Screening for Albuminuria: A Case for Estimation of Albumin in Urine

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The usefulness of bromcresol green for estimating albumin in urine was evaluated by comparison with the Laurell "rocket" technique. In contrast to the bromcresol green method applied for urinary albumin, rather doubtful results were obtained with conventional (Microzone) electrophoresis for albumin and with precipitation techniques for total protein estimation. Albumin estimation with bromcresol green is recommended as a more reliable substitute for total-protein estimations in urine. Limitations of bromcresol green are also pointed out.

Additional Keyphrases: albumin vs. total-protein content of urine • routine urine analysis • bromcresol green for screening urines • intermethod comparison

Determinations of protein loss via the kidneys are either inaccurate or elaborate. Colorimetric methods with preceding precipitation of the protein require careful standardization of all reagents and manipulations. Doubtful results in our laboratory—and in particular the dependence of the protein values on the type of centrifuge used—caused us to search for an alternative. Because albumin is the most prominent protein leaking through the impaired glomerulus, we attempted to replace routine measurements of total urinary protein by estimations of urinary albumin.

Materials and Methods

Urines. Protein-free and protein-containing urines were obtained from laboratory staff and from hospitalized patients. In general, morning specimens were collected. Some specimens were preserved at −20 °C, with added sodium azide (1 g/liter).

Bence Jones protein. A purified preparation isolated from urines of patients with multiple myeloma was kindly supplied by Dr. H. G. van Eijk, Department of Chemical Pathology, Medical Faculty, Erasmus University, Rotterdam.

Procedure

Laurell "rocket" technique. This was as described by Laurell (1). Anti-albumin was obtained from Behring Werke, Marburg/Lahn. Repeated single determinations on a specimen containing 1.469 g of albumin per liter (mean) showed a coefficient of variation of 0.892% (n = 20).

Total protein content. The protein was precipitated either with trichloroacetic acid (final concentration 0.15 mol/liter) or perchloric acid (final concentration 0.33 mol/liter). After the mixtures had stood for 30 min at room temperature, the precipitates were centrifuged either (a) for 20 min at 1100 × g or (b) for 4 min at 8000 × g. The precipitates were processed and, after reaction with biuret reagent, measured as described in standard textbooks (e.g., 2). When treating the precipitates according to procedure b, repeated single determinations (n = 20) of a urine containing 1.638 g of protein per liter (mean) showed a CV of 5.9%.

Electrophoresis on cellulose acetate was done with Millipore equipment, employing the "Phoroslides" developed by this producer. Details of the procedure are described in Application Report AR-22 (Millipore Corp., Bedford, Mass. 01730).

Albumin in urine by bromcresol green (BCG) technique. After various trials, the following modification of the BCG method of Schirardin and Ney (3) was adopted. The approximate albumin content of the urine was estimated with "Albustix" (Ames Co.,
Elkhart, Ind. 46514). After filtration, the urine was diluted to achieve a concentration of albumin in the range 0-4 g/liter. With the unknowns, we ran three standards of human albumin solutions, 1, 2, and 4 g/liter.

Aliquots (100 µl) of both standards and unknowns were mixed with 1 ml of BCG reagent with an LKB autodiluter. The absorbance at 625 nm could be read immediately, but remained stable up to 5 h at room temperature. Results were calculated with reference to the standard calibration line. The BCG reagent was prepared as described (3). For reasons of economy the standards were made from residues of human albumin concentrates for intravenous administration. The albumin content of the concentrates was calibrated by applying both the BCG method and the Laurell “rocket” technique to human albumin (purchased from AB Kabi, Stockholm, and having a stated purity of >99%). Repeated single determinations of three albuminuric urines and one aqueous solution of albumin showed the following coefficients of variation:

<table>
<thead>
<tr>
<th>mean albumin concn, g/liter</th>
<th>CV, %</th>
<th>n</th>
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<tbody>
<tr>
<td>0.5</td>
<td>6.0</td>
<td>22</td>
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<tr>
<td>1.0</td>
<td>3.5</td>
<td>21</td>
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<td>2.0</td>
<td>2.0</td>
<td>22</td>
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<tr>
<td>4.0</td>
<td>1.0</td>
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**Results**

Intermethod Comparison

Figure 1 illustrates the correlation between results of albumin determinations with the Laurell “rocket” technique and the BCG method. The points, representing 50 urine samples with various protein contents, cluster in two distinct concentration ranges, 0.4-11 and 15-22 g/liter, suggesting a qualitative rather than merely quantitative difference between the two groups of proteinurias. Leaving the seven values of the higher concentration range out of consideration, a narrower correlation is observed (Figure 2), with the regression line passing closer to the origin. The overall correlation depicted in Figure 1 is nevertheless fairly good. It warranted the use of the BCG method as a reference for the evaluation of methods that in our experience had proven less accurate, i.e., estimations by electrophoresis or by precipitation. Figures 3 and 4 show the correlations between total protein values obtained by the biuret method and the albumin values obtained by the BCG method. The following features of the two correlations support the explanation that insufficient packing of the precipitates and loss by decantation account for underestimation of total protein by the precipitation technique:

(a) The slope of both regression lines is less than unity, but the slope obtained with the higher centrifugal force is nearer unity.

