TECHNICAL METHODS

A Comparison of Two Commonly Used "Salt-fractionation" Methods for Differential Plasma Protein Estimation

BY

J. K. FAWCETT AND VICTOR WYNN

From the Surgical Unit, St. Mary's Hospital, London

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The estimation of the levels of plasma albumin and globulin is useful in numerous conditions. In many laboratories, electrophoretic procedures are not yet practised and reliance is placed upon older methods usually depending on precipitation of globulin by strong salt solutions. Howe's (1921) method, in which 22% sodium sulphate is used to precipitate globulin, has achieved wide popularity. It is now known that at this concentration of sodium sulphate part of the albumin is precipitated (Marrack and Hoch, 1949) and some of the α and β globulins are not (Gutman, Moore, Gutman, McClellan, and Kabat, 1941). Howe's method tends to give higher results for albumin than those of electrophoresis, particularly in some pathological sera.

Majoor (1942, 1946), Milne (1947), Kibrick and Blonstein (1948), and others showed that when the final sodium sulphate concentration was 26% the value obtained for plasma albumin was usually closer to the value obtained by electrophoresis than when the concentration was 22%. Since electrophoresis is generally regarded as a reference method for determining the relative concentration of the plasma proteins, it has been argued that a routine method giving results for albumin and globulin close to those of electrophoresis is better than one which does not.

In comparing these two salt-fractionation methods two questions must be taken into account. Is the clinically useful information greater with Majoor's method than with Howe's? Are there technical considerations which favour the use of one method or the other? This paper is concerned with an attempt to elucidate these two questions.

Procedures and Methods

The levels of plasma albumin and globulin were studied by the methods of Howe (1921) and Majoor (1942, 1946) with minor modifications previously described (Fawcett, 1954).

The Subjects.—A single sample of blood from each of 15 healthy young men and 15 healthy young women was analysed, these samples being taken at varying times throughout the day and not necessarily in the post-absorptive state. Also 10 samples of blood were taken from each of five of these healthy subjects over a period of six months.

Fifty-four hospital patients were studied in whom abnormal plasma protein levels were expected. In some of these patients repeated estimations were made over periods up to 105 weeks. Altogether 200 pathological plasmas were examined.

Selection of Hospital Patients.—These were classified in four groups: (Ai) 12 patients with malnutrition, due either to surgical lesions, e.g., pyloric obstruction, to malabsorption, e.g., steatorrhoea, or to unknown causes; (Aii) 13 patients with malignant growths of the gastrointestinal tract or lungs; (B) 12 patients with chronic hepatitis of viral or alcoholic origin, of whom six had ascites and six had recovered from ascites when the blood was taken; (C) 10 patients with nephrotic syndrome, all of whom were severely oedematous but had normal or only slightly raised blood urea levels and no significant hypertension, and three were young children; (D) seven patients with chronic nephritis with severe uraemia and with moderate to severe proteinuria.

The Methods.—The analytical methods were as follows: 10 ml. of venous blood was withdrawn, without venous stasis, into a syringe containing about 0.04 ml. of a solution of heparin (5,000 units per ml.). The blood was centrifuged under liquid paraffin.

Results were calculated from the protein nitrogen content of suitably prepared material, using methods based upon the semi-micro-Kjeldahl procedure and described elsewhere (Fawcett, 1954). The analyses were performed in duplicate.

Results

The reproducibility of the results is as follows. The mean difference between duplicates of all the total protein determinations is 0.07 g./100 ml.; the maximum difference is 0.15 g./100 ml. The reproducibility of results for albumin is equal by both methods, the mean difference of duplicates being 0.07 g./100 ml. and the maximum difference 0.2 g./100 ml. Thus, neither method is superior to the other on grounds of greater reproducibility.

TABLE I

PLASMA TOTAL PROTEIN AND ALBUMIN AND GLOBULIN FRACTIONS DETERMINED BY HOWE'S AND MAJOOR'S METHODS IN 30 HEALTHY SUBJECTS

	Total Plasma Protein (g./ 100 ml.)	Plasma Albumin (g./ 100 ml.) by Howe's Method	Plasma Globulin (g./ 100 ml.) by Howe's Method	Plasma Albumin (g./ 100 ml.) by Majoor's Method	Plasma Globulin (g./ 100 ml.) by Majoor's Method
Arithmetic mean ..	7.31	4.71	2.60	3.89	3.42
Median ..	7.32	4.72	2.60	3.90	3.42
S.D. ..	0.28	0.30	0.32	0.28	0.32
Lowest value ..	6.80	3.95	2.00	3.20	2.70
Highest value ..	7.85	5.15	3.20	4.30	4.00

of results. In practice, however, Howe's method is slightly easier to perform because sodium sulphate is less liable to crystallize from the more dilute solution.

