J. clin. Path., 1970, **23**, 586-589

Separation of the proteins of cerebrospinal fluid using gel electrofocusing followed by electrophoresis

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SYNOPSIS The technique previously applied to serum can, with minor modifications, be applied to cerebrospinal fluid. Good protein patterns have been obtained and similarities as well as differences have been demonstrated between cerebrospinal fluid and serum. The patterns obtained should prove useful in neurological diagnosis.

The technique of isoelectric focusing in polyacrylamide gel has proved to be a sensitive method of separating proteins in solution (Dale and Latner, 1968; Leaback and Rutter, 1968; Awdeh, Williamson, and Askonas, 1968; Fawcett, 1968; Wrigley, 1968; Catsimpoolas, 1968; Beeley, 1969). The problems encountered in interpreting the complex patterns obtained from biological fluids, together with the necessity to remove the carrier ampholytes before staining the proteins, have led to a two-dimensional procedure in which isoelectric focusing was followed by electrophoresis into a polyacrylamide gel slab (Dale and Latner, 1969). The present work deals with the application of this technique to cerebrospinal fluid and the modifications which were found necessary. The specimens were obtained from patients in a neurological ward.

Method

The lower protein level in cerebrospinal fluid necessitated preliminary concentration of the sample. By using ultrafiltration, the protein concentration could be increased without a concomitant increase in salt.

The relatively constant levels of serum proteins allowed a fixed volume of sample to be used (Dale and Latner, 1969). This contrasts with cerebrospinal fluid, in which the protein level shows considerable variation. Results comparable to serum were obtained by applying a constant amount of protein for each separation. In order to maintain uniformity of pore size of the isoelectric focusing gel, an amount of cerebrospinal fluid containing a fixed quantity of protein was concentrated to a fixed volume. A volume of cerebrospinal fluid containing 1,200 μg protein was concentrated to 50 μl by ultrafiltration in a sac made from 8/32 in. Visking tubing (Visking Corp., Chicago). An Ampholine/acrylamide monomer was prepared from the following aqueous solutions: 1 ml 0·8% (v/v) N,N,N′,N′– tetramethylethylenediamine (TEMED), 1 ml 0·004% riboflavine, 2 ml 40% sucrose, 2 ml 28% acrylamide containing 0·735 g methylenebisacrylamide in 100 ml, 0·5 ml 0·001% bromophenol blue, and 0·4 ml Ampholine carrier ampholytes (LKB Produkter, A.B., Stockholm-Bromma, Sweden).

Of this monomer, 0·25 ml was added to the concentrated sample in the sac and mixed to remove any protein solution adhering to the walls. Then 0·2 ml of the cerebrospinal fluid/monomer mixture was transferred to a test tube (50 × 9 mm). A volume of 83 μl Ampholine/acrylamide monomer and 17 μl water was added to the solution remaining in the sac. After careful mixing, the whole amount (0·2 ml) was pipetted into a second test tube. The first tube contained 800 μg and the second 400 μg protein. Each sample was then transferred, using a fine Pasteur pipette, into the glass tubes for photopolymerization. Isoelectric focusing, as described for serum (Dale and Latner, 1969), was continued for

Received for publication 1 January 1970.

three hours using an initial current of 9 mA. At the end of this time, the gel cylinders were embedded in a slab of polyacrylamide gel, subjected to electrophoresis for five hours, and the proteins stained with naphthalene black 12B (B.D.H.).

Results

Examples of the protein patterns obtained from cerebrospinal fluid are shown in Figure 1. Although there was evidence during isoelectric focusing of protein precipitation in the gel cylinder containing the 800 μg sample, the patterns remained very similar, with the larger sample demonstrating more readily some of the trace proteins.

