Evaluation of a Cellulose-Acetate Electrophoresis System for Serum Protein Fractionation

Alex Kaplan and John Savory*

A rapid system for the quantitative fractionation of serum proteins by electrophoresis on cellulose-acetate membranes was evaluated and found to be quite precise. The reproducibility (coefficient of variation) of the routine fractionation of a control serum carried out 40 times during a 15-week period was 2.4% for albumin and 14.2, 6.0, 6.1, and 5.2%, respectively, for the $\alpha_1$-, $\alpha_2$-, $\beta$-, and $\gamma$-globulin fractions.

Normal values are given for serum protein fractions (specimens from nonprofessional blood donors) obtained by cellulose acetate electrophoresis.

Although paper electrophoresis is a widely used technic for the fractionation of serum proteins, cellulose-acetate membranes are superior to paper as a supporting medium (I-7). Some advantages of cellulose acetate over paper are as follows:

1. The regularity of pore size results in sharp resolution of fractions in a relatively short time.
2. There is little adsorption of protein to the membrane, thereby increasing the resolution and eliminating the "trailing" of albumin.
3. The quantitation of protein-bound dye by densitometric scanning is more accurate because the protein is more evenly distributed in the fine membrane structure than in irregular paper fibers.
4. The membranes are rendered transparent easily, thereby increasing the accuracy of the scanning process.
5. Wet cellulose acetate membranes have greater tensile strength.

Despite these advantages, cellulose acetate has not readily replaced paper as a supporting medium in clinical chemistry laboratories because too much time has to be consumed in the individual handling of the cellulose-acetate strips during the dyeing and clearing process. Also

From the University Hospital and Department of Biochemistry, University of Washington, Seattle, Wash. 98105.

We are indebted to Dr. Eloise R. Giblett, Associate Director of the King County Central Blood Bank, for the serum specimens.

Received for publication Apr. 10, 1965; accepted for publication July 14, 1965.

*Postdoctoral trainee supported by Training Grant in Clinical Chemistry, T1 GM 776-01, from the National Institutes of Health.
scanning systems designed for paper electrophoresis are not readily adaptable to the shorter migrating distances of protein bands on cellulose acetate.

Recently, an apparatus has become available which eliminates these shortcomings and introduces several favorable features. The new Microzone* cellulose-acetate electrophoresis system accommodates eight serum samples on a single membrane, thereby, requiring only one-eighth of the manipulations in subsequent processing. It utilizes about 0.2 μl. of serum per sample and requires only 20 min. of electrophoresis to separate the protein fractions. The strip is stained and rendered transparent and then evaluated by means of the Analytrol* densitometer fitted with a new scanning attachment.

The present study was undertaken to evaluate the accuracy and precision of this system for serum protein fractionation.

**Experimental**

**Reagents and Apparatus**

- **Barbital buffer** pH 8.6 and 0.075 ionic strength.
- **Ponceau S solution** 0.2 gm. of Ponceau S dye, 3 gm. of trichloroacetic acid, and 3 gm. of sulfosalicylic acid are dissolved in water and made up to 100 ml.
- **Acetic acid 5% (v/v)** 50 ml. of glacial acetic acid are diluted to 1000 ml. with water.
- **Clearing solution** 20 ml. of glacial acetic acid are added to 80 ml. of methanol.
- **Biuret reagent** Prepared according to Gornall et al. (9).
- **Electrophoresis Apparatus** The Model R-101 Microzone electrophoresis cell, Duostat power supply, Model R-102 scanning attachment, and the Analytrol with B2 cam were employed in this study.

**Procedure**

The details of electrophoresis were very similar to those described by Grunbaum et al. (10), with minor modifications as suggested in the Beckman Instruction Manual. A drop of serum was placed on a nonwettable surface (Parafilm†) and touched with the platinum loop of a sample applicator which picks up and transfers to the membrane about 0.20 μl. of serum. Reproducibility of the application volume is ±5%. Eight serum samples were placed upon the cellulose-acetate membrane and were subjected to electrophoresis for 20 min. at 250 v. The wet membrane

---

*Spinco Division, Beckman Instruments, Inc., Palo Alto, Calif.
†Marathon Division, American Can Company, Menasha, Wis.
was stained in Ponceau S solution for 10 min., excess dye was removed by washing in 5% acetic acid, and the membrane was dehydrated by immersion in 100% methanol. It was placed in the methanol-acetic acid clearing solution, positioned upon a glass plate, and removed after a total of 45 sec. immersion. Excess solution was removed by gently applying a squeegee to the surface of the membrane. The membrane was air-dried for 2 or 3 min. and heated for 20 min. in an oven at 70–80°. After mounting in a plastic envelope, the absorbance of dye in the protein bands was measured by a densitometer at 520 mμ.