Table I reports the plasma total protein and albumin and globulin levels found after precipitation of globulin with sodium sulphate at a concentration of 22% (Howe) or 26% (Majoor) in 30 healthy subjects. As the standard deviation of these values is very similar, irrespective of whether 22% or 26% sodium sulphate is used, the normal range of variation is therefore substantially the same by both methods.

Table II gives the results for the plasma total protein and albumin and globulin concentrations obtained by the same methods as in Table I and their variation when 10 samples of blood were taken from each of five healthy subjects over a period of six months. They show that each individual has characteristic levels which vary less than the variation among the large group of healthy subjects. Thus the standard deviation for the plasma albumin and globulin concentrations determined by Howe's method is, in the case of the individual, about half the value for the healthy group as a whole, and the standard deviation for albumin and globulin determined by Majoor's method is about two-thirds. It is doubtful whether this difference in standard deviation represents a real advantage in Howe's method.

To compare the differential protein results in disease with those in health, and also to compare Howe's method

TABLE II

PLASMA TOTAL PROTEIN AND ALBUMIN AND GLOBULIN FRACTIONS DETERMINED BY HOWE'S AND MAJOOR'S METHODS IN 10 SPECIMENS TAKEN OVER SIX MONTHS FROM EACH OF FIVE HEALTHY SUBJECTS

Subject	Total Plasma (g./100 ml.)		Plasma Albumin (g./100 ml.) by Howe's Method		Plasma Globulin (g./100 ml.) by Howe's Method		Plasma Albumin (g./100 ml.) by Majoor's Method		Plasma Globulin (g./100 ml.) by Majoor's Method	
	Arith-metic Mean	S.D.	Arith-metic Mean	S.D.	Arith-metic Mean	S.D.	Arith-metic Mean	S.D.	Arith-metic Mean	S.D.
A	7.39	0.23	4.53	0.19	2.86	0.24	3.63	0.25	3.76	0.26
B	7.60	0.17	4.81	0.14	2.79	0.13	3.85	0.14	3.75	0.16
C	7.11	0.13	4.63	0.15	2.48	0.09	3.92	0.17	3.19	0.13
D	7.38	0.15	4.84	0.16	2.54	0.21	4.03	0.19	3.35	0.28
E	7.19	0.18	4.89	0.14	2.30	0.14	4.06	0.22	3.13	0.18

with Majoor's, results are plotted in Figs. 1 and 2. (When blood was taken from a patient more than once the initial value is used.) These figures show that the results can be arranged as follows :

A, the group of healthy subjects ; B, the malnutrition and malignancy groups, in which the results have identical distribution and are therefore represented as one group ; C, the chronic hepatitis group, which partly overlaps Group B ; D, the nephrotic group, which is separate from the other groups except for one result which falls in Group B using Howe's method (Fig. 1) ; E, the chronic

FIG. 1.—Albumin (A22) and globulin (G22) levels determined by Howe's method in 30 healthy and 54 diseased subjects.

FIG. 2.—Albumin (A26) and globulin (G26) levels determined by Majoor's method in 30 healthy and 54 diseased subjects.

nephritis group, which overlaps Group B using both methods. Also one result falls among the healthy group using Howe's method (Fig. 1) but not using Majoor's (Fig. 2).

Comparison of Figs. 1 and 2 shows that the separation of these patients into groups is similar by the two methods, but a little more distinct by Majoor's. This method therefore appears to have a slight advantage from the point of view of diagnosis.

When the arithmetic difference between albumin concentrations derived by Howe's and Majoor's methods is calculated (Table III), it is found to vary within narrow limits. The greatest variability is in the nephrotic syndrome and chronic nephritis groups, but even in these the

TABLE III
VARIATIONS OF THE ARITHMETIC DIFFERENCE BETWEEN ALBUMIN (G./100 ML.) DETERMINED BY HOWE'S AND MAJOOR'S METHODS

	Healthy Subjects	Malnutrition and Malignancy	Chronic Hepatitis	Nephrotic Syndrome	Chronic Nephritis
Arithmetic mean ..	0.82	0.78	0.84	0.76	0.89
S.D. ..	0.12	0.18	0.21	0.27	0.25
No. ..	30	25	12	10	7

mean of the arithmetic difference is close to that in the healthy group. The relationship between the two results for the albumin fraction for the 30 healthy and 54 diseased subjects is shown in Fig. 3. Statistical analysis reveals that they are related by the equation $A22$ (albumin

FIG. 3.—Relationship between albumin values obtained by Howe's (A22) and Majoor's (A26) methods.

