

Protein

Normal Physiology

Normally, large quantities of high-molecular-weight (HMW) plasma proteins traverse the glomerular capillaries, mesangium, or both without entering the urinary space. Both charge- and size-selective properties of the capillary wall prevent all but a tiny fraction of albumin, globulin, and other large plasma proteins from crossing.^{[353] [354]} Smaller proteins (<20,000 D) pass readily across the capillary wall.^{[355] [356]} However, because the plasma concentration of these proteins is much lower than that of albumin and globulins, the filtered load is small. Moreover, LMW proteins are normally reabsorbed by the proximal tubule. Thus, proteins such as α_2 -microglobulin, apoproteins, enzymes, and peptide hormones are normally excreted in only very small amounts in the urine.^{[355] [356]} Most healthy individuals excrete between 30 and 130 mg/day of protein, and the upper limit of normal total urine protein excretion is generally given as 150 to 200 mg/day for adults.^{[357] [358] [359]} The upper limit of normal albumin excretion is usually given as 30 mg/day.^[359]

The very small amount of protein that normally appears in the urine is the result of normal tubular secretion. Tamm-Horsfall protein is an HMW glycoprotein (23×10^6 D) that is formed on the epithelial surface of the thick ascending limb of the loop of Henle and early distal convoluted tubule.^{[360] [361]} Tamm-Horsfall protein, also known as uromodulin, binds and inactivates the cytokines interleukin-1 and tumor necrosis factor.^{[362] [363]} Immunoglobulin A (IgA) and urokinase are also secreted by the renal tubule and appear in the urine in small amounts.^[364]

From a consideration of normal physiology, it is apparent that abnormal amounts of protein may appear in the urine as the result of three mechanisms. First, a disruption of the capillary wall barrier may lead to a large amount of HMW plasma proteins that overwhelm the limited capacity of tubular reabsorption and cause protein to appear in the urine. The resulting proteinuria can be classified as glomerular in origin. Second, tubular damage or dysfunction can inhibit the normal resorptive capacity of the proximal tubule, resulting in increased amounts of mostly LMW protein to appear in the urine. Such proteinuria can be classified as tubular proteinuria. Third, increased production of normal or abnormal plasma proteins produced can be filtered at the glomerulus and overwhelm the resorptive capacity of the proximal tubule. These filtered proteins can be especially numerous if they are small or positively charged. Finally, although greater urine protein excretion can also result from increased tubular production of protein, this is rarely the case.

Techniques to Measure Urine Protein

Protein can be measured in random samples, in timed or untimed overnight samples, or in 24-hour collections. Inaccurate urine collection is probably the greatest source of error in quantifying protein excretion in timed collections, particularly 24-hour collections.

However, urine creatinine concentration can be measured to judge the adequacy of the 24-hour collection. If creatinine excretion is similar to that in previous 24-hour samples, then the collection is likely to be reasonably accurate. If no other collections are available for comparison, the adequacy of collection can be judged from the expected normal range of creatinine excretion. For hospitalized men aged 20 to 50 years, this range was found to be 18.5 to 25.0 mg/kg body weight/day, and for women of the same age, 16.5 to 22.4 mg/kg/day ([Fig. 24-4](#)).^[111] These values declined with age, so that for men 50 to 70 years, creatinine excretion was 15.7 to 20.2 mg/kg/day, and for women, 11.8 to 16.1 mg/kg/day (see [Fig. 24-4](#)). Patients who are malnourished or have reduced muscle mass for other reasons can be expected to have lower than normal creatinine excretion rates.

Tests to accurately quantitate total protein concentration in urine rely on precipitation. In the commonly used sulfosalicylic acid method, sulfosalicylic acid is added to a sample

Figure 24-4 Age-related differences in urine creatinine excretion in normal men (*left panels*) and women (*right panels*). Shaded areas represent 95% confidence intervals calculated from the data of Kampmann and co-workers.^[111] Values in the *upper panels* are in mg/day, and values in the *lower panels* are in mg/kg body weight/day.

of urine, and the turbidity is measured with a photometer or a nephelometer. Protein is quantified through comparison of the turbidity of the sample with that of a standard. This method lacks precision, and the coefficient of variation is as high as 20%.^[365] A number of proteins are detected with this method, including γ -globulin light chains and albumin. The method is more sensitive to albumin than to globulins. Trichloroacetic acid can be used in place of sulfosalicylic acid to increase the sensitivity to γ -globulin. False-positive reactions may occur from high levels of Tolectin, tolbutamide, antibiotics, and radiocontrast agents.^{[29] [366] [367]} Total protein content can more accurately be quantified with the use of several monospecific antibodies to different types of urine protein,^[368] but this method is somewhat cumbersome and is seldom used in clinical laboratories.

