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Effect of Sodium Taurodeoxycholate on Biological Membranes: Release of Phosphorus, Phospholipid, and Protein from Everted Rat Small Intestine

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Abstract ☐ Sodium taurodeoxycholate accelerates the release of total phosphorus, lipid phosphorus, and protein from isolated everted rat small intestinal sacs. The effect occurs at concentrations of the physiological surfactant above the CMC. The effect of sodium taurodeoxycholate on components of the biological membrane can be related to an increase in permeability of the everted intestine to phenol red in the presence of various concentrations of the surfactant. The interaction of the surfactant with the biological membrane may produce an acceleration of the loss of structural integrity of the preparation, resulting in an increased permeability to phenol red.

Keyphrases ☐ Sodium taurodeoxycholate—effect on biological membranes, release of phosphorus, phospholipid, and protein from the everted rat small intestine, loss of structural integrity proposed ☐ Membranes, biological, everted rat small intestine—effect of sodium taurodeoxycholate on release of phosphorus, phospholipid, and protein ☐ Surfactants—effect of sodium taurodeoxycholate on release of phosphorus, phospholipid, and protein from the everted rat small intestine ☐ Permeability, everted rat small intestine—effect of sodium taurodeoxycholate on transport of phenol red, release of phosphorus, phospholipid, and protein from membrane, loss of structural integrity proposed

Previous investigators (1–7) showed an increase in drug absorption across biological membranes in the presence of physiological surfactants. An increase was found in the rate of transfer of salicylate (1) and salicylamide (2) across the everted rat small intestine in the presence of micellar sodium taurodeoxycholate. Absorption of orally administered riboflavin in man increased upon coadministration of sodium deoxycholate, an unconjugated bile salt (3). The GI absorption of phenol red in the rat increased when phenol red was administered with sodium deoxycholate (4). Increased absorption of a number of drugs in the presence of bile salts was reported (5, 6) using an *in situ* perfusion technique. The influence of sodium taurodeoxycholate on the pharmacological effect (time required to produce overturn) of pentobarbital and ethanol in goldfish was studied (7). All these effects are presumably due to an alteration in the permeability of the biological membrane to these drugs by the physiological surfactant.

The exact mechanism of action of the physiological surfactants in increasing the permeability of biological membranes has not been elucidated, although it was postulated (1, 2, 4) that the mechanism may involve changes in membrane structure, possibly by solubilizing lipid components of the membrane. Bile salts were shown to solubilize phospholipids (8), a major component of biological membranes (9).

In a later study (10), the addition of lecithin, a phospholipid, or a mixture of fatty acids and esters to the micellar bile salt solution resulted in a reduction of the permeability effect of the bile salt on the everted rat intestine to salicylate ion. It was, therefore, decided to

Table I—Total Phosphorus Released from Everted Rat Small Intestine as a Function of Time and Mucosal Concentration of Sodium Taurodeoxycholate

Solution	Total Phosphorus Released, mcg.[a]			
	30 min.	60 min.	90 min.	120 min.
Buffer	3.17 (1.01)	4.10 (1.14)	4.99 (1.40)	7.63 (2.12)
1 mM[b]	4.51 (0.98)	5.88 (0.55)	7.26 (0.23)	8.69 (1.29)
2.5 mM[b]	5.02 (5.50, 4.56)[c]	7.50 (7.52, 7.48)[c]	9.29 (0.56)[d]	11.1 (0.65)[d]
5 mM[b]	6.17 (0.60)	9.60 (0.39)	13.3 (1.94)	14.3 (1.80)
10 mM[b]	8.62 (1.42)	13.1 (1.32)	14.4 (2.31)	16.1 (2.08)

[a] Mean of at least 10 determinations ± SD. [b] Sodium taurodeoxycholate. [c] Mean of two determinations. [d] Mean of four determinations.

investigate the effect of sodium taurodeoxycholate on the release of components from biological membranes, using the everted rat small intestine as a model biological membrane.

EXPERIMENTAL

Materials—Sodium taurodeoxycholate[1] was certified as chromatographically pure. All other reagents were analytical grade.

