individuals.[374] As many as 20 of these proteins have been identified. A typical pattern seen in these patients is shown in Figure 9.12. The magnitude of tubular proteinuria exceeds 150 mg per day and rarely is greater than 2 g per day.[375–377]

As described previously, the urinary clearance rates of these low molecular weight proteins in normal individuals and in patients with glomerular disease are very low despite the fact that these proteins are filtered readily, suggesting that when the tubules are intact, extensive tubular reabsorption and degradation of these substances occurs.[254,370] By contrast, in patients with primary tubular diseases, the clearance rates of these proteins are markedly increased. In fact, in these patients, the clearance rate correlates closely with the predicted filtration rates of these proteins (estimated from molecular size) if the assumption is made that no tubular uptake occurs.[370] On the basis of these observations, it appears that tubular proteinuria is due to impaired tubular reabsorption of low molecular weight proteins rather than to increased glomerular permeability.[343,370]

Among the low molecular weight proteins excreted in excess in tubular and interstitial diseases are N-acetyl-beta-D-glucosaminidase (NAG), beta-2 microglobulin (B2M), neutral endopeptidase, and lysozyme (muramidase), an enzyme with a molecular weight of 14,600. The finding of increased amounts of these proteins has received attention as a diagnostic aid in identifying tubular and interstitial disease as well as serving as an early marker of acute kidney injury (AKI).[378–381] The cause for increased excretion of these low molecular weight proteins is thought to be ineffective reabsorption and catabolism by the injured, dysfunctional tubules.[382] Lysozyme excretion is increased in patients with tubular damage secondary to infection, transplant rejection, nephrotoxic agents, and Fanconi syndrome. Unfortunately, the diagnostic utility of this determination is limited because many patients with interstitial and tubular disease do not have lysozymuria and because increased excretion of lysozyme occurs in some patients with glomerular diseases.[379] The largest increase in lysozyme excretion occurs in patients with leukemia, presumably secondary to increased production of this protein (see Overproduction Proteinuria).

The interpretation of increased excretion of light chains poses a problem similar to that encountered in patients with lysozymuria—namely, to distinguish between increased excretion secondary to tubular disease on the one hand or to overproduction of the protein on the other. In the case of light chains, a slight increase in excretion and a finding of a mixture of both kappa and lambda fragments points to a primary tubular defect, whereas high levels of excretion (greater than 500 mg per day) and the presence of only a single type of light chain points to accelerated synthesis.[383,384]

Overproduction Proteinuria

When the plasma concentration of a filterable protein is increased beyond the capacity of the tubules to reabsorb it, it then appears in the urine. Enhanced excretion of light chains, heavy chains, and other fragments of immunoglobulins occurs predominantly in the monoclonal gammopathies, including multiple myeloma, macroglobulinemia, heavy-chain disease, and idiopathic light-chain proteinuria (Fig. 9.12). Overproduction with increased filtration rather than a primary tubular defect appears to account for the increased excretion of these substances. Both light-chain and heavy-chain fragments of immunoglobulins are excreted in minute amounts in the urine of normal individuals.[352,385] Normally, only 3 mg or so of light chains are excreted daily and the ratio of kappa to lambda light chains is approximately 3 to 1.[386–388] Approximately 25% are present as monomers and the remainder as dimers, even though light chains are normally synthesized as monomers.[386,389,390]

Because light chains are small in size and a substantial amount is filtered, it follows that an increase in their delivery into the glomerular filtrate will result in increased excretion unless tubular reabsorption is concomitantly increased. In fact, the clearance of light chains in patients with multiple myeloma is quite high and is inversely related to molecular size—a finding consistent with the view that tubular reabsorption is readily saturated.[391] In these patients, light chains have clearances ranging from one tenth to one half that of creatinine, depending on the size of the specific protein destined for excretion (Table 9.8). Light-chain proteinuria is most often found in patients with multiple myeloma and, in this disease, some patients have a daily excretion greater than 15 g. Although a mild increase in albumin excretion is common in patients with monoclonal gammopathies, the excretion of light chains usually predominates, unless the glomerular lesion of renal amyloidosis (or light-chain deposition disease) supervenes. Increased excretion of lysozyme in acute leukemia,[392] amylase in pancreatitis, myoglobin in muscle injury, and hemoglobin following hemolysis are other examples of overproduction proteinuria. The quantity of urine proteins can serve as an index of clinical disease. In the case of light chains, the quantity of light-chain excretion is a reflection of tumor burden and is used clinically as a biomarker of remission and relapse after treatment.

Overproduction proteinuria can have important clinical consequences. Patients with light-chain proteinuria can develop acute or chronic kidney failure, and others manifest the Fanconi syndrome,[393,394] distal renal tubular acidosis,[384] nephrogenic diabetes insipidus, or various combinations of these disorders.[383,384] The association between light-chain

proteinuria and tubular nephropathies has led to the speculation that light chains are toxic to renal tubular cells.[384,393,394] Because some patients with increased excretion of light chains have no abnormalities of tubular function, the specific factors producing tubular dysfunction remain to be defined.[383] Similarly, the lysozymuria associated with leukemia has been implicated as a cause of renal potassium wasting seen in some patients.[395] However, in view of studies demonstrating potassium wasting in some leukemic patients in the absence of lysozymuria, this thesis must be considered unproven.[396,397] It should be noted that lysozymuria also occurs in experimental glomerulonephritis and that its excretion is in direct proportion to the magnitude of the albuminuria.[398]

The Interpretation of Proteinuria

Proteinuria is central to the detection, evaluation, and management of CKD (Table 9.10). The pattern of proteinuria can be assessed by first determining whether the urine protein contains albumin. A positive dipstick test is strongly suggestive of albuminuria, which, when substantial, most likely indicates glomerular proteinuria. Quantification of albumin excretion provides a clue to diagnosis, prognosis, and response to therapy. A negative dipstick test in the presence of elevated total urine protein excretion suggests nonalbumin protein due to tubular proteinuria or overload proteinuria. Electrophoresis or other tests should be performed to detect light chains or other low molecular weight proteins when suspected.

NKF KDOQI guidelines on CKD define persistent proteinuria for 3 months as a marker of kidney damage, which is sufficient for the detection of CKD, even in the absence of other markers of kidney damage or decreased GFR. Persistent albumin excretion (>30 mg per day, roughly equivalent to an albumin-creatinine ratio >30 mg per g) is widely acknowledged to be the earliest sign of diabetic nephropathy. Persistent albuminuria in this range is generally required for the diagnosis of diabetic nephropathy, and precedes the decline in GFR in most patients.[399] The literature indicates that albuminuria in this range is one of the earliest markers of kidney damage in hypertension, but does not occur in all patients prior to the reduction in GFR.[400,401] However, there is a substantial heterogeneity in the presentation of these diseases, likely due to the substantial coexistence of type 2 diabetes and hypertension. In these diseases and others, it seems likely that albuminuria is associated with systemic endothelial dysfunction in addition to altered glomerular permselectivity, which may relate to its increased risk for cardiovascular disease. Using this definition of albuminuria as a marker of kidney damage has enabled studies of the prevalence of earlier stages of CKD, regardless of specific cause, in large populations.[402]

A large number of clinical practice guidelines now suggest routine testing for urine albumin-to-creatinine ratio. The National Kidney Foundation recommends testing for albuminuria in all individuals at increased risk for CKD, including those with hypertension, diabetes, a family history of kidney disease, or advancing age.[1] The American Diabetes Association also endorses routine testing for albuminuria in all diabetic subjects as part of their evidence-based guidelines.[403] Furthermore, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) recommended routine urinalysis in the evaluation of all patients with hypertension with the option of measuring urine albumin excretion as well.[404] Increasingly, guidelines focus on the urine albumin-to-creatinine ratio as the screening test of choice, and the optimal way to quantitate proteinuria in CKD.

9.10 Importance of Proteinuria in Chronic Kidney Disease

Interpretation	Explanation
Marker of kidney damage	Spot urine albumin-to-creatinine ratio >30 mg/g or spot urine total protein-to-creatinine ratio >150 mg/g for ≥3 months defines CKD
Clue to the type (diagnosis) of CKD	Spot urine total protein-to-creatinine ratio >500–1000 mg/g suggests diabetic kidney disease, glomerular diseases, or transplant glomerulopathy
Risk factor for adverse outcomes	Higher proteinuria predicts faster progression of kidney disease and increased risk of CVD
Effect modifier for interventions	Strict blood pressure control and ACE inhibitors are more effective in slowing kidney disease progression in patients with higher baseline proteinuria
Hypothesized surrogate outcomes and target for interventions	If validated, then lowering proteinuria would be a goal of therapy

ACE, angiotensin-converting enzyme; CKD, chronic kidney disease; CVD, cardiovascular disease.

