New Colorimetric Method for Quantitative Determination of Protein in Urine

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Total urinary protein is rapidly precipitated at room temperature by tannic acid. The tannic acid/protein precipitate, dissolved in aqueous triethanolamine/ferric chloride solution, gives a purple-violet color of high absorptivity. Absorbance at 510 nm is linearly related to concentration from 0.05 to 1.50 A for a protein content of 0.05 to 1.50 g/liter, and less than 5 mg/liter can be detected. The CV and analytical recovery ranged from 0.5 to 1.8% and 98 to 103%, respectively. Nonprotein urinary constituents do not interfere.

Additional Keyphrases: tannic acid precipitation with Fe³⁺ colorimetry of the solubilized precipitate • normal values

Quantitative determination of protein in urine is complicated by the low concentration of protein to be measured and the presence of many interferences. This report presents a new method for determining protein in urine based on the use of tannic acid as a convenient and efficient precipitant. Protein is measured indirectly by a rapid, stable, and sensitive color reaction between the tannic acid in the precipitated tannic acid/protein and a solubilizing ferric chloride solution added to this precipitate. Either known protein or tannic acid solutions can be used in standardization, which greatly simplifies the method. The method is easy, rapid, accurate, and more practical than any other method for routinely quantitating protein in urine.

Materials and Method

Apparatus

A Model 202-UV Spectrophotometer was used (Cecil Instruments Ltd., Cambridge CB4 4 AZ, U. K.).

Reagents

Sodium chloride, 1.5 mol/liter.
Tannic acid (Merck, mol wt approx. 1700), 1 mmol/liter of water containing 1 g of benzoic acid.
Ferric chloride, 10 mmol/liter in equal volumes of triethanolamine and water.

All reagents are stable for at least two months at room temperature.

Protein solutions, 0.05–2.00 g/liter, were prepared by diluting with physiological saline a crystallized pure preparation (approx. 99%) of human albumin, bovine albumin, human γ-globulin, bovine γ-globulin, or a stock bovine albumin, 100 g/liter (Sigma Chemical Co., St. Louis, Mo. 63178).

Procedure

Transfer 0.5 ml of filtered urine, 0.5 ml of the sodium chloride solution, and 0.5 ml of tannic acid (1 mmol/liter) to the bottom of a 15 × 60 mm centrifuge tube. Mix, and after 5 min centrifuge for 10 min at 3000 rpm. Decant the supernatant liquid and invert the tube on filter paper to drain thoroughly. Add 5 ml of the sodium chloride solution and, after suspending the precipitate, centrifuge and remove the wash solution as above. Add 2 ml of water and 0.5 ml of the ferric chloride reagent to the precipitate, and mix. After 5 min, measure the absorbance at 510 nm vs. a blank consisting of 2 ml of water and 0.5 ml of ferric chloride (the color is stable for hours) with a 10-mm pathlength cuvet. Absorbance is linearly related to protein concentration up to 1.5 g/liter (Figure 1). For samples with higher concentration repeat the determination with urine diluted 10-fold in physiological saline.

Results and Discussion

Previous studies have demonstrated that normal and abnormal proteins in urine can be precipitated by tannic acid, isolated by adding caffeine to the precipitate, which replaces the protein because it forms a less-soluble compound with tannic acid, and then detected immunoelectrophoretically or quantitated by nitrogen determination (1–4). The quantity of protein so recovered from urine of normal subjects was found to range from 76 to 151 mg/liter (4).

In the present method, urinary protein is precipitated with tannic acid, but determined colorimetrically by the very sensitive reaction between tannic acid complexed to protein and ferric ions added to the precipitate, after removal of excess tannic acid by washing the precipitate.
Fig. 1. Relation between protein concentration and absorbance

with sodium chloride (1.5 mol/liter), which does not remove tannic acid bound to protein.

The tannic acid/protein complex is fully precipitated on 5-min incubation at room temperature. No changes were observed on incubating a longer time (up to 2 h) at room temperature or at 4 or 37 °C in experiments with normal or hyperproteinuric urine. Under the conditions of the procedure described above, 0.1 µmol of tannic acid is complexed to 0.333 µg of human albumin.

Specimens of 0.05, 0.10, 0.15, and 0.20 ml of the tannic acid precipitant reagent, diluted 2 ml with water and mixed with 0.5 ml of the ferric chloride reagent, yielded absorbances of 0.333, 0.666, 1.000 and 1.333, respectively, corresponding to those obtained with pure human albumin solutions of 0.333, 0.666, 1.000, and 1.333 g/liter (Figure 1). Results for other proteins in the range of 0.05–1.50 g/liter were not notably different. There was no appreciable difference in the case of pure bovine albumin or the stock bovine albumin; pure human and bovine γ-globulins gave about 10% lower values, which I think is not a clinically important difference.

Absorbance was linear from 0.05 to 1.50 A for protein concentrations of 0.05 to 1.50 g/liter. The detection limit is 5 mg/liter (Figure 1). Analytical recovery was complete (98–103%) for 0.5, 1.0, and 1.5 g of human or bovine albumin per liter, added to urine specimens. The results for duplicate analyses of 2-, 4-, 6-, 8-, and 10-fold dilutions of urine containing 1.25 g/liter averaged 1.25 ± 0.025 (SD) g/liter. The coefficient of variation was 0.5 to 1.8% for 10 concurrent determinations of six urines, containing 0.1, 0.2, 0.5, 0.8, 1.1, and 1.4 g of protein per liter.

In an endeavor to detect sources of interference from urinary nonprotein constituents, I applied the method to ultrafiltrate from a filter retaining any substance with molecular weight greater than 10 000 (PM-10; Amicon Co., Lexington Mass. 021173). Absorbances for such ultrafiltrates of urine from 10 normal subjects and 10 proteinuric patients were negligible, ranging from 0.000 to 0.010. Studies for possible interference from commonly used drugs are in progress. Results for some antihypertensive, sedative, and tranquilizer drugs have been negative. Caffeine was not found to interfere 4 h after an intake of 0.75 g by each of five normal subjects and five proteinuric patients, probably because it is excreted as 1-methyluric acid.

Results by the present method were not directly compared with those by any other method. There is no universally acceptable comparison method. I have measured protein in the urine of normal subjects by the new method. The 24-h excretion by two groups of 50 apparently healthy adults of both sexes, 18–40 and 40–88 years old, was 85 mg/day (range 56–148) and 118 mg/day (range 70–192), respectively. These results accord with those observed by others who used accurate methods for total urinary protein, including mucoprotein: 90–297 mg/day (5), 40–150 mg/day (6), and 82–207 mg/day (7, 8).

References