Polyacrylamide Gel Electrophoresis of Spinal-Fluid Proteins

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Twenty-three control samples of CSF were analyzed by vertical column electrophoresis on acrylamide gel. Quantitation results using a microdensitometer are presented. The normal protein pattern shows 15 constant bands which coincide in mobility and in many cases in staining pattern with serum proteins. The haptoglobin peaks, except for the band of the 1-1 type and the leading band of the 2-1 type were not seen in control fluids.

Acrylamide gel column electrophoresis (disc gel electrophoresis), introduced in 1959 by Ornstein and Davis (3, 4, 9) and by Raymond and Weintraub, (13, 14) provides a high-resolution technic for electrophoretic separations of the protein components of various biological fluids. The facility of this method for use with small volumes of dilute protein solution makes it ideal for electrophoresis of cerebrospinal fluid (CSF).

In 1964, Cunningham published five examples of electrophoretic separation of CSF proteins by disc gel electrophoresis (2). These determinations distinguished as many as 22 bands. Monseu and Cumings (8) applied the column technic in a qualitative and semi-quantitative analysis of 59 CSF samples (including 30 controls), finding about 15 consistent bands.

The present study was designed to explore the possibility of using disc gel electrophoresis as a diagnostic aid in the laboratory. A microdensitometer for use with disc electrophoresis is commercially avail-
able,* and this instrument was employed for quantitative analyses of the gels.

This paper presents results of a study of CSF electrophoretic proteins from control subjects prior to application of the method in a study of organic neurologic diseases.

Materials and Methods

Sample Collection

Cerebrospinal fluid was obtained by atraumatic lumbar puncture from 23 control subjects. These subjects were selected from the general hospital service and satisfied the conditions that: (1) they had no organic neurologic or systemic disease; and (2) the total protein of their CSF was less than 45 mg./100 ml., and the cell count less than 5 lymphocytes per cubic millimeter. CSF samples were frozen immediately after collection and stored at −20°. We do not have evidence of protein denaturation during the frozen-storage state.

Electrophoretic Technic

Electrophoresis was carried out within 3 weeks of collection. Total protein was estimated by the trichloroacetic acid technic of Meulemans (7). Following this, aliquots of the sample, usually 3 ml., were concentrated 10–30 times by pressure dialysis against 0.01 M phosphate-buffered saline at pH 7.5. Concentration time was usually 12 hr., and a final volume of 0.1–0.3 ml. was obtained.

Electrophoresis was carried out on vertical gel columns using a 7.5% polyacrylamide gel for band separation. This system is essentially that recommended by Davis and Ornstein (3), and is commercially available.* Following the technical recommendations of the manufacturer, a spacer gel and a sample gel were layered above the polyacrylamide lower gel. The sample gel contained a 0.1 ml. aliquot of concentrated CSF, adjusted with normal saline to contain 250 µg. protein, plus 0.1 ml. gel. Following polymerization of this system, electrophoresis was carried out for about 75 min. at a constant current of 3.5 mAmp. per column. Buffer reservoirs were filled with tris (Hydroxymethyl) aminomethane (0.05M)-glycine (0.5M) buffer at pH 8.3. Progress of the run was followed by addition of small amounts of the tracking dye, bromphenol blue. A migration of 1.7 cm. for the albumin band was found most satisfactory for purposes of quantitation.

Following electrophoresis, the gels were stained with amido black.

*Canal Industrial Corp. (Canalco), Bethesda, Md.
Densitometric tracings of the stained gels were obtained using a micro-
densitometer.*

Special stains were employed as follows. Hemoglobin and hemoglobin-
haptoglobin complexes were determined by staining with benzidine hy-
drochloride. Color reaction was immediate and these gels were also
scanned in the densitometer. Glycoproteins were determined by use of
periodic acid-Schiff stain.

**Gel Quantitation**

An electrophoretogram of a control CSF and its densitometric tracing
are shown in Fig. 1. To allow quantitation of these traces, the follow-
ing technic was adopted.

![Control CSF electrophoretogram and densitometric tracing.](image)

The trace was divided into five zones: (1) prealbumin zone (prealbu-
min peak or peaks); (2) albumin zone (albumin plus some globulins
migrating with albumin); (3) A zone (between the albumin peak on the
anodal side and the ceruloplasmin peak on the cathodal side); (4) B
zone (including the ceruloplasmin peak on the anodal side and extend-
ing to the band G-30, a glycoprotein, on the cathodal side); and (5)
G zone (including the G-30 peak and extending to the origin of the
lower gel).

At concentrations optimal for globulin analysis, the albumin peak was

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*Model E, Canal Industrial Corp.*
above the absorbance (A) of 2.0 and hence off-scale. To allow an approximation of this peak, it was assumed that the concentration distribution in the peak is symmetrical. In this system, the protein bands enter the gel as a very sharp peak or lines. As migration proceeds, the peaks formed by monodisperse components spread out primarily as a result of diffusion. Such a process produces a nearly symmetrical peak. If the gels are run at a dilution where the albumin peak as developed has an absorbance less than 2.0 at its greatest density, the peak is indeed symmetrical.*

An approximate value for the albumin peak area was obtained by drawing the tangents to the ascending and descending lines of the peak at the inflection points, extending these tangents to the base line below and to their meeting point at an apex above the base line. The area of this triangle was taken as the area of the albumin peak.