Total serum protein was measured by the method of Reinhold (8), with use of the biuret reagent of Gornall (9).

Results and Discussion

Precision

The reproducibility of the electrophoretic system was tested (1) by separating 40 fresh sera in quadruplicate and calculating the coefficients of variation of the replicates and (2) by separating a control serum 40 times over a period of 15 weeks and calculating the coefficients of variation. The latter approach was a test of the precision of the method under routine operating conditions of the laboratory. Table 1 summarizes the results of the quadruplicate fractionation of the 40 sera. Duplicates of each of four different sera were run on each membrane. Thus, each serum was fractionated on two different membranes. The standard deviations (expressed as per cent of total protein) were 1.54, 0.55, 0.79, 1.17, and 0.95, respectively, for the albumin, α1-, α2-, β-, and γ-globulin.

Table 1. Precision of the Electrophoretic Fractionation on Cellulose Acetate of Forty Sera in Quadruplicate

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Standard deviation*</th>
<th>Coefficient of variation†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>1.54</td>
<td>2.6</td>
</tr>
<tr>
<td>α1-globulin</td>
<td>0.55</td>
<td>15.7</td>
</tr>
<tr>
<td>α2-globulin</td>
<td>0.79</td>
<td>7.8</td>
</tr>
<tr>
<td>β-globulin</td>
<td>1.17</td>
<td>9.8</td>
</tr>
<tr>
<td>γ-globulin</td>
<td>0.95</td>
<td>6.4</td>
</tr>
</tbody>
</table>

* \[
\sqrt{\frac{\sum x^2 - \left(\frac{\sum x}{N}\right)^2}{N}}
\]

where \(x\) = individual values for per cent of protein, \(\left(\sum x\right)^2 = \) the square of the summation of replicate values in each quadruplicate set, and \(N = 4\).

† S.D. \(\times 100\), where S.D. = standard deviation and \(\bar{x}\) = mean value for each protein fraction in the entire series of 40 sera.
fractions. The coefficients of variation ranged from 2.6 for albumin to 15.7 for $\alpha_1$-globulin.

In order to measure the precision and accuracy of the electrophoresis system under routine operating conditions of the clinical chemistry laboratory, a commercial control serum (Versatol*) was reconstituted, and 0.1-ml. portions were sealed in capillary tubes and preserved at $-20^\circ$. One capillary tube was thawed for each run and applied to a single spot on a membrane. The results of the evaluation of 40 separate determinations done over a 15-week period are shown in Table 2. The respective standard deviations (as per cent of total protein) were 1.46, 0.46, 0.60, 0.67, and 0.76 for the albumin, $\alpha_1, \alpha_2, \beta,$ and $\gamma$-globulin fractions, respectively. The respective coefficients of variation were 2.4, 14.2, 6.0, 6.1, and 5.2%.

The reproducibility of the protein fractionation in the multiple routine measurement of the same serum was slightly superior to that of quadruplicate fractionations of numerous sera. The differences were not great and may be ascribed to variations in the position of the sera on the membrane. In the quadruplicate series, the sera were placed in random order on the membrane, while in the routine operation the control serum always was placed in the Number 1 position on the membrane. The precision of protein fractionation in both of the above series of experiments, however, is superior to that reported by Bartlett (5) for a different cellulose-acetate system and by Sunderman and Sunderman (11) for a system with paper as the supporting medium.

