Technical Papers

Improved Hanging-Strip Paper-Electrophoresis Technique

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The hanging-strip paper-electrophoresis technique for protein separation (*1*) is coming into wide use, particularly for clinical purposes (*2*). In this technique, the rack supporting the strip is removed from the cell at the end of an experiment and placed in an oven to dry and fix the protein pattern on the strips before they are dyed. It has been recognized for some time that distortions of the patterns occur during this drying stage. Attempts have been made to avoid this difficulty by placing the strips immediately in a dye-coagulant solution without the drying step (*3*). This expedient has the drawback that the proteins may not be thoroughly "denatured," which may lead to variations in dye uptake (*4*, Chap. 18). For separation involving substances that cannot be chemically coagulated or precipitated *in situ,* some drying procedure usually must be employed.

The degree of pattern distortion due to liquid redistribution incident to drying was conveniently visualized by the following procedure (Fig. 1, top). Reference circles were marked with a pencil on dry strips; the paper was saturated with buffer and then subjected to the electrophoresis procedure. Immediately prior to the drying step, spots of dye solution (acid fuchsin) were rapidly applied from a pen point at the reference marks. The strip was lifted from the buffer and was oven dried while it was still apically supported on the rack. The movement of the dye spots away from the reference marks then gave an indication of the magnitude of fluid redistribution and incidental pattern distortion. Similar experiments with serum proteins followed by dyeing showed distortions of like magnitude.

Pattern distortion can be substantially eliminated Fig. 1, bottom) if the following conditions, which are listed in their probable order of importance, are fulfilled. (i) Excess liquid at the dependent ends of the strips must be removed as soon as the vapor-saturated enclosure is opened. (ii) The application of heat to all areas of the paper strip should be uniform. (iii) The strip should not be dried in contact with any support within or near the region of the pattern. (iv) Drying should be carried out at a rapid rate to arrest fluid movement as quickly as possible.

When a rack is lifted from a cell, a drop of electrolyte is dependent from the ends of the strips (Fig. 2*B*). The drop of fluid acts as a reservoir continuously feeding electrolyte upward toward the apex of the strip in a manner analogous to ascending chromatography. This effect varies in magnitude along the length of the strip, being greatest near the ends and zero at the apex (Fig. 1, top). As a consequence, distortion of the protein pattern results with loss of resolution; and, further, observed mobilities are less than actual mobilities (*4*, Chap. 15).

An obvious solution to this difficulty would be to blot the ends of the strips prior to placing them in the oven. We tested a strip rack that carried a plastic support bar, against which the strip ends could be blotted after the rack was removed from the cell. It was found to be difficult to blot each strip end in a reproducible manner, and that downward displacement into excessively blotted ends sometimes occurred.

Another solution to this difficulty is to cut off the dependent ends of the strips prior to placing them in the oven. It has been found, however, that by employing a heavy filter-paper wick between the strip ends and the electrolyte reservoir, the desired effect is produced in a more convenient and reproducible manner (Fig. 2 *C, D*) as the strips are removed from the cell.

A support rod touching the strip during drying (*5*) produces a characteristic artifact presumably due to local shading and alteration of the drying rate owing to the heat capacity of the support. The strip should therefore not be dried in contact with any support in or near the pattern area.

The cell design illustrated in Fig. 2 *A* permits drying to be carried out without pattern distortion and preserves the advantages of the hanging-strip procedure, which utilizes gravity to promote the attainment of reproducible capillary equilibrium. The paper strips are removed at the end of the experiment on the detachable folding rack, which pulls them horizontal and almost taut, the pattern area of the strip hanging free from contact with the central support.

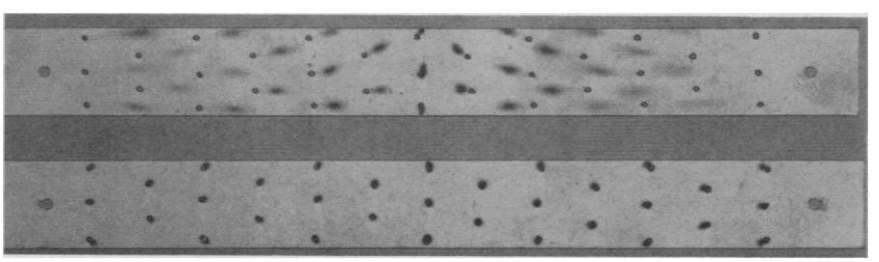

Fig. 1. Pattern distortion incidental to drying. (Top) Strip was dried over apical rod without removal of dependent drop (Fig. 2*C*). (Bottom) Strip was dried on the rack shown in Fig. 2 after removal from intermediate wick, without formation of dependent drop.