The pattern produced was essentially similar to that previously described for serum (Dale and Latner, 1969). Amongst the features which distinguished cerebrospinal fluid from serum were the prominent pre-albumin spot and a spot featured in most of the specimens examined close to transferrin. This spot had an electrophoretic mobility slightly less than that of transferrin with a more alkaline isoelectric point. Like the two transferrin spots, this latter also stained for iron with 2,4-dinitroso-1,3-naphthalenediol (Ornstein). The haptoglobin spots were frequently undetectable and, unlike serum, the most common type seen was Hp 1-1 (Smithies and Walker, 1956), which is shown in Figure 2. The IgG zone showed more variation than in serum; in some cases the whole area was increased and in others there were local variations in intensity as shown in Figure 3. IgA was not readily demonstrated unless the total protein content was raised. A protein spot was frequently present which, during electrophoresis, migrated slightly ahead of albumin, but had a more acid isoelectric point (Fig. 2).

Contamination of cerebrospinal fluid with blood significantly increased the protein concentration and could give rise to additional spots, notably haemoglobin, when lysis of the erythrocytes took place, as shown in Figure 4.

Discussion

Proteins, such as gamma globulin, are present in cerebrospinal fluid in relative amounts which are considerably less than those in serum. In order to study these globulin fractions, it has therefore proved necessary to take an amount of cerebro-

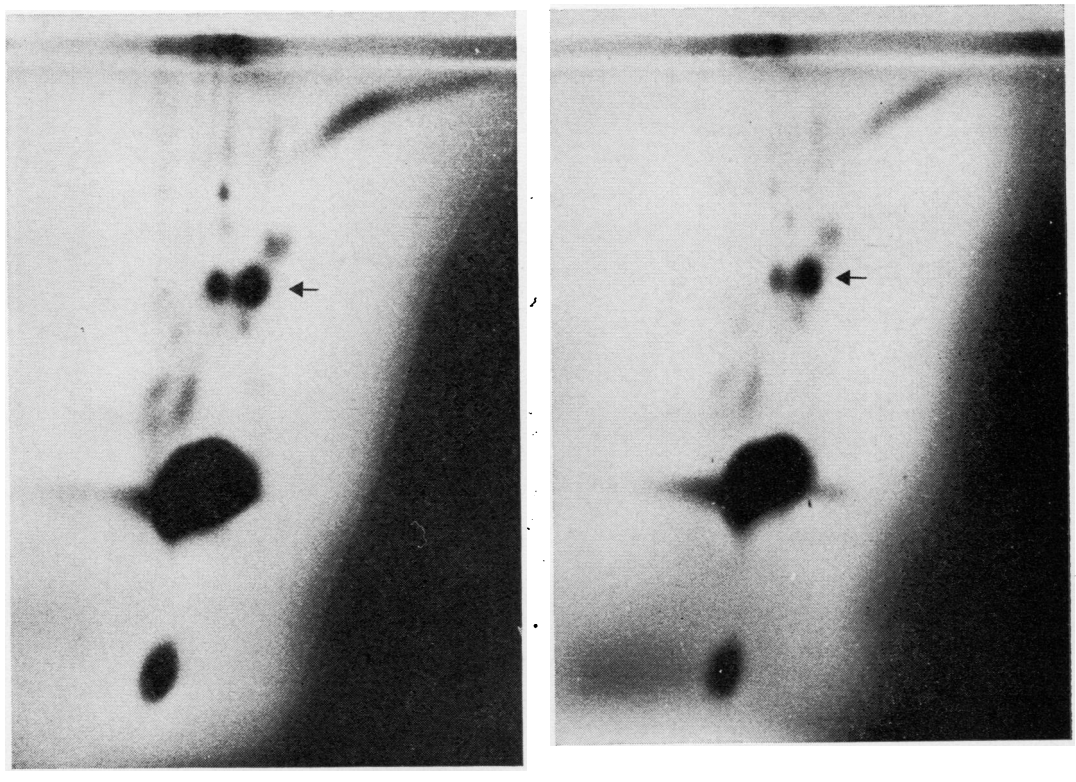


Fig. 1 *Cerebrospinal fluid protein patterns obtained with* (left) *800 μg total protein;* (right) *400 μg total protein. The pair of transferrin spots is indicated by an arrow.*

