(see Figures B and C[8] and Figures 1, 3, 5, 8–10, 14, 15a, 16–20)[9]. ISHIDA and CHANG transfer the eggs to a slide between four vaseline spots, anchoring them between slide and coverslip. Thereafter the edges of the coverslip are sealed with nail-polish. This method is rather complicated, and in their photographs as well, the eggs show some deformation (see Figures 1 and 2)[9].

Compared with the techniques of pasting the eggs before or after the reaction, those described by ISHIDA and CHANG[10] and by us show several advantages for delicate histochemical reactions: isotonicity is preserved during the mounting, uneven adhesion to the slide is excluded and protein-precipitants or other pasting agents, as needed for the pasting technique, are avoided. Furthermore, the techniques are applicable without modification for all preparations and with any mode of fixation, as long as the reaction can be done on free eggs and the reaction product is not water-soluble.

In comparison with the technique of ISHIDA and CHANG, it appears to us that ours offers some advantage in that it is more simple. In addition, the eggs lie completely free and they can be dislocated for purposes of observation by sliding the coverslip many hours after mounting. One disadvantage must be mentioned: experience is required for a presentable demonstration of all the eggs found in one animal[11].

Zusammenfassung. Anisotonische Lösungen verursachen bei der Durchführung histochemischer Reaktionen an freien Eiern von Nagern Artefakte. Es wird eine einfache Montagetechnik nach Durchführung der Reaktion beschrieben, bei der intrazelluläre Veränderungen vermieden und weder Form noch Grösse der Eier durch Anisotonie verändert werden. Die Technik erlaubt jede Art von Fixation und jede Reaktion, solange das Reaktionsprodukt wasserunlöslich ist.

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[11] This investigation was supported by a research grant from the Ford Foundation.

Purification of Ferritin-Labelled Immunoglobulins

Ferritin has thus far proved to be the most commonly used label for identifying immunoglobulins in the electron microscope. This probably owes much to the careful work of its first proponents[1], and to the ease and certainty with which labelled molecules can be detected. Purification of the crude mixture obtained after the coupling reaction is, however, rather tedious. RIFKIND et al.[2] recommend three sequential centrifugations at 100,000 *g* for 4 h to remove the uncoupled γ-globulin, free ferritin being allowed to remain. BOREK and SILVERSTEIN[3] suggested the use of continuous flow paper electrophoresis to isolate the conjugate, but this has not been much used (see, however, BAXANDALL et al.[4]). The present note describes the use of a zone electrophoretic separation which forms a convenient preparative method for obtaining the conjugate free of impurity.

Ferritin (twice crystallized; Nutritional Biochemicals Corp.) was coupled to γ-globulin by means of toluene 2,4-diisocyanate (K. and K. Laboratories). The method used was derived by combination of those described by SINGER and SCHICK[1] and RIFKIND et al.[2]. After the crude reaction mixture has been dialysed into 0.05 *M* phosphate buffer pH 7.5, it contains, in addition to the conjugate, unreacted γ-globulin, free ferritin, and ferritin which has reacted with diisocyanate alone. These can be removed by zone electrophoresis using polyvinyl powders[5,6], because γ-globulin is slow-travelling and remains near the origin; ferritin, on the other hand, travels faster than the conjugate, while ferritin-diisocyanate is the fastest travelling of the three major contaminants.

The procedure used is that described by FAHEY and MCLAUGHLIN[6]; Pevikon C 870 being obtained from the Shandon Scientific Co. Ltd., 65 Pound Lane, London N.W. 10, and Breon Vinyl 425, said to be the equivalent

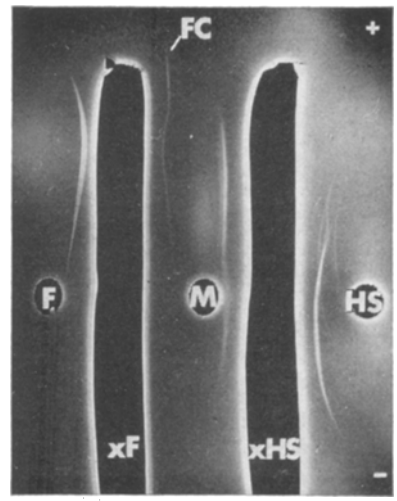

Fig. 1. Immunoelectrophoresis on agar gel in veronal buffer pH 8.2 ionic strength 0.05. Plate photographed after drying, fixing, and staining with Ponceau red. F, purified horse ferritin; FC, presumed ferritin-diisocyanate complex; xF, goat anti-ferritin antiserum; M, crude reaction mixture; xHS, rabbit anti-human serum antiserum; HS, human serum.

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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

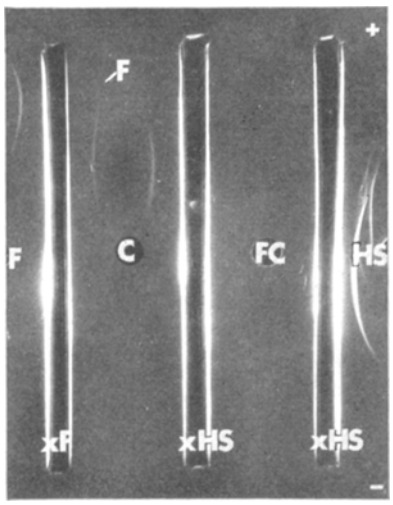

Fig. 2. Immunoelectrophoretic plate photographed wet. Lettering as for Figure 1.

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.

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