Initial and repeated measurement of protein excretion is a valuable guide in following the course of patients with kidney disease. Several studies have established that the magnitude of proteinuria is directly correlated with the risk of progressive decline in kidney function, regardless of the cause or type of kidney disease.[405–407] This is also true, independent of the level of GFR.[408]

Secondary analysis of recent clinical trials demonstrates that the beneficial effects of lowering blood pressure and inhibition of the renin-angiotensin system are greater in patients with higher levels of proteinuria at the beginning of therapy.[409–413] Consequently, the magnitude of proteinuria is central to the new guidelines for the management of hypertension in chronic kidney disease.[400] For patients with persistent albumin-to-creatinine ratio >30 mg per g, the guidelines recommend a lower target blood pressure (<130/80 mm Hg) and initial antihypertensive therapy using an ACE inhibitor or ARB. Furthermore, evidence has mounted to demonstrate that the degree of reduction in proteinuria serves as an important guide to the prognosis of patients treated with ACE inhibitor or ARB therapy. Studies demonstrate that the better the antiproteinuric response to therapy, the better the outcome.[409,414,415] This may lead to the selection of agents and dosing based on the antiproteinuric response. This strategy would need to be tested in a prospective fashion, in various kidney diseases, before being broadly adopted, as adverse effects of higher dose or combination agents may outweigh their benefits.[416]

URINALYSIS

Urine examination has been used in medicine for more than 6,000 years.[417] Ancient Hindu, Babylonian, and Egyptian physicians are known to have used urine for diagnosing illnesses, and the connection between sweet urine and diabetes was made as early as 600 BC.[417] Early western physicians like Hippocrates (460–355 BC) and Galen (129–200 AD) have written about the associations between different urinary characteristics and disease states.[417] During medieval times (500–1500 AD), urinalysis established its place as an unrivalled diagnostic tool and was also widely used by unscrupulous profiteers to prognosticate future events.[418]

The modern urinalysis techniques took a foothold in the 19th century with the use of microscopy; description of casts; discovery of chemical analysis methods for urine, glucose, bile acid, protein, and blood; and the development of urinary test strips.[419,420] Dipsticks became commercially available in 1956 and, since the early 21st century, the manual reading of dipsticks is increasingly being replaced by sophisticated semiautomatic or automatic strip readers with both chemical and sediment analysis capabilities.[300] In spite of advances in microscopy, chemical analysis, and automation, direct examination of urine by physicians provides invaluable diagnostic information and remains indispensable for evaluating patients who are suspected of having acute or CKD.

Urinalysis entails macroscopic, dipstick, and microscopic examinations of the urine. The standards for urinalysis have not yet been uniformly applied and there is a wide inter-operator variability in the interpretation of both macroscopic and microscopic findings.[298,299,421–423] A number of organizations have recently developed guidelines to make laboratory urinalysis more uniform, but most nephrologists perform this procedure in their office outside of laboratory purview.[424,425]

Urine Collection

Urine specimens can be obtained by voiding into a container, urethral catheterization, or suprapubic needle aspiration of the bladder. Voiding into a container is the most commonly used method. Urethral catheterization is usually reserved for patients who are unable to void due to urinary obstruction, incontinence, or impaired consciousness. Suprapubic needle aspiration of the bladder is mostly done in infants and is only used in older children or adults if urine cannot be obtained by any other means. Regardless of the method used, every effort should be made to avoid specimen contamination with the contents of skin, urethra, or vagina. Urethral catheterization and suprapubic aspiration should be done using a sterile technique to prevent introduction of infection into the bladder.

A clean catch sample is essential for accurate assessment. Patients should be instructed to wash their hands before sample collection. Men should retract the foreskin and wash or use sterile wipes to clean the external genitalia. For uncircumcised men, the foreskin should be held back during the entire collection time. Women should separate the labia and clean the area around the urethral meatus with sterile wipes going from front to back. The same procedures are used for children. For infants and very young children, external genitalia are cleaned and dried using wipes and towels, and a sterile urine collection bag is placed over the area using adhesives to adhere to the skin. A mid-stream clean catch sample is preferred as this is least likely to be contaminated. The specimen should be examined as soon as possible after the collection as the chemical composition of urine changes and formed elements degenerate over time. Highly concentrated and acidic urine favor cellular preservation. Refrigeration is acceptable but leukocytes break down rapidly and cell counts performed after 2 to 4 hours may be questionable even with refrigeration.[426] Microbiologic investigations should be done within 2 hours and if more than 2 hours of delay is expected then the sample should be refrigerated at 4°C.[424]

The first morning urine is traditionally considered to be the standard specimen for urinalysis as it best correlates with a 24-hour urine sample.[1] The first morning specimen is least likely to be affected by prior food or fluid intake, movement, and also allows for preservation of formed elements as it is usually most concentrated and acidic.[424] A random urine sample is an acceptable alternative to the first morning urine in an acute setting, and when the collection, storage, and timely transportation of the first morning specimen is difficult.

Macroscopic Examination

Normal urine color is determined by the concentration of the pigment urochrome. Urochrome is the product of hemoglobin metabolism in the liver. Urine is pale yellow when it is dilute and dark yellow or amber colored when it is concentrated. Occasionally, the precipitation of phosphate crystals in alkaline urine and urate crystals in acidic urine may give rise to cloudy urine in the absence of any disease. The appearance of urine changes in certain disease states, and with the ingestion of certain foods or drugs.[300] Cloudy urine may be seen in urinary tract infection or in the presence of significant pyuria. Foamy urine suggests moderate to heavy proteinuria. Reddish urine indicates hematuria, hemoglobinuria, myoglobinuria, or the intake of rifampin, phenytoin, phenazopyridine, or beet-root. Yellow-brown urine may be seen in hyperbilirubinemia, or following the ingestion of chloroquine, nitrofurantoin, senna, or rhubarb. White milky urine suggests chyluria, and dark or black urine may be seen in alkaptonuria, porphyria, or malignant melanoma.

Urine odor usually does not have much clinical significance. A pungent odor may indicate bacterial ammonia production. Sweet or fruity odor suggests ketonuria. Certain rare hereditary metabolic diseases may give rise to strong unusual urine odor: maple-syrup urine disease, maple syrup odor; phenylketonuria, musty or mousy odor; isovaleric academia, sweaty feet odor; hypermethioninemia, rancid butter or fishy smell; Oasthouse urine disease, brewery odor; tyrosinemia, cabbagelike or fishy odor; trimethylaminuria, stale fish odor; and hawkinsinuria, swimming pool odor.[427]

Dipstick Examination

Use of a single- or multiple-test reagent strip, commonly called a dipstick, allows for a rapid and convenient chemical screening of urine specimens. The dipstick method uses a paper or plastic strip embedded with pads that contain reagents for different chemical reactions. Reactions in these pads result in color change when a particular analyte is present in the urine. The degree of color change in each pad is then compared against the range of colors on brand-specific color charts to get a semiquantitative result for the analyte in question.

Although the dipstick method is simple to perform, certain precautions need to be taken to obtain accurate and reliable results.[300] Reagent strips should not be exposed to the extremes of temperature and must be stored in a dry place away from direct sunlight. Only the container provided by the manufacturer should be used for storage as these containers are light-sealed and have desiccants to prevent moisture. While performing the test, the reagent strip should be dipped in urine in one continuous motion and the excess urine needs to be removed by touching the edge of the strip to the urine container as mixing or dilution of reagents gives rise to false results. The color change in reagent pads takes time and reading should be done only after the manufacturer specified wait time. Strips usually have a control pad and the color change in that pad must always be compared with the standard before interpreting reactions in other pads.

Dipsticks commonly include tests for specific gravity, pH, blood, protein, glucose, ketones, bilirubin, urobilinogen, nitrites, and leukocytes. The composition of reagents and detection limits vary with different brands and manufacturers. Table 9.11 lists chemical reactions, detection limits, and conditions associated with false-positive and false-negative results for a typical dipstick. Dipstick results provide important diagnostic clues and abnormal dipstick findings usually lead to further evaluation with confirmatory tests (Table 9.12).

Dipstick reactions perform variably when compared to confirmatory tests. The dipstick specific gravity reaction detects ion concentration rather than particle mass, thus the dipstick will show a lower value for urine specific gravity than hygrometry or refractometry when nonionized molecules like glucose and radiocontrast dye are present.[428,429] The dipstick specific gravity, however, correlates fairly well with urine osmolality in most situations. Specific gravity is the relative mass of the urine compared to water, and thus reflects the total number of particles in solution and their size and density, whereas osmolality reflects only the number of particles. Therefore, a solution of glucose (molecular weight = 180 daltons) equal in osmolality to a solution of urea (molecular weight = 60 daltons) has a higher specific gravity (Fig. 9.13). For total protein, dipsticks have low sensitivity and variable specificity and positively charged proteins like immunoglobulin light chains may escape detection even when concentrations are high.[300] Dipsticks perform poorly as a screening tool for diabetes as fasting urine glucose testing has sensitivity of only 17% even though specificity can be as high as 98%.[430] Similarly, dipsticks cannot be used solely to estimate the level of ketosis as beta-hydroxybutyrate, the most abundant serum ketone during ketosis, is not detected by dipstick. Dipsticks perform well for white blood cells (WBCs) and are usually positive when more than five WBCs are present per high power field (HPF).[431,432] Dipsticks are also reliable for detecting red blood cells (RBCs) with the sensitivity and specificity of 80% to 95% and 95% to 99% for more than three RBCs per HPF, respectively.[433–435] For the detection of urinary infection, negative dipstick results for leukocytes and nitrites are likely sufficient to exclude microscopic and culture abnormalities.[432,436–438] It has been shown that 95% of urines with negative dipstick results for protein, glucose, ketones, blood, leukocytes, and nitrites have normal microscopic examination and, in most cases, microscopy can be reserved for only those with abnormal dipstick results.[432,439] Discolored urines and urine samples of patients with urinary tract symptoms or kidney disease, however, are best examined microscopically as the dipstick examination alone may not pick up all potentially relevant abnormalities.[436]

Microscopic Examination

Microscopic examination of the urine is primarily performed to identify cells, casts, crystals, and microorganisms. The examination can be a qualitative or semiquantitative procedure.

