Reproducibility and Validity of Sodium Sulfate Fractionation of Proteins in Plasma and Knee Joint Fluid

O. H. Gaebler, W. T. Beher, J. W. Sigler, and R. R. Galpin

Simple and rapid methods of determining plasma proteins, which yield results comparing favorably with those obtained by electrophoresis, have been sought by several investigators. The procedure of Milne seemed of particular interest to us, since it differs from the widely used one of Howe only with respect to the concentrations of sodium sulfate employed. The dual purpose of the present study was, first, to secure an additional series of normal values for serum proteins determined by the method of Milne, and second, to ascertain whether his reported agreement between salt fractionation and electrophoretic separation of serum applies to knee joint fluid. A component moving more rapidly than albumin was noted in all knee joint fluids examined, and was studied further by means of electrophoresis-convection (1).

MATERIALS AND METHODS

Blood samples were drawn from the cubital vein, during mid-morning, from 16 apparently normal staff members who were seated during venepuncture. About an hour after clotting, serum was separated by centrifugation. Analyses were made in duplicate or triplicate by the method of Milne.

Knee joint fluids were brought to the laboratory shortly after aspirations had been performed in the Arthritis Clinic of the Hospital. Veronal buffer, *p*H 8.6 and ionic strength 0.1, was used in electrophoretic studies.

From the Edsel B. Ford Institute for Medical Research, and the Arthritis Division, Henry Ford Hospital, Detroit, Michigan.

We are indebted to Dr. James C. Mathies for carrying out the electrophoresis-convection experiment; to Mr. R. B. Scott and Mr. Ernest J. Schoeb of the Research Laboratories, Parke, Davis and Company, for electrophoretic analyses; and to the Michigan Chapter, Arthritis and Rheumatism Foundation, for a grant in support of this study.

Received for publication July 24, 1954.

Each specimen was diluted 1:1 with this buffer and dialyzed against it for 16 to 24 hours before being run. The Aminco apparatus was used, and duration of electrophoresis was 120 minutes. Electrophoresis-convection had previously been applied, in this laboratory, to purification of alkaline phosphatase by Mathies, who carried out the experiment reported in this paper.

RESULTS

In Table 1 are presented the data on serum. The finding of Milne that there is no difference between results for males and females was confirmed. Our normal values also coincide with his, and, as already stated, represent values in persons who were seated for a few minutes in the course of their regular duties. Although Rowe in 1916 recognized that exercise and posture alter plasma protein values, and Wolfson and Cohn have quite recently stated that the change from a standing to a recumbent position

Table 1. VALUES FOR FIBRINOGEN, BY THE METHOD OF WU, AND FOR SERUM PROTEINS BY THE SODIUM SULFATE FRACTIONATION PROCEDURE OF MILNE, IN NORMAL ADULT MALE AND FEMALE HUMAN SUBJECTS

