THE ESTIMATION OF ALBUMIN AND GLOBULIN IN BLOOD SERUM

II. SEPARATION OF FRACTIONS BY CENTRIFUGATION WITH THE ANGLE CENTRIFUGE*

By HOWARD W. ROBINSON, J. WAIDE PRICE, and CORINNE G. HOGDEN

(From the Children's Hospital Research Foundation and the Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati)

(Received for publication, August 1, 1938)

In the clinical methods for the quantitative estimation of serum proteins, filtration, through filter paper, is the usual procedure for the separation of the globulin precipitated from albumin by a 1.50 M sodium sulfate solution. On account of the nature of the precipitate, a highly retentive paper is needed, and also, with most sera, the filtrate must be refiltered many times before it is clear. In a previous communication (1) the authors showed that filter paper adsorbs a definite amount of the soluble protein. Therefore, it is necessary to discard the first portion of the filtrate, because there is a loss of albumin. Later portions are uniform in nitrogen concentration and contain the protein that is soluble in this salt concentration. This protein adsorption was suggested as the cause of the discrepancies in nitrogen values on duplicate albumin filtrates which have been experienced by many workers in employing the micromethod of Howe (2). A detailed procedure for the filtration was outlined in Paper I (1), which, in our hands, has always given like results on duplicate filtrates. Unfortunately, the method requires at least double the amount of serum and, when the quantity of solution that is filtered is increased, the operation becomes very time-consuming. Under these conditions it seemed desirable to search for a substitute or a means of elimination of the filter paper.

*An abstract of this paper was presented before the Thirty-second annual meeting of the American Society of Biological Chemists at Baltimore, March, 1938.

207
Centrifugation of the globulin precipitate had been suggested as a quick way to obtain a clear albumin solution. Before the development of our present filtering procedure (1), we had attempted to separate the protein constituents in the ordinary centrifuge (International, size No. 1). The serum-sodium sulfate mixtures were placed in 50 cc. Pyrex centrifuge tubes and the eight-place combination head was employed. Centrifugation was carried out in a temperature-controlled room at 38°. The results were very unsatisfactory, as in some cases there was very little sedimentation in a reasonable time and in none were clear supernatant solutions obtained. At the time we were much disturbed by the possibility that the effect of heating of the solution in the centrifuge might influence the solubility of the protein and also cause convection currents that would hinder sedimentation. When the angle centrifuge was brought to our attention, we decided to give it a trial on this problem.

This centrifuge1 is a small portable instrument in which the centrifuge tubes revolve at an angle of 40° with the vertical. According to the manufacturer, the principle of this machine was discovered by Dr. Ragnar Lundgren, of St. Goran Hospital, Stockholm. He found that the speed of sedimentation is very much greater with the tube in this position. On centrifuging the 1.50 M sodium sulfate-protein mixture for 1 hour, a clear supernatant solution is obtained which, with reasonable care, can be pipetted from the globulin precipitate and the albumin determined on an aliquot. On a series of blood sera, albumin determinations were made on solutions obtained by our filtration procedure with filter paper and on those obtained by centrifugation. The results are shown in Table I. The difficulties caused by filter paper can be avoided and the determinations made in a shorter time and on smaller samples of serum by centrifugation of the globulin precipitate.

Methods

Human, dog, and rabbit sera were used in these experiments. The rabbit blood was obtained from the heart without anesthesia and the dog and human bloods by venous puncture. A solution

1 The angle centrifuge is imported and sold by Ivan Sorvall, 210 Fifth Avenue, New York.
of 22 per cent sodium sulfate was added to the serum in the proportion of 30 parts to 1 part of serum. For the comparison study, at least 3.5 cc. of serum were treated with 105 cc. of the salt solution. All the precipitations, filtrations, and centrifugations were carried out in a 38° constant temperature room. As a matter of convenience the serum-sodium sulfate mixtures were allowed to stand overnight at 38°, although comparable results could be obtained after standing for 4 hours. 75 cc. of the serum-sodium sulfate mixture were filtered through one sheet of 9 cm. No. 00 Munktell paper in the manner described before ((1) p. 496). Four 5 cc. aliquots for nitrogen determinations were measured from the last 30 cc. of solution that came through the filter. These determinations were made by the micro-Kjeldahl digestion and distillation procedure described before (1).

