Interpretation of Cerebrospinal Fluid Proteins by Gel Electrophoresis

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ABSTRACT

The use of polyacrylamide gel electrophoresis (PAGE) for the separation of proteins in cerebrospinal fluid (CSF) results in greater definition than does a “routine” method such as cellulose acetate electrophoresis. Unconcentrated CSF is easily separated into as many as 18 bands by the use of PAGE. By means of a modified PAGE method described in this paper, unconcentrated and untreated CSF is quickly and conveniently analyzed for protein constituents. This modification involves a continuous buffer environment, a pore-size concentration gradient and CSF in amounts of 0.1 to 0.4 ml. Sucrose addition is not necessary in this procedure. Whereas most central nervous system (CNS) disease states do not yield consistently distinctive protein patterns, some diseases, such as vascular disease, infectious meningitis and some metastatic tumors, yield significantly altered patterns. It is suggested that the chief value of CSF protein electrophoresis at the present time is to follow the course of a CNS disease.

Introduction

Cerebrospinal fluid protein (CSF Prot.) determinations play a significant role in the physician’s ability to diagnose clinical conditions involved in the central nervous system and, concomitantly, in the laboratory’s efforts to provide meaningful data. Protein concentration in the CSF is increased in a variety of pathological conditions involving the meninges, brain and cord. With only passing reference to hemorrhage where the presence of cells, hemolysis and gross elevation of proteins are obvious evidence of this condition, many pathological processes may exist where CSF Prot. are normal, moderately increased or highly increased within the category of nonexudative processes. When evidence of an exudate exists, the qualitative or quantitative examination of CSF, especially by electrophoretic methodology, is without meaning, such as in certain kinds of meningitis where microscopic examination for pus, cells and bacteria are often sufficient. In conditions of lesser inflammatory involvement, the study of CSF Prot. has seemed to offer laboratory aid in an area of difficult differential diagnosis. These conditions include the nonpurulent meningitides, multiple sclerosis,
polyneuritis, cerebral tumors and obstructive disorders among others.

While total CSF Prot. globulin tests (such as Pandy’s test) and the colloidal gold test have their undoubted value, workers in this field of pathology have sought for tests more discriminating and meaningful in the hope that significant differences in CSF Prot. distribution would be pathognomonic for at least some of the CNS pathologies.

Naturally enough the search turned first to the already established method for studying protein distribution, protein electrophoresis, but it is readily apparent that the study of CSF Prot. posed problems not found in the protein electrophoresis of serum. The normal range for CSF Prot. is usually given as 15 to 45 mg per dl, a concentration so small as to necessitate concentration before undergoing the “routine” serum protein electrophoretic technique. Besides the demonstrated alterations caused by concentration techniques, the entire process of concentration plus the electrophoretic process makes the entire procedure one of tediousness and involves many hours of operations.

In addition to the “routine” method of electrophoresis which is meant to be by means of cellulose paper, cellulose acetate or agar gel electrophoresis, workers turned to gel electrophoresis involving polyacrylamide gel or starch gel for the purpose of utilizing the greater resolving power of those media. Most of the work with polyacrylamide gel electrophoresis (PAGE), however, involved concentration of the CSF Prot. prior to electrophoresis. A current thrust in PAGE, which is the one discussed here, is to use unconcentrated CSF in conjunction with PAGE, a procedure which confers the resolving power of PAGE along with a relatively quick procedure thus relieving the laboratory of the tediousness and time consuming requirements of other procedures. In addition to the methods discussed here which have to do with gel electrophoresis, attention may be brought to cellulose acetate electrophoresis on unconcentrated CSF.

Methods

While this discussion does not concern itself with a complete literature survey of PAGE (or starch gel) of CSF Prot., some key publications will be cited along with a discussion of the procedures described.