The areas of the globulin curves were obtained by direct reading. No correction for differential dye absorption among the various peaks was made.

For each gel scan, values for the following were obtained: (1) prealbumin area, (2) albumin area, (3) A-zone area, (4) B-zone area, (5) G-zone area, and (6) total area (the sum of 1 through 5). Using these values, two ratios were calculated, an albumin/globulin ratio:

$$\frac{\text{Albumin area}}{\text{Total of A + B + G + Prealb. areas}}$$

and a G/B ratio:

$$\frac{\text{G-zone area}}{\text{B-zone area}}$$

The percentages of each fraction compared with the total were also calculated.

**Mobility Measurements**

Mobility was determined for each fraction with reference to the mobility of transferrin C and albumin. These mobilities were determined as:

$$R_T = \frac{\text{Migration of Fraction X}}{\text{Migration of Transferrin C}}$$

and

$$R_{A1b} = \frac{\text{Migration of Fraction X}}{\text{Migration of albumin}}$$

*It has been observed for this system that asymmetric traces of the albumin peak are obtained at absorbance values greater than 2.0. Pert and Pinteric report a similar phenomenon in starch gel systems (11). Standardization with bovine serum albumin showed that the protein content vs. peak area relationship for such traces is nonlinear. Conversely, using the triangulation described, a plot of protein content vs. peak area is linear to a total content of 250 µg.*
where migration is measured in centimeters from the origin. For purposes of $R_{A1b}$ calculation, the position of the albumin band was taken as the midpoint between the intercepts of tangents to ascending and descending curves with the base line of the scan. Reference to the front cannot be used for mobility descriptions since the relative mobility of the front with respect to protein components varies with the age and amount of reuse of the buffer.

**Results**

**Qualitative**

Exclusive of the haptoglobin bands, 15 bands were commonly observed in spinal fluids. Figure 2 shows a normal electrophoretic pattern with a numbering system, based on our quantitative method, adopted for description of the bands observed. In mobility and staining characteristics, the patterns were identical with serum components described

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*During preparation of this paper, our attention was drawn to a study by Peacock et al. (10) of serum electrophoresis on vertical polyacrylamide gel slabs in which transferrin C is employed as a marker for mobility measurements.*
in this system (Fig. 3). Three bands, prealbumin I, B-10, and B-20 occurred with relatively high intensity in spinal fluid as opposed to serum.

Table 1 describes 16 commonly observed fractions in terms of mobility and gives a tentative identification based on mobility and staining. The mobility measurements are averages of 10 determinations

where the average albumin migration from the origin of the lower gel was 1.7 cm. The coefficient of variation for these measurements was 3%.

In control fluids, haptoglobin was present in very low concentration. Using CSF samples having abnormally high protein concentration, the mobilities of these bands and their hemoglobin complexes were investigated and found to be essentially as described for serum in this system (5). The haptoglobin band of the 1-1 type has a mobility with reference to transferrin C of 1.08. Smithies and Connell (15), using purified haptoglobins and starch gel electrophoresis, showed that haptoglobins of
the 2-1 and 2-2 types and their complexes with hemoglobin show mobility values which fit into a geometric series. In our system these mobilities can be described by the formula:

\[ R_T = R_T K^n \]

where \( R_T \) is the mobility of any band in the series, preferably the leading (closest to the anode) band, and the exponent \( n \) equals 0, 1, \(-1\), etc. The constant, \( K \), varies with the system considered. \( K \) and \( R_T \) values are given in Table 2 for the 2-1 and 2-2 types and their hemoglobin complexes.

### Quantitative

The quantitative results (percentages of total) obtained on control fluids, 23 samples, with the standard deviation of each determination were, for prealbumin, 5.5 (2.0 S.D.); albumin, 50.0 (7.5 S.D.); A zone, 9.0 (1.4 S.D.); B zone, 20.0 (3.4 S.D.); G zone, 15.5 (3.3 S.D.); the al-