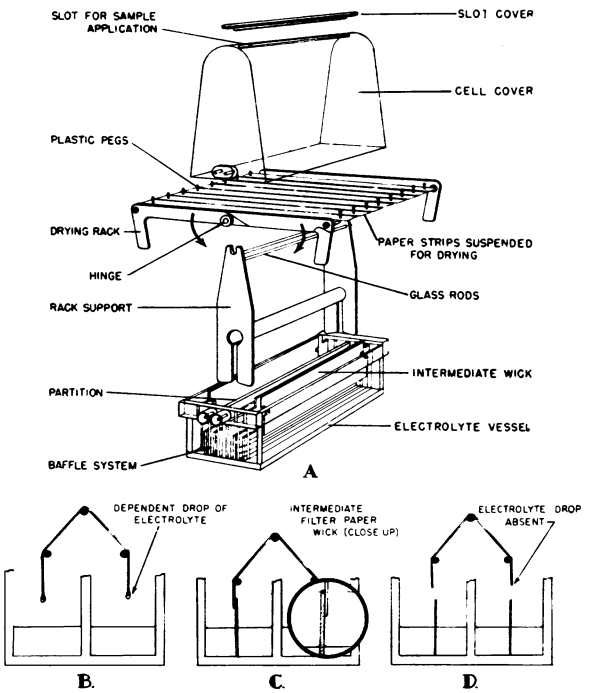

Fig. 2. Hanging-strip paper-electrophoresis cell and rack. (*A*) Cell with parts separated for visualization. (*B*) Formation of dependent drop of electrolyte. (*C, D*) Elimination of drop by intermediate wick.

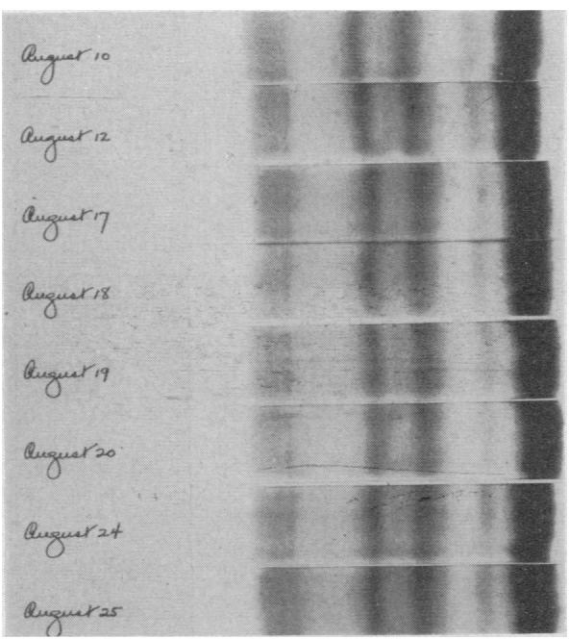

Fig. 3. Reproducibility study. Strips from eight different experiments, performed on the dates indicated, with the cell shown in Fig. 2. The experimental conditions were Whatman No. 3MM filter paper, eight strips 29 by 300 mm, 0.01 ml of serum applied at the apex, barbital buffer pH 8.6, $\mu = 0.075$, 16-hr duration, constant current of 8 ma (for eight strips), and stained with bromphenol blue (*4, 6*).

Under these conditions the strips dry with practically no shift of the resolved zones. This procedure also prevents the formation of apical creases, which are difficult to avoid when any of the heavier papers (for example, Whatman No. 3MM) are dried over an apical rod. Since these creases are reproduced as spikes by most photoelectric scanners, they may obscure the "application artifact" which is caused by the presence of particulate material, traces of fibrinogen, and other substances in the serum that are precipitated or otherwise bound at the origin. The application artifact probably has clinical significance.

Figure 3 illustrates the reproducibility and resolution obtainable with apparatus of this design when Whatman No. 3MM strips, 29 by 300 mm were used. The same serum was studied during eight different experiments over the course of 2 wk.

References and Notes

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5. If support rods are small in diameter and are treated with a silicone antiwetting agent, no meniscus forms at the point of contact between the support rod and the paper during electrophoresis, and no distortion of field or other artifact is detectable.
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18 February 1955

Use of Narrow Holes for Producing Glow-to-Arc Transitions

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When, in experimental apparatus designed to confine the cathode spot on the surface of a mercury pool to a small area (*1*), a pinhole is left in a funnel-shaped glass member joined to the envelope in a ring-seal (*2*), a glow-to-arc transition usually results in an arc passing through the pinhole. These observations are presented as unmistakable evidence that a concentration of current in the positive column of a glow discharge is a condition that favors the establishment of a cathode spot and the resulting transition to the arc. If the hole is in the millimeter-size range, the arc takes the path through it in the majority of cases over a considerable range of gas pressures. In a glow discharge, the current density through a hole of fixed size can be increased by adding appropriately placed conducting extensions to the cathode,