Modified Krebs bicarbonate buffer, with no potassium dihydrogen phosphate included, was made by dissolving the sodium chloride, potassium chloride, and sodium bicarbonate in distilled water. The pH of the solution was adjusted to pH 7.4 with dry ice. When sodium taurodeoxycholate was included in the buffer, it was dissolved before the pH was adjusted with dry ice. In all cases the sodium-ion concentration was adjusted to 150 mM by the proper addition or omission of sodium chloride.

The Fiske–Subba Row (11) reagent was prepared by adding 0.125 g. of 1-amino-2-naphthol-4-sulfuric acid, with mechanical stirring, to 50 ml. of freshly prepared 15% sodium bisulfite (anhydrous), followed by 0.25 g. of anhydrous sodium sulfite. The solution was filtered, stored in a dark bottle in a refrigerator, and freshly prepared weekly.

Preparation of Everted Rat Intestinal Sacs—The procedure developed by Crane and Wilson (12) was used with the following modifications. Male, Sprague–Dawley descent rats[2], weighing 250–350 g. and fasted for 20–24 hr. (water allowed ad libitum), were anesthetized with ether. A midline abdominal incision was made, the entire small intestine was removed, and the rat was sacrificed. The intestine was rinsed with several portions of normal saline and cut 15 cm. from the pylorus. The 15-cm. portion was discarded and the intestine was everted over a glass rod. Four consecutive sacs were made by stretching the intestine with an 8-g. weight and tying off consecutive 5-cm. segments. The segments were separated and filled with 1 ml. of buffer at pH 7.4 through a syringe and blunt needle. The sacs were then individually incubated in a mucosal solution at 37° consisting of 20 ml. of buffer alone or buffer containing various concentrations of sodium taurodeoxycholate. The mucosal solution was oxygenated continuously with a mixture of 95% oxygen–5% carbon dioxide. One-milliliter samples of the mucosal solution were taken at 30-min. intervals for 2 hr.

Intestinal Transfer Rate Measurements—The Crane and Wilson (12) procedure was used with the modifications previously described (1). Two segments of everted intestine, each 10 cm. in length, were taken. Each segment was attached to a cannula as previously described (1) and was suspended in 80 ml. of mucosal solution, which consisted of buffer at pH 7.4 containing 0.1 mg./ml. phenol red in the presence or absence of various concentrations of sodium taurodeoxycholate. Samples of the serosal solutions were taken at appropriate intervals and assayed for phenol red content.

Assay Procedure—Total Phosphorus—The assay procedure described by Bartlett (13) was used with slight modifications. The mucosal samples were diluted to 10 ml. with distilled water. One-milliliter aliquots were taken and placed in 15-ml. conical centrifuge tubes, and 0.5 ml. of 10 N sulfuric acid was added to each tube. The tubes were placed in an oven at 160° for 3 hr. After the initial 3-hr. heating period, 3 drops of 30% hydrogen peroxide were added to each tube and the tubes were returned to the oven for another 3 hr. at 160°. After the samples were allowed to cool, 4.4 ml. of dis-

tilled water, 0.2 ml. of 5% ammonium molybdate, and 0.2 ml. of Fiske–Subba Row reagent were added. The samples were then heated for 10 min. at 100° and allowed to cool, and the solution was read at 830 nm. on a colorimeter[3] equipped with a red-sensitive phototube. A calibration curve was made by dissolving a known amount of potassium dihydrogen phosphate in distilled water and diluting appropriately to various concentrations of phosphorus equivalents. Each solution was assayed for phosphorus content by the assay previously described, and a calibration curve was constructed. Sodium taurodeoxycholate did not interfere with the assay procedure.

Phospholipid Phosphorus—Phospholipids in the mucosal solution were assayed by a modification of the Zilversmit and Davis (14) method. To 1 ml. of the mucosal solution in a centrifuge tube, 3 ml. of 10% trichloroacetic acid was added drop by drop with swirling. The tubes were allowed to stand for 2 min. and then were centrifuged at approximately 2000 r.p.m. for 5 min. The supernate was decanted and the precipitate was washed twice with 3-ml. portions of 10% trichloroacetic acid. After centrifugation and decantation of the supernate, 1 ml. of distilled water and 0.5 ml. of 10 N sulfuric acid were added to each tube and the assay procedure for total phosphorus was followed as outlined previously.

