THE FRACTIONATION OF SERUM PROTEINS USING SODIUM SULPHATE

BY

N. H. MARTIN, R. MORRIS, AND M. SMITH

From St. George's Hospital Medical School, London

(RECEIVED FOR PUBLICATION APRIL 4, 1950)

Before the development of the moving boundary technique of electrophoresis it had been the custom to classify the circulating serum proteins into three broad groups, albumin, pseudoglobulin, and euglobulins, based on their solubilities in salt solutions of varying concentrations. Howe (1921), using sodium sulphate in varying concentrations, defined three zones in which there was little or no alteration in the amount of protein precipitated. These lay between 13.5 and 14.5%, 16.4 and 17.4%, and between 21 and 22%. The technical developments in electrophoretic analysis associated with the name of Tiselius (1939) have popularized the classification of the serum proteins on the basis of their mobilities in an electrical field, the broad groups being designated as albumin and α , β , and γ globulin.

Majoer (1947) carried out a careful study of the solubility of serum proteins over a wide range of concentrations of sodium sulphate and compared these with electrophoretic analysis. Four sera were examined: one normal, one from a patient suffering from chronic hepatitis, one from a patient suffering from myeloma, and one from a patient suffering from chronic nephritis. A study of his curves demonstrates zones of precipitation occurring between concentrations of sodium sulphate of 14 to 18 g. per 100 ml. of water, 18 and 26 g. per 100 ml., and between 26 and 34 g. per 100 ml. As a result of his investigations Majoer has suggested that concentrations of 18.5 g. sodium sulphate per 100 ml. water and 26.8 g. sodium sulphate per 100 ml. water would give a convenient and sufficiently characteristic division of the serum proteins for routine clinical use. Such a procedure gives three fractions, the most soluble being almost pure albumin, the next most soluble largely α and β globulins, and the least soluble principally γ globulin. Majoer admits that there is an overlap of the three fractions and that no absolute separation is obtained by such a simple procedure. Milne in 1947 examined 11 sera from different diseases using 26.8 g. sodium sulphate to 100 ml. water and 19.6 g. sodium sulphate to 100 ml. water. These concentrations, he claims, give separation into three fractions corresponding to albumin, the α globulins, and the combined β and γ globulins. His analyses show remarkably good agreement, but it is noteworthy that no sera are included from patients suffering from nephritis, in which increases in the α and β globulins are frequently observed. Kibrick and Blonstein (1948) have used 15.75 g. sodium sulphate per 100 ml. water to precipitate γ globulin but noticed occasional gross deviations from the electrophoretic analysis. Over the past three years studies have been continuing in this laboratory on convenient means of protein fractionation and their application to the study of disease. The data analysed here were collected

in part as an ancillary to a study reported by Martin and Morris (1949) on the precipitation of total globulin. Two objectives were borne in mind: first, could a reasonable correlation be obtained between analysis by electrophoresis and analysis by simple salt fractionation over a diverse range of clinical conditions, and, second, could a simple fractionation yield material suitable for further detailed study?

Material and Methods

Venous blood was drawn, under paraffin, from fasting patients suffering from a variety of clinical conditions, into clean centrifuge tubes without any anticoagulant. It was allowed to clot and then spun at 2,500 revolutions, the clear serum being withdrawn for analysis. In each instance the electrophoretic analysis and salt fractionations were all carried out on one serum sample.

The electrophoretic analyses were carried out in the Tiselius apparatus under the same conditions as detailed in an earlier paper (Martin and Morris, 1949). Precipitation of the globulins was carried out with precautions outlined in the same paper. The concentrations of sodium sulphate used are listed in Table I.

TABLE I
CONCENTRATIONS OF SODIUM SULPHATE

	Concentration (g./litre)	Approximate Final Molar Concentration before Precipitation	Temperature	Range of Protein Concentrations (g. per 100 ml.)	Precipitation Time (Hours)
Sodium sulphate:					
Na ₂ SO ₄ *	130	0.9	37° C.	0.14-0.29	24
Na ₂ SO ₄	150	1.06	37° C.	0.14-0.29	24
Na ₂ SO ₄	190	1.34	37° C.	0.14-0.29	24
Na ₂ SO ₄	260	1.77	37° C.	0.14-0.29	24

* In each instance the proportion of serum to sodium sulphate solution was 1 to 29.