In 1964 Cunningham described a procedure involving PAGE and unconcentrated CSF ("native" CSF) and claimed that the method was a rapid, simple and labor-saving technique. Starting out with the basic Ornstein and Davis technique for PAGE, Cunningham modified the method to eliminate the sample gel step but instead layered the spacer gel with unconcentrated CSF containing an addition of sucrose to a final concentration of 25 percent. In common with Ornstein and Davis' technique, a small pore separating gel was used as the separation gel (electrophoretic medium). Cunningham utilized an 11 cm glass tube filled to a height of 6 cm with small pore gel, an unspecified height of spacer gel (presumably similar to Davis' technique) and a CSF-sucrose volume of up to 0.8 ml of fluid such that the protein content would be from 250 to 300 μg. Electrophoresis was carried out with 2 ma per tube for about two hours by which time the free dye (bromophenol blue) had migrated to the cathodic end of the gels. Bromophenol blue dye has been used extensively in this type of electrophoresis to mark the migration of both the electrophoretic front and the albumin fraction (as an albumin-bromophenol blue complex). Staining of the protein fractions was accomplished with 1 percent Amido Black B in 7 percent acetic acid (the time was unspecified but is usually from one to three hours). Elimination of excess dye (destaining) was done electrophoretically.

Monseu and Cumings utilized the methods of Ornstein and Davis and Cunningham since they perceived advantages in the PAGE technique not realized by either
cellulose paper or starch gel methodologies. In a more recent paper Cumings et al. continued the use of PAGE, omitted the spacer gel as being unnecessary, but retained the use of a sucrose-CSF sample. The sucrose-CSF fluid was applied directly to the top of the separation gel but otherwise their method followed the Cunningham procedure.

Evans and Quick also used PAGE in their study of CSF Proteins in various neurological disorders. Their method however differed from the previous authors' in that they followed Ornstein and Davis' procedure closely. That is, their gel system consisted of a small pore separation gel, a large pore spacer gel and a large pore sample gel. Their CSF was concentrated by pressure dialysis to the point where about 0.1 ml of fluid contained 200 to 250 µg of protein. A 0.1 ml aliquot of fluid was mixed with the photopolymerizing stock sample gel. Otherwise, their electrophoretic procedure was similar to the methods already mentioned.

Cudney and Wald in 1969 also investigated various neurological disorders by applying essentially the Cunningham technique for PAGE. The present authors applied their more simplified technique to the study of CSF Proteins. This method has been applied in the past to the study of serum haptoglobins, serum alkaline phosphatase, and serum proteins. It differs from the previous methods and from Ornstein and Davis' method in some important aspects: (1) a continuous system of buffer and pH is used; (2) an easily made pore size gradient is made at the top (loading point and primary separation section) of the gel column; (3) a single gel—the separation gel—is used; and (4) unconcentrated CSF without sucrose addition is used in amounts ranging from 0.1 ml to 0.4 ml depending on the protein concentration of the CSF. The authors believe that these simplifications result in an extremely simple and convenient method which does not sacrifice the resolution of the other methods already alluded to.

Principle of the Continuous Gel Gradient Method (Proposed Method)

PAGE (as used in this procedure) is a process of vertical electrophoresis that takes place in a molecular sieve polyacrylamide gel matrix and allows proteins to be separated on the basis of both molecular size and molecular net charge. Since the process utilizes a basic buffer, the migration of proteins is anodic in direction. The gel does not exhibit electroendosmosis so all proteins which possess an isoelectric point (pI) below 9.2 will migrate into the gel with a speed dependent on charge; however, large molecules will be relatively retarded owing to the molecular sieving effect. Thus, the separation pattern will be somewhat different than in conventional agarose or cellulose acetate electrophoresis. The use of a vertical direction of migration allows the application of a large volume of CSF. A thin-starting zone is achieved in the process since the migration of proteins in the fluid CSF supernatant is rapid to the point of entering the separating-gel matrix. The proteins pile up on the surface of the gel column in a thin zone, perhaps as little as 50 microns thick, and then a slower migration with separation takes place. Thus, unconcentrated CSF may be sampled directly and yield multiple protein zones that are clearly discernable after the entire electrophoretic and staining procedures are completed.

