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Electrophoresis of normal lumbar cerebrospinal fluid proteins in the African*

P. O. CHUKE

Department of Medicine, University Teaching Hospital, Lusaka, Zambia

Summary

Lumbar cerebrospinal fluid obtained from Zambian patients suspected of suffering from central nervous system conditions was concentrated over a hundred-fold and subjected to small-scale cellulose acetate electrophoresis (Millipore). The percentage of each protein fraction was determined by scanning. Only twenty samples were considered normal out of a total of 150 studies.

These normal values were not statistically different from those of published data on Europeans and North American Caucasians. The high serum gamma globulin in African subjects is not reflected in the cerebrospinal fluid.

Résumé

Du liquide céphalo-rachidien obtenu par ponction lombaire chez des patients Zambiens suspects de souffrir d'atteintes du système nerveux central, a été concentré au dela de 100 fois et soumis à une électrophorèse (petite bande, sur cellulose acétate), (Millipore). Le pourcentage de chaque fraction protéique etait déterminé par évaluation visuelle. 20 échantillons seulement sur un total de 120 échantillons éxaminés ont été considérés normaux.

Ces valeurs normales n'étaient pas statistiquement différentes des données publiés sure des Européens et der Caucasiens Nord-Américains. La haute valeur en gamma-globuline sérique des sujets Africains n'est pas reflétée dans le liquide céphalo-rachidien.

The proteins of normal cerebrospinal fluid (C.S.F.) are identical with those of normal plasma. By modern concept C.S.F. is formed at the choroid plexuses of the lateral, third and fourth ventricles. The fluid escapes into the subarachnoid spaces at the level of the fourth ventricle and is reabsorbed through the venous system of the meninges, perhaps mainly by way of the Pacchionian granulations. Studies with ^{131}I-labelled albumin and gamma-globulin (Frick & Scheid-Seydel, 1958) support the concept of the plasma origin of normal C.S.F. proteins. The proteins diffuse into the C.S.F. at the site of its formation and the concentration in the C.S.F. will depend on the concentration in the serum and the molecular weight of the individual proteins (Rosenthal & Soothill, 1962).

Previous studies have shown that the normal adult African has a higher gamma-globulin and lower albumin concentration in the serum than European subjects (Edozien, 1957; Ezeilo, 1970). If this higher serum gamma-globulin is reflected in the C.S.F., a reassessment of some of the various precipitation tests used in the detection of central nervous system disease may have to be made. It is important therefore that the normal values of proteins in the C.S.F. in the adult African subjects be determined.

The present paper is a preliminary and exploratory study, the two-fold purpose of which is to determine the concentration limits of the normal spinal fluid proteins in Africans and to note any deviation from the normally accepted values for European subjects.

Materials and method

The C.S.F. used in this study was collected by lumbar puncture of adult in-patients who were initially suspected of suffering from a disease of the central nervous system for which examination of C.S.F. was indicated. No specimen with red blood cells or with more than five white blood cells in the high

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Correspondence: Dr P. O. Chuke, Ministry of Health, P.O. Box 205, Lusaka, Zambia.

power field was included in the study. The fluid from patients subsequently found not to have a condition that will affect the value of the constituent proteins were labelled as normal. One hundred and fifty specimens were studied and only twenty met these rigid criteria. It has been clearly demonstrated that the spinal fluid protein concentration must be considered in relation to the age of the subject. Newborn infants have a high content presumably due to a poorly developed blood brain barrier (Levison, 1950), while a similar tendency is observed over the age of sixty (Madonick & Weissman, 1955). In the present study the ages of the subjects ranged from 8 to 60 years. The samples can be considered to be random and the mean age was 39·87 years and the range of ± one standard deviation being 25·46 to 54·22 years.

Technical aspects

Electrophoresis of C.S.F. proteins requires a preliminary concentration of the fluid in order to raise its usually very low value (normally about 20 mg per 100 ml) to the same range as that of plasma. A hundred-fold or higher concentration was achieved for this study by dialysis against a concentrated solution or polyvinyl-pyrrolidione. The desired concentration was achieved after about 6 h by continuous automatic agitation of the cellophane bag containing the C.S.F. using the 'Oxford dialyser'. It is assumed that protein characteristics were not altered during dialysis. This is valid since no denaturation was observed during electrophoresis judging by the absence of accumulated material at the starting point. The same observation has been noted by Brackenridge (1962).

The final products were then subjected to small-scale cellulose acetate electrophoresis (Millipore). Using a barbital buffer of pH 8·6 and a constant voltage of 100 V, good separation was achieved in 20 min. After staining with diluted Panceau-S concentrate the strip was rinsed three times in 5% acetic acid and dried completely. Clearing was performed with a mixed solution of thirty parts of ethyl acetate and seventy parts glacial acetic acid after preliminary soaking of the strip in N-propanol. Each C.S.F. sample was run side by side with the respective serum on the same strip for clear identification of the various fractions. A phoroscope densitometer was used for the determination of the relative values of the separated fractions.