Protein Determinations—Protein determinations of the mucosal samples were made by the method of Lowry et al. (15). The solutions were read[3] at 750 nm. against an appropriate blank. Sodium taurodeoxycholate did not interfere with the assay procedure.

Phenol Red Assay—The serosal samples were diluted appropriately with distilled water alkalinized with 3 N sodium hydroxide, filtered[4] if necessary, and assayed for phenol red spectrophotometrically at 560 nm. Neither sodium taurodeoxycholate nor "blank" serosal fluid interfered with the assay procedure.

RESULTS AND DISCUSSION

The effect of sodium taurodeoxycholate on the release of total phosphorus from the everted rat intestine as a function of bile salt concentration and time is shown in Table I. At all time periods studied, with increasing concentrations of sodium taurodeoxycholate there was an increase in the total amount of phosphorus released from the everted intestinal sac. Comparison of the total amount of phosphorus released at 2 hr. after incubation reveals that at all concentrations of sodium taurodeoxycholate above 2.5 mM the amount of phosphorus in the mucosal fluid was significantly[5] different than that of control values. No statistical difference existed between control studies and the 1 mM concentration of sodium taurodeoxycholate as determined by the Student t test (16).

Since a possible mechanism for the increase in permeability of the everted rat intestine to drugs after exposure of the membrane to bile salts involves the interaction of the surfactant with lipid components of the biological membrane, the release of lipid-associated phosphorus from the everted intestinal sac in the presence of various concentrations of sodium taurodeoxycholate was measured. The results (Table II) show an increase in the amount of lipid phosphorus released from the everted rat intestine at 2 hr. after incubation in mucosal solutions containing sodium taurodeoxycholate in concentrations above 2.5 mM when compared to the control buffer and 1 mM bile salt solution.

Since biological membranes consist of both lipid and protein, it

Table II—Lipid Phosphorus Released from Everted Rat Small Intestine as a Function of Time and Mucosal Concentration of Sodium Taurodeoxycholate

Solution	Lipid Phosphorus Released, mcg.[a], at 120 min.
Buffer	0.34 ± 0.12
1 mM^b	0.53 ± 0.20
2.5 mM^b	0.77 (0.74, 0.80)[c]
5 mM^b	1.55 ± 0.41
10 mM^b	2.32 ± 0.39

[a] Mean of four determinations ± SD. [b] Sodium taurodeoxycholate. [c] Average of two determinations.

was important to determine if sodium taurodeoxycholate had an effect on the protein composition of the everted rat intestinal sac. The released protein was measured as bovine serum albumin equivalents (Table III). Table III lists the milligrams of protein released from the everted intestinal sac at 60 and 120 min. after incubation as a function of sodium taurodeoxycholate concentration. As can be seen from the data, with increasing concentration of sodium taurodeoxycholate there was an increase in the total amount of protein released.

The next phase of the study examined the influence of sodium taurodeoxycholate on the transfer of a drug across the everted rat small intestine. This was done to determine if any correlation existed regarding the release of membrane components and the alteration in permeability of the membrane to a drug substance. Phenol red was chosen as the model drug, and the effect of various concentrations of sodium taurodeoxycholate on the transfer of the drug across the everted rat intestine was examined (Table IV). The total amount of phenol red transferred across the intestinal segments at 60 and 120 min. increased with increasing concentrations of sodium taurodeoxycholate. The increase in the amount of phenol red transferred in 2 hr. was significantly different from control values for all bile salt concentrations except 1 mM sodium taurodeoxycholate.

