

Definition

Small quantities of albumin are normally filtered at the glomerulus and reabsorbed by the proximal tubule. The protein that escapes reabsorption does not exceed 150 mg/24 hr so that proteinuria in excess of this amount is regarded as pathologic.

Technique*Albumin*

Two semiquantifiable methods are generally employed to detect albumin: the dipstick method and sulfosalicylic acid precipitation. (The older heat test, while still valid, is not generally used. It involves the demonstration of a ring of precipitate when urine in a test tube is exposed to a flame confined to a narrow segment of the tube.)

The dipstick method (colorimetric reagent strip) is based on the ability of proteins to alter the color of certain acid-base indicators, such as tetrabromophenol blue, without changing the pH. When the dye is buffered at pH 3, it is yellow; the addition of increasing concentrations of protein changes the color to green and then to blue. The developed color is compared with a color chart which allows the protein concentration to be graded from trace to 4+, corresponding to concentrations from 1 to 10 mg/dl to greater than 500 mg/dl.

The sulfosalicylic acid test requires centrifugation of the urine followed by addition of 2.5 ml of the supernatant to 7.5 ml of 3% sulfosalicylic acid. The degree of turbidity is quantified as follows:

Protein (mg/dl)	Degree of Turbidity
0	Clear
1-10	Opalescent
15-30	Can read print through tube
40-100	Can read only black lines
150-400	No visible black lines
>500	Flocculent

Both tests are subject to the caveat that a *concentration* of protein is being tested. The more dilute the urine, the lower will be the concentration of protein for any absolute rate of excretion. Each test is also subject to false negative and false positive reactions. The sulfosalicylic acid test gives a false positive reaction when x-ray contrast media, penicillins, tolbutamide, sulfasoxazole, and *p*-aminosalicylic acid are present in the urine. The test, appropriately, will show a positive reaction in the presence of light chains. The dipstick, on the other hand, will not detect light chains since it is specific for albumin.

Quantitative measurements are performed on an accurately timed 24-hour urine collection. If the patient is well hydrated by drinking at least 1.5 L of fluid during this period, the accuracy of the collection will be enhanced.

Immunoglobulins

The urinary excretion of increased amounts of kappa or lambda light chains, also known as Bence-Jones protein, is suggested by a positive sulfosalicylic acid test with a negative dipstick test. The positive sulfosalicylic acid test indicates the presence of globulins (if the false positive reactions are excluded). To determine the presence of light chains, a heat test is required. This test is based upon the fact that these proteins precipitate out at 56°C and redissolve as the temperature is raised to the boiling point.

To perform the heat test, the urine must first be acidified by adding 1 ml of 2 M acetate buffer to 4 ml of filtered urine. The mixture is heated to 56°C in a water bath for 15 minutes. If, after heating the mixture for 3 minutes at 100°C, clearing of the precipitate occurs, Bence-Jones protein is present. The boiling mixture can then be filtered and should become cloudy as it cools. If large amounts of albumin are present, the disappearance of the Bence-Jones protein precipitate may be obscured; similarly, if the amount of Bence-Jones protein is excessive, it may not dissolve on boiling and the urine needs to be diluted to obtain a positive result.

The definitive tests for immunoglobulins in urine are electrophoresis and immunoelectrophoresis. In normal individuals albumin constitutes about a third of urinary protein and alpha-1 and alpha-2 globulins about half. The beta and gamma components include the microglobulins as well as light chains. A monoclonal protein appears as a dense narrow band which is generally wider than that observed in serum. In tubular abnormalities the urine electrophoresis shows a small amount of albumin and significant amounts of light chains. Glomerular disease is characterized predominantly by albuminuria but globulins may also be present in the urine.

The identity of an abnormal protein in the urine is best determined by immunoelectrophoresis of concentrated urine. This procedure may be positive even when the standard electrophoresis is negative and is the definitive test to establish the presence or absence of light chains and whether they are monoclonal or polyclonal.

