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THE ALBUMIN/GLOBULIN RATIO: A TECHNICAL STUDY

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In 1885–6 Kauder observed that globulin was precipitated from serum when the concentration of ammonium sulphate reached 24 to 29 per cent of full saturation and that its precipitation was complete when the concentration reached 36 to 46 per cent of complete saturation. Thereafter no further precipitation took place until the concentration reached 64 per cent of full saturation, when albumin started to precipitate, its precipitation being complete at 90 per cent saturation. The simple qualitative differentiation of albumin from the globulin by their behaviour in the presence of ammonium sulphate is based on this and like observations.

Howe (1921), investigating the properties of sodium sulphate as a precipitant, demonstrated three zones in which variations of the concentration of the sodium sulphate did not produce any significant change in the amount of protein precipitated. These lay between 13.5 and 14.5 per cent, between 16.4 and 17.4 per cent, and between 21 and 22 per cent, using anhydrous sodium sulphate. This last concentration precipitated approximately the same amount of protein as did half saturation with ammonium sulphate. It was assumed to represent the point at which the globulins had been completely precipitated from solution. The use of the sodium salt for the precipitation enabled the estimation of the residual soluble protein to be carried out by the Kjeldahl technique, and soon the method was adopted widely as a routine procedure in the estimation of the "albumin/globulin ratio."

The application of the more delicate physico-chemical techniques to the study of the serum proteins, and in particular the accumulation of data derived from analyses made by the electrophoretic technique of Tiselius, makes it desirable to check how far the common practical routine methods yield results comparable with those obtained by the more delicate physico-chemical techniques.

Dole (1944) demonstrated clearly that the albumin-globulin ratio of normal plasma calculated from electrophoretic data was constantly at variance with that obtained by the Howe fractionation technique. Subsequently one of the writers, in studying sera from diseased persons, noted not only that there was divergence but that the discrepancy was on occasion such as to suggest that the cruder technique was yielding meaningless results in terms of the electrophoretic data. Other workers (Pillemer and Hutchinson, 1945; Majoor, 1947; Milne, 1947) have been exercised by the same problem.

If the routine procedure of reporting the albumin/globulin ratio was to retain any meaning at all it seemed highly desirable to adopt a procedure which, while simple enough for routine procedures in a busy laboratory, gave results which bore some reasonable relationship to the figures obtained by more elaborate methods of analysis. With this end in view some of the common laboratory techniques of separation and modifications of them were examined.

Materials

The sera were drawn from patients with a wide variety of clinical conditions in whom qualitative and quantitative disturbances of circulating proteins were anticipated.

Methods

The following single-stage methods of protein fractionation were examined:

1. (*a*) Precipitation of the globulin fraction using sodium sulphate according to the technique of Howe; (*b*) precipitation of the globulin fraction using 1.83 molar sodium sulphate (26 per cent sodium sulphate) at 37° C. (Majoor, 1947).

2. Precipitation with sodium sulphite according to the technique originally outlined by Campbell and Hanna (1937).

3. Precipitation with magnesium sulphate following the technique outlined by Popják and McCarthy (1946).

4. Precipitation by methanol in an acetate buffer according to the technique of Pillemer and Hutchinson (1945).

The conditions of precipitation are shown in Table I.

Robinson and others (1937) have observed that repeated filtration results in adsorption of protein

TABLE I

CONDITIONS FOR PROTEIN PRECIPITATION

	*p*H	Conc. g./L	Approx. final molar conc. before ppt.	Alcohol conc.	Temp. °C.	Range of protein conc. before ppt. (in g. per 100 ml.)	Time allowed for complete precipitation (hours)
Sodium sulphate Na_2SO_4	6.4 ± 0.04	225	1.53	nil	37	0.38–0.14	16
Sodium sulphate Na_2SO_4	6.5 ± 0.04	260	1.77	nil	37	0.38–0.14	16
Sodium sulphite $Na_2SO_3 7H_2O$	9.6 ± 0.04	420	1.59	nil	20	0.58–0.210	⅙
Magnesium sulphate saturated sol.	7.0 ± 0.2	360	3.00	nil	20	1.45–0.53	24
Methyl alcohol in sodium acetate buffer	6.7 ± 0.05		0.06*	42.5%	0–2	2.3–0.85	½

* Approximate molarity of acetate buffer.

from solution on the filter paper. Filtrations, therefore, were carried out once only. If the filtrate was not clear it was rejected. The nitrogen estimations were carried out by the micro-Kjeldahl technique, which was standardized by the estimation of the nitrogen content of weighed amounts of crystalline bovine albumin.

The protein values calculated from the nitrogen estimations were correlated with an electrophoretic analysis of the same sample of serum after dialysis against a phosphate buffer and run at *p*H 8.0 in a 0.2 M-sodium phosphate buffer, the protein concentration being adjusted to 2.0 g. per 100 ml. buffer.

