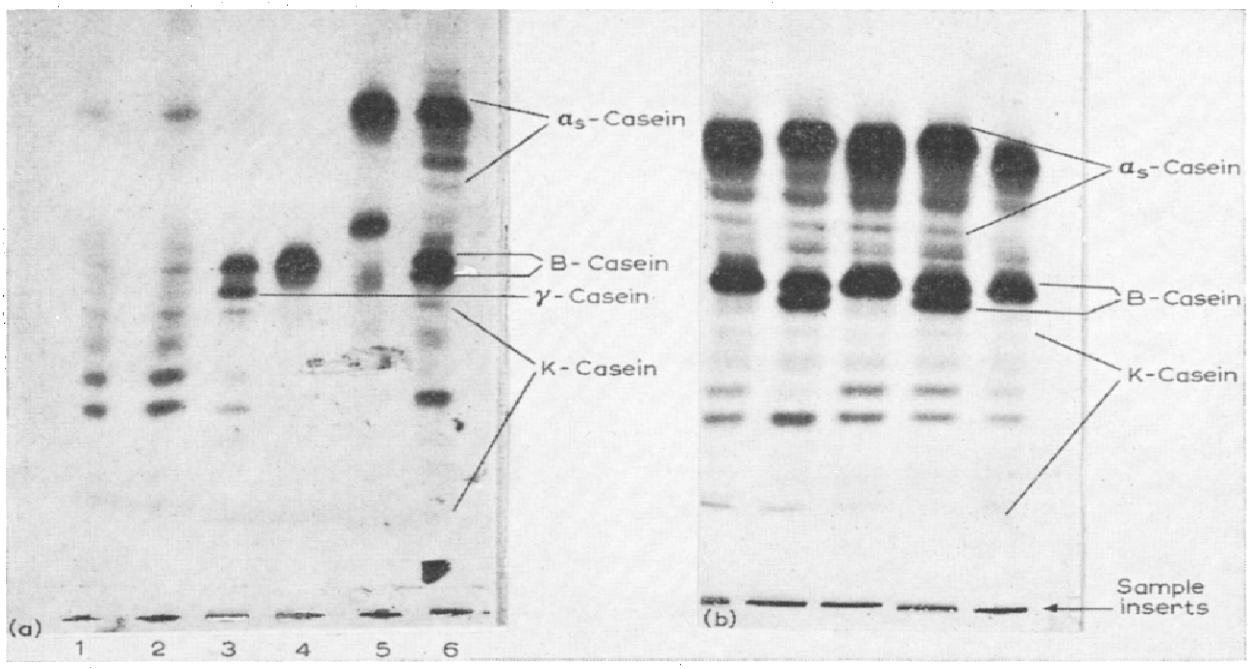

Fig. 2. Starch-gel electropherograms of casein and its components. (a) 1 and 2 = K-casein; 3 = γ-rich casein fraction; 4 = B-casein; 5 = α_s-casein and 6 = whole casein. (b) Five samples of whole casein from individual cows.

The genetic variants of α_s, B-, and K-caseins are clearly demonstrated in these patterns. The starch gel technique used for preparation of these electropherograms has been previously reported by the author[2].

Consistently reproducible starch gel electropherograms have been obtained in the author's laboratory using the above discussed migration chamber.

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The influence of Tween 60 on the microelectrophoretic patterns of human serum on nitrocellulose and acetylcellulose membranes

In a preliminary note[1] on the microelectrophoresis of human serum on nitrocellulose membranes[2] it was shown that a pretreatment of the membranes with polyglycol sorbitol monostearate (Tween 60) was necessary for successful separations

of protein fractions. Various types of nitrocellulose membranes, differing by their pore sizes, have been tested[3] and microelectrophoresis on this supporting medium was used for the characterisation of modified bovine serum[4]. In comparison with electrophoresis on acetylcellulose strips[5], a smaller electro-endo-osmotic flow was observed on nitrocellulose impregnated with Tween 60[1,4] and also certain differences in the protein patterns were noticed. According to other orientation experiments[3], a part of the Tween 60 is firmly adsorbed on the nitrocellulose, most probably forming a film which covers the inner surface of the membrane pores; thus the interfering adsorption of proteins on nitrocellulose[1,2] was prevented. Another part of the Tween 60, however, seemed to be only mechanically occluded in the microporous structure of the membrane under the conditions used.

