Zone Electrophoresis in a Starch Supporting Medium. (19516)

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Recent investigations employing filter paper have demonstrated the value of electrophoresis in a supporting medium(1,2). The complete separation of components, rather than the partial separation occurring in the unpacked channel of the Tiselius cell, makes this method useful for preparative purposes. However, certain limitations of the filter paper technic have become apparent. First, the capacity is low making it difficult to isolate substantial amounts of material. Second, adsorption and consequent trailing has been noted with certain substances, particularly the basic proteins and peptides. Third, elution of material from the paper may be difficult and low recoveries are often obtained. As a result, a search was made for other types of supporting media that might be free of these disadvantages. Certain of these had previously been used for similar purposes(3,4).

Among the materials investigated were glass beads, glass powders, special sands, gels, acid and basic resins and starch. Some separation of applied protein could be achieved when each of these materials, mixed with buffer, was molded into a block and placed in an electric field. The block was made just dry enough to preserve its form and was encased in wax paper. A very large electroosmotic flow of water to the cathode was noted in most cases and represented a disadvantage. Albumin, colored yellow by excess bilirubin bound to it, and hemoglobin, were used to test the separation and the electroosmotic flow. No separation of these 2 proteins was obtained by simple water flow through the medium without an electric current. The electroosmotic flow could be calculated for each supporting medium from the relative position of each of the two colored proteins to the origin and to each other(2).

The approximate relative mobility of electroosmotic flow could also be calculated from the observed mobilities of these 2 proteins in free solution, 6.5 in the case of albumin and 3.4 in the case of hemoglobin (Table I). In some instances the flow of liquid to the cathode was almost as great as the migration of albumin to the anode, resulting in an almost stationary position for this protein. Adsorption was tested with a solution of lysozyme and β_1 lipoprotein which had been found to adsorb to filter paper leaving a trail. In general, considerable adsorption of these substances was observed with those materials giving a high electroosmotic flow. This could be observed by simply shifting the protein by passing buffer through the block with a siphon-like arrangement without an electric current, or from the trailing in an electric field. Fig. 1 shows the absence of adsorption of lysozyme on starch as compared with Whatman 3 MM filter paper. On filter paper this preparation of crystalline lysozyme gave such trailing that it was impossible to determine the electrophoretic homogeneity. No significant adsorption was observed with the starch for any of a large group of proteins and peptides that were tested. Because of this finding, and in view of the relatively low electroosmotic flow, it was employed as the main supporting medium for subsequent studies.

TABLE I. Electroosmotic Flow for Various Media Expressed in Terms of Mobility (μ_{el}) and in Relation to the Observed Distance of Migration of Albumin. (Barbital buffer pH 8.6, μ .1).

Medium	$\mu_{el} \times 10^5$	$\frac{d_{el}}{d_{alb}}$
Filter paper (Whatman 3 mm)	1.5	.30
Potato starch (Amend)	2.5	.62
Washed sea sand	4.3	1.95
Ground glass (Corning 35-60 mesh)	5.1	3.64
" " (" 150-200 ")	5.9	9.87
Soft glass beads (200 mesh)	5.6	6.20
Agar (1%)	4.7	2.60

Fig. 2 illustrates a barely moist block of starch* encased in wax paper between 2 glass plates resting on the lips of electrode vessels. Contact of the starch block with the buffer solution was obtained with plastic sponges or moist cloth. After the starch block had been

* Amend potato starch.

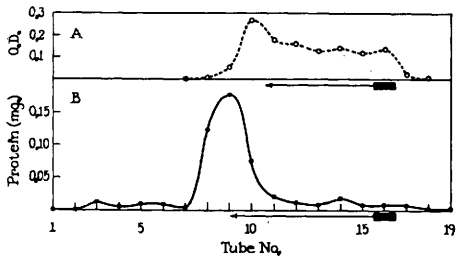

FIG. 1. Curves obtained for lysozyme employing electrophoresis with filter paper (A) and starch (B) in phosphate buffer at pH 7.6, μ .1. Protein in A was determined by the bromphenol blue elution method on paper segments, in B by the modified Folin procedure.

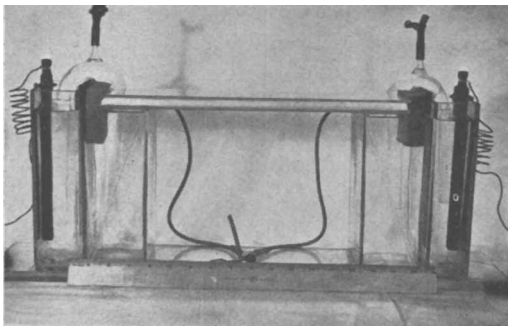

FIG. 2. Photograph of electrophoresis apparatus with a starch block between glass plates resting on the lips of electrode vessels. Contact with the buffer vessels was made through plastic sponges.

