Use of Tris (Hydroxymethyl) Aminomethane Buffer in Moving-boundary Electrophoresis of Serum

Julius Sendroy, Jr., F. Lee Rodkey, and Maxime MacKenzie

A tris-borate buffer solution of $\mu = 0.1$ and pH 8.9 is shown to be satisfactory for use in the electrophoresis of human and bovine sera by the moving-boundary technic. Comparison of the patterns obtained by the use of tris-borate and barbital buffer is presented. Similar numbers of electrophoretically distinct components are found by the use of either buffer. Although the calculated mobility of albumin is slightly lower in tris-borate than in barbital, mobilities of the other serum components are similar in the two buffers. Several practical advantages of the tris-borate over the barbital mixture make the former buffer more than satisfactory as a replacement of the latter.

Various studies of the effects of buffer solvent composition and pH on the electrophoretic separation of serum and plasma proteins have been made (1–6). From these, it appears that the choice from among a limited number of available buffers (glycine, acetate, phosphate, barbiturate, and borate), is determined empirically, depending on the component protein under study and the extent of separation desired. Thus, in earlier work, Longsworth (1) recommended the use of diethylbarbiturate (barbital) buffer of $\mu = 0.1$ and pH 8.6, for a separation of $\alpha_1$ globulin from albumin in human plasma, more adequate than that which could be achieved in phosphate buffer at pH 7.7. Moore (2) concurred and suggested that the choice of buffers for serum electro-

---

From the Division of Chemistry, U. S. Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.


The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Received for publication Dec. 7, 1962.
phoresis is also dependent in part on the species from which the sample is taken. Hoch (4), however, obtained no clinically significant differences in results with these two buffers at pH 8.6. More recently, tris (hydroxymethyl) aminomethane, (tris) has been used for measurements of electrophoretic mobility (7) and, in mixtures with other buffers, for paper (8) and moving-boundary (9) electrophoresis. The present study shows that a mixture of tris and tetraborate may advantageously be substituted for barbital as a buffer solvent (at pH 8.6–8.9).

Methods and Procedure

Serum separated at 5° from freshly drawn samples of defibrinated ox blood* or clotted normal human blood† was diluted with buffer solution to a protein concentration of 2.5%, then dialyzed for 15 hr. against 400 volumes of the same solution, at 5–7°. Final adjustment of the serum protein to 2% concentration, by the addition of the buffer dialysate, was controlled with a hand (Hitachi) refractometer.

Buffers were prepared from reagent grade chemicals (Mallinckrodt, except for tris, which was obtained from the Fisher Scientific Co.). A barbital buffer solution (μ = 0.1) used for comparison contained 0.0152M diethyl barbituric acid and 0.1M concentration of its sodium salt. Tris-borate buffer solution (μ = 0.1) contained 0.05M tris, 0.005M HCl, 0.01M sodium tetraborate, and 0.085M NaCl.† The pH of these solutions, measured by glass electrode at 25°, was 8.7 and 8.9 for the tris borate and barbital, respectively.§

Moving-boundary electrophoresis was performed at 2°, with a Per-
kin-Elmer, Model 38, instrument. Standard cells were used in the so-called open system, and boundaries were observed by schlieren-scanning technic. The electrophoretic separations were carried out with a constant potential gradient of 7.5 v/cm. for 105 min., unless otherwise indicated. For mobility calculations, the specific conductivity of the buffer dialysate was measured at 2°, and the distance of migration was obtained from the scanning diagrams (patterns). The measurement of the latter was made, before passage of current, from the starting point obtained upon making junction between the protein solution and the overlying buffer. In accordance with Longsworth and MacInnes (13), the patterns of the descending side were used for mobility calculations. The same patterns were used for the calculation of fractional composition from the areas under the curve, exclusive of the concentration anomaly. Areas were measured by the photoelectric procedure of Sendroy and Cecchini (14).

Results

Bovine serum was subjected to electrophoresis in several buffer systems, some of which contained Tris (Table 1). The results, illustrated in Fig. 1, showed marked dissimilarity of the ascending and descending patterns observed when the tris buffer system was present alone (A), with ethylenediaminetetraacetic acid (EDTA) (B), or with boric acid (C). Use of the latter buffer mixture with EDTA, as described by Aronsson and Grönwall (8) for paper electrophoresis, resulted in extremely asymmetric patterns with an increased number of inadequately separated components (D). More satisfactory separations were

Table 1. Composition of samples for which electrophoretic patterns are shown in Fig. 1.