Results

The results of 10 analyses are shown in Table II. In the table the electrophoretic analyses have been summed so that direct comparison may be made with salt fractionation. There is reasonable agreement between the residual soluble protein after precipitation with 26% sodium sulphate and albumin determined electrophoretically. Analysis of the supernatant after precipitation with 19% sodium sulphate gives a reasonable approximation to the electrophoretic values for the sum of albumin and α globulin in seven out of the ten sera; in the other three, all from patients suffering from nephritis, there is a gross discrepancy. Analyses of the supernatant after precipitation with 15% sodium sulphate agree well with electrophoretic values for the sum of albumin and α and β globulins in sera 3 and 6, while analysis after precipitation with 13% sodium sulphate agree well for sera 1, 4, and 7. In sera 8, 9, and 10, all from cases of nephritis, there is little agreement, salt fractionations usually giving higher figures for γ globulin than those arrived at by electrophoresis. In serum 2, from a case of hepatitis, the mean value of the two salt fractionations gives a good approximation to the γ globulin content estimated by electrophoresis. In serum 5 both salt fractions give low values for γ globulin compared with electrophoresis.

TABLE II
COMPARISON OF ELECTROPHORETIC AND SODIUM SULPHATE ANALYSIS

No.	Disease	Serum Total Protein (g./100 ml.)	Analysis by Electrophoresis			Soluble Protein after Precipitation with Sodium Sulphate (g./100 ml.)				γ Globulin by Electrophoresis	γ Globulin by Difference (15% Sod. Sulphate Precipitation)	γ Globulin by Difference (13% Sod. Sulphate Precipitation)
			Albumin	Albumin + α Globulin	Albumin + α and β Globulin	26%	19%	15%	13%			
1	Hepatitis	7.8	2.85	5.45	6.8	2.8	5.4	6.6	6.8	1.05	1.2	1.05
2	Hepatitis	7.2	2.0	5.0	6.0	2.1	4.8	5.7	6.2	1.1	1.5	1.0
3	Cirrhosis	6.4	1.47	2.09	3.10	1.45	2.05	3.0	3.2	3.3	3.4	3.2
4	Cirrhosis	7.6	2.8	3.35	4.85	2.7	3.4	4.5	4.7	2.75	3.1	2.9
5	Cirrhosis	8.2	2.0	2.6	3.4	1.9	2.55	3.85	5.28	4.8	4.35	2.92
6	Myeloma											
7	Myeloma diffuse	8.5	1.06	1.08	1.95	1.15	1.2	2.0	2.5	6.55	6.5	6.0
	Myeloma solitary	5.1	2.3	3.22	4.00	2.1	3.3	3.8	4.0	1.1	1.3	1.1
8	Nephritis	4.4	0.98	3.25	3.77	0.96	1.55	2.9	3.4	0.63	1.5	1.0
9	Nephritis	4.6	0.4	1.6	2.8	0.35	1.9	2.1	3.35	1.8	2.5	1.25
10	Nephritis	6.23	2.1	4.8	5.2	2.39	4.35	4.5	5.4	1.03	1.73	0.85

Discussion

Majoor (1947) and Milne (1947) have both studied the problem in normal and a limited number of abnormal sera. The present series deals entirely with abnormal sera. It demonstrates that while reasonable correlation exists between albumin determined electrophoretically and determined by salt precipitation, the correlation of the globulin fractions is a more difficult matter. Milne showed good agreement between analysis obtained by precipitation with 19.6% sodium sulphate and the electrophoretic analysis of albumin and α globulin combined. Our figures using 19.0% sodium sulphate also show reasonable agreement with the exception of the sera from patients with nephritis, where there are gross discrepancies. Milne's series includes no such cases. It is precisely these cases, in which there is a marked increase in the α and β globulins, that form a critical test of the procedure. The deviation is not unexpected when it is recalled that electrophoretic analyses by the Tiselius technique are dependent on the refractive increment, whereas the analyses of salt fractionation are based on nitrogen determinations. Armstrong, Budka, and Morrison (1947), in an exhaustive study of material isolated by the more elaborate techniques of cold alcohol fractionation (Cohn, Strong, Hughes, Mulford, Ashworth, Melin, and Taylor, 1946), have shown the extent to which commonly accepted nitrogen factors must be corrected when dealing with concentrations of individual serum protein fractions, especially when, as in the case of α and β globulins, they contain a relatively high percentage of lipid.

