Electro-osmosis on glass fiber filter paper

In an attempt to find a stabilizing medium other than cellulose paper for the separation of serum glycoproteins by ionography, glass fiber filter paper was employed. It was observed that the order of magnitude of electroosmosis was much greater on this glass paper than that which has been observed on cellulose paper[1]. A few experiments were set up to determine the extent of the electroosmosis.

The Precision Ionograph* was used for all separations. All runs were made using a veronal buffer at pH 8.6, ionic strength 0.05, a temperature of 4° C and a potential gradient of five volts per centimeter. The current carried by the glass paper was observed to be twice that carried by the cellulose paper under otherwise identical experimental conditions. The cellulose paper used was Whatman No. 1 (0.5 inch width) and the glass fiber paper was No. X-934-AH (made by Hurlbut Paper Co. and distributed by H. Reeve Angel and Co., Inc. New York 7, N.Y.). The latter sheets, 12 × 15 inches in size, were cut into strips 0.5 × 15 inches. In the first set of experiments (1 in Fig. 1) 5 μl of a 0.01 % solution of bromphenol blue was used as the migrant.

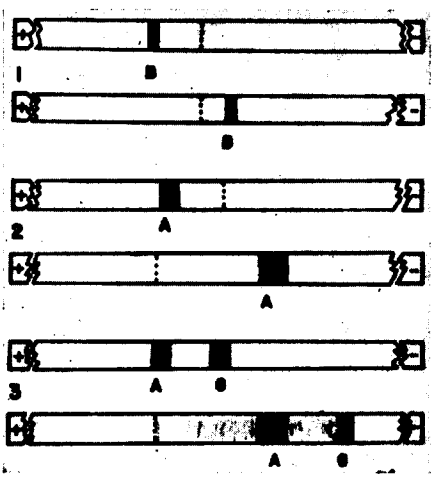

Fig. 1. A = bovine plasma albumin; B = bromphenol blue; G = bovine plasma γ-globulin; vertical broken line = point of application of migrant to strips. The second strip in each set is the glass filter paper. In the glass strip in 3, the lightly shaded portion indicates distribution of globulin all the way from the origin.

In all cases the migrant was added as a thin streak across the full width of the strips midway between the ends. Six strips were run simultaneously, three of cellulose paper and three of glass. The mobility of the bromphenol blue was + 0.77 microns/second/volt/cm (the + sign indicating migration to the positive electrode) on the cellulose paper, and —0.44 on the glass paper. In the next experiment, (2 in Fig. 1) five μl of a 1 % solution of crystalline bovine plasma albumin was applied to each of the six strips. The mobility of the bovine plasma albumin was + 0.26 on the cellulose paper and —0.64 on the glass paper. The location of the protein bands in each case was determined by use of the bromphenol blue technique[1].

An attempt was then made to separate a mixture on glass paper. The migrant in the next series of experiments (3 in Fig. 1) was a solution containing 1 % crystalline bovine plasma albumin and 1 % crystalline bovine plasma γ-globulin (Fraction II). In all cases the protein samples were obtained from the Research Division, Armour and Company, Chicago. The mobilities of the bovine plasma albumin and bovine plasma γ-globulin on cellulose paper were + 0.26 and + 0.02 respectively. On glass paper the mobilities were —0.64 and —0.91 respectively. The mobility of dextran, which has often been used as a electroosmotic indicator in ionographic research, was found to be —14. The location of the dextran zone on the glass filter paper strip was determined by immersing the strip in warm concentrated sulfuric acid which resulted in a brown charred zone. Since the glass paper, as contrasted with cellulose paper, does not yield a colored background with carbohydrate-detecting agents, the latitude of choice of developing agent is a wide one. Upon scanning the stained ionograms with an automatic scanning device[1], used to determine the amount of material in a given band, it was found that the glass paper absorbs almost three times as much light as Whatman No. 1 paper (1.4 optical density units and 0.93 optical density units respectively).

The bromphenol blue gave as sharp a band on the glass paper as it did on the cellulose paper, but the albumin band was slightly more diffuse on the glass paper than on the cellulose paper. The γ-globulin patterns were quite diffuse on the glass paper strips, with the material being spread out in the fashion of a continuous spectrum all the way from the point of origin. If on further examination the different portions of the broad zone on the ionograms are found to represent real differences in the character of the γ-globulin, this electrosmotic fractionation may prove to be of value in the study of globulin heterogeneity. At first it might appear that substances

* Precision Scientific Company, Chicago 47, Illinois.

with a lower net negative charge yield diffuse zones on the ionograms, but dextran, which exhibits only a very low electrophoretic mobility in free solution, gave a sharp band.

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[1] H. J. MCDONALD, R. J. LAPPE, E. P. MARBACH, R. H. SPITZER AND M. C. URBIN, *Ionography; Electrophoresis in Stabilized Media*, Year Book Publishers, Chicago, Ill., 1955.

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Elution apparatus for quantitative paper chromatography

In the course of recent studies on plasma corticosteroids it was necessary quantitatively to elute large numbers of paper strips 7 × 1.5 cm in a minimal volume of solvent. The apparatus illustrated greatly facilitated the procedure.

In place of micropipettes, 1.5 mm bore glass capillary tubing was used to collect the eluate, one end being bent up at a right angle and flared to receive the end of the paper; each tube was calibrated at 0.1 and 0.2 ml.

The tank and inner trough are constructed in stainless steel*. The trough is mounted on a screw at each end, by means of which its height is adjusted to accommodate strips of different lengths. The slope of the tubes may be varied; they are attached by clips to short sections of steel tubing mounted on a 5 mm rod, and these may be rotated on the rod by slackening a fixing screw.

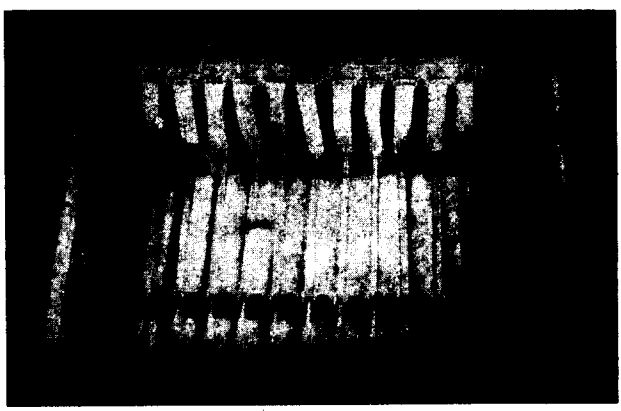

Fig. 1.

Papers are held between a single thin glass sheet below and small rectangles of 7 mm glass plate above. The tank is covered by a glass lid which rests on a sponge-rubber rim.

The bottom of the tank is covered with the eluting solvent, and after inserting the papers the trough is half filled with solvent; it is unnecessary to await equilibration.

Using this procedure 2 μg cortisol was eluted into 0.05 ml absolute ethanol without detectable loss in ten recovery experiments.

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* The stainless steel apparatus was constructed by the Marks Engineering Works Association, Capetown.