of the Geon used by FAHEY and MCLAUGHLIN, from British Geon, Devonshire House, Piccadilly, London.

The whole of the reaction mix was incorporated into the block. After an overnight run with a current of about 20–30 mA at 300 V, the ferritin-diisocyanate impurity is not far from the anodal end, and forms a brown, recognizably separate band. Behind is found a wide brown band, rather streaky and covering a distance of some 6 cm. The most advanced part of this is probably free ferritin, the rest conjugate freed of ferritin and globulin

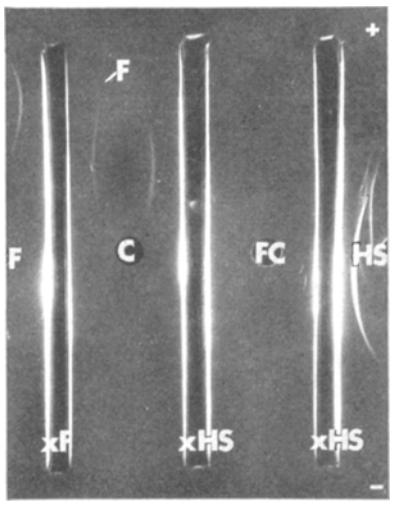

Fig. 2. Immunoelectrophoretic plate photographed wet. Lettering as for Figure 1.

contamination. Globulins remain in the vicinity of the origin.

Most of the brown area identifying the conjugate is then removed, some of the leading and trailing edge being sacrificed. The conjugate is eluted with successive aliquots of 0.05 *M* phosphate pH 7.5, and transferred to 8/32 Visking tubing to be concentrated by ultrafiltration. It is then passed through a 0.45 μ millipore filter and kept in a sterile vial.

Typical immunoelectrophoretic plates of the reaction mixture before and after zone electrophoresis are shown in Figures 1 and 2.

Figure 1, using the crude reaction mixture in the middle well, shows the presence of ferritin-γ-globulin conjugate together with free γ-globulin and the faster-travelling, presumed ferritin-diisocyanate, component. Figure 2, using the isolated conjugate, shows that it contains a trace of free ferritin but is devoid of free γ-globulin and ferritin-diisocyanate. The additional well, into which was put isolated ferritin-diisocyanate, shows no reaction between this and the rabbit antiserum against human serum, indicating that no γ-globulin has been conjugated.

Zusammenfassung. Die Reinigung eines durch Markierung von Immunglobulin mit Ferritin hergestellten Rohmaterials wird beschrieben. Zonenelektrophorese mittels Polyvinylpulvers ergibt ein ferritin- und globulinfreis Endprodukt.

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Comparative Microelectrophoresis of Different Sera on Nitrocellulose Membranes Impregnated with Tween 60

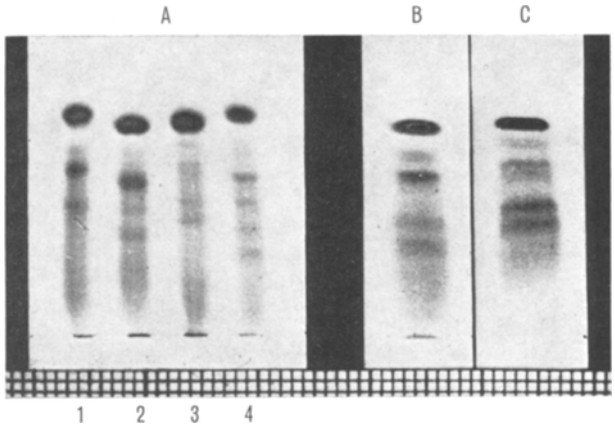
Nitrocellulose membrane filters pretreated with Tween 60 (polyglycolsorbitolmonostearate) were shown recently as being a suitable supporting medium for the rapid microelectrophoresis of proteins[1–4], whereas untreated membranes were not suitable for this purpose. Electrophoresis on Tween-impregnated nitrocellulose proved to be an adequate alternative to the electrophoresis on acetylcellulose strips[5,6], especially for the analysis of ultramicro-amounts of material. This was confirmed by good separations of human serum[1–4] as well as by a rapid characterization of different batches of a modified bovine serum[2]. A search for the most convenient conditions for a quantitative evaluation of the microelectropherograms by direct photometry is under experimentation now.