Total protein concentration in urine can be rapidly estimated with chemically impregnated plastic strips. Most dipstick reagents contain a pH-sensitive colorimetric indicator that changes color when negatively charged proteins bind to it.^[369] However, positively charged proteins are less readily detected. Positively charged immunoglobulin light chains, for example, may escape urine dipstick detection even when present in large amounts in the urine.^[370] A very high urine pH (>7.0) can also give false-positive results, as can contamination of the urine with blood. The dipstick technique is sensitive to very

small urine protein concentrations (the lower limit of detection is 10 to 20 mg/dL).^[370] However, at these low levels, the major constituent of urine protein may be Tamm-Horsfall protein, and thus, a positive test result may not reflect kidney injury. This situation is especially likely to occur when the urine volume is low and the concentration is high. When urine volume is high and the urine is maximally dilute, however, a relative large amount of protein can go undetected. Indeed, total protein excretion approaching 1 g/day may not be detected if urine output is high. If, for example, urine volume is 10 L/day, then the concentration of 1 g of protein would be 10 mg/dL, or below the limit of detection for most reagent strip tests for total protein.

The consistency of results with the same sample assessed repeatedly, or the precision, of reagent strip tests of urine total protein concentration is generally poor.^{[370] [371]} Variability in interpretation both by the same technologist and among technologists has been examined and has been found to be relatively high. For example, at low levels of urine protein concentration (e.g., 6 to 39 mg/dL), inconsistent results among different technologists were seen in 19% to 56% of the determinations.^[370] At higher concentrations (e.g., 196 to 328 mg/dL), inconsistencies were seen in 19% to 44%.^[370] Similar findings were reported in a later study that also found that inconsistencies depended somewhat on the experience of the operator and the type of reagent strip. Inconsistencies were found among experienced technologists in up to 33% of cases, and among inexperienced technologists in up to 93% of cases.^[371]

The sensitivity and specificity of reagent strip protein tests have also been assessed with more accurate quantitative determinations used as gold standards. Interestingly, the sensitivity of these tests appears to be higher when assessed through the use of samples prepared by adding albumin and globulin to normal, protein-free urine than when assessed through the use of actual patient specimens.^{[370] [371]} This difference likely reflects the inability of reagent strips to react to many of the heterogeneous proteins found in human urine. When 20 to 25 mg/dL is used as the limit of detection in clinical specimens, the sensitivity of reagent strips has been found to be only 32% to 46%, and the specificity to be 97% to 100%.^{[370] [371]} The effect of the sensitivity and specificity on the utility of these reagent strip tests also, of course, depends on the prevalence of proteinuria in the population being screened. In a population with a low prevalence of disease, the low sensitivity of the reagent strip tests suggests that the majority of individuals with proteinuria would be missed.^[371]

Urine albumin concentrations can be quantified by a number of techniques. The most commonly used are as follows:

1. Radioimmunoassay can be carried out using a double-antibody technique. Albumin in a urine sample competes with a known amount of radiolabeled albumin for fixed binding sites of antibodies. Free albumin can be separated from bound albumin by immunoabsorption of the (albumin-bound) antibody. Albumin concentration in the sample is inversely proportional to the radioactivity.^{[372] [373] [374]}
2. The immunoturbimetric technique depends on the turbidity of a solution when albumin in a sample of urine reacts with a specific antibody. The turbidity is

- measured with a spectrophotometer, and the absorbency is proportional to the albumin concentration.^{[375] [376]}
3. When it reacts with a specific antibody, albumin in the urine sample forms light-scattering antigen-antibody complexes that can be measured with a laser nephelometer. The amount of albumin is proportional to scatter in the signal.^{[377] [378]}
 4. The competitive enzyme-linked immunoassay (ELISA) has also been used to measure urine albumin.^[379]

The correlation between most of these quantitative techniques is very high. For example the correlation coefficients (r values) between radioimmunoassay and immunoturbidimetry and between radioimmunoassay and nephelometry were both 0.98.^[380] Intra-assay coefficients of variation for immunoturbidimetry and nephelometry were found to be 6.6% and 11.5% at low concentrations and 11.1% and 4.1% at high concentrations, respectively.^[380] Interassay coefficients of variation were 11.4% and 11.5% at low concentrations and 5.4% and 1.4% at high concentrations, respectively, for these two techniques.^[380] In another study, the intra-assay coefficients of variation for radioimmunoassay and nephelometry were 1.7% and 7.7%, respectively, at low albumin concentrations and 3.7% and 6.3%, respectively, at high albumin concentrations. Corresponding values for interassay coefficients of variation were 6.7% and 8.9% at low concentrations, and 8.1% and 11.0% at high concentrations.^[381] The within-run coefficient of variation for an immunoturbidimetric method was found to be 3.5% at low albumin concentrations and 2.4% at high albumin concentrations.^[382] The day-to-day coefficient of variation for the same assay was 5.1% at both low or high albumin concentrations.^[382] These results are similar to those reported by others for the intra-assay and interassay coefficients of variation for nephelometric urine albumin determinations.^[383] Thus, the

precision of these different methods appears to be similar, and choosing among them is largely determined by issues of accuracy, cost, and convenience.