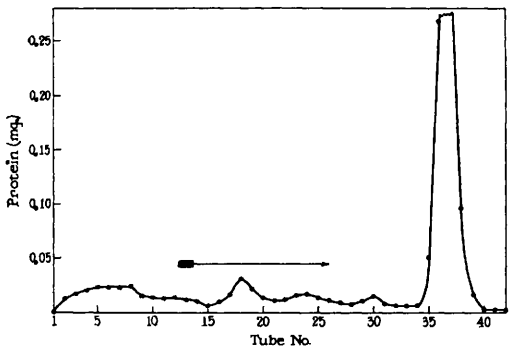

FIG. 3. Curve obtained for a normal serum separated in the starch medium with analyses for protein on the saline extract of the starch segments by the modified Folin method. The arrow and block indicate the origin and direction of migration. Barbital buffer pH 8.6, μ .1.

formed by pouring the starch solution into a mold lined with wax paper, permitted to settle and partially dried with heavy filter paper, the protein was applied either directly with a pipette into a narrow slit in the starch block or by removing a rectangular segment of starch and replacing it with a barely moist mixture of protein and starch. Although various sizes of the starch block were employed, a 38 x 10 x 1.5 cm size was used most frequently. Following the conclusion of an experiment (usually 24 hours at 400 volts at low temperatures) the block was dried slightly, cut into 1 cm segments, shaken with saline and aliquots taken after the starch had settled. Good recoveries could be obtained by displacement filtration of the starch on a sintered glass funnel under suction.

In most experiments employing the starch block, 1-5 cc of material were applied to the origin. Fig. 3 illustrates the pattern obtained for 1 cc of normal serum separated in a 50 cm block. An aliquot representing 4% of the material from each starch segment was used for protein analysis by the modified Folin tyrosine method(2). This was found to have some advantage over the ninhydrin method although both were used. The main components observed by classical free electrophoresis can be seen. The heterogeneity of the gamma globulin is quite apparent. Since only 4% of the material of each tube was used for the protein curve, the remainder was available for other analyses. The best patterns for serum were obtained with volumes below 2.5 cc. Larger volumes presented more difficulties but some separation could be obtained. The α_1 peak often was not visible when more than 4 cc of serum were used. Curvatures in the bands represented a limiting factor of the technic and became more pronounced with larger volumes of serum. This could be seen from the natural pigments of serum, particularly the yellow albumin, when a light was placed under the starch block. Three pigment bands were usually observed for normal human serum. The best results were obtained with serum diluted to $\frac{2}{3}$ concentration or less with buffer.

The major application of the zone electrophoresis method in the present study was to the problem of the lipoproteins of serum. The α and β lipoproteins were readily separated in the starch medium and could be followed by cholesterol and phospholipid analyses on the starch segments. Fig. 4 illustrates the cholesterol and phospholipid curves obtained

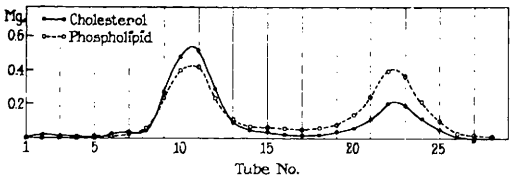

FIG. 4. Curves obtained by cholesterol and phospholipid analyses of starch segments for a normal serum. The two peaks represent the α (tube 22) and β (tube 11) lipoproteins with different cholesterol-phospholipid ratios. Barbital buffer pH 8.6, μ .1. Origin in tube 8.

for an essentially normal serum at pH 8.6, μ 0.1. The high cholesterol-phospholipid ratio for the β-lipoprotein and the lower ratio for the α-lipoprotein are apparent. A further subdivision of these lipoproteins was obtained at lower pH and ionic strength. Trailing of the β-lipoproteins was obtained with various types of filter paper and glass powders, but was not observed with the starch. The details of the lipoprotein studies have been reported separately(5).

Discussion. Certain of the other supporting media that were tested possessed specific advantages over the starch. For example, agar gave a very low supporting medium—liquid ratio, an important factor in increasing the capacity of the system. The starch system usually gave a ratio of approximately 1, with the starch occupying one-half of the total volume. However, difficulties with the agar system, particularly in isolating components at reduced temperatures, overcame this advantage. The filter paper system possessed the advantage of the extreme sensitivity and simplicity of the protein staining technic with bromphenol blue. No success was achieved in adapting this to the starch. A further disadvantage of the starch was that certain impurities sometimes interfered with specific analyses on the segments, although some of these, such as the ninhydrin positive materials, could be washed off. Starch did not represent the ideal supporting medium but because of the homogeneous packing achievable, the lack of adsorption, good recoveries and the relatively low electroosmotic flow, it proved useful for many purposes. Further advantages have been derived with the starch system in narrow vertical columns employing a liquid displacement method rather than the segmentation technic.

Summary. 1. A procedure was described for the electrophoretic separation and isolation of materials employing various types of supporting media. 2. Starch proved particularly useful because of its low adsorption of proteins and peptides in aqueous buffers. 3. A comparison was made of the electroosmotic flow in various media under similar conditions. 4. The separation of 1-4 cc of serum with isolation of the components could be carried out employing the starch system. 5. The α- and β-lipoproteins of serum could be determined quantitatively by phospholipid and cholesterol analyses of the serum fractions.

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1. Durrum, E. L., *J. Am. Chem. Soc.*, 1950, v72, 2943.

2. Kunkel, H. G., and Tiselius, A., *J. Gen. Physiol.*, 1951, v35, 89.

3. Gordon, A. H., Keil, B., Sebesta, K., Knessl, O., and Sorm, F., *Coll. Czechoslovak. Chem. Comm.*, 1950, v15, 1.

4. Svensson, H., and Brattsten, I., *Arkiv. Kemi*, 1950, v1, 401.

5. Kunkel, H. G., and Slater, R. J., *J. Clin. Invest.*, in press.

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