<table>
<thead>
<tr>
<th>Concentration*</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D (%)</th>
<th>E (%)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris (M)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.5</td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Borate (M)</td>
<td></td>
<td></td>
<td>0.01</td>
<td>0.075</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>EDTA (M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>NaCl (M)</td>
<td>0.09</td>
<td>0.03</td>
<td>0.10</td>
<td></td>
<td>0.088</td>
<td></td>
</tr>
<tr>
<td>Barbital (M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>pH (25°)</td>
<td>8.67</td>
<td>8.77</td>
<td>9.05</td>
<td>8.86</td>
<td>8.51</td>
<td>8.6</td>
</tr>
<tr>
<td>μ</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>(0.1)</td>
<td>0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>Protein, %</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.2</td>
<td>1.2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*Electrolyte or total buffer content.
obtained with the borate buffer (E) used by Brandt et al. (6) and with the tris-barbital buffer (F) of Rapp and Memminger (9). The patterns in Fig. 1 were all obtained at comparable pH (8.6–8.9), ionic strength (μ = 0.07–0.1), and total protein concentration (1-2%). Simi-

Fig. 1. Electrophoretic patterns of bovine serum in various buffers containing tris and/or borate (composition given in Table 1). Starting points of the ascending (left) and descending (right) patterns are indicated by the vertical line at the end of the arrows.

lar asymmetric patterns were obtained with other buffers, of tris alone or combined with various concentrations of EDTA or boric acid, of which the pH, ionic strength, or protein concentration was altered.

More satisfactory diagrams were observed in a tris buffer containing sodium tetraborate. The most nearly enantiographic ascending and descending patterns were obtained with 0.05M tris buffer of pH 8.9 containing 0.01M sodium tetraborate and sufficient NaCl to increase
the ionic strength to 0.1. The patterns observed when this buffer was used in the electrophoresis of bovine and human sera are compared in Fig. 2 with those obtained for identical serum samples analyzed in barbital buffer. The degree of separation and the number of compo-

![Fig. 2. Electrophoretic patterns of bovine (Column A) and human (Column B) sera after electrophoresis in barbital (top row) and tris-borate (bottom row) solutions. Composition of solutions and other conditions described under Methods and Procedure.](image)

nents observable is similar in both buffer systems. Although the separation of the α₁ component from the albumin of both species is better in barbital buffer, the "beta spike," of the descending pattern of human serum is much less noticeable in the tris-borate system.

The calculated mobilities of the various components and the fractional composition of bovine serum are given in Table 2. The number of components was arbitrarily taken as given by Hogness et al. (3) for bovine serum. Calculated mobilities of the several components were slightly less when tris-borate buffer was used but the fractional composition calculated from the two data was nearly the same.
Table 2. Electrophoretic Separation of Bovine Serum in Buffers with $\mu = 0.1^*$

<table>
<thead>
<tr>
<th>Component</th>
<th>Mobility (cm$^2 \times 10^8$ V$^{-2}$sec$^{-1}$)</th>
<th>Composition (%)</th>
<th>Mobility (cm$^2 \times 10^8$ V$^{-2}$sec$^{-1}$)</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma_1$</td>
<td>1.7</td>
<td>7.3</td>
<td>1.2</td>
<td>5.5</td>
</tr>
<tr>
<td>$\gamma_2$</td>
<td>2.2</td>
<td>14.7</td>
<td>1.6</td>
<td>16.7</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>3.0</td>
<td>11.9</td>
<td>2.6</td>
<td>10.3</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>1.5</td>
<td>1.5</td>
<td>3.5</td>
<td>4.3</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>4.7</td>
<td>10.6</td>
<td>4.3</td>
<td>12.4</td>
</tr>
<tr>
<td>$\alpha_1$</td>
<td>5.7</td>
<td>4.5</td>
<td>5.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Albumin</td>
<td>6.8</td>
<td>49.5</td>
<td>6.0</td>
<td>44.9</td>
</tr>
</tbody>
</table>

*Fractional composition and mobilities calculated from the descending patterns with boundary anomalies excluded.