Reagents

SOLUTIONS

Acrylamide solution (Reagent A). Exactly 28.0 g of acrylamide and 0.56 g of N,N'-methylenebisacrylamide (BIS) are dissolved in about 50 ml of water and made up to final volume of 100 ml. This solution is stable at 4°C.

Buffered TEMED solution (Reagent B). Precisely 1.94 g of 2-amino-2-(hydroxymethyl)-1, 3-propanediol (TRIS) and 0.68 g glycine are dissolved in water and made up
to a final volume of 100 ml; 0.23 ml of N, N, N', N'-tetramethylethylenediamine methylethylene diamine (TEMED) is then added. The solution is mixed thoroughly and stored at 4° C where it is stable.

Stock buffer. Exactly 19.4 g of TRIS and 6.8 g of glycine are dissolved in water and made up to final volume of 1 L. The final pH should be approximately 9.2.

Working buffer. Two hundred-fifty ml of stock buffer are diluted to 1 L with water.

Ammonium persulfate solution (Reagent C). Precisely 0.140 g of reagent grade ammonium persulfate is diluted with water to a final volume of 100 ml. This is stable for one week at 4°C.

Water. It is understood that either deionized or distilled water is used.

Stain solution. One percent Amido Black B (Naphthol Blue Black) in 7 percent acetic acid is used.

Destaining acetic acid solution. Seven percent acetic acid is used.

Special Apparatus

Sample columns. Pieces of 7 mm (O.D.) glass tubing are cut to 75 ± 2 mm lengths. The inner diameter (I.D.) of this tubing should be about 4.8 to 5 mm. The ends of the tubes may be smoothed with a file, but should not be fire-polished.

Column base caps. Rubber stoppers from Vacutainer (#3204) tubes make suitable base caps.

Loading syringe. A 20 to 30 ml syringe fitted with a Wintrobe hematocrit needle makes an ideal loading syringe. Alternately, a 75 mm length of plastic tubing attached to a blunt tip needle may be used instead of the Wintrobe needle.

Gel extrusion needle. A 75 mm long blunt tip needle which is used in conjunction with a 20 to 30 ml syringe is needed.

Injector needle. A 35 mm piece of 0.030" × 0.048" polyethylene tubing is fitted over the length of a blunt tip 20 gauge ½" needle. A 10 mm piece of 22 gauge platinum wire is introduced into the free end of the tubing until it is totally inside the tubing. The tubing and its platinum wire are bent at right angles at a distance about 4.5 mm from the end in order that this right angle bend may be entered into the 7 mm (O.D.) glass tubing without binding. The injector needle is used in conjunction with a 5 ml syringe. The needle assembly is modified from one of our previous papers in order to accommodate the longer glass tubing used in this procedure.

Electrophoretic equipment. Many manufacturers make available the necessary modules needed for this procedure. The authors have used Canalco equipment consisting of the Model 100 constant current power source and the Model 1200 electrophoretic buffer box-electrode system (bath assembly). Canalco also provides many of the ancillary equipment used in disc gel electrophoresis and mentioned in this procedure. Davis' article may also be consulted for basic technique and description of apparatus.

Procedure

1. Up to 12 glass sample columns are fitted into rubber base caps and set upright on the bench so that there is no apparent angle of tilt.

2. Five ml of the refrigerated reagents A and B and 10 ml of reagent C are transferred into separate test tubes.

3. Reagents A, B and C are poured into a 50 ml beaker and mixed. The contents of the beaker are drawn into a 30 ml syringe and any air in the syringe is expelled. With the aid of the 75 mm plastic tubing or a Wintrobe needle, the tubes are filled with gel reagent to a distance of 51 mm from the bottom. This mixing of reagents and filling of tubes must be done without delay to avoid premature gelation. After the filling step, syringe and needle or tubing are rinsed quickly with water.