For centrifugation, 15 cc. portions of the serum-sodium sulfate mixture were placed in the special 15 cc. oval Pyrex centrifuge tubes and the tubes were covered with rubber caps. This tube is a special tube made for the centrifuge which takes full advantage of the principle of the machine, as it reduces to a minimum the distance the precipitated particle travels across the tube. The type SP angle centrifuge head was used with the aluminum adapters to fit the 15 cc. tubes. If the centrifuge is run at a speed of 4200 r. p. m. for 1 hour in the 38° room, the temperature of the mixture reaches approximately 40°. With longer periods of centrifugation the temperature does not increase above 40°.

So far we have obtained clear supernatant solutions from all mixtures of serum in 1.50 M sodium sulfate solutions after centrifuging for 1 hour. However, there may be some pathological sera, such as those from patients with lipoidal nephrosis, with which it may be impossible to make the separation by centrifugation.

The precipitated globulin is thrown to the bottom and lower end of the outer side of the tube from the axis of rotation. The precipitate does not tend to take a horizontal position, as do red blood corpuscles from plasma, when the tube comes to rest in the

2 We lost a number of determinations through breakage of the glass tubes before we learned the desirability of keeping the aluminum adapters in shape by means of a steel form. These adapters are made of very thin material and lose their shape after a few weeks of centrifuging at high speed.
oblique position. The tube is carefully removed from the centrifuge in the inclined position and turned so that the precipitate is on the lower side of the tube. The rubber cap is removed and a 5 cc. Mohr pipette, whose end is attached to a rubber suction tube, is carefully introduced into the clear solution. About 11 cc. of the supernatant can be withdrawn without disturbing the precipitate. 5 cc. aliquots of this solution are measured into 100 cc. Kjeldahl flasks for the nitrogen determinations. In this study all centrifugations were made in two 15 cc. tubes and two samples for analysis were obtained from each supernatant solution.

In most cases the precipitated globulin tends to settle readily in the 1.50 M sodium sulfate solution. At higher salt concentrations the precipitate remains dispersed in the solution or may rise to the top. In a series of experiments to determine solubility curves, we have been able to centrifuge some precipitates from 1.70 M Na₂SO₄ solution and obtain clear supernatants.

For centrifugation of precipitate the 1.50 M solution of sodium sulfate is a much better medium than the 2.025 M potassium phosphate buffer mixture (K₂HPO₄:KH₂PO₄ = 2:1), which has the same salting-out properties (3), because the density of the former solution is not as great as the latter.

Results

The results of a representative group of the determinations are presented in Table I. The rabbits and dogs except Dog D-5 were apparently normal animals that had not been subjected to any other laboratory procedures. Dog D-5 had been on the modified protein-deficient diet of Weech, Goettsch, and Reeves (4) since December 10, 1937. The human subjects were hospital patients; H-1 had a catarrhal jaundice, H-2 subacute bacterial endocarditis, H-3 subacute nephritis of the nephrotic type, and H-4 nephritis. In all our experiments the difference between the two methods has never been greater than 0.07 gm. of protein per 100 cc. of serum. All the values in column (b) in Table I were made from supernatants which were obtained after centrifuging the mixture for 1 hour. This time interval may be longer than that required in most cases, for we have also obtained good results by a half hour of centrifugation. However, a firmer packing of
precipitate is obtained with the longer centrifuging, which makes
the withdrawal of clear solution much easier. The results indi-
cate that the use of filter paper may be avoided and determina-
tions of serum albumin made in a shorter time on smaller samples

**Table I**

**Serum Albumin Determinations**

Comparison of serum albumin concentrations made from solutions when
globulin precipitate was removed by (a) filtration at 38° through paper by
"correct value" procedure (1); (b) centrifugation at 38° in the angle cen-
trifuge.