FIG. 4.—Plasma total (T) and albumin determined by Howe's (A22) and Majoor's (A26) methods during the recovery phase of a patient with the nephrotic syndrome.

determined by Howe's method) = $1.03 \times A26$ (albumin determined by Majoor's method) + 0.73. In only seven cases, the result by Howe's method varies from the value calculated from that determined by Majoor's method by more than 0.3 g./100 ml., and in only two by as much as 0.4 g./100 ml.

In the individual, the relationship between the albumin concentrations determined by Howe's and Majoor's methods respectively is even more constant despite considerable variation in the concentrations of albumin and globulin during the course of the disease. This is shown in Fig. 4 in the case of an adult with the nephrotic syndrome who recovered in 16 weeks.

Discussion

So long as albumin and globulin estimations continue to be used in clinical biochemistry, the use of several methods giving different results can only lead to confusion. It was to avoid such confusion that we hoped to demonstrate that either Howe's method or Majoor's possessed a considerable advantage over the other.

We were able to demonstrate only a slight advantage, because the reproducibility of results is identical, the normal ranges are equal, and the separation into diagnostic patterns is only a little better by Majoor's method. Because the arithmetic difference between albumin concentrations determined by Howe's and Majoor's methods is relatively constant, it scarcely matters which method is adopted, because one albumin value is reasonably predictable from the other. For an individual patient this difference between them is even more constant than amongst patients as a whole, so that the determination of a change of albumin according to one method is accompanied by a practically equal change according to the other.

Since, however, albumin and globulin values obtained by electrophoresis are likely to become more widely used in clinical work, and since Majoor's method gives results said to be closer to those of electrophoresis than Howe's method, this alone would seem to be a good reason for employing Majoor's.

Summary

Howe's (using 22% sodium sulphate concentration) and Majoor's (using 26% sodium sulphate concentration) methods for differential plasma protein estimation were compared by means of parallel assays on a series of normal and pathological plasmas.

We found that there is little advantage in one method over the other, because the reproducibility of results is identical, the normal ranges are equal, and the separation of results into diagnostic patterns is only slightly better when Majoor's method is used. The arithmetic difference between the two albumin levels is relatively constant in the conditions studied, and therefore either albumin level is reasonably predictable from the other.

Since Majoor's method is said to give results closer to those of electrophoresis than Howe's, this, together with the slight advantage indicated by our own results, would seem to be a good reason for employing Majoor's method.

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The Value of Formol-Ether Concentration of Faecal Cysts and Ova

BY

D. S. RIDLEY AND B. C. HAWGOOD

From the Hospital for Tropical Diseases, London

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It is recognized that protozoal cysts and helminth ova will be detected in stools more frequently if they have been concentrated before the search is made. Of the methods used for the recovery of both cysts and ova, the formol-ether technique (Ritchie, 1948) avoids the faults in the popular zinc sulphate centrifugal flotation method (Faust, D'Antoni, Odom, Miller, Peres, Sawitz, Thomen, Tobie, and Walker, 1938; Faust, Sawitz, Tobie, Odom, Peres, and Lincicome, 1939) that cysts are liable to distortion while some ova are too heavy to be brought to the surface. The formol-ether method has been evaluated (Wykoff and Ritchie, 1952) and compared favourably with the zinc sulphate method (Ritchie, Pan, and Hunter, 1952); the object of this paper is to draw attention to its value as a routine diagnostic measure and to describe a simplified procedure.

Method

Stools from 670 unselected patients referred to this hospital were examined directly and again after concentration and the findings compared. For the direct examination the whole of one microscope cover-slip was searched carefully for cysts and ova; a concentrated specimen was then searched in the same way by the same technician. A number of highly skilled technicians were employed, all of whom had received at least six months' whole-time training in the searching of stools, and who, in most cases, had had two or more years' experience.

The simplified formol-ether concentration is carried out as follows:

About 1 g. of faeces is thoroughly emulsified with about 7 ml. of 10% formol-saline, and strained through wire gauze (40 mesh per inch) into a centrifuge tube. Ether, 3 ml., is added and the mixture shaken vigorously for one minute. It is then centrifuged, accelerating slowly and gradually over a period of two minutes to a speed of 2,000 r.p.m., and then allowed to come to rest. The debris on the surface and at the interface between the two liquids is loosened from the wall of the tube with a stick and the supernatant is decanted, the last drop or two being allowed to run back. The upper part of the tube is wiped clear of fatty debris. The small deposit is shaken up and poured on to a slide. Some practice is required to obtain optimum results.