Subject	*Sex*	*Fibrinogen* (Gm. %)	*Serum proteins* (Gm. %)				*Serum proteins* (% total serum protein)			*Serum N.P.N.* (mg. %)
			Total	*Euglobulin*	*Pseudoglobulin*	*Albumin*	*Euglobulin*	*Pseudoglobulin*	*Albumin*	
O. H. G.	M		7.22	1.76	0.86	4.60	24.4	11.9	63.7	32.6
M. J. S.	M	0.27	7.56	1.56	1.10	4.90	20.6	14.6	64.8	29.8
W. T. B.	M	0.31	7.60	1.81	1.07	4.72	23.8	14.1	62.1	29.6
R. S.	M		7.78	1.78	1.30	4.70	22.9	16.7	60.4	28.0
W. T. H.	M		7.88	2.06	1.26	4.56	26.2	16.0	57.8	27.8
P. D. B.	M	0.33	7.70	1.69	1.16	4.85	21.9	15.1	63.0	33.8
J. C. M.	M	0.36	7.40	1.86	0.92	4.62	25.1	12.4	62.5	26.8
H. H. S.	M	0.25	7.77	1.61	1.58	4.58	20.7	20.3	59.0	31.6
F. J. O.	M	0.23	6.91	1.81	0.92	4.18	26.2	13.3	60.5	28.9
E. H. W.	F	0.26	7.75	2.44	1.25	4.06	31.5	16.2	52.3	20.8
E. M. C.	F	0.28	7.46	1.51	1.42	4.53	20.2	19.0	60.8	24.2
B. M.	F	0.29	7.85	2.18	0.85	4.82	27.8	10.8	61.4	30.2
E. G.	F	0.31	7.16	2.04	0.97	4.15	28.5	13.5	58.0	25.4
B. C.	F	0.32	7.47	1.91	0.69	4.87	25.6	9.2	65.2	23.6
Mean, males		0.29	7.54	1.77[a]	1.13	4.63	..	..	..	..
Mean, females		0.29	7.54	2.02[a]	1.04	4.49	..	..	..	..
Mean, all subjects		0.29	7.54	1.86	1.10	4.58	24.7	14.5	60.8	28.1
Standard deviation		±0.04	±0.29	±0.25	±0.25	±0.27	±3.3	±3.0	±3.35	..

[a] Significance of difference in means, $t = 1.94$; $p > 0.05, < 0.1$.

Table 2. Analyses of Knee Joint Fluids by Sodium Sulfate Fractionation and Electrophoresis

Sample number	Albumin (Gm.%)		Pseudoglobulin by sodium sulfate (Gm.%)	α_1 plus α_2 globulins by electrophoresis (Gm.%)	Euglobulin by sodium sulfate (Gm.%)	β plus γ globulins by electrophoresis (Gm.%)
	Sodium sulfate	Electrophoresis				
1-L[a]	1.85	1.78	0.79	0.56	2.19	2.49
2-R[a]	1.98	2.00	0.70	0.70	2.12	2.12
3-L	1.40	1.91	1.52	0.55	1.95	2.41
4-R	1.76	2.14	1.04	0.62	2.07	2.10
5-L	1.35	1.99	1.14	0.52	2.06	2.04
6-R	1.64	1.89	0.87	0.70	2.15	2.08
7-L	1.18	1.92	1.44	0.57	2.08	2.20
8-R	1.65	2.00	1.17	0.63	2.02	2.18

[a] L left knee; R right knee.

has hydrostatic effects which may alter serum protein values 15 per cent, this information is still received with skepticism at times. In Table 1 fibrinogen values, determined by the method of Wu, are included for completeness. Specimens for this purpose were oxalated with 2 mg. of potassium oxalate per ml.

In Table 2 are presented the values for proteins in knee joint fluid. Actually, we analyzed 18 knee joint fluids by the method of Milne, but only 8, in which comparisons between the electrophoretic and salt fractionation results were made, are included in the table. These were all obtained at different visits from the same patient. In Samples 3 to 8, albumin values obtained by the sodium sulfate method were well below the electrophoretic ones, and pseudoglobulin considerably exceeded the sum of the α_1 and α_2 electrophoretic fractions. This suggests entrainment of albumin, when the high concentration of sodium sulfate required for precipitation of pseudoglobulin is used in the analysis of knee joint fluid. Whatman #50 filter paper was used throughout, since it has been reported (2) that this minimizes errors due to adsorption of albumin on filter paper. Results for euglobulin compared fairly closely with the sum of β and γ fractions, in six of the eight samples.