Table 3. Electrophoretic Separation of Human Serum in Buffers with $\mu = 0.10^*$

<table>
<thead>
<tr>
<th>Component</th>
<th>Mobility (cm$^2 \times 10^8$ V$^{-2}$sec$^{-1}$)</th>
<th>Composition (%)</th>
<th>Mobility (cm$^2 \times 10^8$ V$^{-2}$sec$^{-1}$)</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$e$</td>
<td>$-0.5$</td>
<td>6.1</td>
<td>1.0</td>
<td>7.8</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>1.2</td>
<td>13.7</td>
<td>2.8</td>
<td>15.1</td>
</tr>
<tr>
<td>$\beta$</td>
<td>2.7</td>
<td>10.4</td>
<td>3.9</td>
<td>11.6</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>3.8</td>
<td>6.8</td>
<td>5.2</td>
<td>4.4</td>
</tr>
<tr>
<td>$\alpha_1$</td>
<td>5.1</td>
<td>63.0</td>
<td>5.8</td>
<td>61.1</td>
</tr>
</tbody>
</table>

*Fractional composition and mobilities calculated from the descending patterns.

Similar mobility and composition measurements for human serum are recorded in Table 3. In both buffer systems, each component had a mobility 5–10% higher in the ascending limb than in the descending, but the greatest difference lay in the values for albumin. Owing to the somewhat arbitrary element involved in the calculation of fractional composition, no consistent difference was evident in concentrations calculated from the ascending and descending patterns. Furthermore, the values obtained by the use of tris-borate were similar in most cases to those obtained with barbital buffer.

Discussion

An ideal buffer for use in electrophoresis of a protein mixture such as serum does not alter the protein-protein interactions within the mixture or change the charge on the protein molecules through interaction
with buffer anions or cations. It is doubtful that a single buffer system exists which completely fulfills these criteria for all sera. Indeed, any electrophoretic separation of a mixture of proteins will itself tend to change the magnitude of all interactions between various protein molecules and buffer ions which depend on electrostatic conditions.

Serum albumin reacts with many anions commonly used in buffer solutions, including chloride, acetate, and barbital (15, 16), but other serum proteins are less reactive toward anions. To the extent that such reactions occur, the amount and kind of buffer anion will affect the mobilities of the component serum proteins. Thus, the higher mobility of the serum albumin in barbital than in tris-buffer, may be partially attributable to the binding of barbital anion by the albumin.

Detailed comparison of the patterns obtained from both human and bovine serum by use of barbital and tris-borate buffer shows that, for any given serum sample, the same number of components are observed in both buffers. The $\alpha_1$ component is more completely separated from albumin for both species when the former is used, owing to the slightly greater mobility of albumin in this buffer. On the other hand, the $\delta$ and $\epsilon$ anomalies are not only less pronounced when tris-borate is used, but they also have less tendency to migrate from the starting points.

Several species differences are evident in the patterns of Fig. 2. One of the major effects observed when barbital is used is the presence in the $\beta$ region of the descending pattern for human serum, of a marked "spike" which is absent from that for bovine serum. There is also a better separation of the $\alpha_1$ component from albumin in the case of human serum. These differences are likewise present to some degree when tris-borate buffer is used.

Although Rapp and Memminger (9) have rejected the use of tris alone as being unsatisfactory for clinical work, they have combined it in mixtures with barbiturate, which has shortcomings of its own. Several practical advantages of the tris-borate over a barbital mixture are the relative ease of preparation, its inert nature as compared with the pharmacologically potent barbital, and the fact that the cost of the latter buffer is 6–7 times that of the tris-borate solvent.

References


Addendum

An o-Toluidine Method for Body-Fluid Glucose Determination, by Kurt M. Dubowski

Since publication of the above paper, we have found that a procedure similar to the one described for determination of aldosaccharides in body fluids using o-toluidine in glacial acetic acid solution was described by Eric Hultman in Nature 183, 108 (1959). A modification of Hultman’s procedure has recently been described by A. Hyvärinen and E. A. Nikkilä in Clin. Chim. Acta 7, 140 (1962).

The omission of reference to Dr. Hultman’s paper was entirely unintentional. Our own procedure was, of course, derived entirely experimentally, based upon Dr. O. M. Forsell's suggestion to us of Mar. 13, 1960, that we should consider the use of o-toluidine as a glucose reagent; and this suggestion is properly credited in the paper as a personal communication. The papers by Hultman and by Hyvärinen and Nikkilä nicely confirm some of the significant experimental details and results of our procedure; but obviously the Hultman paper has priority with respect to this principle.