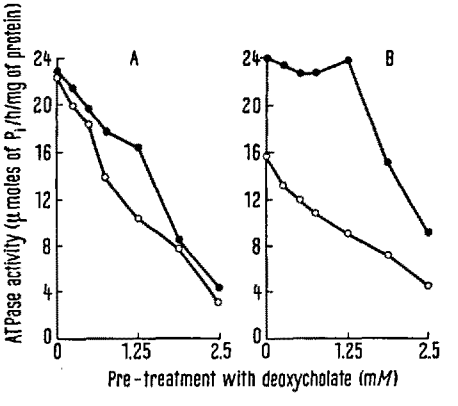
The Effect of Deoxycholate on Microsomal Adenosine Triphosphatase Prepared from the Intestine of Goldfish Acclimatized to Different Temperatures

Deoxycholate has long been used as a natural detergent to facilitate the dispersion of water-insoluble substances in aqueous solvents[1]. But dispersion of particulate fractions prepared from tissue homogenates in deoxycholate invariably causes some loss of enzyme activity. Microsomal Mg^{2+}-activated adenosine triphosphatase (ATPase) is found to be more labile than the $Mg^{2+}+ Na^{+}+ K^{+}$-activated enzyme under these conditions and treatment of membrane fractions with deoxycholate always causes the $Mg^{2+}+ Na^{+}+ K^{+}/Mg^{2+}$ activity ratio to increase[2]. Previous experiments with the goldfish intestine, where the mucosal tissue was homogenized in media containing deoxycholate, suggested that the $Mg^{2+}+ Na^{+}+ K^{+}/Mg^{2+}$-activity ratio depended on the previous environmental temperature of the fish[3]. The effect of deoxycholate on goldfish intestinal ATPase activity has now been determined separately using fish acclimatized to 2 different temperatures. Deoxycholate inhibited both the Mg^{2+}- and the $Mg^{2+}+ Na^{+}+ K^{+}$-activated parts of the ATPase system but this effect could readily be distinguished from other changes in the system associated with acclimatization of the fish to different temperatures.

Goldfish weighing 20–50 g were kept in aerated water at 8° or 30 °C for 2–8 weeks before use. Groups of 8°-acclimatized fish were alternated with 30°-acclimatized fish throughout, so that comparisons between the 2 temperatures might remain independent of seasonal changes. The procedure for killing fish and for collecting mucosal scrapings from the anterior intestine has already been described in detail[4]. Homogenization was in 10 parts (v/w) of 0.25 *M*-sucrose solution containing 5 m*M*-diaminoethanetetraacetic acid (EDTA), adjusted to pH 7.1 by the addition of *Tris*-(hydroxymethyl)-amino methane. Each homogenate, containing the pooled mucosal scrapings from 3 fish, was centrifuged first at 10,000 *g* for 15 min to sediment nuclei together with most of the mitochondria. This sediment was discarded and a second pellet sedimented from the first supernatant by a further centrifugation at 20,000 *g* for 60 min. Both centrifugations were carried out in the cold using a Spinco model L preparative ultracentrifuge (rotor AH50). The second pellet, found previously to consist almost exclusively of membrane-like material[4], was resuspended in 10 m*M* histidine, pH 7.1, for immediate estimation of ATPase activity. The amount of Na^{+} and K^{+} in these preparations was determined by flame photometry (Evans Electro Selenium, EEL). Protein was estimated colorimetrically using crystalline bovine plasma albumin as standard[5].

The ATPase activity of each microsomal fraction was determined after a preliminary dilution and incubation with an equal volume of 10 m*M* histidine or with various histidine-deoxycholate mixtures, all at a pH of 7.1. Tubes containing these enzyme-deoxycholate mixtures were shaken for 15 min at 37 °C with no Na^{+} or K^{+} present, 0.1 ml samples then being transferred to incubation tubes containing 5 m*M* $MgCl_2$, with or without 40 m*M* NaCl plus 20 m*M* KCl, made up to a constant volume of 0.9 ml with 10 m*M* histidine, pH 7.1. The final incubation was started by the addition of 0.1 ml, 30 m*M*-*Tris* ATP. Incubation was for 15 min at 37 °C when hydrolysis was stopped by the addition of 0.1 ml, 50% w/v trichloroacetic acid. Deoxycholic acid was insoluble in the acidified medium and this, together with the precipitated protein, was sedimented by centrifugation at 1400 *g* for 5 min. Inorganic phosphate (P_i) was estimated[6] in 0.8 ml samples of the clear supernatants and a correction applied for the small amount of P_i present in the *Tris*-ATP, incubated under the same conditions but with no enzyme present. The amount of microsomal protein used in these experiments varied from 40–95 µg. ATPase activity was expressed as µmoles of P_i produced/mg of protein/h incubation.

The effect of deoxycholate on the ability of these preparations to hydrolyse ATP is shown in the Figure. The Mg^{2+}-activated ATPase was inhibited by pre-treatment with the lowest concentration of deoxycholate used (0.25 m*M*), a concentration far below the critical micellar concentration quoted for this detergent when used to solubilize simple organic molecules in an aqueous phase[7]. Acclimatization to a low temperature increased the resistance of the Mg^{2+}-activated enzyme to inhibition by deoxycholate. The estimated concentrations of deoxycholate needed to cause 50% inhibition were 1.2 and 1.6 m*M* for fractions prepared from 30°- and 8°-acclimatized fish respectively. Small amounts of deoxycholate were pipetted with the enzyme into the final incubation mixture and this might have led to a slight over-estimate of the inhibitory action of deoxycholate at the highest concentration used. This would not apply to other estimates of



Inhibition of goldfish intestinal ATPase by pre-treatment with deoxycholate. Each enzyme preparation, consisting of a 20,000 *g* sediment of homogenized intestinal mucosa, was pre-treated with various concentrations of deoxycholate for 15 min at 37 °C at a pH of 7.1. Samples of these solutions were then incubated for a further 15 min at 37 °C in medium containing 5 m*M* $MgCl_2$, 3 m*M* *Tris*-ATP and 10 m*M* histidine, pH 7.1, (—o—) or in the same medium containing, in addition, 40 m*M* NaCl and 20 m*M* KCl (—•—). Each point represents the mean of 3 estimates in duplicate, each estimate being made on a pooled preparation derived from the intestinal mucosa of 3 fish. (A) and (B), fish acclimatized to 30° and 8 °C respectively.

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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.

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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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