The *p*H of the various precipitating solutions was estimated using the standard glass electrode, measurements being made before and after precipitation of the globulin fraction. The *p*H of the solutions is shown in Table I.

Results

The results of the various analyses are compounded in Table II. The electrophoretic analyses of the soluble proteins after precipitation by the Howe technique (22.5 per cent sodium sulphate) has been shown by Perlmann to consist of appreciable amounts of α and β globulins. By increasing the concentration of sodium sulphate to 26 per cent a fractionation was obtained which approximated much more closely to the albumin/globulin ratio obtained by electrophoretic analysis (compare columns b, g, and i in Table II). Electrophoretic analysis of the soluble

TABLE II

TABLE OF ANALYSIS

No.	Serum	Total protein by micro-Kjeldahl g./100 ml.	Electrophoretic analysis fractions as g./100 ml.					After precipitation with Na_2SO_4		After precipitation with MeOH with acetate	After precipitation with Na_2SO_3	After precipitation with $MgSO_4$
			Globulins									
			Alb.	α_1	α_2	β	γ	26%	22.5%			
		(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
1	E (ter)	6.20	1.27	0.23	0.39	1.01	3.3	1.2	2.15	1.25	2.4	1.9
2	M_2	7.85	2.85	0.7	1.9	1.4	1.0	2.8	3.4	2.9	4.0	3.35
3	E_2	7.6	2.8	0.55		1.5	2.6	2.7	3.2	2.7	2.9	3.1
4	Br_1	5.1	2.3	0.92		1.02	0.82	2.1	2.55	2.35	2.8	2.35
5	R_{11}	5.1	2.55	1.27		0.76	0.51	2.3	2.5	2.5	2.8	2.6
6	P	4.2	1.85	0.3	0.34	0.82	0.8	1.8	2.3	1.9	2.4	
7	S	4.4	0.97	1.8	0.48	0.52	0.67	0.96	2.7	1.0	2.8	
8	E_1	8.8	2.8	0.5		1.8	3.6	2.7	3.2	2.7	3.4	
9	II_3	6.8	1.7	1.36	1.63	1.5	0.60	1.8	3.2	1.75	3.0	
10	Cr	7.2	3.2	0.22	1.2	1.73	0.72	3.2	4.0	3.4	3.7	
11	II_2	6.0	2.55	0.5	0.75	0.95	1.2	2.6	2.9	2.7	3.0	
12	B_1	11.6	1.21	—	0.98	—	9.35	1.2	2.1	1.25	1.7	
13	M_1	8.5	4.15	0.76	0.85	1.53	1.02	4.2	5.1	4.4	4.8	
14	Cl	7.6	3.6	0.4	0.7	1.75	1.2	3.6	4.3	3.55		
15	B_2	8.5	1.06	—	0.9	—	6.5	1.2	2.0	1.03		
		(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)

portion in fact demonstrated that a small but appreciable portion of α globulin remained in solution when precipitation was complete. This is of fundamental importance when considering properties attributable to this soluble fraction. The soluble proteins obtained by the alcohol acetate fractionation of Pillemer were also examined electrophoretically, and it will be seen from Table II, column i, that they, too, gave results approximating well with the electrophoretic analysis.

Discussion

Because the amount of serum from individual patients was limited, the majority of estimations was concentrated on two techniques which in our hands seemed to give results corresponding most consistently with electrophoretic data.

It will be seen that both the classical Howe technique and the technique of Campbell and Hanna, using sodium sulphite, are at considerable variance with the albumin/globulin ratio deduced from electrophoretic analysis. A further objection to the use of sodium sulphite is that the *p*H of the precipitating solution lies so far to the alkaline side of neutrality that there is risk of denaturation of some of the proteins under examination and of alteration in their solubility. Though Cohn and others (1946) have shown that if the temperature of the mixture is kept low the use of the organic solvents is permissible in affecting separation, they stress the caution with which such solvents must be used. Our examination of the soluble fraction from the acetate methanol separation suggested that on occasion some slight degree of denaturation may occur. We do not believe that this is frequent or extensive if care is taken in maintaining a low temperature during precipitation. We mention it to stress the care that must be exercised when employing organic solvents in separation. Apart from this defect the method advocated by Pillemer was clean, rapid, and convenient. That, and the separation by 26 per cent Na_2SO_4 along the lines outlined here and as laid down by Majoor (1947), have in our hands offered the most convenient and most accurate routine procedures for the estimation of the albumin/globulin ratio in a clinical laboratory.

Summary

Salting-out methods for determination of serum proteins have been compared with the figures obtained by electrophoretic methods.

By the use of 26 per cent sodium sulphate or methanol precipitation, close agreement with the electrophoretic values was obtained.

Twenty-two per cent sodium sulphate, sodium sulphite, or magnesium sulphate gave results which differed significantly from the electrophoretic values.

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