9.11 Urine Dipstick Testing

Test	Reaction in Dipstick Pad	Detection Limit[a]	Associated with False-positive Results	Conditions Associated with False-negative Results
Specific gravity	Urinary cations compete with H+ bound to polyionic polymer causing a release of free H+ that alters pH of a pH-sensitive dye	1.000–1.030	Heavy proteinuria Acidic urine	Alkaline urine
pH	H+ reacts with methyl red and bromthymol blue	pH 5.0–9.0	Prolonged storage (falsely alkaline) Formaldehyde (falsely acidic)	—
Protein	Proteins (primarily albumin) alter pH of a pH-sensitive dye (commonly tetrabromophenol blue)	≥18–32 mg/dl (albumin)	Concentrated urine Alkaline urine Phenazopyridine Polyvinylpyrrolidone (blood substitute) Chlorhexidine	Dilute urine Acidic urine
Blood	Hemoglobin or myoglobin oxidizes ortho-toluidine and organic peroxidase	≥5–20 RBC/μl	Oxidizing detergents Dilute urine Alkaline urine Hemoglobinuria Myoglobinuria Bacteria with pseudo-peroxidase activity (Enterobacteriaceae, staphylococci, streptococci)	Ascorbic acid Formalin preservative Acidic urine
Glucose	Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. Hydrogen peroxide then reacts with chromogen.	≥30–40 mg/dl	Oxidizing detergents	Ascorbic acid Keto-acids Aspirin Bacteria Concentrated urine
Ketone	Acetoacetate and acetone reacts with nitroprusside reagent	≥5–15 mg/dl (acetoacetic acid) ≥70 mg/dl (acetone)	Ascorbic acid Phenazopyridine Levodopa Mesna Free sulfhydryl group (N-acetylcysteine, captopril)	Improper storage
Bilirubin	Conjugated bilirubin reacts with aniline dye	≥0.5–1.0 mg/dl	Fecal contamination Chlorpromazine Phenazopyridine	Ascorbic acid

(continued)

9.11 Urine Dipstick Testing (continued)

Test	Reaction in Dipstick Pad	Detection Limit[a]	Associated with False-positive Results	Conditions Associated with False-negative Results
Urobilinogen	Urobilinogen reacts with dimethylaminobenz-aldehyde (Ehrlich's reaction)	≥0.4–2.0 mg/dl	Sulfonamides Phenazopyridine Procaine Alkaline urine	Prolonged storage
Nitrite	Bacteria with nitrate reductase activity reduces urinary nitrates to nitrites. Nitrites react with p-arsanilic acid, forming a diazonium compound.	≥0.05–0.10 mg/dl (typically >10 organisms/mL)	Phenazopyridine	Prolonged storage Short bladder incubation (<4 hours) Ascorbic acid Low vegetable diet Bacteria without nitrate reductase activity (Enterococcus, Neisseria, Mycobacterium)
Leukocyte esterase	Pyrrole amino acid esters are cleaved forming free pyrrole that reacts with diazonium compound.	>25–35 WBC/μl	Vaginal contamination Beets Formaldehyde Imipenem Meropenem Clavulanic acid	Concentrated urine Ascorbic acid Glycosuria Heavy proteinuria Cephalexin Gentamicin Tetracycline Nitrofurantoin

[a]Based on Chemstrip® 10 MD COBAS® of Roche Diagnostics, Indianapolis, IN 46256, USA.
RBC, red blood cell; WBC, white blood cell.

Data from references: Lam MH. False 'hematuria' due to bacteriuria. Arch Pathol Lab Med. 1995;119(8):717–721; Brigden ML, Edgell D, McPherson M, et al. High incidence of significant urinary ascorbic acid concentrations in a west coast population—implications for routine urinalysis. Clin Chem. 1992;38(3):426–431; Mundt LA, Shanahan K, eds. Graff's Textbook of Routine Urinalysis and Body Fluids, 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2011; Wallace J, ed. Interpretation of Diagnostic Tests, 8th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2007.

Preparation of the urine sediment and examination methods are of the utmost importance. About 10 mL of fresh or properly stored urine is centrifuged in a conical tube at approximately 2,000 revolutions per minute for at least 5 minutes. The supernatant is carefully decanted and the pellet is resuspended in a small amount of urine that remains in the tube by gentle agitation. A pipette is then used to transfer a drop of this resuspended pellet onto the microscope slide. A coverslip is gently placed on top of the urine before transferring the slide to the microscope. The sediment is usually examined unstained. Papanicolaou stain may be used to enhance details, and Wright's or Hansel's stain is used in special circumstances to identify eosinophils.

Microscopic examination of the urine is most commonly performed using a bright field microscope. Polarized light is used to identify some crystals and fat droplets, and phase-contrast microscopy is occasionally used when detailed examination of cell membranes is required.[439,440] The use of interference contrast microscopy, scanning electron microscopy, and transmission electron microscopy have also been reported but there use is mostly limited to research settings.[441–443] The examination is first done under low magnification (×100) to identify formed elements. Higher magnification (×400) is important to differentiate the types of casts, cells, crystals, or other abnormalities. Laboratories provide quantification by counting and averaging the

9.12 Clinical Significance and Confirmatory Tests for Urine Dipstick Results

Test	Clinical Significance	Confirmatory Test
Specific gravity	1.000–1.005: Excess water intake, diabetes insipidus 1.010 (Isosthenuric urine): Acute tubular necrosis, severe CKD >1.030: Volume depletion, glycosuria, extrinsic osmotic agent	Refractometry or hygrometry Urine osmolality
pH	<5.0: Metabolic acidosis of nonrenal cause, volume depletion, hyperaldosteronism, high protein diet >6.0–6.5: Type I renal tubular acidosis, low protein diet, infection with urea-splitting organisms (e.g., >Proteus)	pH-electrode under oil emulsification
Protein	Persistently positive in CKD with elevated albuminuria	Further quantification and qualification using a timed or spot urine sample (see Measurement of Urine Protein)
Blood	Spot staining: Hematuria Diffuse staining: Marked hematuria, hemoglobinuria, myoglobinuria	Urine microscopy
Glucose	Positive in proximal tubular dysfunction, or when serum glucose concentration is more than renal glucose threshold (180 mg/dl)	Serum glucose (diabetes); Urine amino-acid, phosphorus and uric acid (proximal tubular dysfunction)
Ketone	Positive in diabetic ketoacidosis, alcoholic ketoacidosis, starvation, and severe volume depletion	Serum keto acids
Bilirubin	Positive in conjugated hyperbilirubinemia Positive bilirubin and negative urobilinogen may indicate intestinal obstruction with conjugated hyperbilirubinemia	Serum bilirubin and liver enzymes, abdominal imaging if obstruction is suspected
Urobilinogen	Positive in conjugated hyperbilirubinemia	Serum bilirubin and liver enzymes
Nitrite	Positive in urinary infection with nitrate reducing bacteria	Urine culture
Leukocyte esterase	Positive in interstitial nephritis and urinary infection	Urine microscopy and urine culture

CKD, chronic kidney disease.

number of elements seen in at least 10 fields in different areas of the sample.[424] The number of casts is usually reported as a number of each type seen per low power field (LPF). The number of cells, crystals, or bacteria is usually reported as a number of each type seen per high power field (HPF). In the office setting, however, physicians use a variety of nonspecific terms like occasional, few, rare, frequent, many, and numerous to quantify formed elements.

Cells

Red Blood Cells. RBCs in the urine originate either from the kidney parenchyma or the urinary tract. Occasionally they may be seen in the absence of kidney or urologic diseases, especially when specimens are obtained during fever, menstruation, or following exercise.[444–446] The upper limit of normal for the number of RBCs in the urine is unclear. Addis was the first to report that healthy people may excrete

