ATPase activity carried out after pre-treatment with lower concentrations of deoxycholate.

The sensitivity of Mg^{2+} + Na^+ + K^+-activated ATPase to inhibition by deoxycholate also depended on the previous environmental temperature of the fish. Preparations from 8°-acclimatized fish were more resistant to inhibition by this detergent than were preparations from 30°-acclimatized fish. No inhibition was seen with 8°-adapted preparations pre-treated with deoxycholate up to and including a concentration of 1.25 mM. This change from no inhibition to inhibition, seen with 8°-adapted preparations, was not found with the 30°-acclimatized fish. In this case all concentrations of deoxycholate inhibited the enzyme but the degree of inhibition became increasingly dependent on the presence of deoxycholate over the concentration range 1.25–2.5 mM. The critical micellar concentration for several bile salts, tested with azobenzene and water, is of the order of 2 mM[8]. Possibly the discontinuity mentioned above represents the point at which deoxycholate begins to solubilize membranes containing the ATPase system.

The most obvious difference between intestinal microsomal fractions prepared from warm- and cold-adapted fish was the ability of the ATPase system to be activated by Na^+ + K^+. Addition of these monovalent ions to microsomal fractions prepared from cold-adapted fish nearly doubled the rate of ATP hydrolysis. Preparations from warm-adapted fish were much less sensitive to these ions. This remained true after pre-treatment with various concentrations of deoxycholate, though this always increased the Mg^{2+} + Na^+ + K^+/Mg^{2+} activity ratio. The difference in Na^+ + K^+-activation was not caused by changes in the amounts of Na^+ and K^+ found in the microsomal pellets. The mean concentrations of Na^+ and K^+, obtained from fractions prepared from fish acclimatized to both temperatures, were 0.3 mM and 0.4 mM respectively. Enzyme preparations were diluted 20-fold before assay and the final concentrations of ions from this source would be most unlikely to change the overall ATPase activity.

An interesting finding in this work was the ability of the Mg^{2+} + Na^+ + K^+-activated ATPase, prepared from 8°-adapted fish, to remain fully active at a time when the Mg^{2+}-activated enzyme was being depressed by deoxycholate. Preparations from warm-adapted fish did not show this effect, though the fall in ATPase activity was less when the medium contained Na^+ + K^+. Further information about the molecular events which lead to this type of difference could provide a useful way of studying the reorganization which must take place in a membrane to allow it to function normally at widely different environmental temperatures.

Riassunto. L'attività di ATP-asi è stata studiata nella frazione dei microsomi preparata dalla mucosa intestinale del pesce dorato, acclimatato a 8° o 30 °C. La differenza notata potrebbe essere una consequenza dell'alterazione nella struttura della membrana lipida plasmatica provocata per cambiamento della temperatura ambiente.

M. W. SMITH and PAT BROWN[9]

Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge (England), 10 May 1968.

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Paramagnetism of Human Serum Proteins Demonstrated by Two-Stage Electromagnetophoresis

In this preliminary report there is described a new molecular property among some of the human normal serums proteins (HNSP), paramagnetic susceptibility, by use of a new technique, two-stage electromagnetophoresis, in polyacrylamide gel systems. Simultaneous electrophoretic techniques reported by others[1–5] describe methods and results quite different from that discussed in this paper. To our knowledge the paramagnetism of HNSP has not previously been recorded.

Methods. The technique of two-stage electromagnetophoresis is as follows: a transparent plastic chamber, open at both ends, was filled by a polyacrylamide gel by the method of ORNSTEIN[6] and DAVIS[7] modified to the extent of eliminating the spacer gel and using sodium persulfate to induce polymerization. At one side on the top surface of the rectangular prism gel in a slight depression 0.1–0.5 ml of human normal serum, protein was deposited. The system was then electrophoresed for 30–45 min using a current of 30 mA with a voltage range of 130–320 V delivered by a variable voltage power supply as a source of direct current. Following the completion of electrophoresis (first stage), the chamber with the intact gel was placed for 1 h in a non-uniform magnetic field (second stage) generated by a 4-inch Varian electro-magnet[8]. Typical values of the product of a magnetic field times the magnetic field gradient were about 25×10^6 Oersteds/cm^2. The proteins so partitioned were visualized when the gel was stained with 0.1% amido-black and destained electrophoretically.

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Discussion. Typical patterns produced under these conditions are shown in Figure 1. From such patterns it is evident that HNSP may be partitioned as a function of paramagnetism and that 2–3 different species of paramagnetic protein molecules may be present in normal serum.