Reagent strip methods have been developed to qualitatively screen for urine albumin excretion. The Albustix (Bayer Diagnostik, Munich, Germany) reagent strip uses a protein error of indicators method that causes color changes in the presence of albumin.^[381] Trace reactions indicate urine albumin concentrations between 50 and 200 mg/L. Thus, more positive reactions can be used to indicate albumin concentrations higher than those generally found in patients with microalbuminuria. In one study, the sensitivity and specificity of the Albustix strip were found to be only 0.81 and 0.55, respectively.^[381] Thus, there is almost a 50% chance of a false-negative result with the Albustix method.

Screening methods have been developed to measure albumin concentrations low enough to detect albumin excretion rates that are abnormal but below the level of detection with standard reagent strips (i.e., in the microalbuminuria range).^{[380] [384] [385] [386] [387] [388] [389] [390] [391] [392] [393] [394] [395] [396] [397] [398]} One of the most extensively investigated methods to screen for microalbuminuria is the immunometric dipstick Micral-Test (Boehringer Mannheim,

Mannheim, Germany).^{[380] [385]} The strip is made up of a series of reagent pads through which the urine sample passes sequentially. Urine is first drawn into a wick fleece and then passes into a buffer fleece that adjusts the sample pH. Next, it passes into a third pad, where albumin in the sample is bound by a soluble conjugate of antibodies linked to the enzyme β -galactosidase. Excess antibody is then adsorbed on immobilized albumin in the next pad, so that only albumin bound to antibody and enzyme reaches the color pad. There the β -galactosidase reacts with a chemical substrate to produce a red dye, the intensity of which is proportional to the bound albumin concentration. The test strip must be read at precisely 5 minutes.^{[380] [385]}

Another qualitative test that has been examined in several investigations is the Micro-Bumintest (Ames, Miles, Elkhart, IN). This test uses a reagent tablet containing the indicator dye bromphenol blue. The intensity of the bluish-green color produced after a drop of urine is placed on the surface of the tablet is proportional to the concentration of albumin.^[380] A latex agglutination method, Albusure (Cambridge Life Sciences, Cambridge, UK), binds albumin in the urine sample to latex.^[381] Agglutination occurs when the albumin is mixed with sheep antihuman antibody. When urine albumin concentrations are greater than 20 mg/L, agglutination is inhibited (antigen excess). Thus, agglutination indicates a urine albumin concentration of less than 20 mg/L.

A number of studies have examined the sensitivity and specificity of screening methods designed to detect very low levels of albumin in urine.^{[380] [384] [385] [386] [387] [388] [389] [390] [391] [392] [393] [394] [395] [396] [397] [398]} Because these tests are only semiquantitative (i.e., nonparametric), a true coefficient of variation cannot be determined. Nevertheless, in one evaluation of the Micral-Test method, an estimated coefficient of variation of the same sample interpreted by different technologists was 12.4%.^[384] Experience in reading the Micral-Test strip was shown to be important.^[397] Observer concordance for the Micro-Bumintest was found to be 95% in one study.^[394] A new version of the Micral-Test, Micral-Test II, has been developed^{[399] [400]}; it is designed to react faster, to be less dependent on timing, and to allow a better color comparison to reduce observer variance. Indeed, in one study, the interobserver concordance was 93% with the Micral-Test II.^[400]

Several studies have examined the sensitivity and specificity of the newer reagent strips that measure very low concentrations of urine albumin. Most of these investigations studied patients with diabetes, and most examined the Micral-Test,^{[380] [385] [386] [387] [388] [389] [397] [401] [402] [403]} the Micro-Bumintest, or both.^{[380] [390] [391] [392] [393] [394] [396]} In general, these albumin reagent strip tests are more sensitive than standard dipsticks, but they also have a relatively high rate of false-positive results. Moreover, it should be remembered that for the most part, these reagent strips were tested in populations of diabetic patients with a high prior probability of a positive result. The number of false-positive results would be expected to be much higher in populations in which the prevalence of albuminuria was lower.