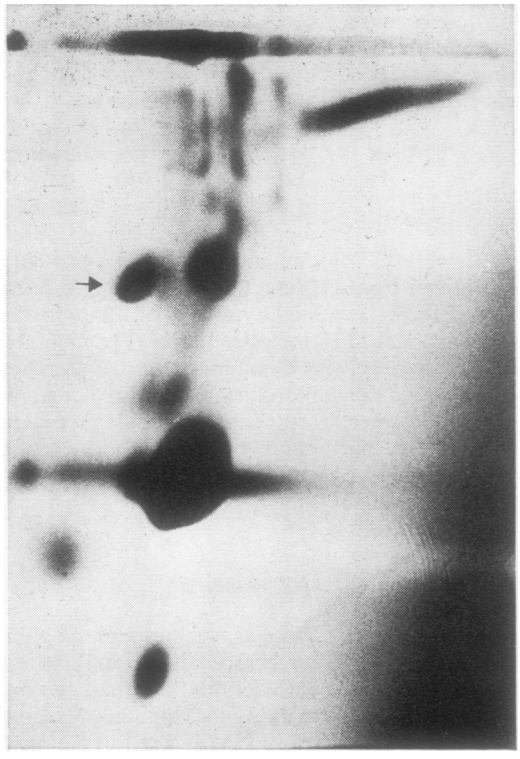


Fig. 2 *Well marked haptoglobin spot (type 1-1) shown by an arrow.*

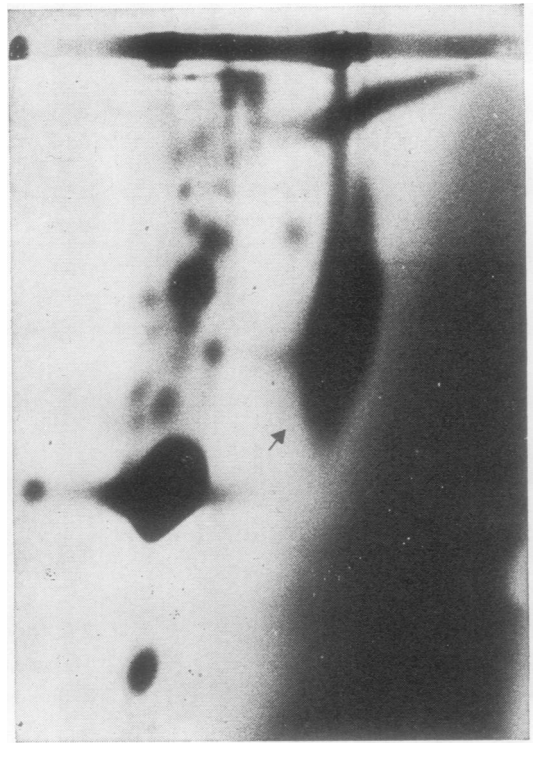
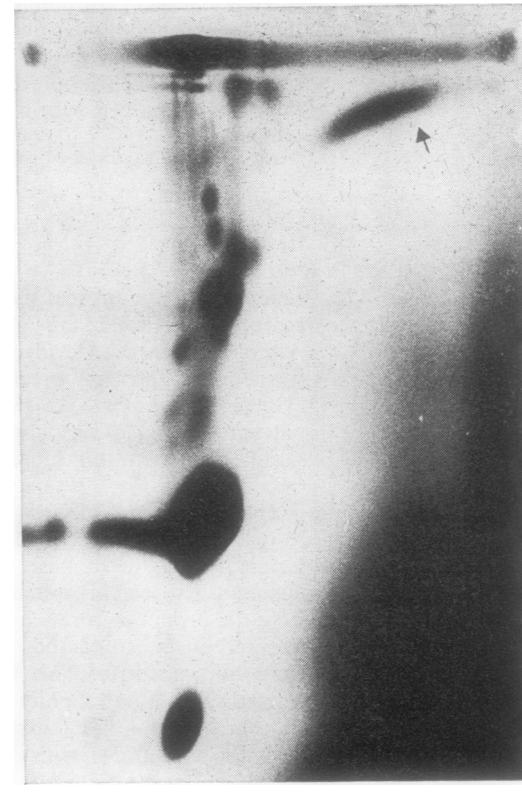