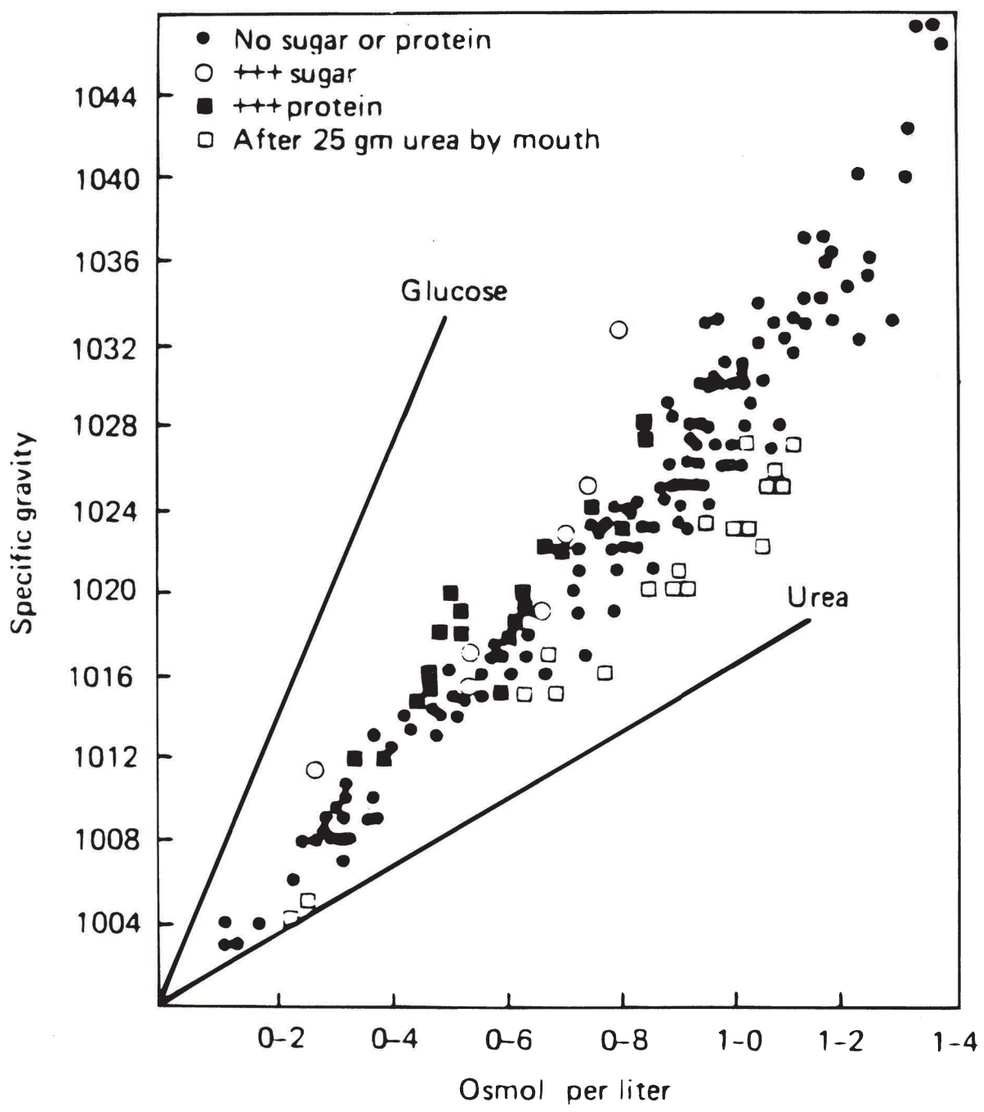


FIGURE 9.13 Relationship between specific gravity and osmolality of the urine. Different urine samples are shown as follows: *small filled circles*, with no sugar or protein; *large open circles*, 3+ sugar; *small filled squares*, 3+ protein; *large open squares*, after 25 g of urea by mouth. The lines show the relation between specific gravity and osmolality for glucose and urea solution. (From Miles BE, Paton A, de Wardener HE. Maximum urine concentration. *Br Med J.* 1954;2:901, with permission.)

up to 425,000 RBCs in urine in a 12-hour period.[444] Subsequent investigators have reported excretion rates in healthy individuals that range from 5,000 to 8,000 RBCs per mL of urine.[447,448] One study revealed that RBCs in healthy individuals typically exhibit a dysmorphic pattern (speculated, crenated, or with cell membrane blebs or folding), suggesting that RBCs enter the urine through the glomeruli.[449] It is believed that RBCs lose their typical biconcave structures as they pass through glomerular basement membrane and get exposed to osmolality changes in renal tubules.[450,451] The presence of more than two to five RBCs per HPF in the urine on two or more occasions is considered by most to warrant further evaluation.[447,452]

The differential diagnosis of hematuria is broad and it is useful to differentiate between hematuria of glomerular origin and nonglomerular origin. The presence of RBC casts or heavy proteinuria points toward glomerular causes for hematuria. Similarly, the presence of a high number of dysmorphic RBCs (particularly acanthocytes) suggests glomerular hematuria (Figs. 9.14 and 9.15).[453,454] Dysmorphic RBCs are best visualized using a phase-contrast microscope.[455,456] The number of dysmorphic RBCs depends on the type of glomerular disease and is considerably higher in proliferative than in nonproliferative glomerular processes.[457]

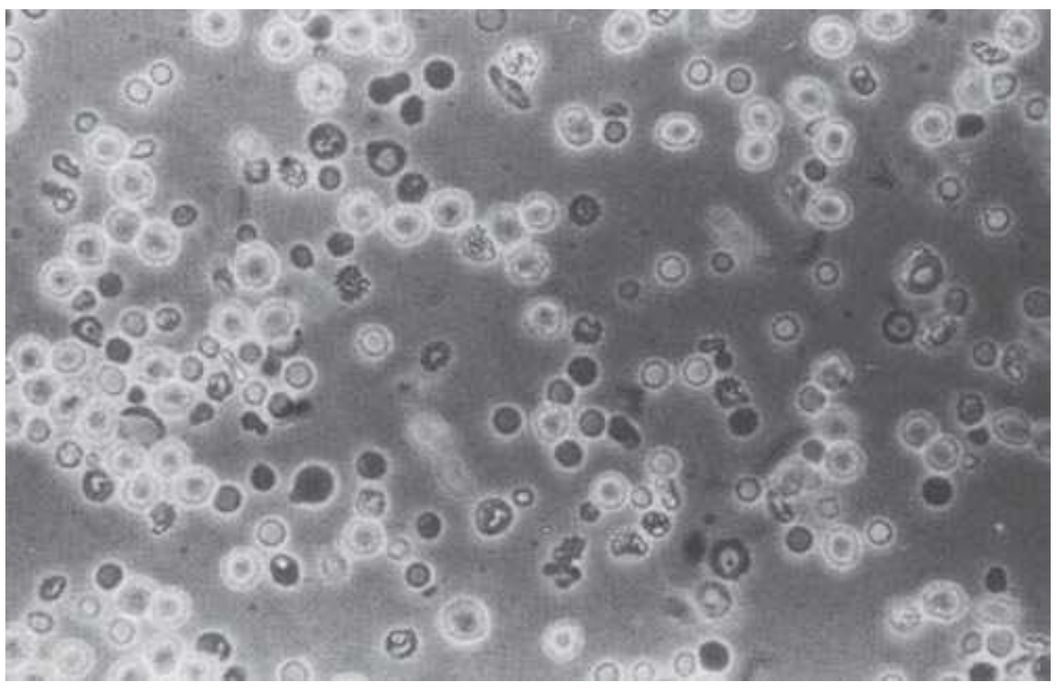


FIGURE 9.14 Urine from a patient with immunoglobulin A glomerulonephritis. Monomorphic and dysmorphic red blood cells are seen. The coexistence of a nonglomerular source of bleeding needs to be considered in such a case, but this "mixed" pattern may be seen in glomerulonephritis in a setting of marked hematuria.

Manual detection of dysmorphic RBCs has a high interobserver variability,[458] and a meta-analysis of 21 studies reported that the average sensitivity and specificity of this technique in detecting glomerular disease in referral centers is between 86% and 90% and 93% and 97%, respectively.[459] Automated red cell volume analysis and urinary flow cytometry have been evaluated as an alternative to the manual examination. The sensitivity and specificity of these techniques in distinguishing between glomerular and nonglomerular hematuria ranges from 98% to 100% and 80% to 91%, respectively, for red cell volume analysis, and 90% to 100% and 87% to 93%, respectively, for urinary flow cytometry.[459,460]

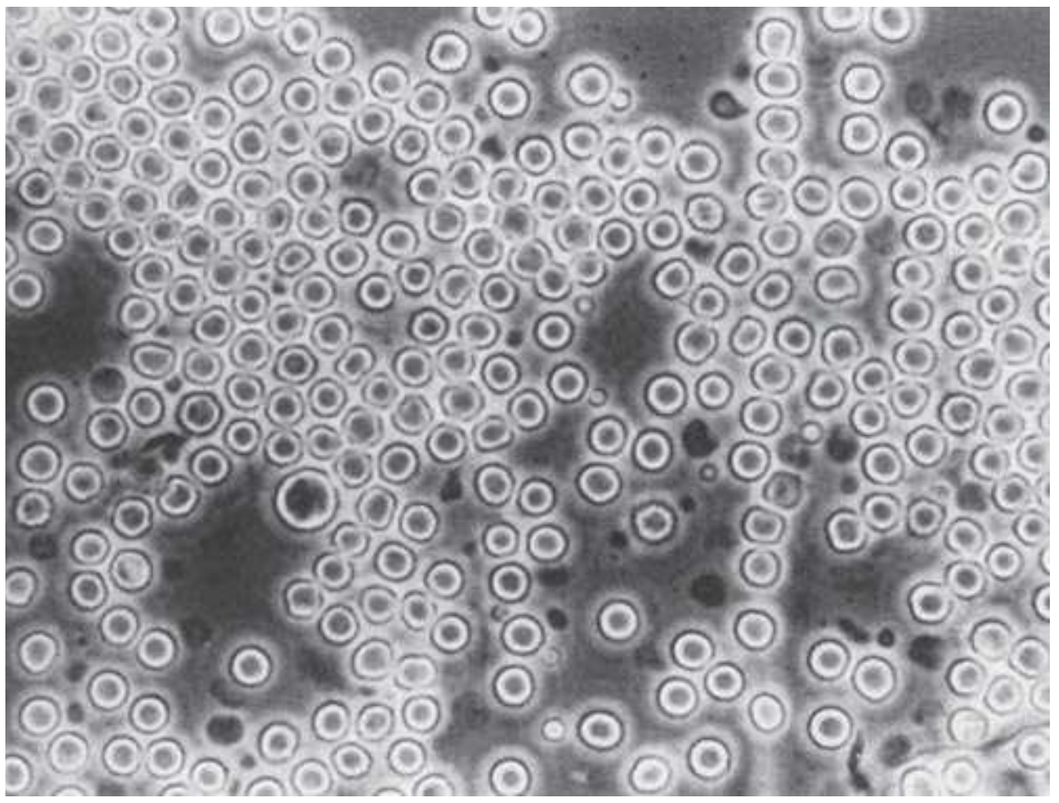


FIGURE 9.15 Monomorphic red blood cells seen in nonglomerular hematuria. Tumors, stones, menstrual contamination, lower urinary tract infection, or contamination of urine sample with blood may give this picture.

White Blood Cells. Small number of WBCs can be seen in urine in the absence of any disease.[461] The presence of five or more WBCs per HPF is usually considered abnormal and requires further evaluation.[462,463] Pyuria, or the presence of WBCs in the urine, implies inflammation in or around the kidneys or urinary tract. The most common cause of pyuria is urinary tract infection.[464] In addition, pyuria may also be seen in interstitial nephritis, glomerulonephritis, acute allograft rejection, and during conditions such as gastroenteritis or acute appendicitis that cause inflammation around the urinary tract. The presence of casts or heavy proteinuria suggests kidney involvement.