In the course of further research work with Tween-impregnated nitrocellulose membranes, we wanted to test whether this micro-technique was sensitive enough to distinguish between the electrophoretic patterns of sera of different species.

Nitrocellulose membrane filters HUFS (pore size 0.3–0.5 μ) and VUFS (pore size 0.1–0.3 μ) (VCHZ Synthesia, Uhříněves, Czechoslovakia)[3,7] were used in these experiments. Electrophoresis was done in a moist chamber[1] with a bridge gap of 3.5–4.0 cm at 0.4–0.5 mA/cm and 15–20 V/cm, using a veronal (25 m*M*)-citrate (2.5 m*M*)-oxalate 1.0 m*M*) buffer at pH 8.6. Each electrophoretic run lasted 15 min. After drying at 75–85 °C for 10 min the electropherograms were stained by nigrosine[1,5]. The impregnation of the membranes before electrophoresis was done in the usual way[1–4], using a 2% solution of Tween 60 in the veronal buffer for 5 min followed by a thorough washing of the strips with 5–10 ml of the detergent-free buffer on a funnel to remove the excess of unbound Tween 60[4]. Sera of man, horse, dog, pig, rat, ox, rabbit and cock were taken from samples stored at − 20 °C and were applied on the starts by means of a wick of acetylcellulose[4] or of HUFS nitrocellulose pretreated with Tween 60. The sample volumes were of the order of 10^{-5}–10^{-4} ml.

[1] T. I. PŘISTOUPIL, Biochim. biophys. Acta *117*, 475 (1966).
[2] T. I. PŘISTOUPIL, Clinica chim. Acta, in press.
[3] T. I. PŘISTOUPIL, Nature, in press.
[4] T. I. PŘISTOUPIL, J. Chromat., in press.
[5] J. KOHN, Ärztl. Lab. *10*, 233 (1964).
[6] R. O. BRIERE and J. D. MULL, Am. J. clin. Pathol. *42*, 547 (1965).
[7] Ultrafilters UFS, VCHZ Synthesia, Uhříněves, 1962.

Characteristic 'species specific' microelectropherograms were achieved with all the sera mentioned above. For documentation, however, the sera of horse, pig, man and dog seemed to be preferable (Figure, strip A) because of the greater amount of fractions (cf. [8]). The patterns were distinct and easy to observe even when micro-amounts of samples were analysed. The difference between the electrophoretic patterns of dog serum on strips B and C (Figure) could be interpreted by the presence of unbound Tween 60 on strip C, according to similar results achieved in a previous study with human serum[4]. On both strips, however, a very fine resolution of zones was observed, especially of the doubled α_1-fraction of dog serum.



Microelectrophoresis of different sera on nitrocellulose. Strip A: HUFS membrane impregnated with Tween 60, excess of the detergent removed. 1, horse; 2, pig; 3, man; 4, dog. Strip B: VUFS membrane impregnated with Tween 60, excess of the detergent removed; dog serum. Strip C: VUFS membrane impregnated with Tween 60, excess of the detergent not removed; dog serum. Veronal-citrate-oxalate buffer, pH 8.6; 0.4–0.5 mA/cm, 15–20 V/cm; 15 min runs; stained with nigrosine. A comparative millimetre scale is added below.

The results presented here might serve as a further example of the applicability of the simple and rapid microelectrophoresis on nitrocellulose membranes impregnated with Tween 60.

Zusammenfassung. Durch mikroelektrophoretische Trennungen von Serumproteinen verschiedener Tierarten wurden weitere Anwendungsmöglichkeiten der Methode an mit Tween 60 (Polyglykolsorbitolmonostearat) imprägnierten Nitrocellulose Membranen geprüft. Die verwendete einfache Mikrotechnik erwies sich als besonders geeignet, charakteristische Unterschiede der Seren zu erfassen.

T. I. PŘISTOUPIL

Institute of Hematology and Blood Transfusion, Prague (Czechoslovakia), January 13, 1966.

[8] T. BEDNAŘÍK and H. CAJTHAMLOVÁ, Hoppe Seyler's Z. physiol. Chem., in press.

CONGRESSUS

Canada

Symposium on Synthesis

Banff (Alberta, Canada), August 31–September 2, 1966

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