<table>
<thead>
<tr>
<th>Date</th>
<th>Type of serum</th>
<th>Total serum protein</th>
<th>Non-protein N</th>
<th>Albumin per 100 cc. of serum</th>
<th>Difference (a) - (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm. per 100 cc.</td>
<td>mg. per 100 cc.</td>
<td>gm.</td>
<td>gm.</td>
</tr>
<tr>
<td>1953</td>
<td></td>
<td></td>
<td></td>
<td>Filtered (a)</td>
<td>Centrifuged (b)</td>
</tr>
<tr>
<td>Jan. 12</td>
<td>R-1</td>
<td>5.95</td>
<td>29</td>
<td>4.62</td>
<td>4.65</td>
</tr>
<tr>
<td>&quot; 24</td>
<td>R-2</td>
<td>7.24</td>
<td>47</td>
<td>5.28</td>
<td>5.29</td>
</tr>
<tr>
<td>Feb. 14</td>
<td>R-3</td>
<td>7.72</td>
<td>48</td>
<td>5.26</td>
<td>5.24</td>
</tr>
<tr>
<td>Jan. 4</td>
<td>D-7</td>
<td>6.00</td>
<td>28</td>
<td>3.81</td>
<td>3.80</td>
</tr>
<tr>
<td>&quot; 24</td>
<td>D-8</td>
<td>5.99</td>
<td>27</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>&quot; 12</td>
<td>D-9</td>
<td>6.20</td>
<td>28</td>
<td>3.88</td>
<td>3.86</td>
</tr>
<tr>
<td>Feb. 4</td>
<td>6.89</td>
<td>28</td>
<td>4.28</td>
<td>4.34</td>
<td>-0.06</td>
</tr>
<tr>
<td>Jan. 7</td>
<td>D-6</td>
<td>6.53</td>
<td>25</td>
<td>3.83</td>
<td>3.82</td>
</tr>
<tr>
<td>&quot; 18</td>
<td>D-18A</td>
<td>6.80</td>
<td>32</td>
<td>4.20</td>
<td>4.24</td>
</tr>
<tr>
<td>&quot; 17</td>
<td>D-5</td>
<td>4.27</td>
<td>20</td>
<td>2.52</td>
<td>2.56</td>
</tr>
<tr>
<td>Feb. 8</td>
<td>4.17</td>
<td>19</td>
<td>2.22</td>
<td>2.27</td>
<td>-0.05</td>
</tr>
<tr>
<td>Jan. 17</td>
<td>D-9</td>
<td>6.52</td>
<td>25</td>
<td>4.05</td>
<td>4.12</td>
</tr>
<tr>
<td>&quot; 18</td>
<td>H-1</td>
<td>7.63</td>
<td>29</td>
<td>4.37</td>
<td>4.42</td>
</tr>
<tr>
<td>&quot; 18</td>
<td>H-2</td>
<td>5.95</td>
<td>30</td>
<td>3.63</td>
<td>3.63</td>
</tr>
<tr>
<td>&quot; 19</td>
<td>H-3</td>
<td>4.95</td>
<td>25</td>
<td>2.80</td>
<td>2.86</td>
</tr>
<tr>
<td>Feb. 7</td>
<td>5.05</td>
<td>25</td>
<td>2.88</td>
<td>2.83</td>
<td>0.00</td>
</tr>
<tr>
<td>Jan. 27</td>
<td>H-4</td>
<td>6.24</td>
<td>59</td>
<td>3.45</td>
<td>3.43</td>
</tr>
</tbody>
</table>

* R = rabbit, D = dog, H = human.

of serum by employing the angle centrifuge for the separation of
the precipitated globulin.

**Summary**

1. The separation of the precipitated serum globulin from the
albumin in 1.50 M sodium sulfate solution can be made by centri-
fugation with the angle centrifuge.
The results of the comparable determinations of serum albumin made by filtration and centrifugation have always been within 0.07 gm. per 100 cc.

3. The advantages of the procedure are, first, that by elimination of the filter paper, the protein adsorption error is avoided and, therefore, smaller amounts of serum may be used for the determination, and, secondly, that centrifugation is much faster than the filtering procedure.

BIBLIOGRAPHY

THE ESTIMATION OF ALBUMIN AND GLOBULIN IN BLOOD SERUM: II. SEPARATION OF FRACTIONS BY CENTRIFUGATION WITH THE ANGLE CENTRIFUGE

Howard W. Robinson, J. Waide Price and Corinne G. Hogden

J. Biol. Chem. 1938, 126:207-212.

Access the most updated version of this article at http://www.jbc.org/content/126/1/207.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/126/1/207.citation.full.html#ref-list-1