adsorption of the solute on a cellulose phase[1] when the mobile solvent phase is absent.

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*Zusammenfassung*

Bei der papierchromatographischen Prüfung bestimmter reiner Stoffe wurde die Bildung mehrerer Flecken beobachtet. Derartige «ghost»-Flecken entstehen mit gewissen herzaktiven Glykosiden und auch mit dem Antibiotikum Monamycin.

[1] A. J. P. MARTIN, Ann. Rev. Biochem. *19*, 517 (1950).

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## Paper Electrophoretic Separation of Tuberculin Constituents

In the course of our investigations into the immune response of the host to tuberculous infection and anti-tuberculosis vaccination, the problem arose as to how to determine the purity of the isolated antigenic fractions by a quick method and on a microscale. The recent successful application of zone electrophoresis on paper for the separation of serum proteins, enzymes, etc.[1] induced us to investigate this method for our purposes. Some of the results are presented in this preliminary note.

Two types of apparatus were used–one similar to that described by KUNKEL and TISELIUS[2] in which the paper is placed horizontally between two glass plates, and the other similar to the one used by GRASSMANN and HANNIG[3] but with room for 6–8 horizontally placed paper strips (35 × 4 cm).

Of the different buffers used, phosphate buffer pH 8·3, ionic strength $\mu = 0{\cdot}1$ and veronal/veronal sodium buffer pH 8·6, ionic strength $\mu = 0{\cdot}11$, gave the best results, the latter yielding better separations in the GRASSMANN and HANNIG apparatus. Citrate buffers of pH 5·5 and 6·3 afforded no good separations, and in addition some of the proteins tended to precipitate out from their solutions in these buffers, whereas 2–10% solutions of most fractions could easily be dissolved in the alkaline buffers. Buffers of much higher pH values have not been tried since denaturation of tuberculo-proteins occurs under such conditions. A thick filter paper (Munktell 20/150) was used.

The experiments were run for 19 h at 200 V and 14–24 mA at + 4°C, applying 10–20 $\mu$l of 2–10% solutions of the antigens. The proteins were identified by the bromphenol blue method according to DURRUM[4], and for quantitative measurement eluted with 0·01 *n* NaOH and measured in the BECKMAN spectrophotometer at 595 m$\mu$. Crystalline bovine albumin was chosen as standard substance. In a parallel run the polysaccharides were directly identified on paper after periodate oxidation with fuchsin sulphite[5], or eluted with water and determined with anthrone according to MORRIS[6].

[1] For a review, see A. TISELIUS and P. FLODIN, Adv. Protein Chem. *8*, 461 (1953).

[2] H. G. KUNKEL and A. TISELIUS, J. Gen. Physiol. *35*, 89 (1951).

[3] W. GRASSMANN and K. HANNIG, Hoppe-Seyler's Z. Physiol. Chem. *290*, 1 (1952).

[4] E. L. DURRUM, J. Amer. Chem. Soc. *72*, 2943 (1950).

[5] E. KÖIW and A. GRÖNWALL, Scand. J. Clin. Lab. Invest. *4*, 244 (1952).

[6] D. L. MORRIS, Science *107*, 254 (1948).

The photographic reproduction in Figure 1 illustrates the separation of two fractions and a mixture of these into their protein components. The result suggests that there is one component which is common to both fractions.

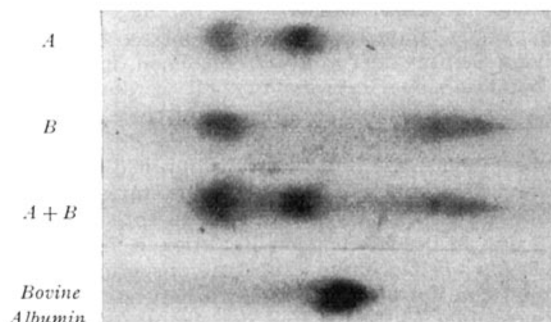


Fig. 1

Figure 2 indicates the usefulness of the method, in showing the heterogeneity of one particular fraction, thus confirming serological results which will be published shortly[1]. It is obvious that the polysaccharide part of the fraction moves rather slowly towards the anode, thereby affording a separation from proteins with higher mobilities. The curve for the proteins suggests the presence of three different components.

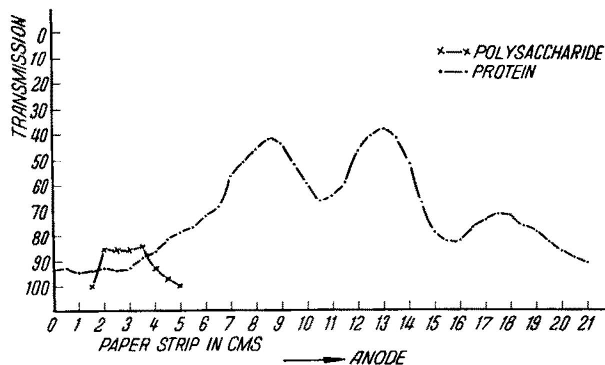


Fig. 2

There are, however, some antigens or antigenic mixtures which cannot yet be satisfactorily separated under these conditions. Heated fractions, e.g. PPD, are not separated into single spots, although they are known to contain at least two proteins with distinct serological specificities. Some unheated proteins, such as a highly purified protein corresponding to SEIBERT's C protein, although serologically nearly pure, show considerable tailing, probably due to strong absorption into the paper. They do not separate into single spots with the electrophoresis method as described above.

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*Zusammenfassung*

Methoden zur papierelektrophoretischen Trennung von antigenen Komponenten von Tuberkulin werden beschrieben.

[1] S. V. BOYDEN and E. SORKIN, To be published.