THE DETERMINATION OF THE ALBUMIN AND GLOBULIN CONTENTS OF HUMAN SERUM BY METHANOL PRECIPITATION

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Although the determination of the albumin and globulin contents of human serum by neutral salt fractionation, as in Howe's method (1), has proved useful because of its simplicity, such methods have definite limitations. Butler (2) has shown that the albumin-globulin separation by neutral salt precipitation is not sharp and that these fractions overlap one another grossly. Leutscher (3) has also pointed out that salting-out methods are most inaccurate in cases in which they are of greatest clinical interest, and that a change in the technique of the salting-out methods is indicated.

While it is impossible to defend any of these techniques as necessarily yielding absolute albumin and globulin values, accumulated evidence indicates that electrophoretic data have more meaning than have results obtained by any salting-out method. The method described in the present paper has been found to yield albumin and globulin values which check closely with the results obtained by the electrophoretic method.

The method is based on the observation of the present authors that normal human serum may be satisfactorily separated into its globulin and albumin components by appropriate treatment with methanol. Electrophoretic analyses of whole serum, and of the separated albumin and globulin fractions, showed that at 0° almost all of the serum albumin remains soluble at a concentration of 42.5 per cent methanol, in the pH range 6.7 to 6.9, and at ionic strength of about 0.03, while the globulins are almost quantitatively precipitated.

A comparison of the albumin-globulin ratios obtained electrophoretically and by the methanol technique as well as by sodium sulfate separation is given in Table I and indicates that the results obtained with the first two methods agree within 5 per cent for normal serums, and within 5 to 10 per cent for abnormal serums. The results by the sodium sulfate method are in far less satisfactory agreement with the electrophoretic analysis, confirming the recent observation of Dole (4) that albumin-globulin ratios, measured electrophoretically, are roughly two-thirds the ratio found by chemical fractionation.

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ALBUMIN AND GLOBULIN DETERMINATION

Procedure

Reagents—

Methanol reagent. 607 ml. of c.p. methanol are added with mixing to 393 ml. of distilled water. The mixture is cooled to 0°, and finally made up to 1 liter with cold methanol.

Acetate buffer. 72 ml. of 1 M acetic acid and 12 ml. of 1 M NaOH are diluted to 1 liter with distilled water.

Comparison of Albumin-Globulin Ratio

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>Albumin, gm. per 100 ml.</th>
<th>Globulin, gm. per 100 ml.</th>
<th>Albumin-globulin ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum No.</td>
<td></td>
<td>Electro-</td>
<td>Methanol</td>
</tr>
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<td>------------------------</td>
</tr>
<tr>
<td>1</td>
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<td>3.29</td>
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<td>3.95</td>
<td>4.00</td>
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</tbody>
</table>

Electrophoretic analyses were performed by Dr. J. W. Williams, University of Wisconsin; Dr. D. Gordon Sharp, Duke University; Dr. L. G. Longsworth, The Rockefeller Institute for Medical Research; Dr. John A. Leutscher, Johns Hopkins Hospital; and at the Department of Physical Chemistry, Harvard Medical School.

* Serums 10 to 13 from normal adults.

Method

The serum and all reagents should be maintained at all times between 0° and +1°. This may be achieved either by working in a 0° cold room or with the aid of a small ice bath, or by the use of a low temperature liquid bath.

2.0 ml. of fresh serum are pipetted into a 15 ml. conical centrifuge tube and 1.0 ml. of acetate buffer is added with stirring, the temperature being maintained as stated above. To this mixture, 7.0 ml. of cold methanol reagent are added with stirring. After being thoroughly mixed, the tube is allowed to stand at 0° for 12 hours, during which time the globulins will have precipitated, while the albumin remains in solution.
Either of two methods for the removal of the precipitate may be followed, depending upon the equipment at hand. If a refrigerated centrifuge is available, the suspension may be centrifuged at 0° to +2° at 3000 R.P.M. for 15 minutes, after which the clear supernatant is decanted. A sample of the supernatant is analyzed for nitrogen; the precipitated proteins are discarded. If such a centrifuge is not available, the following procedure has been found to yield equally satisfactory results.

A small funnel fitted with a fluted No. 42 Whatman filter paper is thoroughly chilled in a cold room or ice box. The cold methanol-protein suspension is then filtered and the clear filtrate used for nitrogen analysis. The actual filtration may be done at room temperature, provided sufficient filtrate for analysis, at least 3 ml., is obtained within 5 to 7 minutes after removal of the material from the cold. In our experience, the temperature of the suspension does not rise above 7° during this time, and results so obtained have been satisfactory. Because of the volatility of methanol, aliquots for nitrogen analysis are immediately taken and transferred to digestion flasks.

The albumin filtrates from normal serum usually contain from 1.2 to 1.5 mg. of nitrogen per ml. of filtrate, while abnormal serums have varied from 0.8 to 1.8 mg. of nitrogen per ml. of filtrate.

Calculations

\[
\frac{500 \times \text{mg. } N \text{ per ml. filtrate}}{\text{Gm. albumin } N \text{ per 100 ml. serum}} = \frac{\text{gm. albumin } N \text{ per 100 ml. serum}}{\text{Gm. total protein } N \text{ per 100 ml. serum}} = \% \text{ albumin}
\]

\[
\frac{\text{Gm. total protein } N \text{ per 100 ml. serum} - \text{gm. albumin } N \text{ per 100 ml. serum}}{\text{gm. globulin } N \text{ per 100 ml. serum}} = \% \text{ globulin}
\]

\[
\% \text{ Albumin} - \% \text{ albumin} = \% \text{ globulin}
\]

\[
\% \text{ Globulin} = \frac{\text{albumin-globulin ratio}}{100}
\]

SUMMARY

A method employing methanol under controlled conditions is presented for the determination of the albumin and globulin contents of human serums. Values obtained by this procedure compare favorably with results obtained electrophoretically.

BIBLIOGRAPHY

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