In the present paper, we wanted to reexamine these results in more detail and to test whether the contact of serum proteins with Tween 60 during electrophoresis would cause some changes in the electrophoretic patterns of certain fractions known to be sensitive to the detergent[6]. The results of the experiments on nitrocellulose were compared again to those obtained on acetylcellulose both untreated and treated with Tween 60.

Experimental

Material and methods. Nitrocellulose membrane filters "VUFS" (produced by VCHZ Synthesia, n.p., Uhříněves, Czechoslovakia)[2] and "Oxoid" electrophoresis strips (produced by Courtaulds, Ltd., Coventry)[5] were used as supporting media for electrophoresis. A veronal (25 m*M*)–citrate (2.5 m*M*)–oxalate (1 m*M*) buffer[7], pH 8.6, was used throughout these experiments. Human serum was taken from a sample stored at —20°.

5 × 1 cm strips were soaked before electrophoresis[1,5] either with the buffer alone or with a solution of 2 % Tween 60 in this buffer, for 5 min[1,3]. The strips were then rinsed on both sides as before[1,3,4], either with 5 ml of the detergent-free buffer, or thoroughly washed with 5–10 ml of the buffer on a Büchner funnel, to remove all excess of "unbound" detergent. Electrophoresis was performed without cooling in a moist chamber[1] with a bridge gap of 3.5 cm, using 0.4–0.5 mA/cm and 15–20 V/cm for 15 min. Sample volumes of the order of 10^{-4} ml were applied from the tip of a square wick of acetylcellulose soaked with sample and through which a perforation had been made by a pin (Fig. 1). The area between the tip and the perforation was blotted gently with a filter paper, while above the perforation a small droplet of the serum (stained with bromophenol blue) was left. This method of application gave the best and most reproducible results among various techniques tested even on acetylcellulose strips. After electrophoresis the strips were dried at 75–85° for 10 min and stained with nigrosine (Ed. Gurr, Ltd.)[1,5].

The adsorption of Tween 60 to the membranes was tested by ascending chromatography, using the electrophoretic buffer as solvent. About 10^{-3} ml of a 2 % solution of the detergent was applied at the start as a streak close behind the advancing front of the solvent. Dragendorff's reagent was used to detect the detergent on the strips[3,8].

Results

Fig. 2 shows schematically the strong adsorption of Tween 60 on the "VUFS" nitrocellulose strip during ascending chromatography and the minimal adsorption

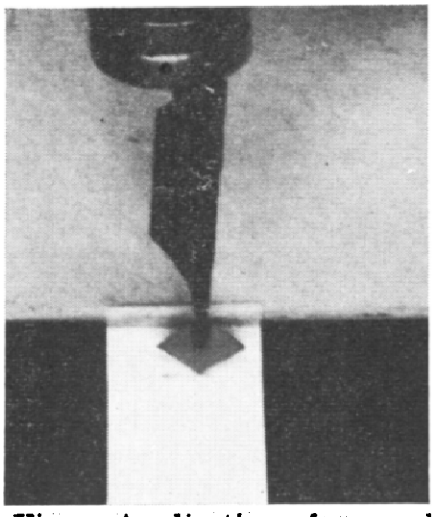

Fig. 1. Application of a sample on the start line. A wick of "Oxoid" membrane soaked with the sample was fitted to a steel pen.

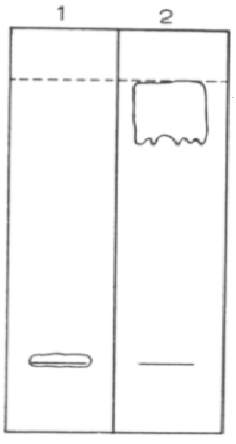

Fig. 2. Ascending chromatography of Tween 60. 1 = nitrocellulose membrane; 2 = acetylcellulose membrane. Solvent: veronal–citrate–oxalate buffer, pH 8.6. Detection: Dragendorff's reagent[8].

of this detergent on the "Oxoid" acetylcellulose. Some strips were pretreated with Tween 60 in the usual way and then only slightly rinsed with buffer, and then the excess detergent was eluted until no more detergent could be detected in the eluted solution. However, even after this operation a strongly positive reaction with Dragendorff's reagent was obtained with nitrocellulose membranes, indicating the presence of firmly bound Tween 60.