(b) With a centrifugal force of 1100 X g, the regression line cuts the ordinate at y = 0.241; i.e., in the range of low concentration, albumin values surpassed the corresponding values of total protein content.

(c) The regression line becomes more nearly linear with use of the higher centrifugal force.

Figure 5 shows the bad correlation between values for albumin as determined by electrophoresis and by the BCG method.

Influence of Color of Urines

From among the urine samples collected, we selected a number of the more intensely colored specimens, to assess the possible interference of color with the estimation of albumin. "Intensely colored" urines usually showed an absorption spectrum running in smooth, steep curves from absorption values between 0.1 and 0.15 at 600 nm up to 1.0 at about 400 nm. Protein-free intensely colored urine showed an apparent content of albumin. Intensely colored albuminuric urines yielded higher values for albumin by the
BCG method than did the Laurell "rocket" technique (Figure 6). Interference from the original color of the urine was shown to depend on the more alkaline pH of those urines, which could not be sufficiently neutralized by the buffer contained in the BCG reagent. Various unsuccessful attempts were made to devise a modified BCG reagent that would ensure a final pH of 4.2, even with strongly alkaline urines, either by adjusting the pH of the reagent to 3.8, by increasing the concentration of the buffer, or by replacing the acetate by biphthalate or succinate. The only way to eliminate the effect of strong urine color appeared to be by individually adjusting the pH of the urine samples to 4.2, by adding HCl, before adding BCG reagent (Figure 6).

Effect of Bence Jones Protein

To assess the affinity of Bence Jones protein for BCG and the possible interference of Bence Jones protein with the albumin determination, we did two sets of experiments:

1. To a series of urines with equal albumin concentration, various amounts of Bence Jones protein were added (0 to 60 g/liter).

2. To a series of urines with different albumin concentrations, equal quantities of Bence Jones protein were added (final concentration, 25 g/liter). Bence Jones protein showed an apparent binding capacity for BCG amounting to 1% of the binding capacity of albumin, but this could be accounted for by a 1% albumin contamination present in the Bence Jones protein preparation.

Discussion

Our experiments confirm the notorious unreliability of precipitation methods for routine determination of urinary protein content (Figures 3 and 4).

Quantification of albumin by electrophoresis yielded even worse results. A more elaborate method of electrophoresis as described by Webster et al. (4), involving extracting the stained albumin bands after electrophoresis of previously concentrated urines, would certainly have yielded more reliable measurements—but such methods would hardly recommend themselves for routine urinalysis. The BCG method, which had initially been considered as highly specific
for the albumin fraction of serum protein, has been shown in certain cases of pathological sera to result in overestimations of albumin, attributable to a considerable affinity of BCG for \( \alpha \) - and \( \beta \)-globulins (4–6). Obviously, this drawback of the BCG method would apply much less to urinalysis; protein excretion patterns in cases of glomerular damage usually include only minute amounts of \( \alpha \) - and \( \beta \)-globulins, even in cases of rather low selectivity grades. Moreover, if the estimation of albumin in urine is intended as a substitute for total protein estimation, a slight overestimation could hardly be objectionable. A more serious overestimation of albuminuria with the BCG method was observed with intensely colored urines. We failed in our attempts to correct for the original color of the urine by reading against a blank obtained by diluting the urine with saline or with the buffer incorporated into the BCG reagent (without BCG dye); the color intensity of the urine/BCG/albumin combination proved to be nonadditive, i.e., the original color of the urine changed in the presence of albumin and BCG. There appeared to be no simple alternative to previously adjusting the pH of the urine to 4.2.

The recent decade has witnessed a very considerable refinement of techniques for differential estimation of protein fractions in urines. Among these the most outstanding are immunological and gel-filtration techniques (7). While refinement of techniques has greatly contributed to increasing the diagnostic possibilities of urinalysis, it could not render the time-honored estimation of total urine protein dispensable. Yet each of the well known total protein determinations has its shortcomings (8). As long as this state of affairs prevails, a laboratory of clinical chemistry must continue to do urinary protein estimations both by total-protein screening tests and by elaborate, more specific methods.

Screening should be as accurate as is compatible with simplicity. Our method of choice, the BCG technique, a compromise halfway between total and specific methods, reasonably satisfies the requirements of simplicity and accuracy for a day-to-day routine testing. In most cases of proteinuria it would cause no serious underestimations. This appears to hold for a large variety of nephrogenic proteinurias as well as for patients with a high excretion of fibrin degradation products. Only in diseases where massive amounts of small paraproteins—such as Bence Jones protein—are excreted, would the BCG method be a bad substitute for total protein estimations.

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References