The data presented in Table V show the ratio of the total amount of membrane component released after a 2-hr. exposure to various concentrations of sodium taurodeoxycholate compared to the amount released in control studies. The increase in the release of membrane phosphorus, lipid phosphorus, and protein corresponded to the increased permeability of the everted intestine to phenol red. In all cases except for the protein experiments, the amount of membrane component released in the presence of 1 mM sodium taurodeoxycholate was not significantly different from control values as determined by the Student t test (16). Similar findings can be seen in the phenol red permeability measurements. The ratio of total amount of membrane component released after a 2-hr. exposure to various concentrations of sodium taurodeoxycholate plotted against the ratio of total amount of phenol red transferred in 2 hr. for each bile salt concentration is presented in Fig. 1. At 2.5, 5, and 10 mM concentrations of sodium taurodeoxycholate, a relationship between total phosphorus, lipid phosphorus, or protein released from the membrane and permeability of the membrane to phenol red appears to exist.

The critical micelle concentration (CMC) of sodium taurodeoxycholate is approximately 1.2 mM at 37° (17). Thus, it appears that the effect of the bile salt on the release of membrane phosphorus and lipid phosphorus occurs at concentrations of the surfactant above the CMC. This effect on permeability at concentrations of

Table III—Protein Release from Everted Rat Small Intestine as a Function of Time and Mucosal Concentration of Sodium Taurodeoxycholate

Solution	Protein, mg.[a,b] 60 min.	120 min.
Buffer	3.61 ± 0.14	5.44 ± 0.12
1 mM^c	3.71 ± 0.17	6.55 ± 0.12
2.5 mM^c	5.99 ± 0.11	9.69 ± 0.15
5 mM^c	7.22 ± 0.23	11.7 ± 0.11
10 mM^c	10.5 ± 0.20	12.1 ± 0.21

[a] Mean of five determinations ± SD. [b] Calculated as bovine serum albumin equivalents. [c] Sodium taurodeoxycholate.

Table IV—Cumulative Amount (Micrograms) Phenol Red Transferred at 60 and 120 min. in the Presence of Various Concentraions of Sodium Taurodeoxycholate

Solution[a]	Cumulative Amount, mcg.[b] 60 min.	120 min.
Control (7)	36.5 ± 3.8	118 ± 12
1 mM^c (4)	39.2 ± 2.8	120 ± 1.8
2.5 mM^c (5)	45.7 ± 3.4	179 ± 13.1
5 mM^c (5)	52.2 ± 13.2	211 ± 8.3
10 mM^c (6)	54.9 ± 6.0	229 ± 38

[a] Number of experiments in parenthesis. [b] Mean ± SD. [c] Sodium taurodeoxycholate.

Table V—Ratio of Total Amount of Membrane Component Released after a 2-hr. Exposure to Various Concentrations of Sodium Taurodeoxycholate

Solution	Ratio (Sodium Taurodeoxycholate/Control) Total Phosphorus	Lipid Phosphorus	Protein	Phenol Red
1 mM^a	1.14[b]	1.55[b]	1.20	1.02[b]
2.5 mM^a	1.46	2.26	1.78	1.52
5 mM^a	1.88	4.56	2.16	1.79
10 mM^a	2.11	6.82	2.22	1.94

[a] Sodium taurodeoxycholate. [b] Not significantly different than control values ($p > 0.05$).

surfactant above the CMC was reported previously (1, 2, 10) and occurred in the transfer of phenol red in the present study.