Neutrophils are the predominant WBCs found in the urine. They are easy to identify due to the presence of granular cytoplasm and multilobed nuclei (Fig. 9.16). Urine lymphocytes may be seen in acute allograft rejection but their detection requires special staining that is not widely used in clinical practice.[465,466] Urine eosinophils are known to be associated with allergic interstitial nephritis and can be identified using either Hansel's or Wright's stain.[467] Hansel's stain is preferred as it is much more sensitive than the standard Wright's stain.[468] Urine eosinophils are reported either qualitatively or as a percentage of total cells in the sediment. Greater than 1% is considered positive and the predictive value for acute interstitial nephritis increases with higher percentage.[467] For more than 1% urine eosinophils, the sensitivity and specificity for acute interstitial nephritis have been reported as 63% and 93%, respectively.[468] More recent data, however, suggests that sensitivity may be much lower and range between 25% and 40%.[469,470] These inconsistent data have raised doubts about the utility of testing for urine eosinophils.[470] It is advisable that clinicians consider urine eosinophil count as only one of the many diagnostic clues for allergic interstitial nephritis and interpret the results in the context of the patient's clinical presentation, drug exposure history, and other urinalysis and laboratory findings.

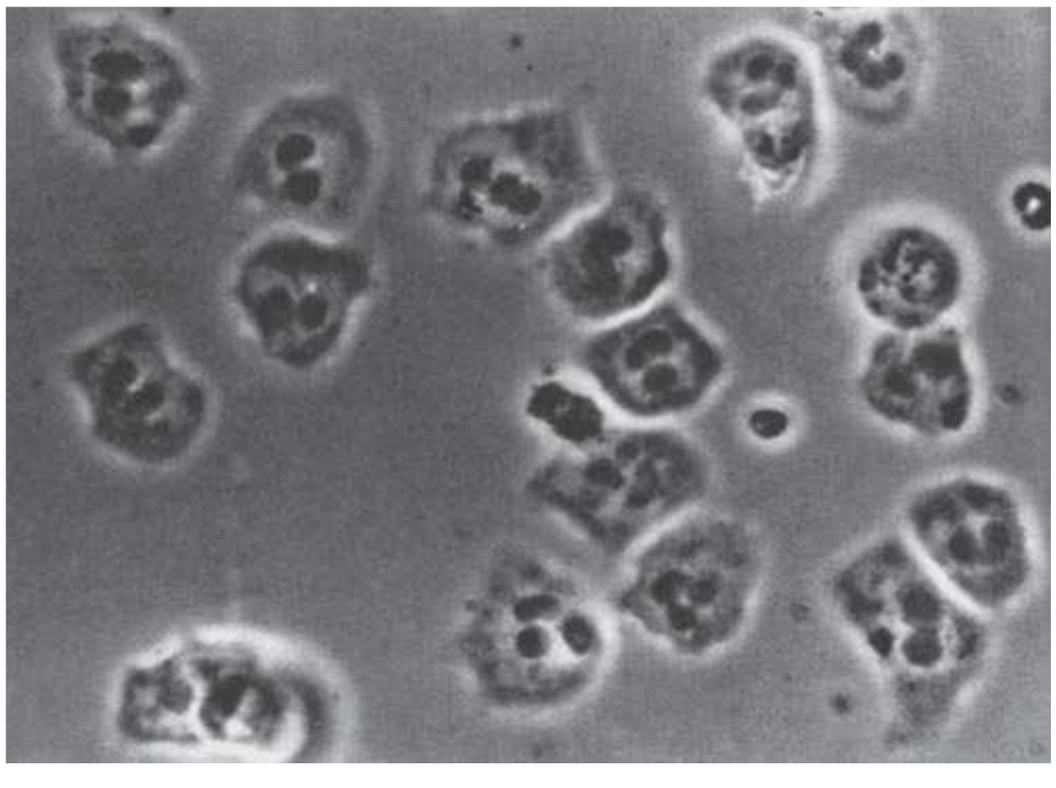


FIGURE 9.16 The multilobed nuclei of the leukocytes, clearly seen on phase-contrast microscopy.

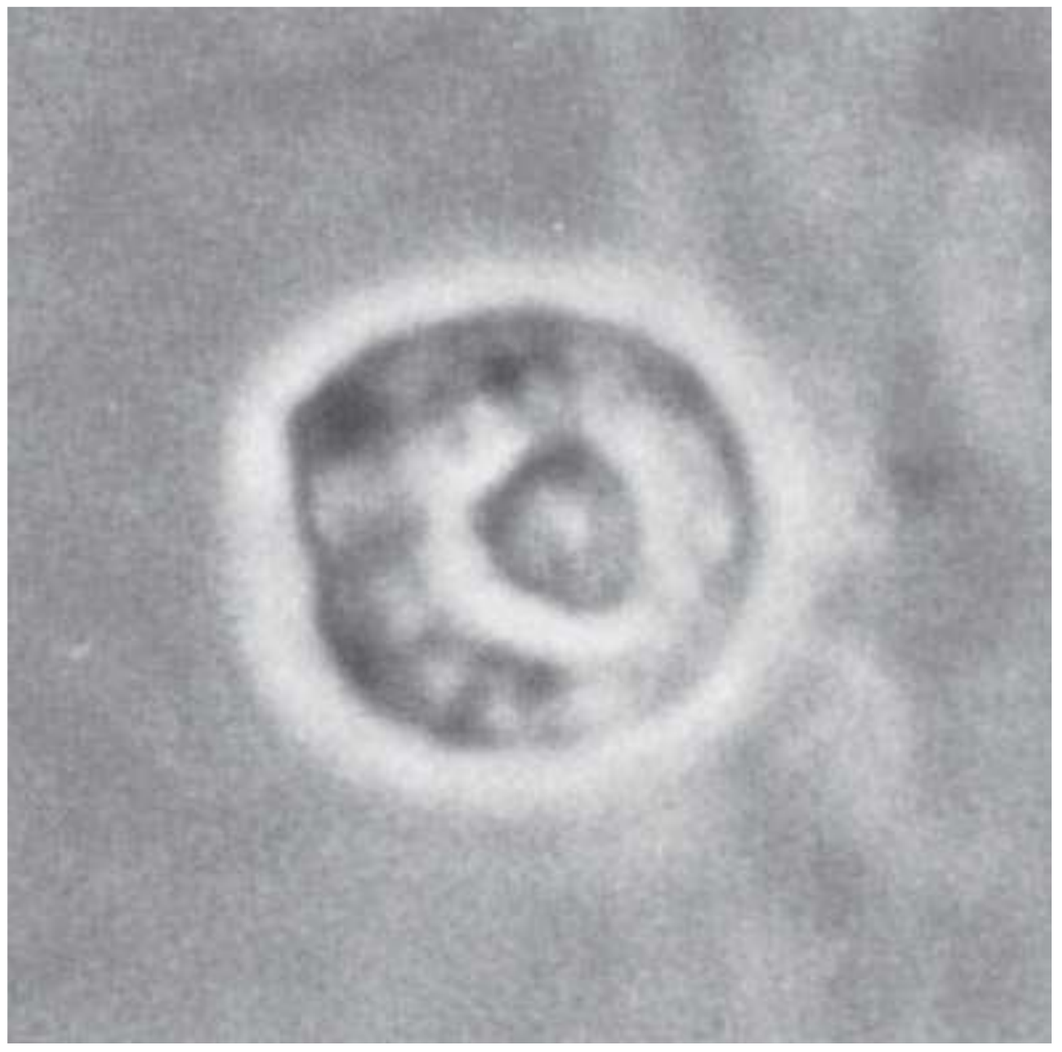


FIGURE 9.17 Renal tubular epithelial cell with a single nucleus, clearly seen on phase-contrast microscopy.

Epithelial Cells. Small numbers of epithelial cells may be present in normal urine. Renal tubular epithelial cells originate at the level of nephrons. They are mononuclear and are larger than neutrophils (Fig. 9.17). Higher numbers of tubular cells indicate tubular damage from conditions like acute tubular necrosis, interstitial nephritis, and allograft rejection. Transitional epithelial cells are derived from renal pelvis, ureters, or bladder. A higher number of transitional epithelial cells is associated with urinary tract infections, tumors, and stones. Squamous epithelial cells are of urethral or vaginal origin. They are large, flat cells with small nuclei. A higher number of squamous epithelial cells indicates urine contamination with the contents of skin, urethra, or vagina.

Casts

Casts are cylindrical structures formed when Tamm-Horsfall glycoprotein (uromodulin) secreted by the epithelial cells of the thick ascending limb of loop of Henle precipitates and takes the shape of renal tubules.[250] Casts are formed in distal tubules and collecting ducts as these are the areas where precipitation is most likely to occur. High tubular fluid concentration, low urinary flow rate, high sodium concentration, heavy proteinuria, and acidic milieu favor cast formation. Trapping of cells, other proteins, and fat within the cast matrix give rise to different types of casts with variable clinical significance (Table 9.13).

Hyaline Casts. Hyaline casts are colorless and are composed of THP alone. They may be seen in the absence of any kidney disease, especially during periods of volume depletion, diuretic use, fever, exercise, or stress.[250,471,472] Hyaline casts are usually seen with other types of cells or casts during disease states.

