Measurement of Total Protein and Albumin in Serum with a Centrifugal Analyzer

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We describe methods for measuring total protein and albumin with the centrifugal analyzer. The biuret reaction for total protein is done in the transfer disc, thus increasing analysis rate. Albumin is measured by the brom cresol green reaction. Precision of both methods is excellent and correlation studies demonstrate the validity of the procedures.

In most clinical chemistry laboratories, mechanized procedures are essential for measurement of serum total protein and albumin. The biuret reaction is well established as the most practical approach to mechanized total protein measurements (1–4); albumin has posed a more difficult problem, with several procedures being proposed, some of which have involved salt fractionation (1–4), electrophoresis (5), dye binding (6–9), or total globulin measurements (10, 11). Dye binding offers the most convenient procedure for automation, but suffers inherent inaccuracies. 2-(4′-Hydroxybenzeneazo)-benzoic acid has been used extensively (6, 7) but is quite inaccurate (11). The brom cresol green (BCG) procedure is more accurate (8, 9), although a recent report questions the specificity of the reaction (12). However, it is easily adapted to automation and at the present time appears to be the reagent of choice. Automated continuous-flow systems are widely used to measure total protein and albumin. Another approach to automation, however, is the centrifugal analyzer, which offers a great deal of flexibility to the clinical laboratory. These instruments are ideally suited to kinetic measurements but can also be applied to conventional end-point determinations. A kinetic total protein procedure has been reported (13) for use with the centrifugal analyzer, but in our hands the method was found to be relatively imprecise and subject to errors in analyses of sera from patients with dysproteinemias. More conventional approaches to adaptation of the biuret reaction for use with the centrifugal analyzer have also been reported (14, 15).

Here, we evaluate procedures adapted to the centrifugal analyzer for serum total protein and albumin, by the biuret reagent and BCG, respectively.

Materials and Methods

Apparatus

We used the Aminco “Rotochem II” (American Instrument Co., Silver Spring, Md. 20901). The Aminco “Rotofill II” diluter was used for sample and diluent delivery and some reagents. Other reagents were dispensed with Eppendorf microliter pipets (Brinkmann Instruments, Inc., Westbury, N.Y. 11590).

Reagents

Biuret reagent. The working reagent contained sodium potassium tartrate (9 g/liter), cupric sulfate (3 g/liter), sodium hydroxide (8 g/liter), potassium iodide (5 g/liter), and ARW-7 wetting agent (1 ml/liter), and was used as purchased from Fisher Scientific Co., Fair Lawn, N.J. 07410.

Total protein blank reagent. Nine grams of sodium potassium tartrate was dissolved in 500 ml of distilled water, 8.0 g of sodium hydroxide and 5.0 g of potassium iodide were added and dissolved, and the solution was diluted to 1 liter.

Brom cresol green reagent. This reagent (from Fisher Scientific Co.) contained brom cresol green (146 mg/liter), citric acid (8.1 g/liter), and trisodium citrate dihydrate (17.1 g/liter). To 1 liter of this reagent was added 2.0 ml of Dow-Corning “Antifoam B” (Fisher Scientific Co.), and the contents were mixed thoroughly. This reagent must be mixed just before it is to be used.

Albumin blank reagent. This reagent was an aqueous solution of citric acid (8.1 g/liter) and trisodium citrate dihydrate (17.1 g/liter).

Quality control sera. Lyophilized control sera used in the precision studies, from Hyland, Costa Mesa, Calif. 92626, were filtered through a 0.45 μm (av. pore size) disposable filter before use ("Milllex"; Millipore Corp., Bedford, Mass. 01730).

Standards

Total protein. The standard (Armour Pharmaceutical Co., Phoenix, Ariz. 85077) contained about 62.5 g of crystalline bovine albumin per liter.

Albumin. The standard contained about 45 g of human albumin, fraction V, per liter (Sigma Chemical Co., St. Louis, Mo. 63178), in physiological saline and preserved with sodium azide (0.2 g/liter). Owing to the difficulty of weighing and solubilizing a specified amount of albumin accurately, the concentration of this standard was established by a manual reference procedure (16) involving the biuret reaction, with crystalline bovine albumin as a standard. This working standard was stable for one month when stored refrigerated in tightly stoppered containers.

Procedures

Table 1 summarizes the conditions for the total protein and albumin methods.

Total protein. The automatic diluter is adjusted to deliver 20 μl of standard (cuvette 1) or sample (cuvettes 2–14) and 90 μl of saline diluent to the large reagent well of the transfer disc, followed by addition of 500 μl of biuret reagent to these same reagent wells. Cuvette zero contains 20 μl of water, 90 μl of saline, and 500 μl of biuret reagent. After incubation at room temperature for 10 min, the contents of the transfer disc were analyzed in the centrifugal analyzer.