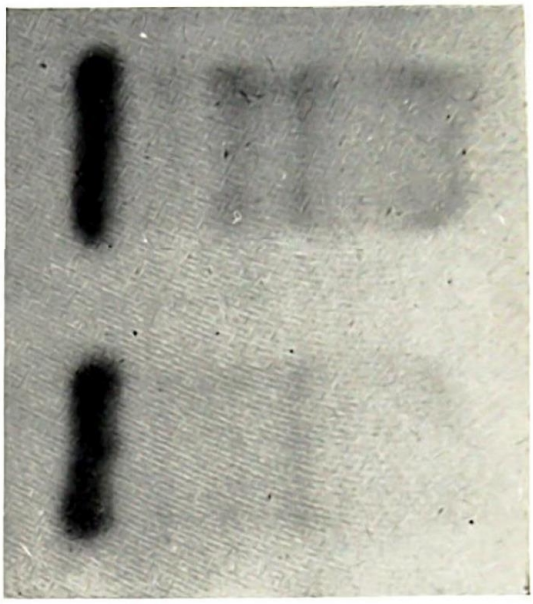

FIG. 1. Normal electropherotograms of serum (upper) and concentrated lumbar C.S.F. (lower) of one subject.

Results

Fig. 1 shows the typical electropherotograms obtained for both C.S.F. and serum. The gamma-globulin is diffuse and the beta-globulin displays two parts; a slower and a faster moving position.

Scanning of the electrophoretic strips yielded the results shown in Table 1.

TABLE 1. Electrophoretic distribution of proteins in normal lumbar C.S.F.

Fractions	% of total
Pre-albumin	6·58± 3·11
Albumin	50·97± 13·66
Alpha$_1$-globulin	9·26± 6·95
Alpha$_2$-globulin	11·79± 5·66
Beta-globulin	14·37± 9·31
Gamma-globulin	11·28± 7·63

Pre-albumin is conspicuous and the dominant globulin is the beta-globulin. The pre-albumin and the albumin fractions together account for more than 56% of the C.S.F. proteins. When these figures are compared with the values of these fractions in serum, notable differences are seen as shown in Table 2.

TABLE 2. Normal serum and lumbar C.S.F. electropherograms

Specimen	Number of sample	Pre-albumin	Albumin	Globulins			
				$Alpha_1$	$Alpha_2$	Beta	Gamma
C.S.F.	20	6·58	50·95	9·26	11·79	14·39	11·28
Serum	18		43·47	3·92	13·64	15·67	23·28

TABLE 3. Electrophoretic distribution of proteins in C.S.F.*

Author	Number of cases	Pre-albumin	Albumin	Globulins			
				$Alpha_1$	$Alpha_2$	Beta	Gamma
Cawley		3·3± 1·1	55·6± 2·6	5·6± 2·3	8·4± 0·4	16·5± 3·8	10·5± 3·0
Bauer	26	4·2± 2·1	59·4± 6·7	13·4± 3·4		13·4± 3·3	9·4± 3·3
Hill *et al.*	21	4·6± 1·3	49·5± 6·5	6·7± 2·0	8·3± 2·1	18·5± 4·8	11·2± 2·7
Chuke	20	6·6± 3·1	50·9± 13·7	9·3± 3·9	11·8± 5·7	14·4± 9·3	11·3± 7·6

* Normal lumbar fluid.

Results in percentage of total± one s.d.

Pre-albumin is not detected in serum cellulose acetate electrophoresis and the relative value of gamma-globulin in C.S.F. is about half its value in serum. There is a higher relative value of alpha-globulin and beta-globulin in C.S.F. than in serum.

Table 3 is the comparison of the results of the present study and those of other authors who had studied European subjects. Again the dominant globulin in all the published data is the beta-globulin. The pre-albumin in the first three sets of data is slightly lower than in the present study. These earlier authors had used paper electrophoresis which is known to give a low value for pre-albumin (Brackenridge, 1962). The most interesting feature is the almost identical value for gamma-globulin in these data and the present study. None of the differences in all the fractions is statistically significant ($P<0·05$). The higher value of gamma-globulin in the normal serum of the African subject is not reflected in the normal C.S.F. despite the fact that the C.S.F. protein is derived by filtration from plasma.

Discussion

The lumbar C.S.F. not only contains more protein than either the ventricular or the cisternal fluid but less relative value of pre-albumin and albumin. This is due to back-diffusion of plasma proteins from the venous re-absorptive sites in the meninges. Comparative studies must therefore be made of fluids obtained at identical sites of the neuroaxis.

The major gamma-peak of the C.S.F., quantitated by scanning electrophoretic diagrams, consists of a protein immunologically identical with serum gamma G-immunoglobulin (Kabat, Glusman & Knaub, 1948; Colover *et al.*, 1963) of molecular weight 156,000–161,000. Albumin has a molecular weight of only 69,000. Rosenthal & Soothill (1962) using immunochemical determination method, calculated the C.S.F./serum concentration ratios for albumin, transferrin, gamma G-immunoglobulin, $alpha_2$-macroglobulin and beta-lipoprotein. These relative values, when plotted against the respective molecular weights, ordered themselves on a line with descending slope, indicating that plasma proteins appeared in the fluid in proportion to their concentration in the serum and in inverse proportion to their molecular weight except for transferrin. It is therefore not surprising that the relative value of gamma-globulin in the C.S.F. is very low and that the higher relative value of gamma-globulin in the normal African serum is not reflected in the C.S.F.

Transferrin (mol. wt 90,000), the predominant protein in the beta-globulin fraction, has a relatively higher 'clearance' value than even albumin, probably due to the fact that the slower fraction of beta fraction (B_2) is devoid of four neuraminic acid residues per molecule (Parker & Bearn, 1962).

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