### Table 1. Protein Bands Observed in Electrophoretic Separation

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tentative identification</th>
<th>( R_T )</th>
<th>( R_{14b} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-10</td>
<td>7 S immunoglobulin, (IgG)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>G-20</td>
<td>Macroglobulin, (IgM)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>G-30</td>
<td>Glycoprotein, S ( \alpha )-macroglobulin*</td>
<td>.460</td>
<td>.235</td>
</tr>
<tr>
<td>B-10</td>
<td>None</td>
<td>.600</td>
<td>.300</td>
</tr>
<tr>
<td>B-20</td>
<td>None</td>
<td>.770</td>
<td>.390</td>
</tr>
<tr>
<td>B-30</td>
<td>None</td>
<td>.800</td>
<td>.400</td>
</tr>
<tr>
<td>B-40</td>
<td>Transferrin C</td>
<td>1.00</td>
<td>.510</td>
</tr>
<tr>
<td>B-50</td>
<td>Haptoglobin 1-1 or 1st band, type 2-1</td>
<td>1.08</td>
<td>.560</td>
</tr>
<tr>
<td>B-60</td>
<td>Ceruloplasmin</td>
<td>1.15</td>
<td>.590</td>
</tr>
<tr>
<td>B-70</td>
<td>None</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A-10</td>
<td>None</td>
<td>1.30</td>
<td>.680</td>
</tr>
<tr>
<td>A-20</td>
<td>Group-specific component</td>
<td>1.50</td>
<td>.740</td>
</tr>
<tr>
<td>A-30</td>
<td>Glycoprotein, group-specific component</td>
<td>1.54</td>
<td>.780</td>
</tr>
<tr>
<td>Albumin</td>
<td>Albumin</td>
<td>1.95</td>
<td>1.00</td>
</tr>
<tr>
<td>Prealbumin II</td>
<td>Orosomucoid</td>
<td>2.23</td>
<td>1.15</td>
</tr>
<tr>
<td>Prealbumin I</td>
<td></td>
<td>6.61</td>
<td>1.33</td>
</tr>
</tbody>
</table>

*Terminology of Poulak and Smithies (12).
†Insufficient accurate measurements.

### Table 2. Calculation of Haptoglobin and Hgb-Haptoglobin Mobilities

<table>
<thead>
<tr>
<th>Haptoglobin type</th>
<th>( R_T )</th>
<th>( K )</th>
<th>( R_T )</th>
<th>( K )</th>
</tr>
</thead>
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<tr>
<td></td>
<td>( R_T )</td>
<td>( K )</td>
<td>( R_T )</td>
<td>( K )</td>
</tr>
<tr>
<td>Free haptoglobins</td>
<td>Principal values of ( n )</td>
<td></td>
<td>Principal values of ( n )</td>
<td></td>
</tr>
<tr>
<td>1-1</td>
<td>1.08</td>
<td>—</td>
<td>—</td>
<td>0.800</td>
</tr>
<tr>
<td>2-1</td>
<td>1.08</td>
<td>.618</td>
<td>0, 1, 2, 3, 4</td>
<td>.800</td>
</tr>
<tr>
<td>2-2</td>
<td>.570</td>
<td>.725</td>
<td>0, 1, 2, 3</td>
<td>.240</td>
</tr>
</tbody>
</table>
bumin/globulin ratio was 1.0 (0.30 S.D.); and the G/B ratio was 0.75 (0.16 S.D.). Total protein was 31 mg./100 ml. (8 S.D.).

Precision

In this series, care was taken to obtain nearly equal loading of columns and relatively constant migration paths. Using 20 duplicate analyses, the precision obtained (95% confidence limits) was \pm 15-25\% for all fractions except prealbumin, where the precision was \pm 45\%. The use of a sample in gel was preferred over the technic reported using an ungelled sample, since the migration of the front through a liquid was subject to vibrational and convectional disturbances, resulting in poor resolution.

Discussion

In work previously published on CSF using the technic described above, Cunningham (2) identified protein bands using an integral numbering system beginning with that prealbumin closest to the anode. Monseu and Cumings (8), on the other hand, numbered integrally from the origin of the gel. Because these workers did not furnish identifying mobility data, it was not possible to compare in detail the pattern obtained in this study with those reported. The numbering system adopted here allows for further insertions as required.

The quantitative values reported here are unique to this system with the exception of the albumin/globulin ratio. Our value for this ratio is 1.00, which is comparable to values obtained using other technics (6). Because of the imprecision of albumin estimation, which is reflected in the variance of the albumin/globulin determination, the globulin ratio G/B may be a more reliable index of protein distribution for the polyacrylamide method. The use of a globulin ratio in interpretation of CSF protein distribution has been suggested by Bélanger et al. (1).

Disc gel electrophoresis offers significant advantages in spinal fluid protein separation: (1) The volume of sample required is small. For example, with a total protein concentration of 15 mg./100 ml., a successful analysis can be performed with a sample of 1.5 ml. (2) The electrophoretogram produced has extremely high resolution. (3) The technic is simple, and the working solutions are relatively stable. (4) A method for quantitation of the gel is available.

On the other hand, there are certain disadvantages: (1) Approximation of albumin in the manner described is subject to imprecision and inaccuracy. (2) Because of asymmetry of the bands, which occasionally occurs, gel scans may be poorly reproducible. (3) Failure to allow for differential dye absorption of the different fractions can lead to signifi-
cant errors in comparing quantitative calculations among gels if the protein distribution in the gels is considerably different. (4) The acrylamide reagents are toxic.

References