The standard Endpoint I computer supplied with the instrument is used for the total protein assay, and calculates the
Table 1. Conditions for Measurement of Total Protein and Albumin

<table>
<thead>
<tr>
<th>Sample vol, μl</th>
<th>Total protein</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>90</td>
</tr>
<tr>
<td>500</td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Computer program</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Wavelength, nm</td>
<td></td>
<td>550</td>
</tr>
<tr>
<td>Reading time, s</td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>

Table 2. Precision of Total Protein and Albumin Measurements

<table>
<thead>
<tr>
<th>Test</th>
<th>No. analyses</th>
<th>Mean g/liter</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-disc precision</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td>13</td>
<td>45</td>
<td>±0.41</td>
<td>0.91</td>
</tr>
<tr>
<td>Albumin</td>
<td>12</td>
<td>29.5</td>
<td>±0.50</td>
<td>1.77</td>
</tr>
<tr>
<td>Within-run precision</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td>52</td>
<td>45</td>
<td>±0.88</td>
<td>1.96</td>
</tr>
<tr>
<td>Albumin</td>
<td>24</td>
<td>30</td>
<td>±0.78</td>
<td>2.57</td>
</tr>
<tr>
<td>Day-to-day precision</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td>91</td>
<td>46.5</td>
<td>±0.90</td>
<td>1.86</td>
</tr>
<tr>
<td>Albumin</td>
<td>86</td>
<td>28</td>
<td>±1.00</td>
<td>3.37</td>
</tr>
</tbody>
</table>

The present procedure allows the reaction to proceed in the transfer disc, and the centrifugal analyzer is used to make only the final absorbance readings, thus minimizing valuable instrument time. The sequence of addition of sample and reagent to the transfer disc is critical to ensure adequate mixing. Sample and flush are added first, followed by biuret reagent. Mixing is obviously adequate, because the precision of the method is excellent (Table 1), and because further mixing by sonication of the transfer disc contents before measurement did not alter the final results. Evaporation is no problem, because the transfer discs have plastic cover plates. The BCG/albumin reaction proceeds very rapidly; a stopped-flow study showed the reaction to be essentially complete in less than 1 s for a sample in the normal range. Thus, conventional mixing and reading in the centrifugal analyzer can be used for albumin measurements.

Blank corrections for all lipemic and moderately icteric and hemolyzed samples are necessary for total protein measurements; they can be made automatically by using a special computer program designed to store the blank absorbance readings and perform a subtraction before the final calculation. Usually only a few samples each day require blank correction, and these can be combined into a single transfer disc. It obviously is important that the blank samples maintain the same cuvette identity as the biuret-incubated samples. Albumin blank corrections are similarly determined. However, the smaller sample volume makes it necessary to apply the correction only for lipemic sera. The biuret reagent can be automatically or manually pipetted, but it is preferable to pipet the BCG reagent by hand, to avoid contamination of the automatic diluter. Cleansing of the transfer disc and cuvettes in the rotor poses no problem when the routine wash maintenance is followed, but the importance of adequate washing of transfer discs and cuvettes between tests cannot be overemphasized. A weekly sequential washing with pepsin (10 g/liter), sodium hypochlorite (50 g/liter), and ethanol is essential. Between-test washing with a 330 g/liter solution of "Contrad 70" (Scientific Products, McGraw Park, Ill. 60085) followed by HCl (10 ml/liter) and water are extremely important, especially after albumin measurements.

We evaluated the precision of the two methods by using

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1 Killingsworth, L. M., personal communication.
lyophilized control sera (Table 2). Precision is excellent and in our laboratory has proved to be more than adequate in providing clinically useful data.

We found early in the study that the BCG reagent without added Antifoam B gave extremely poor precision, because of intracuvette splashing during the centrifugal analyzer vacuum-mix cycle. Addition of Antifoam B with thorough mixing of reagent before use eliminated this problem completely.

**Intermethod comparisons.** The two methods proposed in this report were checked against procedures that are known to give accurate results.

The total protein method was correlated with a manual total protein procedure in which the biuret reaction is also used. The good results of this correlation study (Figure 1) are not surprising, because the basic reaction used in the two methods is the same.

The albumin method was compared to an immunochemical technique involving nephelometric measurements of antigen-antibody complexes. This comparison procedure, although not widely used, offers potentially good specificity, owing to the immunochemical basis of the reaction. The results of the comparison study (Figure 2) indicate that the higher albumin concentrations are underestimated, whereas in the low range the reverse is true. These findings are very similar to those of Webster et al. (12), who compared BCG with the Laurell "rocket" immunochemical method for serum albumin. The explanation given by Webster et al. was that BCG binds nonspecifically to globulins and it is a common clinical finding that hypoglobulinemia is associated with some hyperglobulinemia. The correlation data given in Figure 2 demonstrate that within the range of 18–55 g/liter of albumin the discrepancy should not exceed 3 g/liter. In view of this negligible error, we consider the BCG method to be satisfactory for serum albumin. However, the user of any BCG method should recognize that there is some overestimation in patients with hypoglobulinemia.

The two procedures we propose for total protein and albumin fulfill the needs of the present day mechanized clinical chemistry laboratory. They are extremely rapid, with the potential of performing over 300 analyses an hour. A sample volume of only 25 μl is required, and the methods are therefore ideal for analysis of pediatric samples. The methods exhibit extremely good precision on a day-to-day basis, and their accuracy is quite satisfactory.

**References**

5. Durrum, E. D., Microelectrophoretic and microionophoretic techniques. *J. Am. Chem. Soc. 72, 2933 (1950).*