Estimations of the γ globulins are not complicated by this factor to the same extent. The γ globulins, however, form a large and complex family. Electrophoretic analysis of sera from patients suffering from advanced, long-standing liver damage repeatedly shows the " γ globulin" component as a peak with a wide base suggesting a group of closely associated proteins rather than an excess of an individual protein (Thorn, Armstrong, and Davenport, 1946; Martin, 1949; Sterling and Ricketts, 1949). This is in contrast to the typical analysis of diffuse myelomata in which the excess of γ globulin in a given serum occurs as a well-defined peak of uniform mobility. As might be anticipated analysis of such a serum (No. 6) using 26% sodium sulphate fractionation and 15% sodium sulphate fractionation gives a working approximation of the chief features of the distribution of the serum proteins compared with electrophoresis. The precision is not mirrored in the analysis of the sera from cirrhotics, though a good working comparison may be obtained by using sulphate concentrations of 15 to 13%.

It is evident from our results, as from those of Majoor and Milne, that simple fractionation procedures can be evolved which give an approximation to electrophoretic analysis sufficient for many routine purposes, but that they must always be used with caution. Their correlation will tend to break down in any case where there is gross excess of α or β globulins or of atypical lipoproteins. Good correlation is not to be expected therefore in sera from patients with conditions such as subacute nephritis or leptospirosis. Provided these limitations are borne in mind and provided always that the method is treated as an approximation there can be no objection to its use.

In this paper we deliberately chose sodium sulphate concentrations differing from those used by Majoor, by Milne, and by Kibrick and Blonstein, so that workers

interested might have observations over a range of concentrations. Milne has used 19.6% sodium sulphate and Majoor 18.5% sodium sulphate. Majoor maintains that at this concentration albumin and α and β globulin remain in solution. We think that for many abnormal sera 18.5% sodium sulphate is too high a concentration to give the optimum point of separation of α and β globulins. We are equally certain that with 13% sodium sulphate a significant amount of γ globulin remains in solution with the β globulin in many abnormal sera. We have favoured 15% sodium sulphate as striking a balance between the two levels, but it is clear that even at this level one may get marked discrepancies with some sera. We have not satisfied ourselves that the method of sodium sulphate fractionation is a convenient one for preparing material for further detailed study of either albumin or globulin fractions. We have obtained globulin by precipitation with 13% sodium sulphate which could be brought smoothly into solution, but this does not happen in every instance. The α and β globulins have proved even more difficult to handle by sodium sulphate precipitation. The preparation of albumins for detailed study by sodium sulphate fractionation we found objectionable for two reasons: the necessary 30 times dilution of the serum and the introduction of a salt solution more than 10 times the molarity of physiological saline. This entails prolonged dialysis and subsequent concentration before the albumin is in convenient concentration for further detailed study. The dilution is not objectionable if chromatographic studies of amino-acids are contemplated, though dialysis to reduce the high salt concentration would still be necessary even for these studies.

Simple fractionation of serum proteins using 26% and 15% sodium sulphate when used with discretion does offer an approximation to the broad electrophoretic distribution of serum proteins in a number of conditions. It breaks down absolutely in the study of sera from patients suffering from subacute nephritis and may be expected to be of little value where more precise differentiation of the α and β globulins is required.

REFERENCES

- Armstrong, S. H., jun., Budka, M. J. E., and Morrison, K. C. (1947). *J. Amer. chem. Soc.*, **69**, 416.
 Cohn, E. J., Strong, L. E., Hughes, W. L., jun., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L. (1946). *Ibid.*, **68**, 459.
 Howe, P. E. (1921). *J. biol. Chem.*, **49**, 115.
 — (1923). *Ibid.*, **57**, 235, 241.
 Kibrick, A. C., and Blonstein, M. (1948). *Ibid.*, **176**, 983.
 Majoor, C. L. H. (1947). *Ibid.*, **169**, 583.
 Martin, N. H. (1949). *Brit. J. exp. Path.*, **30**, 231.
 — and Morris, R. (1949). *J. clin. Path.*, **2**, 64.
 Milne, J. (1947). *J. biol. Chem.*, **169**, 595.
 Sterling, K., and Ricketts, W. E. (1949). *J. clin. Invest.*, **27**, 1459.
 Thorn, G. W., Armstrong, S. H., jun., and Davenport, V. D. (1946). *Ibid.*, **25**, 304.
 Tiselius, A. (1939). *Harvey Lect.*, **35**, 37.