Microelectrophoresis of serum proteins on nitrocellulose membranes

Nitrocellulose membrane filters impregnated with Tween 60 have been used recently as a suitable supporting medium for microelectrophoresis and microimmunoelectrophoresis of proteins[1–4]. Satisfactory electrophoretic patterns have been achieved within 10–15 min. with sample volumes of 10^{-5} to 10^{-4} ml of serum. Further experiments, however, were necessary to get more information on this method. The present communication refers to microelectrophoresis of human serum on nitrocellulose membranes having different pore sizes impregnated with different detergents under different conditions. The effect of the position of the start was also investigated.

Experimental

Materials and methods. The following membranes (produced by VCHZ Synthesia, n.p., Uhříněves, Czechoslovakia)[5] were tested (the mean pore sizes according to producer's data are given in the parentheses): "RUFS" (1.2 μ). "AUFS" (0.6–0.9 μ), "HUFS" (0.3–0.5 μ) and "VUFS" (0.1–0.3 μ). Strips 5 × 1 cm or 5 × 2.5 cm were treated usually with 2 % solutions of the detergents in a veronal (0.025 *M*)–citrate (0.0025 *M*)–oxalate (0.001 *M*) buffer, pH 8.6, for 5 min[1–3], rinsed with 5 ml of the detergent-free buffer, blotted slightly with Whatman No. 1 filter paper and placed in position in the moist chamber of the electrophoretic apparatus. Human serum stained slightly with bromophenol blue was applied immediately on the start by a small wick of "Oxoid" acetylcellulose[2,3]. The sample volumes were of the order of 10^{-5} ml. The bridge gap was usually 3.5–4.0 cm, the current was 0.4–0.5 mA/cm and the effective voltage 15–20 V/cm. No cooling was used. The time of an electrophoretic run was 12–15 min. The strips were dried at 75–85° for 10 min and 0.002 % nigrosine (Ed. Gurr, Ltd., London) in 2 % acetic acid was used to stain the protein zones[1,6].

The following detergents were used:

Anionic type: primary *n*-propyl sulphate, *n*-butyl sulphate, *n*-amyl sulphate, *n*-octyl sulphate.

Cationic type: dimethyllaurylbenzylammonium bromide (ajatin).

Nonionic type: polyoxymethylene sorbitol monostearate (Tween 60), oleyl and cetyl alcohol with 20 *M* ethylene oxide (EO), a mixture of aliphatic alcohols C_{10}–C_{16} with 13 *M* EO, lauryl alcohol with 4 *M* EO, stearic acid with 6 *M* EO, ricinoleic acid with 15 *M* EO.

In the course of investigation of the behaviour of various detergents on nitrocellulose membranes using the chromatographic technique (*cf.* ref. 3), Dragendorff's reagent[7] and cyanocobaltic acid solution[8] were used for the detection of the nonionic detergents. Iodine vapours proved useful for the detection of anionic detergents and bromophenol blue[8] for the detection of ajatin on the membranes.

Results and discussion

The electrophoretic patterns of human serum had a similar character on all types of the membranes impreganted with Tween 60 (*cf.* ref. 1–3). A finer zone formation, however, was achieved on membranes with smaller pore sizes *e.g.* "HUFS" and especially "VUFS" on which it was usually observed that 8–10 fractions were resolved. This seemed to be due to the fact that the serum could be applied on these strips in a much finer start-line than on "AUFS" or "RUFS" strips. The "HUFS"

and "VUFS" membranes were also easier to manipulate because of their firmer mechanical constitution.

The general electrophoretic pattern and the relative positions of the individual fractions of human serum were not influenced significantly by the position of the start line on the "VUFS" strip impregnated with Tween 60 (Fig. 1). A slight shift, however, of the γ-globulins towards the cathode was observed when the start was nearer to the anode (*cf.* ref. 9). This shift, which was much less on nitrocellulose than on acetylcellulose[1,3], seemed to be connected not only with electroendosmosis but also partly with the increased evaporation of the buffer from the centre of the strip during electrophoresis and the consequent stream of new buffer from both sides.