9.13 Clinical Significance of Cells and Casts in Urine Sediment

Clinical entity	Urinalysis finding									
	Hyaline casts	RBC	RBC Casts	WBC	WBC casts	Tubular cells	RTE casts	Granular casts	Fat[a]	Waxy casts
No kidney or urinary tract disease	+/−	−	−	−	−	−	−	−	−	−
Urinary tract disease not involving kidney	+/−	+/−	−	+	−	−	−	−	−	−
Cystic kidney diseases, urinary tract or kidney neoplasms	+/−	+	−	+/−	−	−	−	−	−	−
Tubulointerstitial nephritis, pyelonephritis	+/−	+/−	−	+	+	+/−	+/−	+/−	−	−
Acute tubular necrosis	+/−	+/−	−	+/−	−	+	+	+	−	−
Hereditary nephritis	+/−	+	+/−	−	−	+/−	−	+/−	−	−
Small vessel disease (microangiopathy)	+/−	+	−	−	−	+	+/−	+	−	−
Proliferative glomerulonephritis	+/−	+	+	+/−	+/−	+/−	+/−	+/−	+/−	+/−
Heavy proteinuria, nonproliferative glomerular diseases	+/−	+/−	−	−	−	+/−	+/−	+/−	+	−
Medium vessel diseases, noninflammatory tubulointerstitial disease	+/−	−	−	−	−	−	−	−	−	−
Severe chronic kidney disease	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+

[a]Free fat, oval fat bodies, fatty casts.
RBC, red blood cell; WBC, white blood cell; RTE, renal tubular epithelial cells.

Granular Casts. Granular casts are formed when proteins or cellular debris are trapped in the Tamm-Horsfall matrix.[473] In fine granular casts, granules are predominantly composed of filtered proteins and appear small and regular. In coarse granular casts, granules are predominantly composed of degenerated cells and appear large and irregular. A few fine granular casts may be seen in the absence of any kidney disease, but the presence of more than a few fine granular casts or of coarse granular casts indicates kidney disease.

Granular casts are usually nonspecific and can be seen in many different glomerular or tubular disorders. A special type of granular cast, called deeply pigmented or muddy-brown cast, is considered to be the characteristic urine sediment finding of acute tubular necrosis.

Red Blood Cell Casts. RBC casts are formed when RBCs leak into the tubules through damaged glomerular or tubular basement membrane gets trapped in the Tamm-Horsfall matrix.[443] RBC casts are considered pathognomonic for glomerular disease, and many RBC casts suggest glomerular inflammation associated with a proliferative glomerulonephritis. RBC casts can occasionally be seen in other parenchymal diseases like pyelonephritis and renal infarction. A careful examination of the sediment is essential when glomerular hematuria is suspected as RBC casts may be very sparse and contain only few cells. The cells within the casts may also show varying degrees of disruption and degeneration (Fig. 9.18) making it difficult to distinguish from more common coarsely granular casts.

White Blood Cell Casts. WBC casts are formed when WBCs leak into the tubules through damaged tubular or glomerular basement membrane and are trapped in the Tamm-Horsfall matrix.[443] The presence of WBC casts suggests tubulointerstitial inflammation, either from infection in the case of pyelonephritis or from toxins or drugs in the case of interstitial nephritis. Rarely, WBC casts may also be seen in acute glomerulonephritis.

Renal Tubular Epithelial Cell Casts. Renal tubular epithelial cell casts are formed when tubular cells slough from the tubular basement membrane and are trapped in the Tamm-Horsfall matrix. These casts are markers of tubular injury and most commonly are seen in acute tubular necrosis or interstitial nephritis.

Pigment Casts. Hemoglobin, myoglobin, bilirubin, and, rarely, melanin may form casts. Hemoglobin casts have a characteristic brownish hue and are coarsely granular in appearance. These casts are formed either from the degradation of RBC casts, or from free hemoglobinuria in patients with intravascular hemolysis. Myoglobin casts appear similar to hemoglobin casts and are associated with rhabdomyolysis. Bilirubin casts are yellow-brown in color and may be seen in patients with hyperbilirubinemia. Melanin casts are extremely rare and may be seen in patients with melanemia and melanotic tumors. They are coarsely granular and have dark brown or black color.

Broad and Waxy Casts. Broad casts are usually colorless and four to five times wider than typical hyaline casts. They are formed in tubules that are dilated as a result of atrophy and fibrosis of surrounding interstitium. Waxy casts (Fig. 9.19) are similar to broad casts in size, highly refractile, smooth, and waxy in appearance. Broad casts and waxy casts are seen in people with advanced kidney disease, and may reflect dilated and hypertrophic tubules in this condition.

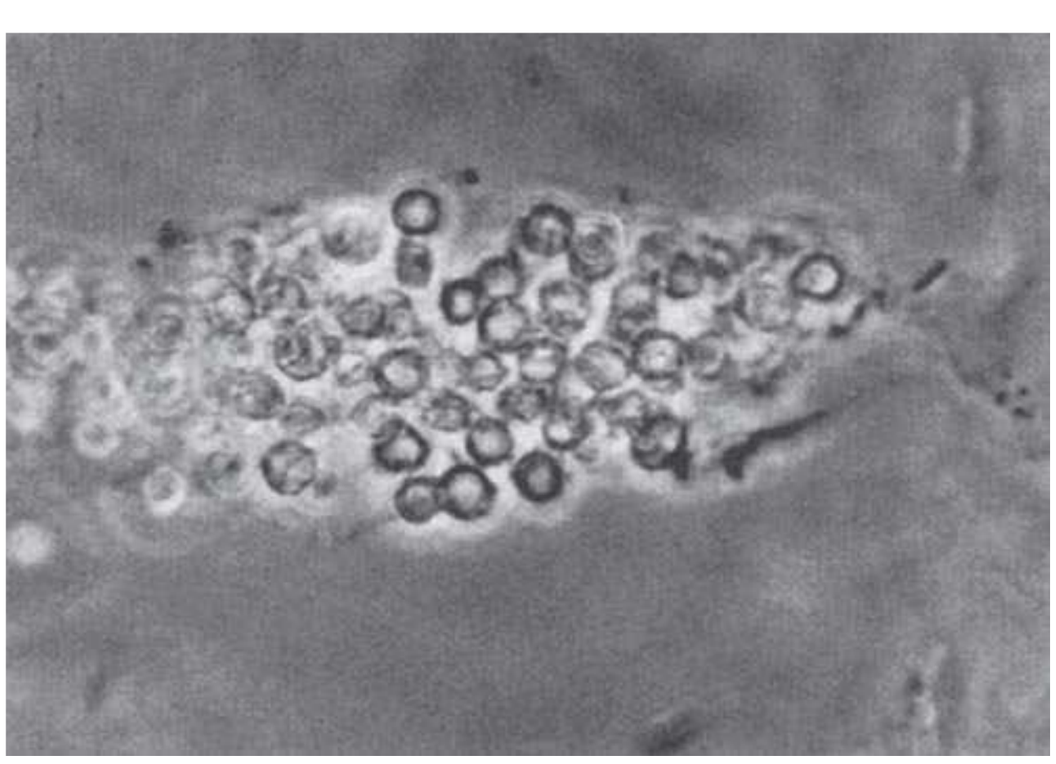


FIGURE 9.18 A red blood cell cast. Much of the hemoglobin from cells has already disappeared.

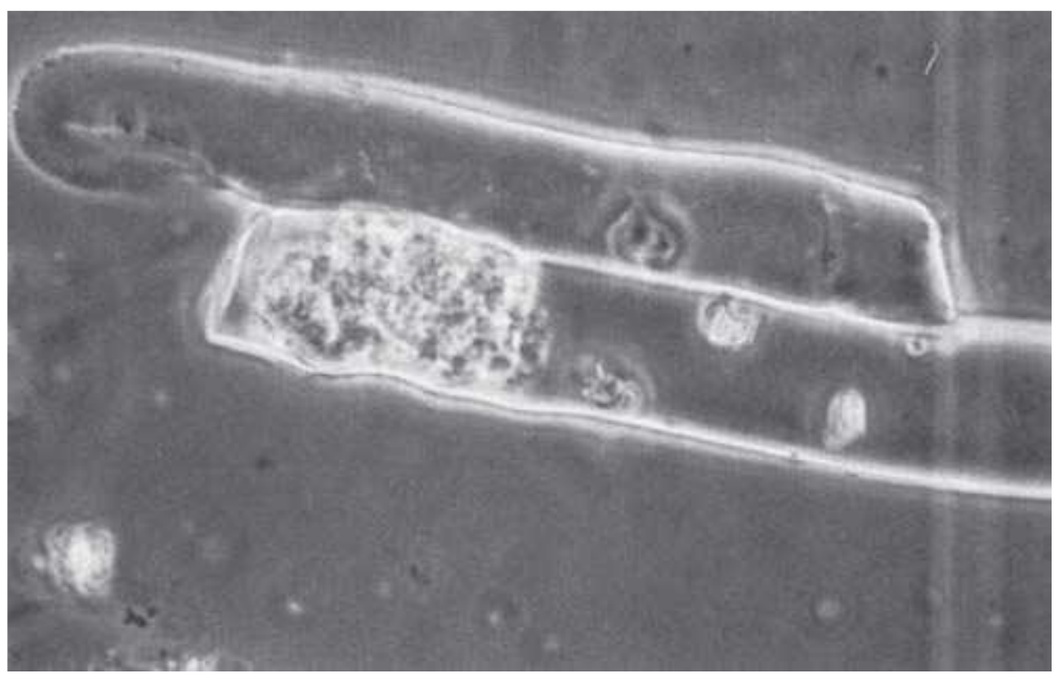


FIGURE 9.19 A waxy cast with sharply defined edges. A clump of disintegrating cells is seen at one end.

Urine Fat and Fatty Casts. Urine fat may be observed in the form of free fat globules, oval fat bodies, or fatty casts. Free fat globules are spherical in shape, yellowish in color, variable in size, and may exist in isolation or as fat clusters. When fat globules are taken up by tubular cells or macrophages, then they are described as oval fat bodies. When fat globules are trapped within the Tomm-Horsfall matrix then they are called fatty casts. Fat globules with cholesterol or cholesterol esters are anisotropic and have a characteristic "Maltese cross" appearance under polarized light (Fig. 9.20). Neutral fats like triglycerides are isotropic, do not polarize, and are identified using special stains like Sudan III or oil red O dye.