All of the qualitative or semiquantitative urine protein and albumin screening tests discussed so far measure only total protein or albumin concentration. The sensitivity and specificity of these tests can be markedly influenced by fluid intake, the state of diuresis, and the resulting urine concentration. Indeed, in one study, albumin concentration had a

low discriminant value for detecting increased albumin excretion in a 12-hour timed urine sample ([Fig. 24-5](#)). In an effort to correct for problems arising out of variability in urine volume and concentration, many investigators have used the protein-to-creatinine or albumin-to-creatinine ratio in random, or timed urine collections. There is a high degree of correlation between 24-hour urine protein excretion and protein-to-creatinine ratios in random, single-voided urine samples in patients with a variety of kidney diseases.^{[404] [405]} It has been suggested that a protein-to-creatinine ratio

Figure 24-5 Comparison of rates of false-positive and false-negative results when urine albumin concentration was used to predict 12-hour (overnight) excretion greater than 15 µg/min in diabetics.

At a urine albumin concentration cutoff greater than 10 mg/L, the false-positive result rate is high. At a concentration cutoff greater than 20 mg/L, the rate of false-positive results is reduced, but that of false-negative results is high. (Data from Kouri TT, Vikari JSA, Mattila KS, Irjala KMA: Invalidation of simple concentration-based screening tests for early nephropathy due to urinary volumes of diabetic patients. *Diabetes Care* 14:591–593, 1991.)

1126

greater than 3.0 or 3.5 mg/mg or less than 0.2 mg/mg indicates protein excretion rates of greater than 3.0 or 3.5 g/24 hours or less than 0.2 g/24 hours, respectively.^{[404] [405]} However, few studies have systematically examined the sensitivity and specificity or defined optimal levels of detection for protein-to-creatinine ratios in large numbers of patients in different clinical settings.

Much of the data on the usefulness of albumin-to-creatinine ratios has been derived from studies of patients with type 1 or type 2 diabetes.^{[391] [406] [407] [408] [409] [410] [411] [412] [413] [414] [415] [416] [417] [418] [419] [420]} In most of these investigations, the sensitivity and specificity of albumin-to-creatinine ratios were determined using albumin excretion rates from timed urine collections as a standard. Data from several studies were combined to examine the rates of true-and false-positive results for albumin-to-creatinine ratios to detect albuminuria in overnight urine.^[421] Independent of the albumin-to-creatinine ratio cutoff used, the sensitivities and specificities appeared to be reasonable.^[421]

Altogether, these data suggest that albumin-to-creatinine ratios may be useful as a screening test for kidney disease in populations in which the expected prevalence of disease is high (e.g., diabetic persons). Less clear is their potential usefulness in other patient populations in which the prior likelihood of disease may be lower than in patients with diabetes.^[422] A cross-sectional study by Ruggenenti and co-workers^[423] found that morning protein-to-creatinine ratios among 177 nondiabetic outpatients with CKD were predictive of declining kidney function. In kidney transplant recipients, protein-to-creatinine ratios have been shown to significantly correlate with measurements of 24-hour urine protein and appear useful as both screening devices and as longitudinal tests for following the level of proteinuria.^[424] Use of the protein-to-creatinine ratio has also proven reliable in detecting significant proteinuria in pregnant women.^{[9] [10]}

Although protein-to-creatinine or albumin-to-creatinine ratios may be more quantitative than a simple dipstick screening procedure, their use has a number of limitations. For example, obtaining protein-to-creatinine or albumin-to-creatinine ratios on morning, first-void samples may under-estimate 24-hour protein excretion because of the reduction in proteinuria that normally occurs at night.^[425] Storage time and temperature may also affect albumin levels in urine,^[426] and specimens should be analyzed as soon as possible after collection. The fact that urine creatinine must be measured in addition to albumin introduces another source of error. Indeed, the combination of the two errors of two measurements is greater than the error of either one alone (the coefficient of variation is the square root of the sum of the two coefficients of variations, each squared). Urine creatinine concentration is extremely variable, so that very different ratios can be obtained in individuals with similar protein excretion rates. Moreover, a number of variables that may interfere with creatinine determinations can affect the ratios.^[427] Despite these limitations, the urine protein-to-creatinine or albumin-to-creatinine ratio may be useful, especially in individuals in whom urine collection is difficult or impossible.

A number of analytic tools have been developed to separate and identify individual urinary proteins.^[428] These techniques include agarose gel electrophoresis, column gel chromatography, polyacrylamide gel electrophoresis, immunoelectrophoresis, and isoelectric focusing. Proteomic techniques employing mass spectrometry and peptide mass fingerprinting have expanded the number of identified urinary proteins.^{[429] [430]} However, these latter techniques are generally designed to identify, but not accurately quantitate, urine proteins. Some have been used in clinical laboratories to determine the selectivity of urine protein or to identify monoclonal immunoglobulin heavy and light chains. Otherwise, they have largely been confined to research applications.