PRELIMINARY NOTES

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Effect of bile salts on transport across brush border of rabbit ileum

In addition to their effects on lipid absorption, conjugated[1-3] and deconjugated[4-6] bile salts have been reported to influence the intestinal transport of a variety of water-soluble substances. The absorption of amino acids, monosaccharides, Na^+ and water by *in vitro* rat jejunum is inhibited by incubation in the presence of bile salts[1,2,6]. In addition, the nonmediated transport of a number of substances, such as urea[1], is also altered. The finding that ATPase activity of intestinal homogenates is reduced following exposure to bile salts has prompted the suggestion that the defects in absorption may be secondary to the inhibition of this enzyme activity[1,2]. This communication presents evidence that bile salts have a direct action on the mucosal border of rabbit ileum that influences the uptake of a variety of water-soluble substances and that this effect cannot be attributed to gross morphologic changes or to inhibition of metabolic processes.

Segments of distal ileum from white rabbits were mounted, mucosal surface up, in an apparatus[7] that permits the exposure of eight defined areas of the mucosal surface alone to solutions of desired composition. Four areas of mucosal surface were exposed to a buffer solution containing 140 mM choline·HCl, 10 mM Tris, 10 mM KCl, 1.2 mM $CaCl_2$ and 1.2 mM $MgCl_2$ (pH 7.4) for 30 min at 37°. Alternate areas of mucosa were exposed to an identical solution that contained, in addition, 10 mM sodium taurodeoxycholate (Calbiochem). These solutions were then withdrawn and the mucosal surfaces were lightly rinsed with buffer solution to remove excess bile salt. The unidirectional influx of a number of solutes was then determined from a mucosal test solution containing the ^{14}C-labeled derivatives of the nonelectrolytes or $^{22}Na^+$ and [^3H]inulin by methods that have been described in detail[7]. The mucosal test solution was either the Na^+-free buffer or an identical solution where all the choline·HCl was replaced by NaCl (Na^+ concn., 140 mM).

The effect of prior exposure of the mucosal surface to taurodeoxycholate on the influxes of a number of solutes is given in Table I. The unidirectional influx of alanine across the brush border of rabbit ileum into the epithelial cells is inhibited both in the presence and absence of Na^+ following exposure to taurodeoxycholate. In contrast, the influx of mannitol, which crosses the brush border of rabbit ileum by a process of simple diffusion[8], is accelerated almost 3-fold. The data on 3-*O*-methylglucose appear more complex because influx is inhibited in the presence of Na^+ but stimulated in the absence of Na^+. GOLDNER *et al.*[8] have observed that the mediated uptake of 3-*O*-methylglucose in rabbit ileum is obligatorily dependent upon the presence of Na^+ in the mucosal test solution; in the absence of Na^+, the influx of 3-*O*-methylglucose resembles that of mannitol and cannot be distinguished from a diffusional process. Thus, the results with 3-*O*-methylglucose are consistent with the

TABLE I

EFFECT OF TAURODEOXYCHOLATE INCUBATION ON SOLUTE INFLUX

J_{me} is the unidirectional influx across the brush border. All experiments comparing the influx of a single solute following incubation in the presence or absence of taurodeoxycholate were performed on tissue from the same animal. Number of determinations is indicated in parentheses. All errors are S.E.

Solute	J_{me} (μmoles/cm^2 per h)	
	Control	*Taurodeoxycholate*
Alanine, 5 mM (Na^+, 0)	1.0 ± 0.1 (2)	0.42 ± 0.03 (2)
5 mM (Na^+, 140 mM)	3.3 ± 0.3 (10)	0.67 ± 0.06 (8)
Mannitol, 5 mM	0.08 ± 0.01 (4)	0.23 ± 0.02 (4)
3-*O*-Methylglucose, 5 mM (Na^+, 0)	0.14 ± 0.01 (2)	0.30 ± 0.01 (2)
5 mM (Na^+, 140 mM)	1.1 ± 0.2 (2)	0.41 ± 0.06 (2)
Na^+, 140 mM	20 ± 0.5 (4)	16 ± 0.8 (4)

conclusion that taurodeoxycholate preincubation inhibits carrier-mediated solute influx while enhancing diffusional processes. This reasoning can likewise be applied to the influx of Na^+ which is inhibited 20 % following taurodeoxycholate incubation. It has been suggested by SCHULTZ *et al.*[7] that Na^+ influx is at least partially mediated since the presence of Li^+ in the test solution inhibits Na^+ influx.

POPE *et al.*[6] have recently presented evidence that the effects observed with commercial conjugated bile salts can be attributed to contamination by deconjugated bile salts. The taurodeoxycholate employed in these experiments is certified by the supplier as greater than 98 % pure by thin-layer chromatography. Paired experiments comparing the effects of 0.5 mM and 1.0 mM deoxycholate with 10 mM taurodeoxycholate on alanine influx indicate a significantly greater inhibition by taurodeoxycholate. Although the possibility that other impurities may contribute to our results cannot be excluded, it is unlikely that contamination of our taurodeoxycholate by deoxycholate alone is entirely responsible for the observed inhibitions.