Urine fat is typically associated with heavy proteinuria and nephrotic syndrome.[474] It may also be

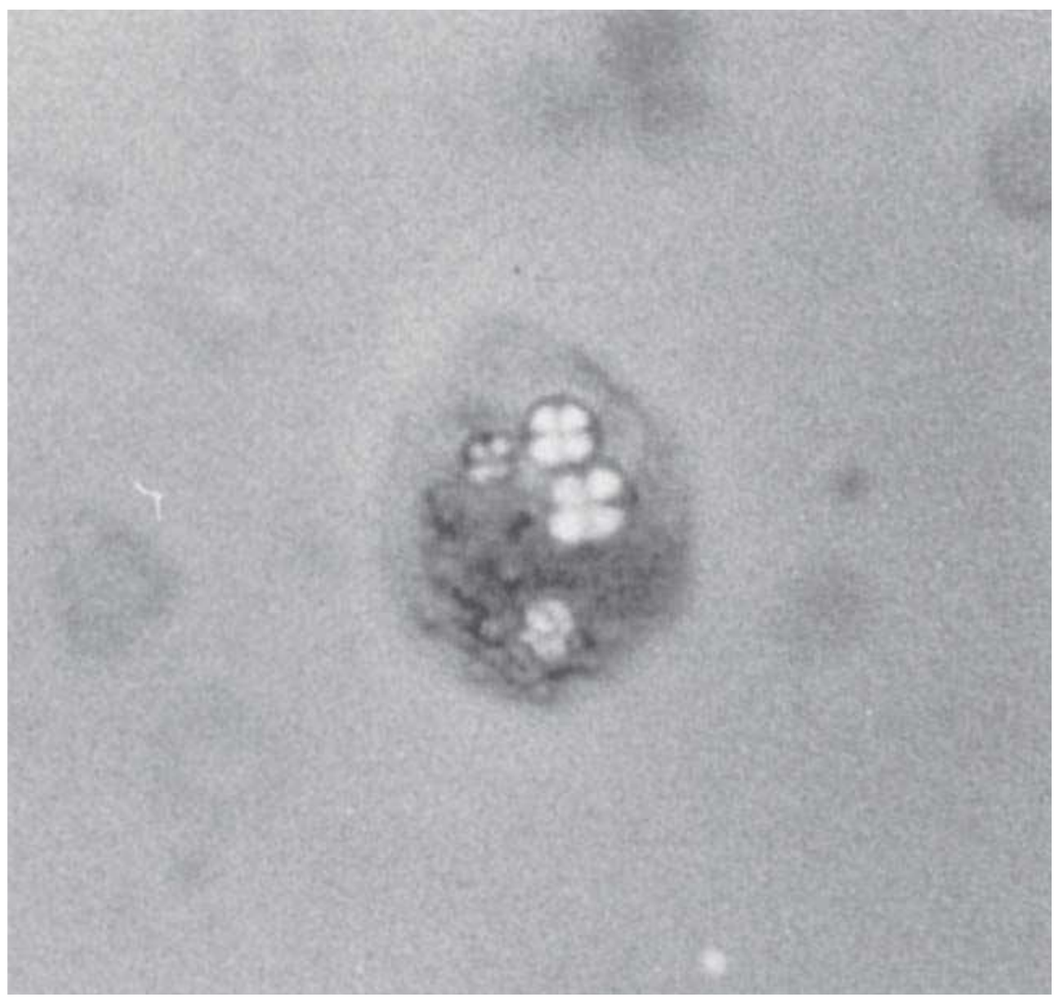


FIGURE 9.20 An oval fat body viewed under polarized light with a classic "Maltese cross" appearance.

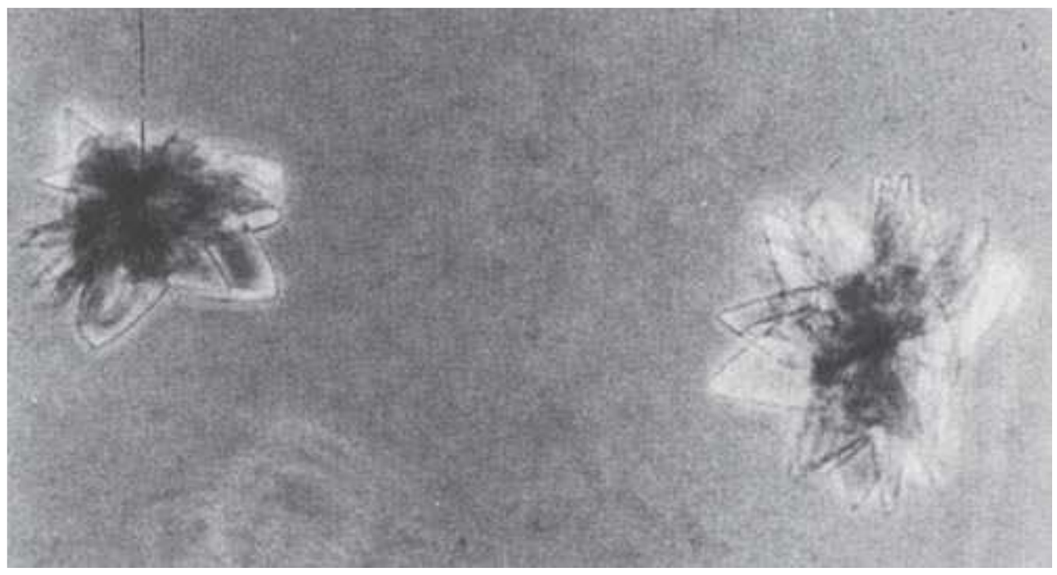


FIGURE 9.21 Uric acid crystals.

seen in polycystic kidney disease and fat embolization syndromes.[475,476]

Crystals

Crystals are common urine sediment findings that are of limited clinical importance in most settings. The identification of crystals, however, can provide important diagnostic clues while evaluating patients with nephrolithiasis, metabolic disorders, or toxin- or drug-induced AKI. Urine pH, crystal morphology, and examination under polarizing light helps to differentiate between different types of urinary crystals (Figs. 9.21 to 9.24). The characteristics of urinary crystals and their associated clinical conditions are described in Table 9.14.

Microorganisms

Bacteria, fungi, protozoa, and parasites may be seen in unstained urine either as a result of contamination or infection. The presence of WBCs suggests infection, and kidney involvement should be suspected if WBC casts or casts embedded with microorganisms are present. Estimation of

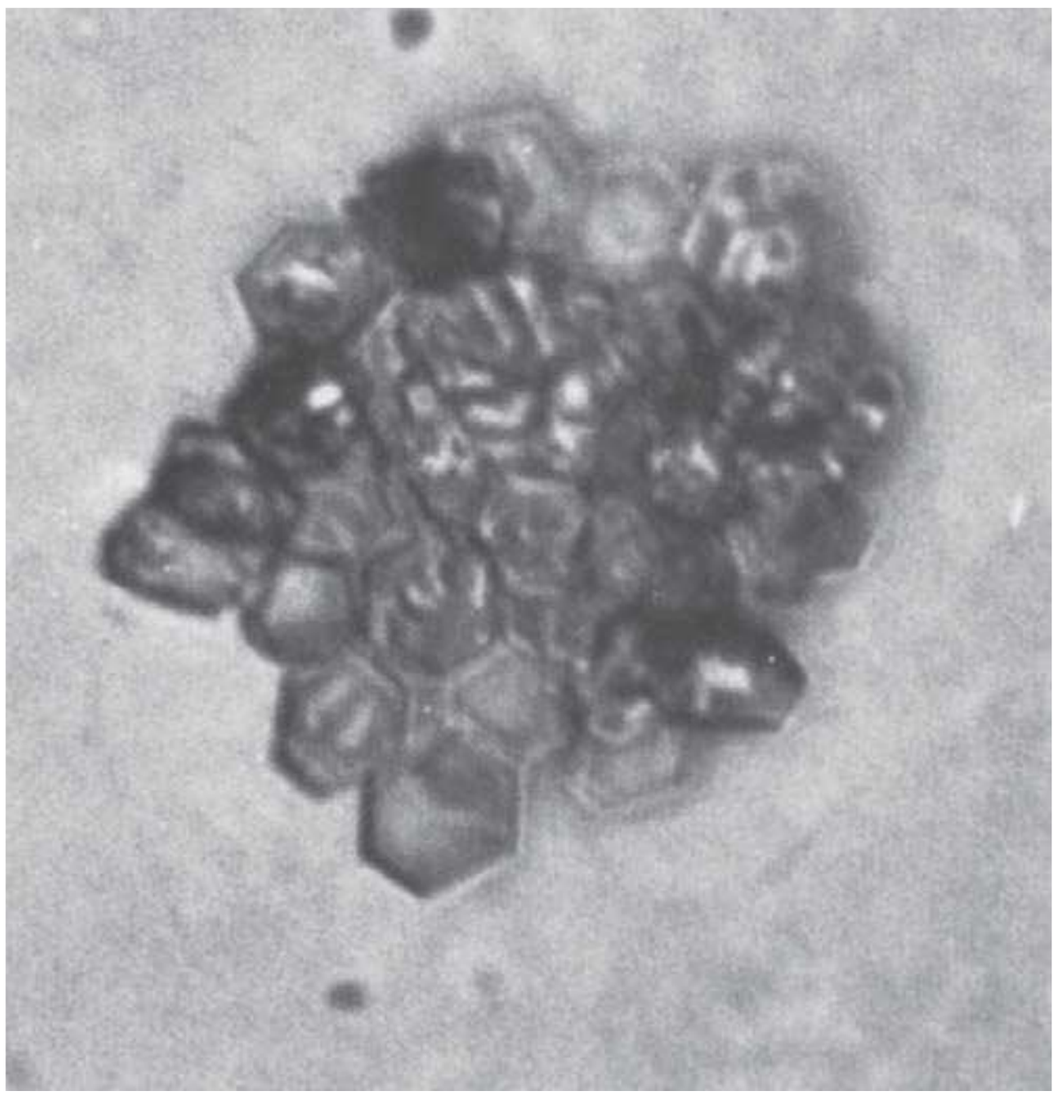


FIGURE 9.23 A conglomeration of cystine crystals from a patient with cystine stones. The typical benzene rings can be seen along the edges.

bacterial count is usually done after Gram staining of the uncentrifuged urine sample. Fungal elements like Candida may be seen both in its yeast and hyphal forms. Trichomonas vaginalis is the most common protozoan found in the urine and has a teardrop shape with motile flagellum. Schistosoma haematobium may be seen in urine in areas of Africa and the Middle East where schistosomiasis is endemic.