**Accuracy**

Moving-boundary (free) electrophoresis has been considered as a standard of reference for the validity of various types of protein fractionation. Data from moving-boundary electrophoretic analysis of the control serum were obtained from the manufacturer and appear in Table 2. The mean values obtained with cellulose-acetate electrophoresis in this study are also shown in Table 2. Agreement between the two methods is best for albumin and $\gamma$-globulin where values of 61.6% and 15.3%, respectively, obtained with moving-boundary electrophoresis correspond to 61.0% and 14.7% with cellulose-acetate electrophoresis. The greatest discrepancy occurs in the $\beta$-globulin fraction where a value of 11.1% was obtained by zone electrophoresis, and 7.3% by moving boundary. The reason for this difference is not known, but may be due to the difference in method of measuring the protein fractions—dye binding versus refraction of light. It is also likely that some denatura-

*General Diagnostics Division, Warner-Chilecott Co., Morris Plains, N. J.
Table 2. Reproducibility of the Electrophoretic Protein Fractionation of a Control Serum Performed 40 Times Over a 15-Week Period

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Mean value (%) of total protein</th>
<th>Standard deviation* (%) of total protein</th>
<th>Coefficient of variation</th>
<th>Moving boundary electrophoresis (%) of total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>61.0</td>
<td>1.46</td>
<td>2.4</td>
<td>61.6</td>
</tr>
<tr>
<td>α1-globulin</td>
<td>3.2</td>
<td>0.46</td>
<td>14.2</td>
<td>4.4</td>
</tr>
<tr>
<td>α2-globulin</td>
<td>10.0</td>
<td>0.60</td>
<td>6.0</td>
<td>11.4</td>
</tr>
<tr>
<td>β-globulin</td>
<td>11.1</td>
<td>0.67</td>
<td>6.1</td>
<td>7.3</td>
</tr>
<tr>
<td>γ-globulin</td>
<td>14.7</td>
<td>0.76</td>
<td>5.2</td>
<td>15.3</td>
</tr>
</tbody>
</table>

* \[ \frac{\sum \text{dev.}^2}{N-1} \]

†Values reported for Batch No. 0767122 by the manufacturer.

Table 3. Normal Values* of Serum Protein Fractions Obtained by Cellulose Acetate Electrophoresis (Specimens Obtained from 40 Healthy Nonprofessional Blood Donors)

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>% of total protein</th>
<th>Amount (gm./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>2 S.D. (95%) range</td>
</tr>
<tr>
<td>Total protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>59.6</td>
<td>52.2-67.0</td>
</tr>
<tr>
<td>α1-globulin</td>
<td>3.5</td>
<td>2.4-4.6</td>
</tr>
<tr>
<td>α2-globulin</td>
<td>10.1</td>
<td>6.6-13.6</td>
</tr>
<tr>
<td>β-globulin</td>
<td>11.9</td>
<td>9.1-14.7</td>
</tr>
<tr>
<td>γ-globulin</td>
<td>14.8</td>
<td>9.0-20.6</td>
</tr>
</tbody>
</table>

The values obtained by Dirstine et al. (6) who employed another type of cellulose acetate system with an elution method for evaluation of the protein fractions were higher for albumin and lower for α2-globulin than those reported here.

Normal Values

Sera of 40 healthy, nonprofessional blood donors were obtained from the blood bank. Total protein was measured and electrophoresis was carried out in quadruplicate by the method described above. The mean values and the 2-S.D. range for both per cent of protein and grams per 100 milliliters of serum are recorded in Table 3. The results agree with those reported by Grunbaum et al. (10) and by Brackenridge (4).
Addendum

The reproducibility of the electrophoretic system was tested further by carrying out 14 replicate determinations on each of 3 grossly abnormal sera. The latter were obtained from patients with nephrosis, multiple myeloma, and carcinomatous invasion of the liver, respectively. The respective mean values for the serum concentration of albumin, $\alpha_1$-, $\alpha_2$-, $\beta$- and $\gamma$ globulin, respectively, were 30.3, 6.5, 33.3, 23.8, and 6.1% for the nephrosis sample; 43.8, 4.2 10.0, 10.6, and 31.5% for the multiple myeloma sample; and 30.0, 9.1, 20.8, 11.7, and 28.3% for the carcinomatous liver sample. A satisfactory degree of reproducibility of protein fractionation was obtained with the abnormal sera. The coefficients of variation for the abnormal group were 3.3 for albumin, 8.5 for $\alpha_1$ globulin, 4.1 for $\alpha_2$ globulin, 8.1 for $\beta$ globulin, and 7.0 for $\gamma$ globulin—values that compare favorably with those shown in Tables 1 and 2 for normal sera.

References