Several experiments were performed to examine the reversibility of the effect of taurodeoxycholate preincubation on alanine and mannitol influxes. Following a 5-min preincubation in 10 mM taurodeoxycholate (which produces 50 % of the effect observed after 30-min preincubation) the solution was withdrawn and replaced by a taurodeoxycholate-free medium. Influx of 5 mM alanine or mannitol was determined after incubation in taurodeoxycholate-free medium for varying lengths of time. The inhibition of alanine influx was not reversed by 45-min exposure to the taurodeoxycholate-free medium. In contrast, mannitol influx decreased to control values following 30-min exposure to the standard buffer solution. These results indicate that the passive permeability of the mucosal border to mannitol is gradually restored after removal of taurodeoxycholate. The failure of alanine influx to return to control values suggests that certain properties of the brush border transport mechanism have been irreversibly altered.

The acceleration of diffusional processes raised the possibility that taurodeoxycholate incubation was removing membrane material from the mucosal surface. To examine this possibility the amount of trichloroacetic acid-precipitable material recovered after 30-min exposure of mucosal areas to mucosal solutions with and without

taurodeoxycholate was determined. The mucosal solutions were aspirated, whole cell debris was removed by centrifugation and ice-cold trichloroacetic acid (final concn. 5 %) was added to the clear solutions. Solutions containing taurodeoxycholate became markedly turbid whereas control solutions remained clear. Dry weight determinations indicate a recovery of 2.2 ± 0.4 mg of trichloroacetic acid-precipitable material per cm^2 mucosal surface in taurodeoxycholate-containing solutions compared to a recovery of only 0.3 ± 0.1 mg/cm^2 in taurodeoxycholate-free solutions. Further, the dry weight of 1 cm^2 of whole-thickness intestine that had been exposed to taurodeoxycholate averaged 17.3 ± 1 mg whereas the dry weight of adjacent 1-cm^2 areas that were not exposed to taurodeoxycholate averaged 20.6 ± 1 mg. Thus, the recovered trichloroacetic acid-precipitable material accounts for 67 % of the decrease in dry weight of the exposed tissue and amounts to approx. 10 % of the control dry weight. Addition of trichloroacetic acid to a solution containing 10 mM taurodeoxycholate that was not exposed to the mucosal surface did not result in a precipitate.

The inhibition of alanine, Na^+ and 3-*O*-methylglucose (Na^+ concn., 140 mM) influxes and enhancement of mannitol and 3-*O*-methylglucose (Na^+ concn., 0) influxes suggest that exposure of the mucosal surface of rabbit ileum to taurodeoxycholate increases permeability of the brush border to passively transported substances while inhibiting carrier-mediated processes. A similar suggestion has been made by ROSENBERG AND HARDISON[5]. These observations cannot be attributed to interference with energy-yielding metabolic processes since alanine and Na^+ influxes across the brush border of rabbit ileum are unaffected by cyanide, dinitrophenol, azide and ouabain[9]. Further, light microscopy reveals minimal gross alterations in epithelial structure and electron microscopy discloses no alteration in brush border or "fuzzy coat" morphology following exposure to taurodeoxycholate. There are no obvious histological changes that could account for a 75 % reduction in alanine influx. Further, any major denudation would be difficult to reconcile with the observation that the permeability to mannitol is restored to normal values following exposure to taurodeoxycholate-free solutions. Two explanations for the action of taurodeoxycholate, which are consistent with detergent actions on lipid and lipoprotein structures, are suggested. First, taurodeoxycholate may irreversibly damage membrane structure or membrane components involved in carrier-mediated influx processes. Second, it may actually remove these components from the membrane structure through its detergent action. In view of the quantities of protein that are recovered in the incubation solution, it is intriguing to speculate that the observed inhibitions may be due to solubilization of membrane components involved in carrier-mediated transport. This possibility is currently under investigation.

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Infrared spectroscopic measurements of phosphatidyl ethanolamine–water liquid crystals

It is well known that phospholipids can exist in liquid crystalline form. CHAPMAN[1] examined the infrared spectrum of DL-α-dipalmitoyl ethanolamine in a KBr disc as a function of temperature from —186 to +140°. The spectrum at low temperature resembled that of a crystalline solid, showing splitting of bands in various spectral regions. This splitting disappeared as the temperature was raised to 140°. At 140° the spectrum resembled that of a fluid rather than a solid phase.

Recent studies[2–4] on many phospholipid–water systems by X-ray diffraction and electron microscopy indicate that these systems exist in a number of well-defined mesomorphic phases (lamellar, hexagonal, cubic). In particular, studies on the phase transitions observed for the phosphatidyl ethanolamine–water system show the existence of the following mesomorphic phases: (1) a lamellar phase at 20°; (2) an inverted hexagonal phase at 55°; (3) at 25–35° simultaneous coexistence of the lamellar and hexagonal phases over a wide concentration range.

In this paper, we report the first infrared spectra of phosphatidyl ethanolamine–water phases (20% water). The phosphatidyl ethanolamine was supplied by Pierce Chemical Co. (bacterial, chromatographically pure), a white powder which darkens at 170°. It was primarily a mixture of palmitic and oleic acid esters, with small amounts of stearic and linoleic acid chains also present. The average molecular weight was 725, slightly lower than that reported by REISS-HUSSON[3], who did not specify hydrocarbon chain composition but only reported a mean molecular weight of 748. Results from electron microscopy[2] have not indicated which phosphatidyl ethanolamine was used in that study, making comparison of results somewhat uncertain. The spectral results obtained lend further strong support to the existence of liquid crystalline phases in these systems, and give some specific evidence regarding the nature of these phases.

Infrared spectra were obtained using a Perkin–Elmer Model 521 double beam grating spectrophotometer. The sample, sandwiched in a thin film (about 10 μ) between Irtran plates, was examined as a function of temperature using a Barnes