Notes

Effect of buffer equilibration on paper electrophoresis

In paper chromatography, equilibration of the paper with the chamber atmosphere and solvents is an important variable (HEFTMANN[1]). The importance of this variable in paper electrophoresis was emphasized when different buffer systems were employed.

Methods

In the experiments to be described, paper electrophoresis was carried out in a Spinco Model R-paper electrophoresis cell, powered by a Duostat, Model RD-2, essentially as described by BIER[2].

Two common buffer systems were tested in this study: (a) The first buffer system was composed of diethylbarbituric acid (0.075 ionic strength) at pH = 8.6. (b) A second buffer according to ARRONSON AND GRONWALL[3], which has a pH of 8.9 was composed of tris-hydroxymethylaminomethane (TRIS), 60.5 g/l (0.5 mole); ethylenediaminetetraacetic acid (EDTA), 6.0 g/l (0.021 mole); and boric acid 4.6 g/l (0.075 mole).

Equilibration of the paper strips was achieved either by pouring the buffer directly over the paper or by capillary action. Saturation of the strips by capillary action was examined at two time intervals. We arbitrarily chose 60 minutes as the minimum time interval and 72 hours for the maximum saturation time interval.

After electrophoretic separation was achieved, the strips were removed from the cell, air dried, and the proteins fixed and stained in the usual manner with Lissamine Green[4].

Results

A summary of the electrophoretic procedures may be seen in Fig. 1. In both the veronal and TRIS buffer systems, we have observed superior excursion and separation of serum proteins on those strips which were saturated by pouring the buffer solution directly on the paper strips before sample application and electrophoresis. Equilibration by capillary action for 1 and 72 hours, tended to distort the migration of the plasma proteins to a much greater extent with the TRIS–borate–EDTA buffer system than with the veronal buffer system.

Discussion

Of the variables influencing the electrophoretic mobility of proteins on paper, the equilibration of the paper strips before sample application is most important. Little attention has been given to this factor in various books (BIER[2] and SMITH[4]) on electrophoresis and by various laboratory technicians (personal communications). A marked difference for the TRIS–borate–EDTA buffer was noticed between equilibration obtained by pouring this buffer over the paper strips and where equilibration was accomplished by capillary action. The chromatographic effects due to saturation by

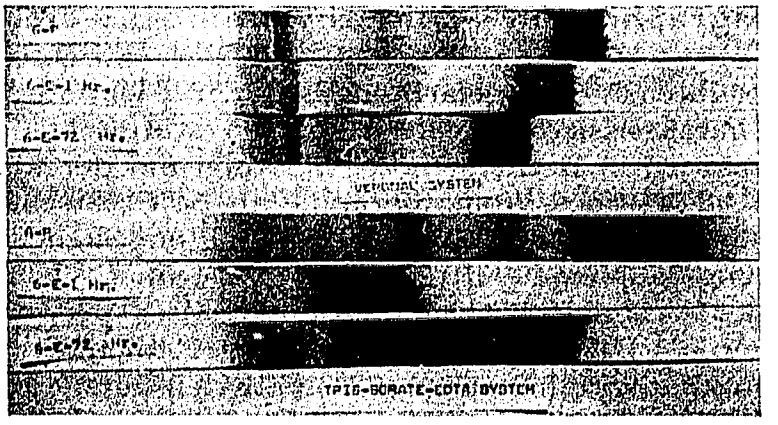

Fig. 1. Comparison was made between two buffer systems in regard to paper saturation before the application of human serum. Papers were saturated by pouring (6-P) and after 1 (6-E-1 h) and 72 hours (6-E-72h) of equilibration. The number 6 refers to the position of the strip in the Spinco Model R cell.

capillary action gave rise to changes in salt concentration in certain parts of the paper. Since conductivity is proportional to buffer concentration, the electrical field will differ along the length of paper. The distortion noted in the present experiments probably was due to these chromatographic effects. The effect differs for the different buffers, being more noticeable with the TRIS–borate–EDTA system (Fig. 1).

Therefore, it is recommended that the procedure of pouring buffer over the strips to attain equilibrium be rigidly followed before sample application. Where these conditions are not scrupulously followed, equilibration by capillary action requires more time for certain buffers and results in poor electrophoretic separations.

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