4. A 5 ml syringe filled with water and
fitted with the injector needle is inserted into each filled tube in turn and water is injected until the liquid column reaches to within 10 mm of the top of the tube. Care is taken in withdrawing the injection needle that the solution is not mixed more than necessary. Creation of the gradient is dependent upon gentle rising of the less dense water with lateral diffusion while rising. When using this technique, water layering is not necessary (see Discussion). Gelation takes place within 30 minutes.

5. After the gels have formed, excess fluid is shaken from the top of the gel column, the column is rinsed once with working buffer and the buffer is then discarded.

6. CSF is pipetted (automatic pipets are satisfactory) into each sample tube. About 0.4 ml of CSF are used.

7. The tubes are inserted into the cathodic buffer box (top) through the grommets that form an integral part of the apparatus. The tops of the glass tubes are brought flush with the tops of the grommets. Working buffer is then added gently to each tube so that liquid fills each tube and forms a rounded surface at the top.

8. With about 500 ml of working buffer in the anodic (bottom) buffer box, the two compartments are joined and buffer introduced into the cathodic buffer box. Gentle addition is advised so that the CSF contents of the tubes are not lost by turbulence.

9. The entire apparatus is then assembled (electrode assembly, safety interlock and connection to the power supply) and the current is supplied to the gel tubes at a rate of 2.5 ma per tube. It is often advisable to add bromophenol blue to the buffer in the cathodic compartment to give a light blue color to the buffer. The addition of dye allows the operator to observe the migration of albumin. If two blue bands are seen during migration the leading band represents free dye while the slower band is due to albumin-dye complex.

10. The albumin band is allowed to migrate for a distance of 30 mm into the gel. The current is then discontinued, the electrode assembly detached and the top buffer box removed to a stand where the sample tubes can be removed. The sample tubes are detached in turn by simultaneously pulling at the bottom of the tube while pushing at the top with a plastic stopper rod. The stopper rod slips into the grommet top as the tube is removed and prevents the buffer from spilling out.

11. The acrylamide gel is removed from within the glass tube by inserting the gel extrusion needle attached to a water-filled 20 ml syringe between the gel and the glass and by rimming the tube while delivering water. The gel is easily loosened in this process and falls into a recipient pan filled with water.

12. Each gel column is transferred to a numbered 12 x 75 mm test tube and covered completely with staining solution. Staining is allowed to proceed for one to three hours or overnight if preferred.

13. Excess dye solution is poured off and the gel rinsed once or twice with water to remove excess surface dye. The gel tubes may then be freed from excess dye by electrophoretic destaining in 7 percent acetic acid. Either the vertical destaining technique of Davis or the horizontal destaining Canalco apparatus (Model 1801) may be used.

14. The destained CSF protein pattern now may be visualized and interpreted by comparison with standard CSF or may be subjected to densitometric analysis. The authors find it convenient both to photograph the gels with a Polaroid camera and to subject the gel to densitometry.

Discussion (Proposed Method)

The original work of Ornstein and Davis serves as the ground work of all PAGE or disc electrophoretic work that followed and produced a voluminous literature. Workers in the field either modified Ornstein and Davis' work slightly or produced methods...
that differed significantly. Matson\textsuperscript{13} demonstrated that the original three layer gel system (separating small pore gel, spacer large pore gel and sample large pore gel) were not necessary and, furthermore, demonstrated that a discontinuous buffer system was unnecessary. Matson used a separating gel and reservoir buffer at a single pH, 9.0 to 9.2. The present authors have adapted Matson's work in our various studies\textsuperscript{6-7,8} and further simplified the system by introducing serum samples directly to the face of the separating gel by means of a microcapillary pipet. The method has been modified in another way\textsuperscript{6} by producing an easily made pore-size gradient at the top of the gel column for the purpose of allowing large molecular size proteins to enter the gel for an appreciable distance thus avoiding stacking-up of slow moving proteins at the gel-buffer interface. This simple technique avoids confusing the gel interface with protein when using densitometry as an adjunct to visual electrophoretic evaluation. Unlike other authors who add sucrose to the CSF to obtain a CSF with a 25 percent final sucrose concentration, the present authors used undiluted and unconcentrated CSF in the procedure. Presumably, sucrose serves as a viscous vehicle giving the operator some assurance that the sample will not be lost in the pre-electrophoretic preparatory steps. The authors have found that with proper technique to avoid turbulence in the loading of the top (cathodic) buffer compartment, even that simple step (sucrose dilution) can be avoided. In summation, with the use of a single gel column, a continuous buffer system and the use of native, undiluted and unconcentrated CSF, the entire electrophoretic procedure takes no longer than the shortest PAGE process for serum proteins.