Basic Science

Although dropsy, or anasarca, was recognized as a common presentation of disease for many centuries, it was Richard Bright who, in 1827, first pointed out that certain cases of edema are due to disease of the kidney ("Bright's disease")

and that the diagnosis of the renal origin of the edema may be made by demonstrating the presence of albumin in the urine. In his classic text "Reports of medical cases with a view of illustrating the symptoms and cure of diseases by a reference to morbid anatomy," he was the first to show systemically that, if heating of urine in a teaspoon led to the formation of a precipitate and if the addition of acid to the urine also caused precipitation, the urine contained "albumen." ("Albumen" was thus spelled to denote the similarity to egg protein; the molecule is now called "albumin".)

Proteinuria may be categorized as being of "glomerular" origin (i.e., caused by an increase in glomerular permeability to macromolecules) or of "tubular" origin, the latter designation usually referring to impaired reabsorption of protein (which is filtered in normal amounts) due to tubular dysfunction. Proteinuria is further categorized according to the nature of the excreted protein, which may be albumin, globulins, kappa or lambda light chains, or Tamm Horsfall protein. The latter is a protein formed in the ascending limbs of the loops of Henle and released when this area of the nephron is damaged, and is thus another form of tubular proteinuria.

Clinical Significance

The differential diagnosis of proteinuria is shown in Table 192.1. The primary diagnostic procedure is concomitant testing of the urine with both sulfosalicylic acid and dipstick. If only the former is positive, albuminuria is excluded and the differential diagnosis is that of monoclonal light chain diseases. If both tests are positive, albumin is present but other proteins including light chains may also be present.

In patients under age 20 it is essential to exclude postural proteinuria, which has a benign prognosis and is associated with only a minor degree of proteinuria. Urine sediment should be normal to make this diagnosis. The test for orthostatic proteinuria involves a urine collection in the evening followed by confinement to bed until the next morning, when a recumbent sample is obtained. The patient is then allowed to ambulate for a few hours and a third sample is obtained. Presence of protein in the first and third sample with absence in the second confirms the diagnosis of orthostatic proteinuria.

"Functional" proteinuria is a term applied to the transient proteinuria that accompanies fever, exercise, or congestive heart failure and is probably hemodynamically mediated within the kidney. Proteinuria rarely exceeds 500 mg/24 hr and resolves when the initiating event is reversed.

If persistent proteinuria is present, a complete urinalysis including a careful inspection of the urinary sediment is mandatory. Red cell casts and oval fat bodies must be

Table 192.1
Differential Diagnosis of Proteinuria

Albuminuria (including other urinary proteins)
Primary renal disease
Glomerulonephritis
idiopathic
secondary to specific antigens (hepatitis, malaria, etc.)
Tubulointerstitial disease (toxic, allergic, vascular, infective, hereditary)
Renal disease associated with systemic disorders
Diabetic glomerulosclerosis
Systemic lupus erythematosus
Amyloidosis
Vasculitis (allergic, polyarteritis, SLE, etc.)
Neoplasia
Toxins (heavy metals, drugs, allergens)
Hemodynamic (congestive heart failure, constrictive pericarditis, renal vein thrombosis)
Monoclonal proteinuria (light chains)
Multiple myeloma (IgG, IgA, IgD, IgE, and free light chains)
Waldenström's macroglobulinuria (primarily IgM)
Amyloidosis (with or without myeloma)
Mu heavy chain disease
Idiopathic monoclonal gammopathy
Lymphoma

searched for carefully; presence of red cells or red cell casts indicates glomerular disease; lipid-laden tubular cells (oval fat bodies) are found in nephrotic syndrome of a variety of causes.

Renal function studies including blood urea nitrogen, serum creatinine concentration, and creatinine clearance should be performed. Bear in mind that gross overestimation of glomerular filtration rate by the creatinine clearance may occur in proteinuric states due to increased tubular secretion of creatinine. To determine the glomerular filtration rate accurately, an inulin clearance or DTPA clearance may be required.

References

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