FRACTIONATION OF SERUM INTO ALBUMIN AND $\alpha$, $\beta$, AND $\gamma$-GLOBULIN BY SODIUM SULFATE

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The values of the albumin and total globulin of serum determined with 22.4 per cent sodium sulfate by the method of Howe (1) are better correlated with the values of one fraction containing the albumin and $\alpha$-globulin and of another containing the $\beta$- and $\gamma$-globulins (2). However, 19 per cent sodium sulfate seems to separate these fractions more efficiently, since it yields results which agree very well with the corresponding fractions determined by electrophoresis (3). Apparently the precipitation of all of the globulin fractions of human serum requires 26 to 27 per cent sodium sulfate (3, 4). The data of Majoor (4) indicate that most of the $\gamma$-globulin is precipitated by 15 per cent of the salt. This is consistent with the findings of Gutman et al. (5) that the $\gamma$-globulin fraction is only partially precipitated at a concentration of 13.5 per cent of the salt, while 17.4 per cent removes a significant amount of $\beta$-globulin in addition to the $\gamma$ fraction. Moreover, 15 per cent sodium sulfate is approximately equivalent to 0.33 saturated ammonium sulfate in respect to salting out. Jager and Nickerson found a good correlation between the amounts of protein precipitated by the latter and the values of $\gamma$-globulin estimated by electrophoresis (6). Hence, it occurred to us that human serum can be analyzed for albumin and for all of the globulin fractions by determining the protein precipitated with 15, 19, and 26 per cent sodium sulfate. The present study was undertaken to determine whether the values estimated by this simple method are consistent with the results of other methods.

EXPERIMENTAL

A series of thirteen sera, obtained from patients with miscellaneous clinical conditions in this hospital, was analyzed by fractionation with sodium sulfate. In addition, the albumin and total globulin were determined with methyl alcohol by the method of Pillemer and Hutchinson (7), and the $\gamma$-globulin fraction was determined with serum of rabbits immunized to this protein. Another series of sera and plasmas of known protein composition determined by electrophoretic analysis was obtained from other laboratories. These were analyzed by fractionation with salt.

1 We wish to thank the following for samples of blood and for the results of the electrophoretic analyses: Dr. Mary L. Petermann and Dr. Nelson F. Young, Sloan-
The fractionation was made by adding 0.5 ml. of serum or plasma to 10 ml. of 15.75, 19.90, and 27.20 per cent sodium sulfate at 37°. About 10 mg. of Hyflo Super-Cel were added from a scoop made from glass tubing, and the mixtures were allowed to stand in the incubator at 37° for 1 hour.² The precipitates were then filtered in covered funnels in the incubator with Whatman No. 50 filter paper, 9 cm. in diameter. Portions of the clear filtrates were added to the biuret reagent, and their protein content was determined by the method of Kingsley (8) as modified by Kibrick and Clements (9).

The biuret reaction was also utilized to determine the total protein of the sera and the albumin in the methyl alcohol filtrates from the method of Pillemer and Hutchinson, as described previously (9).

Serum immune to human γ-globulin was obtained by injecting rabbits twice weekly by ear vein with 1 to 2 ml. of 1 per cent of the protein in glycine solution³ containing 0.05 per cent aluminum ammonium sulfate. After about 6 weeks and a period of 6 days without injections, the animals were bled from the heart and the serum was prepared with 0.01 per cent sodium merthiolate. Traces of antibodies to the other serum proteins were removed by absorption with a solution of human albumin⁴ and with a mixture of the α- and β-globulins.⁵ The determinations were made by adding 2 ml. of the immune serum in centrifuge tubes to 1 ml. of human serum which had been diluted 50 to 150 times with 0.9 per cent sodium chloride. The tubes were allowed to stand in a water bath at 40° for 2 hours and then placed in the refrigerator until the next day. The immune precipitates were centrifuged and washed twice with 3 ml. of cold 0.9 per cent sodium chloride. They were then dissolved in dilute alkali and their content of...
nitrogen was determined by the micro-Kjeldahl method. Several standard tubes containing a dilute solution of \( \gamma \)-globulin were run with each series of determinations. The concentration of \( \gamma \)-globulin was calculated from a standard curve prepared from the results of serial dilutions of a weighed amount of the protein.

**RESULTS AND DISCUSSION**

Table I shows that the values of albumin and total globulin determined with sodium sulfate agree quite well with those determined with methyl alcohol. This is further confirmation that 26 per cent sodium sulfate effects a reliable separation in the serum of subjects with a variety of clinical conditions. The values of the \( \gamma \)-globulin fraction also agree quite well with the results obtained by precipitation with immune serum. Kabat et al. have determined the \( \gamma \)-globulin in cerebrospinal fluid by the latter method (10), but it does not seem so convenient for routine use. Table II shows that the protein precipitated with 15 per cent sodium sulfate is in satisfactory agreement with the \( \gamma \)-globulin fraction estimated by electrophoretic analysis.

We have found that the amount of protein precipitated with 19 per cent sodium sulfate in about 100 different sera is only slightly less than that precipitated with 22 per cent salt by the method of Howe. In many cases...
instances the results were almost identical, but there was a suggestion that better agreement is possible between duplicate determinations with the smaller concentration. There seems to be a definite break in the precipitation of protein at about 19 per cent, which the data of Milne (3) indicate is equal to the sum of the $\beta$- and $\gamma$-globulin fractions. If this is correct, the amounts of the $\alpha$ and $\beta$ fractions, derived from this value and from the values of total globulin and of the $\gamma$ fraction, are also correct. Table II shows that they are in agreement with those found by electrophoresis.

**Table II**

*Comparison between Results of Fractionation with Sodium Sulfate and Those from Electrophoresis*

The results are expressed in gm. per 100 ml.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Salt fractionation</th>
<th>Electrophoresis</th>
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<tbody>
<tr>
<td></td>
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<tr>
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We are grateful to Dr. Joseph Felsen, Director of Laboratories and Research, for advice and encouragement.

**Summary**

1. A simple chemical method is proposed to estimate the fractions of protein in human serum by precipitation with 15, 19, and 26 per cent sodium sulfate. The results of a series of thirteen sera are compared with the values of albumin and total globulin determined with methyl alcohol and with the values of $\gamma$-globulin determined by precipitation with immune rabbit serum. The results in another series of fourteen samples are compared with values derived from electrophoretic analysis.
2. Most of the values of albumin, total globulin, and γ-globulin are within 0.2 gm. of those determined with methyl alcohol and with immune serum.

3. The values of albumin and of α-, β-, and γ-globulin also compare favorably with the results of electrophoresis.

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