Sources of Error

Uneven migration, streaking, blurring of bands. This occurs owing to (1) dirty tubes and (2) faulty reagents. Tubes should be washed scrupulously clean with a good grade detergent. Occasionally, a treatment with acid cleaning solution is required.

Air bubbles in gel. Usually this artifact is not seen, but when it seems to occur regularly, a process of deaeration of the mixed reagents (step 3) is required. After filling the syringe with mixed reagents A, B and C, air is expelled from the syringe; next, holding the syringe nipple firmly on a rubber mat or using one of the rubber base caps, the syringe barrel is drawn up to create a negative pressure and causing air to bubble
out of solution. Air is expelled again and the tubes are quickly filled as in step 4.

Fast gelation. Usually this undesirable occurrence can be avoided by using cold reagents.

Background staining. The buffer and/or the dye solution should be made up fresh.

No gelation. Most probably the cause of no gelation is faulty ammonium persulfate solution. A fresh reagent should be made.

Interpretation of Results

Cunningham reported, in a limited number of patients, that 21 bands were found in a normal CSF. The most prominent bands were 1 or 2 pre-albumins, albumin, bands 8, 9, 10 in the midsection of the gel, identified only by number, followed by slow-moving bands to the cathodic end. Seven of the bands were at the lower limit of detectability. A band that did not exist in the normal CSF was found in the pre-albumin section in a case of multiple sclerosis (post-mortem specimen) and in the post-albumin section in a case of presenile dementia. Cunningham also pointed out that the electrophoretic pattern of unconcentrated CSF differed in some significant respects from that obtained with pressure dialysis.

In Monseu and Cumings' work, normal CSF electrophoretic patterns (30 subjects) yielded from nine to 17 bands (14 on the average). Albumin was considered to be band #1; band #8 was identified as a β-globulin (siderophyllin). Between bands #1 and #8, five bands were found, band #6 being the most intense and most frequent. The authors considered band #6 as the fast α₂-globulin (Fe₂) of starch electrophoresis. Slower than band #8, but in front of the γ-globulins, were one to four bands. Band #9 was encountered with great regularity while band #10 was considered to be the slow α₂-globulin of starch electrophoresis of serum. At the cathodic (slow-moving fractions) end of the gel were a group of up to seven distinct γ-globulin bands (average five). The electrophoretic patterns of abnormal patients showed less difference than expected relative to the normal CSF pattern. Nine cases of multiple sclerosis showed only minor differences, the most notable being the more intense γ-globulins in one case of the nine. In seven cases of polynuclear, some changes in pattern and intensity were seen. In every case, band #2 (fast α₂-globulin) was deeply stained and in one of the patients a parallel increase in the α₂-globulin of serum was seen. There was a small but significant increase in the γ-globulins (as also seen in three cases of widespread secondary carcinomatosis). Ten cases of primary cerebral tumors did not show any abnormalities except for an increase of γ-globulins in three of the cases. Presenile dementia (two cases), cortical atrophy (six cases), and benign intracranial hypertension (four cases) presented no abnormalities in the electrophoretic patterns. The authors concluded that their experiments did not support claims of correlation of electrophoretic pattern with disease, especially in multiple sclerosis, although significant CSF Prot. differences were found in polynuclear. The abnormal band #2 of polynuclear was also found in cases of carcinomatosis and a single case of acute poliomyelitis.