Fig. 4 *Cerebrospinal fluid contaminated with blood. The arrow indicates the large haemoglobin area.*



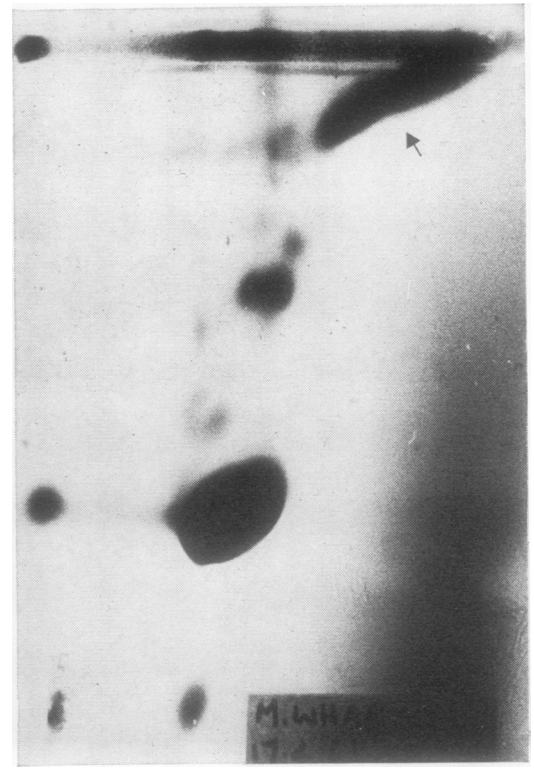


Fig. 3 *Varying presentations of IgG:* (above) *localized increase;* (below) *generalized increase. In each case the IgG area is indicated by an arrow.*

spinal fluid, the total protein of which was greater than that used in previous serum studies.

For serum separations, 250-400 μg protein was applied; using cerebrospinal fluid two samples were run, one containing 400 μg and the other 800 μg protein.

A pronounced pre-albumin spot has been found in all the samples of cerebrospinal fluid, confirming the findings of Kabat, Landow, and Moore (1942) and of Kabat, Moore, and Landow (1942).

The iron-staining protein spot found in the neighbourhood of transferrin could well be the β_2 or τ protein, which has been shown to react immunologically as transferrin (Gavrilesco, Courcon, Hillion, Uriel, Lewin, and Grabar, 1955; Grabar and Burtin, 1955).

Of the immunoglobulins, IgA was demonstrated only in samples having greatly raised initial protein concentrations and was much less prominent than IgG. The latter was usually less prominent in cerebrospinal fluid than serum, but marked variations in the intensity of the protein zone were seen between individual samples.

The haptoglobins were less prominent in cerebrospinal fluid than in serum and the frequency of the various types appeared to be different. The most commonly observed type was Hp 1-1, a finding noted by Blau, Harris, and Robson (1963), who concluded that haptoglobins in cerebrospinal fluid are derived from the plasma. The smaller molecules of type 1-1 gain access to the fluid more readily than the higher molecular weight polymers of types 2-1 and 2-2.

It is of interest to note that the trace proteins appeared with a greater intensity in samples with a low initial protein level requiring a higher degree of concentration. This suggests that the levels of many trace proteins may remain fairly constant despite variations in the total protein.

The technique of isoelectric focusing does not appear to have been reported for cerebrospinal fluid proteins, although polyacrylamide gel has been used as a medium for disc electrophoresis (Cunningham, 1964; Monseau and Cummings, 1965; Evans and Quick, 1966; Felgenhauer, Bach, and Stammler, 1967; Shapiro, Miller, and Harris, 1967). The two-dimensional separation appears capable of demonstrating a large number of proteins, and the patterns obtained from different samples show variations which may prove to be of value in the investigation of neurological disorders.

We are grateful to Dr D. A. Shaw for the provision of specimens of cerebrospinal fluid. One of us (C.F.) was in receipt of a grant from the Medical Research Council.

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