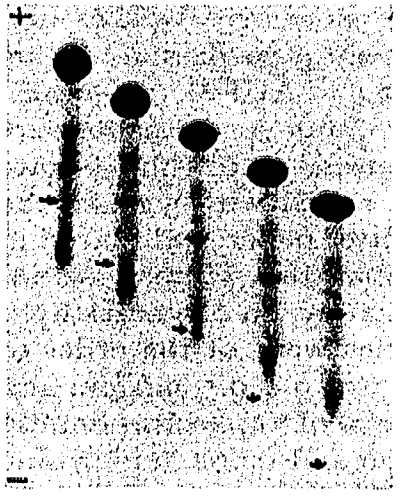

Fig. 1. Microelectrophoresis of human serum on a "VUFS" membrane Veronal–citrate–oxalate buffer, pH 8.6; 15–20 V/cm; 0.4–0.5 mA/cm; bridge gap 4 cm; time: 12 min; sample volumes of the order of 10^{-5} ml; stained with nigrosine. The samples were applied at different distances from the cathode. Start positions are indicated by arrows.

Anionic detergents as well as the cationic ajatin did not prevent strong adsorption of proteins to the membrane because the binding of the detergent to nitrocellulose was smaller than that of protein; the alkyl sulphates, especially, could be readily eluted from the membranes using the electrophoretic buffer. These types of detergents were therefore not suitable for impregnating the nitrocelloluse membranes before electrophoresis. Nonionic detergents, however, were bound more firmly than proteins and some of them, *e.g.* ricinoleic acid with 15 *M* EO, could be used for impregnation of the strips with similar results as Tween 60. Other substances of this group were also found suitable to prevent the adsorption of serum to the membranes, but the respective electrophoretic patterns were not so satisfactory as with Tween 60 or ricinoleic acid with 15 *M* EO.

The effect of a different concentration of Tween 60 used for the impregnation of the nitrocellulose membranes is shown schematically in Fig. 2. The minimal time required for a "sufficient" impregnation of the VUFS membranes at laboratory temperature did not usually exceed 1 min when using 2 % solutions of Tween 60.

The results seem to be in accordance with the assumption that the formation of a compact film of the suitable detergent, having a certain minimal thickness and

covering the whole microporous structure of the nitrocellulose membranes is necessary to make the membranes suitable for electrophoresis (*cf.* ref. 3).

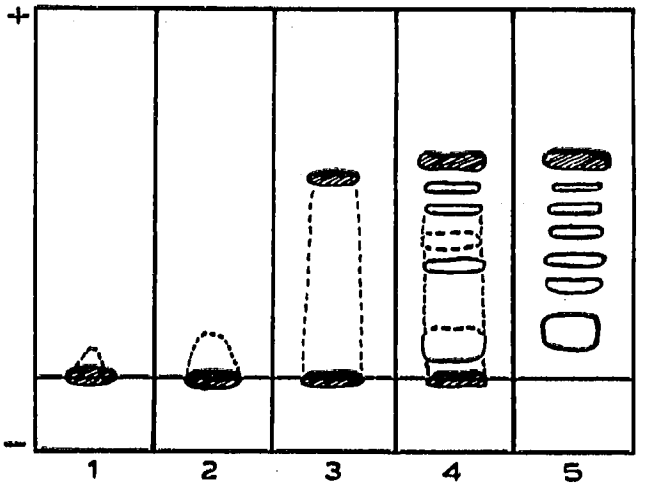

Fig. 2. Electrophoresis on "VUFS" membranes impregnated with Tween 60 of different concentrations. 1 = Not impregnated; 2 = 0.1 % Tween 60; 3 = 0.2 % Tween 60; 4 = 0.5 % Tween 60; 5 = 1.0 % Tween 60. Impregnation lasted 5 min at 22–25°. For the conditions of electrophoresis, see Fig. 1.

Attempts to improve the electrophoretic separations of serum proteins on nitrocellulose membranes by using longer strips, higher voltage, different pH values, in the region 5–10, or discontinuous buffer systems, were unsuccessful under the conditions used.

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Institute of Hematology and Blood Transfusion, Prague (Czechoslovakia)*

T. I. PŘISTOUPIL
V. FRIČOVÁ

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* Director: Prof. J. HOŘEJŠÍ, M.D., D.Sc.