Cumings et al., in a continuation of this study again found from 14 to 18 bands in normal subjects; the proteins most readily determined were prealbumin, albumin, fast α₂-globulin, β-globulin, and two γ-globulins (IgG and IgA). In 26 cases of multiple sclerosis IgG was increased significantly as was also the case in one out of four cases of subacute sclerosis encephalitis. The total γ-globulin content in multiple sclerosis was elevated. It was stated in this paper that the ratio of β-globulins to γ-globulins showed a lowering in almost all cases of multiple sclerosis and that this ratio plus the level of IgG "are almost diagnostic." These elevations of γ-globulins could be seen even when the total protein was relatively low. In Krabbe's disease the β to γ ratio was elevated. CSF
Figure 1. A control CSF electrophoretogram and densitometric tracing. Reprinted by permission of the authors and Clinical Chemistry.

Figure 2. A control CSF electrophoretogram illustrating the zones mentioned in the text and the proteins to be found in the zones. Reprinted by permission of the authors and Clinical Chemistry.
Prot. patterns from a wide range of other neurological disorders, apart from meningitis which was not studied, did not yield any diagnostic patterns.

Cudny and Wald$^2$ could not establish diagnostic criteria in a study of 72 patients and 10 controls although occasionally abnormal patterns were seen. They emphasized, however, that there may be a diagnostic value of the CSF Prot. patterns by PAGE in cases of “blood-brain” barrier disturbances.

The work of Evans and Quick$^9,10$ is the most comprehensive in the area of CSF Prot. analysis by PAGE. They found four characteristic CSF Prot. patterns (identification of zones and of individual proteins are illustrated by figures 1 and 2 and table 1).

1. **Serumization.** In this condition, sharp intense haptoglobin lines are found along with a relative decrease in a prealbumin I, an accentuation of prealbumin II, a decrease of proteins in the B-Zone (zones to be discussed below) as well as an increase in albumin, some of the glycoproteins (A-30 and G-30), the IgG band and the macroglobulin band (G-20). This type is seen following traumatic taps, post-hemorrhagic CSF and in metastatic tumor.

2. **Hemorrhage.** Free hemoglobin and haptoglobin-hemoglobin complexes appear. The CSF appearance is grossly bloody.

3. **Changes associated with obstruction of CSF drainage.** When protein increases, a relative increase of albumin, transferrin C and, occasionally, the B-20 peak occur. Relative decreases of IgG and the G-20 (macroglobulin) peaks are seen. An inverse correlation of prealbumin I and total protein occurs. For example, where total protein was 28 mg per dl a prealbumin of 10 percent was seen; in a CSF with a total protein concentration of 250 mg per dl, a prealbumin of only 2 percent was found.

4. **Immunoglobulin Increase.** Changes in G-10 (IgG) were difficult to evaluate unless increases in protein were extreme.

Evans and Quick divided their electrophoretic patterns into 5 zones: (1) prealbumin zone; (2) albumin zone; (3) A-zone (between the albumin peak and up to but not including the ceruloplasmin peak); (4) B-zone (includes the ceruloplasmin peak and extends to the G-30 band, a glycoprotein); and (5) G-zone (includes the G-30 peak and extends to the cathodic end of the gel, encompassing the γ-globulins and other slow-moving fractions).

Evans and Quick regularly observed 13 bands in their normal control samples. The bands in CSF appeared to be similar to those found in serum. With the use of an albumin/globulin ratio and a G-Zone/B-Zone ratio, significant variations in pattern by a quantitative scanning technique were found in vascular disease with elevated protein (A/G), infectious meningitis with elevated protein (G/B), vascular disease with elevated protein (G/B), and metastatic tumors (G/B). The rises in G-zone proteins in the above condi-