Some changes of the electrophoretic patterns of human serum, caused by Tween 60, can be seen in Fig. 3. The "Oxoid" strip (No. 1) served as the "normal" standard pattern. After treatment of acetylcellulose with Tween 60 (strip No. 2), the prealbumin zone gained in mobility but lost in intensity. Furthermore, there was a gap

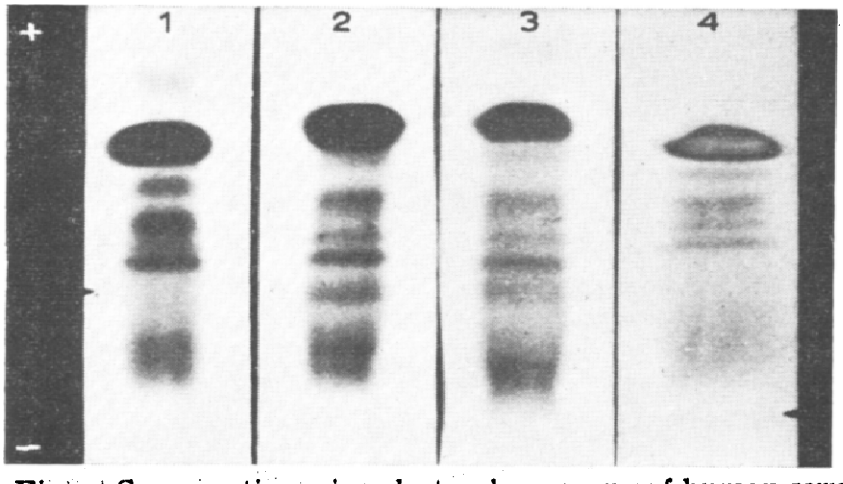

Fig. 3. Comparative microelectropherograms of human serum. 1 = acetylcellulose without detergent; 2 = acetylcellulose pretreated with Tween 60; 3 = nitrocellulose pretreated with Tween 60; 4 = nitrocellulose pretreated with Tween 60, excess of unbound detergent removed. 0.4–0.5 mA/cm, 15–20 V/cm; 15 min runs; nigrosine stain. The position of the start on strips No. 1 and 2 is indicated on the left, on strips No. 3 and 4 on the right.

in the α-globulin region and a new zone appeared in the region of the β-globulins. The γ-globulin also showed the presence of at least two zones. Similar changes were also observed on the nitrocellulose membranes. Thus strip No. 3 (with an excess of unbound detergent present) showed an electrophoretic pattern analogous to that on acetylcellulose strip No. 2. On strip No. 4 (with firmly bound detergent only), however, a "normal" pattern was achieved which could be compared to that on strip No. 1. A somewhat greater adsorption of proteins was observed sometimes on the "VUFS" strips treated in this way as well as a slight coloration of the background by nigrosine. The zones, however, were often more distinct than on acetylcellulose.

Discussion

The results mentioned above led to the conclusion that changes of the "normal" electrophoretic pattern of human serum were most probably caused by the presence of unbound Tween 60 in the membranes. Similar changes have been described[6] with sera incubated with detergents. These artefacts, however, consisting in a changed mobility of certain fractions, might be expected to have a special analytical value, for instance when analysing sera of patients suffering from different diseases.

Nevertheless, it was interesting to find that Tween 60, when firmly bound to the supporting medium, did not cause the changes mentioned above. This result seemed to confirm a previous assumption[3] that the electrophoretic separation of proteins took place in a buffer solution which was supported by the microporous framework of nitrocellulose, coated thoroughly by a film of Tween 60. In this connection it would be possible to speak about an electrophoresis on Tween 60 rather than on nitrocellulose. Further experiments involving quantitative evaluations will be necessary, however, before an adequate interpretation can be given to these findings.

The results presented here reaffirmed our previous observations of a minimal electro-endo-osmotic flow on nitrocellulose as compared to that on acetylcellulose (*cf.* Fig. 3).

It seemed also reasonable to assume that other impregnating substances could be bound to the nitrocellulose membranes instead of Tween 60, to form supporting media with appropriate properties and special binding capacities. Experiments concerning these questions are now under study in our laboratory.

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