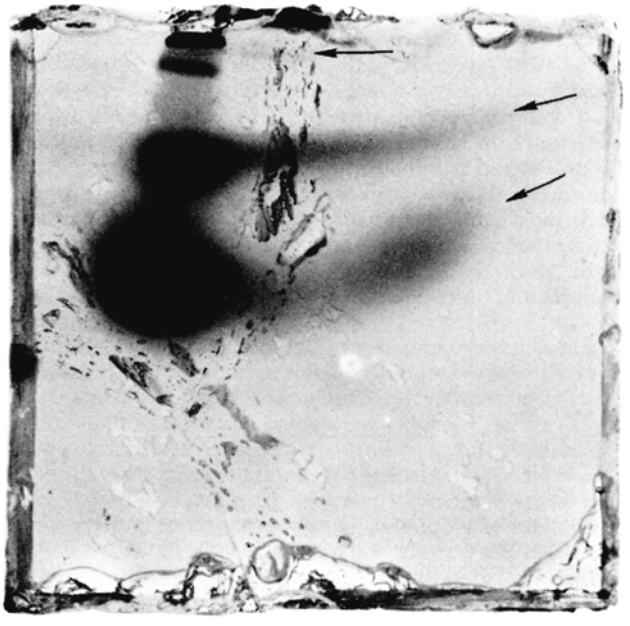

Fig. 1. This is a typical partition pattern of serum protein achieved in polyacrylamide gel by two-stage electromagnetophoresis. Undesirable diffusion phenomena are present. Paramagnetic protein molecules have been displaced laterally in 2 prominent bands and a third faint band (arrows). The over-all dimensions of the gel are 10 × 10 × 2 cm. The electrical polarity during electrophoresis (stage I) was negative at the top of the gel and positive at the bottom of the gel. The non-uniform magnetic fields were applied at right angles to the electrophoretic axis, i.e. from left to right. Control specimens did not show lateral displacement of the proteins.

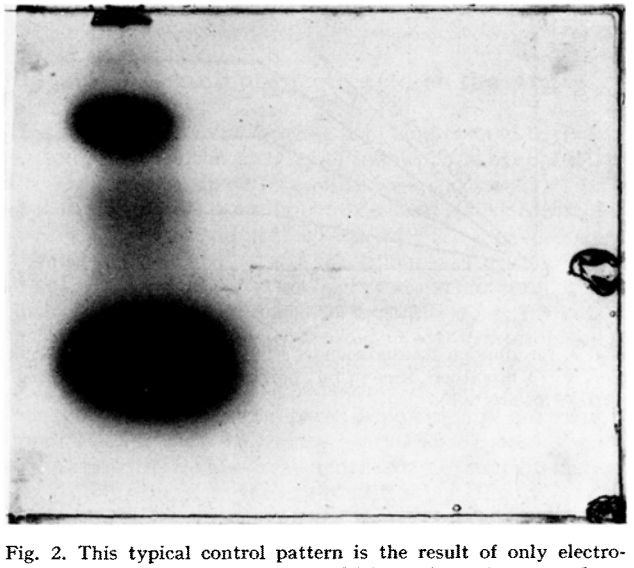

Fig. 2. This typical control pattern is the result of only electrophoresis (first stage). 'Magnetophoresis' (second stage) was not done. Note that no lateral displacement of the electrophoretically partitioned proteins has been effected.

This two-stage electromagnetophoretic technique evolved from a one-stage simultaneous electromagnetophoretic procedure which has been published elsewhere[9]. We postulate that the observed displacement in the direction of the magnetic field gradient of the partitioned HNSP in the second stage of the two-stage procedure is a function of the interaction between the impressed, non-uniform magnetic field and the magnetic dipoles of individual protein molecules. We, therefore, believe that the basis for the observed partition patterns is a well-known physical principle: linear displacement of paramagnetic molecules can be induced in a non-uniform magnetic field parallel to the magnetic field gradient[10,11].

Under the conditions of the experiment, it appears evident that the function of electrophoresis of the specimen simply permits separation by an additional, unrelated parameter of the protein molecules and does not of itself influence in any way the lateral molecular migration. Control specimens showed no lateral displacement patterns of HNSP.

It is implicit under the conditions of the two-stage electromagnetophoresis that the biochemical, bio-physical, and immunological integrity of the partitioned proteins probably remains unaltered. Thus, proteins partitioned in this manner may be used for molecular characterization and molecular manipulation.

On the basis of these preliminary results we wish merely to record our observations and to avoid speculative interpretation[12,13].

Zusammenfassung. Normale Serumproteine wurden mittels zweistufiger Elektromagnetophorese in Polyacrylamidgel, einer neuen Technik, aufgetrennt, wobei die Trennung auf Grund der paramagnetischen Eigenschaften der einzelnen Proteine erfolgt.

R. M. Nalbandian, R. E. Michel and I. Mader

Department of Pathology, School of Medicine, Wayne State University and William Beaumont Hospital Research Institute, Royal Oak, Department of Physics, Research Laboratories, General Motors Corporation, and Department of Medicine, William Beaumont Hospital, Royal Oak (Michigan 48072, USA),
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