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**TABLE I**

Tabulation of Findings in Cerebrospinal Fluid

<table>
<thead>
<tr>
<th>Zone and Component</th>
<th>Tentative Identity</th>
<th>Percent Concentration ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Prealbumin</td>
<td></td>
<td>5.5 ± 2.0</td>
</tr>
<tr>
<td>Prealbumin I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prealbumin II</td>
<td>Orosomucoid</td>
<td></td>
</tr>
<tr>
<td>2. Albumin</td>
<td></td>
<td>50.0 ± 7.5</td>
</tr>
<tr>
<td>3. A-Zone</td>
<td></td>
<td>9.0 ± 1.4</td>
</tr>
<tr>
<td>A-30</td>
<td>Glycoprotein, Gc component</td>
<td></td>
</tr>
<tr>
<td>A-20</td>
<td>Gc component</td>
<td></td>
</tr>
<tr>
<td>A-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. B-Zone</td>
<td></td>
<td>20.0 ± 3.4</td>
</tr>
<tr>
<td>B-70</td>
<td>Ceruloplasmin</td>
<td></td>
</tr>
<tr>
<td>B-60</td>
<td>Haptoglobin 1:1</td>
<td></td>
</tr>
<tr>
<td>B-40</td>
<td>Transferrin C</td>
<td></td>
</tr>
<tr>
<td>B-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. G-Zone</td>
<td></td>
<td>15.5 ± 3.3</td>
</tr>
<tr>
<td>G-30</td>
<td>Glycoprotein, Sg^-macroglobulin</td>
<td></td>
</tr>
<tr>
<td>G-20</td>
<td>Macroglobulin (IgM)</td>
<td></td>
</tr>
<tr>
<td>G-10</td>
<td>7 S Immunoglobulin (IgG)</td>
<td></td>
</tr>
</tbody>
</table>

The data are from Evans and Quick.$^{10}$ The components are listed in terms of diminishing mobility. Those components having no identifying name were then uncharacterized.
Figure 3. Illustrated are four paired CSF electrophoretograms. CSF's 1, 3, 5, 7 and 9 were run unconcentrated and untreated. CSF's 2, 4, 6, 8 and 10 were run unconcentrated but contained 25 percent sucrose. The bands are similar except for an accentuation of B-60 (system of Evans and Quick) in the CSF samples treated with sucrose. Gels 1 and 2 involved a patient with a ruptured disc; gels 3 and 4 involved a patient with a lumbar ruptured disc; gels 5 and 6 involved a patient whose complaint was lower back pain; gels 7 and 8 involved a patient with essential hypertension with transient global amnesia secondary to cerebrovascular disease; and gels 9 and 10 involved a patient with low back pain whose discharge diagnosis was hemorrhoids with mild emphysema.

...tions "were compatible with the assumption of increased permeability of the blood CSF barrier and were not thought to indicate a specific increase of immunoglobulin G." Patients with active infectious meningitis showed a rise in the G-zone which was thought to be the result of an increased IgG production. It was also noted that the rise in CSF of proteins, especially IgG, mirrored the elevation of those proteins in serum. Evans and Quick concluded by stating that CSF Prot. electrophoresis had little value as a single determination but suggested that serial studies during the course of an illness could have value.

While insufficient samples have been run by us using the proposed method to add to the literature concerning patients, some salient points can be made regarding the methodology itself. First, the system utilizes a single buffer for the preparation of gels and for use in the buffer boxes, thus simplifying the procedure by limiting the number of individual reagents to be made. Second, the use of an unconcentrated CSF without the necessity of adding sucrose is a further simplification. An illustration of a series of CSF Prot. separations made with and without the addition of sucrose is shown in figure 3. The use of a non-treated CSF is seen to yield electrophoretic patterns similar to those treated with sucrose although some differences may still be seen leading one to the conclusion that even the simple step of crystalline sucrose addition may alter the electrophoretic pattern. Further, the use of a gradient pore-size gel succeeds in distinguishing protein zones in the body of the gel that would otherwise be concentrated in indistinguishable zones at the cathodic (slow) end of the gel. This improvement in resolution has been noted in previous publications. It is hoped that use of this technique will add significantly to the study of CSF Prot.

References
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3. Cumings, J. N., Shortman, R. C., and Tooley, M.: Polyacrylamide disc electrophoresis of cerebro-