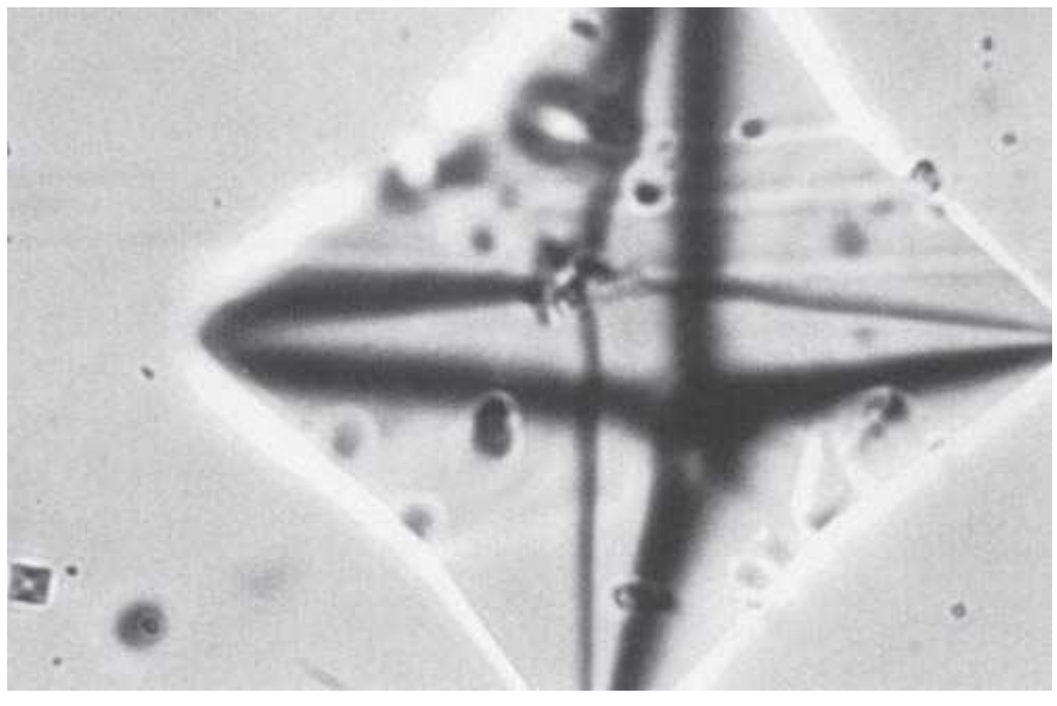


FIGURE 9.22 A very large calcium oxalate crystal. Its large size is obvious when this crystal is compared with the small calcium oxalate crystal (*bottom left corner*).

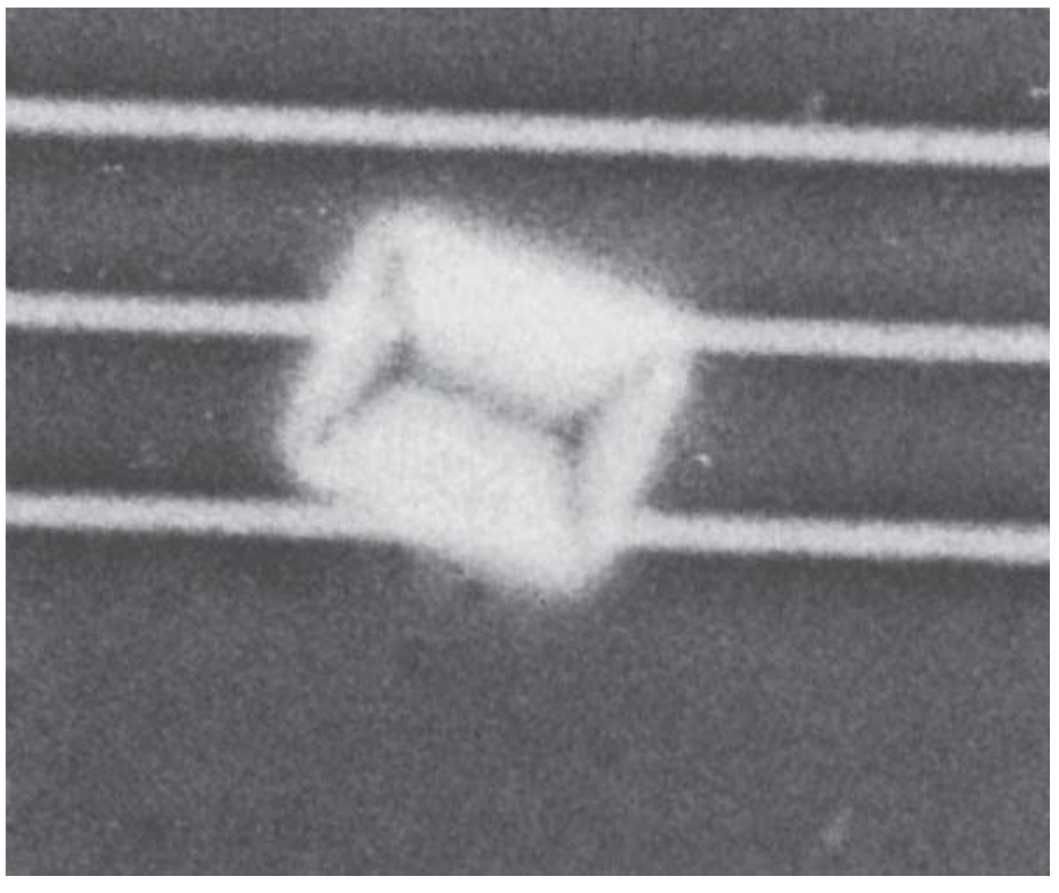


FIGURE 9.24 A triple phosphate crystal with a typical "coffin-lid" appearance, commonly seen in association with infection.

9.14 Urinary Crystals

Type	Description			Associated Clinical Condition
	Shape	Favorable pH	Polarizing Ability (Yes/No)	
Uric acid	Pleomorphic (amorphous, rhomboid prisms, rosettes, needles)	Acidic	Yes	Normal urine, nephrolithiasis (rarely)
Sodium urate	Amorphous	Acidic	Yes	Normal urine, nephrolithiasis (rarely)
Bihydrated calcium oxalate (Weddellite)	Bipyramidal or envelop	Acidic	No	Normal urine, nephrolithiasis (rarely)
Monohydrated calcium oxalate (Whewellite)	Dumb-bell, oval, or biconcave	Acidic	Yes	Nephrolithiasis (rarely), ethylene glycol intoxication
Amorphous phosphate	Amorphous	Alkaline	No	Normal urine, nephrolithiasis (rarely)
Calcium phosphate (hydroxyl-apatite or brushite)	Pleomorphic (prism, starlike, needle)	Alkaline	Yes	Nephrolithiasis (rarely), hyperparathyroidism (rarely), hypercalciuria (rarely)
Magnesium-ammonium-phosphate (triple phosphate or struvite)	Coffin lid or pyramid	Alkaline	Yes	Normal urine, urinary tract infection by urea-splitting bacteria
Calcium carbonate (Calcite)	Dumb-bell, small sphere with radial striations	Alkaline	Yes	Normal urine
Cystine	Hexagonal plate	Acidic	Yes	Cystinosis
Leucine and tyrosine	Yellow sphere (leucine), and brown needles (tyrosine)	Acidic	Yes	Severe liver disease, tyrosinosis, maple syrup disease
Cholesterol	Thin rectangular plate with a square notch corner	Non-pH dependent	Yes (slightly)	Heavy proteinuria
Bilirubin	Needle, red-brown sphere	Non-pH dependent		Conjugated hyperbilirubinemia
Ammonium biurate	Yellow-brown sphere with stria and spicules (thorny apple)	Alkaline	Yes	Normal urine, severe liver disease or portosystemic shunting (rarely)

(continued)

9.14 Urinary Crystals (continued)

	Description			
Type	**Shape**	**Favorable pH**	**Polarizing Ability (Yes/No)**	**Associated Clinical Condition**
Sulfadiazine	Pleomorphic, rosette	Acidic	Yes	Drug use or overdose
Sulfamethoxazole	Sphere, plate, rosette	Acidic	Yes	Drug overdose
Amoxicillin	Broom brush or sheave	Acid	Yes	Drug overdose
Ciprofloxacin	Pleomorphic (needle, sheave, star, fan, butterfly)	Alkaline	Yes	Drug use or overdose
Acyclovir	Needle	Acidic	Yes	Drug use or overdose
Indinavir	Platelike, fan-shaped, starburst	Acidic (may be seen in physiologic pH)	Yes	Drug use, may cause nephrolithiasis
Contrast agent	Platelike		Yes (slightly)	Contrast use

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