Results of electrophoresis-convection were simple. Pooled knee joint fluids from 4 patients were centrifugalized for 30 minutes at 5° and 3300 RPM to remove a flocculent precipitate. A 10 ml. sample (Sample 1) of the supernatant was diluted with an equal volume of phosphate buffer, *p*H 7.2 and 0.1 ionic strength, and dialyzed against four 4 liter portions of this buffer. The supernatant was still faintly turbid, and the solution was viscous. Electrophoresis-convection was carried out for 6 hours at

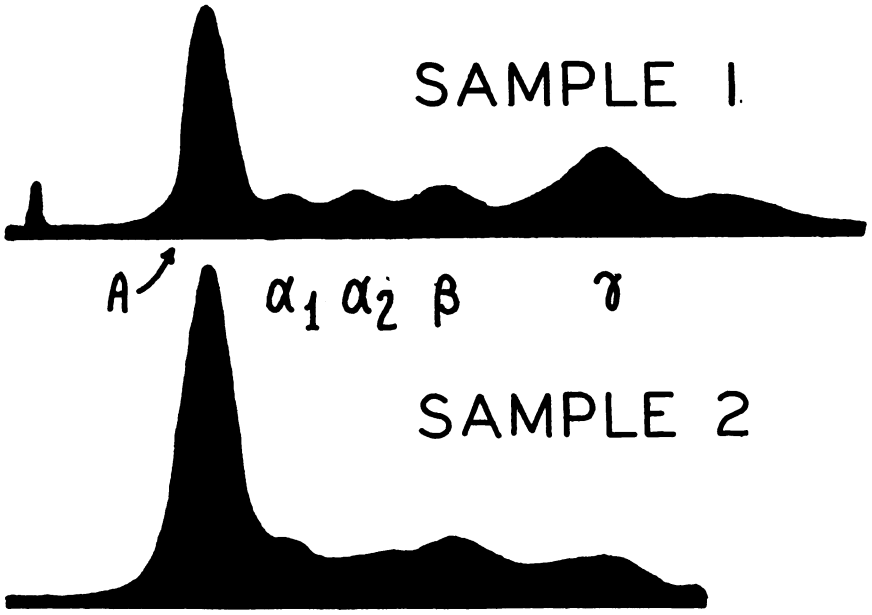

Fig. 1. Electrophoretic patterns (ascending boundaries) of pooled knee joint fluids (Sample 1) and fraction thereof (Sample 2) taken from lower chamber of electrophoresis-convection apparatus.

0.5 amp., the potential drop across the apparatus being 12 v. The bottom fraction was then sampled (Sample 2). The electrophoretic patterns before and after electrophoresis-convection are shown in Fig. 1. The fast moving component was not concentrated in the bottom fraction, as one had reason to expect, but disappeared. Adherence to the membranes of the apparatus is one of several possible explanations. In any case, use of this procedure to concentrate the component for study does not appear feasible. In the 8 samples, this component amounted to only 0.04–0.10 per cent of the total protein.

SUMMARY

Blood was drawn from 14 laboratory workers under conditions similar to those under which blood samples are taken from ambulatory clinic patients. The sera were analyzed for the various proteins by the method of Milne. Eighteen knee joint fluids were analyzed by the same method; and 8 fluids from one patient were also analyzed electrophoretically. Results for these 8 fluids, and for all of the sera, are presented in tabular form and discussed. A component moving faster than albumin was noted in all knee joint fluids. This could not be concentrated from a

pooled lot from 4 patients by electrophoresis-convection, but disappeared instead.

REFERENCES

1. Cann, J. R., Kirkwood, J. G., Brown, R. A., and Plescia, O. J., *J. Am. Chem. Soc.*, 71, 1603 (1949).
2. Harris, R. C., *J. Biol. Chem.*, 127, 751 (1939).
3. Howe, P. E., *J. Biol. Chem.*, 49, 93 (1921).
4. Mathies, J. C., *Science*, 115, 144 (1952).
5. Milne, J., *J. Biol. Chem.*, 169, 595 (1947).
6. Rowe, A. H., *Arch. Int. Med.*, 19, 499 (1917).
7. Wolfson, W. Q., and Cohn C., *Rapid Chemical Micro Methods for the Analytical Fractionation of Serum Proteins*. Ann Arbor, Michigan, The Overbeck Co., 1951.
8. Wu, H., and Ling, S. M., *Chinese J. Physiol.*, 1, 161 (1927).