A previous report (10) presented data to show that the addition of phospholipid or fat digestion products to micellar bile salt solutions results in a decreased permeability response by the everted rat intestine in the presence of the bile salt. To determine whether the addition of phospholipid to the micellar bile salt solution would have an effect on the release of membrane components, 10 mM egg lecithin was added to 10 mM sodium taurodeoxycholate and the release of protein from the intestinal sac was measured. The results are presented in Fig. 2 and clearly show that the addition of lecithin to the micellar bile salt solution results in a decreased amount of protein released when compared to the bile salt alone. This is in good agreement with results from previous permeability experiments (10).
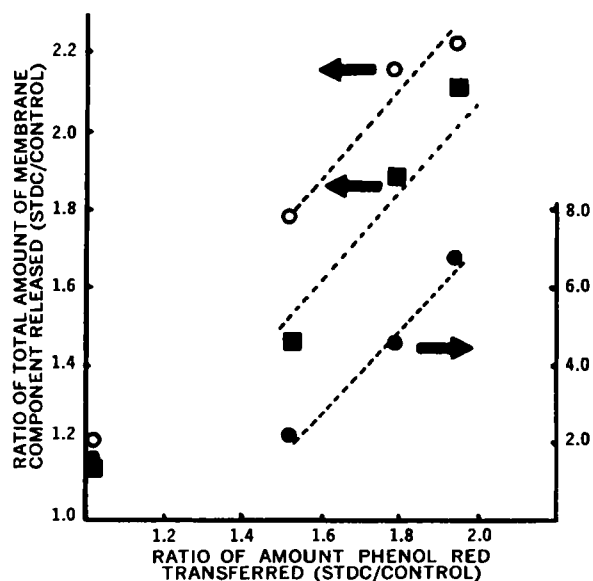
Figure 1—*Plot of the ratio of membrane total phosphorus (■), lipid phosphorus (●), and protein (○) released in 2 hr. in various concentrations of sodium taurodeoxycholate (STDC) to that in controls versus the ratio of phenol red transferred in 2 hr. in various concentrations of sodium taurodeoxycholate to that of controls.*
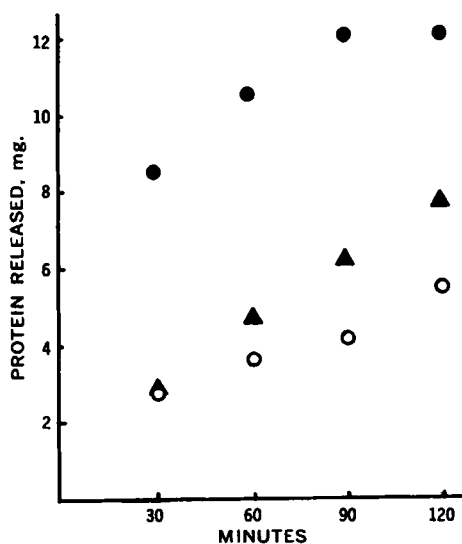
Figure 2—*Plot of total amount of protein released from the everted rat intestinal sac versus time in the presence of sodium taurodeoxycholate, 10 mM (●); sodium taurodeoxycholate, 10 mM + egg lecithin, 10 mM (▲); and control buffer (○).*

It appears that sodium taurodeoxycholate alters the composition of the everted rat intestinal membrane by producing an efflux of membrane components from the everted sac preparation. A similar effect by detergents on a microsomal fraction from mammalian brain was reported by Bradford *et al.* (18). These workers found that sodium deoxycholate, digitonin, and cetrimonium (cetyltrimethylammonium) bromide increased the percentage of protein, cholesterol, and phospholipid phosphorus recovered in the supernatant liquid of microsomal suspensions from brain tissue. The mechanism of action was postulated to involve interaction of the surfactant with the membrane lipo-protein complex. Moreover, Bradford *et al.* (18) did not observe significant increases in recovery of protein, phospholipid phosphorus, or cholesterol until the CMC of each surfactant was exceeded.

The alteration of the biological membrane in the presence of micellar concentrations of sodium taurodeoxycholate appears to be responsible for the increased permeability of the everted rat intestinal membrane to certain drugs after exposure to the physiological surfactant. The exact mechanism of the interaction is still unknown but most likely involves interaction of the bile salt with the protein and/or phospholipid components of the membrane. Recent work (19, 20) suggests a strong possibility for the observed effects; an increase in clearance values of polar drugs across the everted rat intestine over a 2-hr. period as compared to the initial 30-min. values was observed. The increase in rate of clearance of the compounds was partly due to a loss of functional integrity of the preparation. Compounds demonstrating initial clearance values of >1 ml./hr. showed little change in permeability over the entire 2 hr.

Previous studies with sodium taurodeoxycholate show little alteration in the permeability of the everted rat intestine to salicylamide (2) after exposure of the preparation to the bile salt, while a significant effect was noted with salicylate (1) and riboflavin (3). The clearance of salicylamide is 2.8 ml./hr., salicylate clearance is

0.7 ml./hr., and riboflavin clearance is approximately 0.3 ml./hr. (19). Phenol red has an initial 30-min. clearance value of 0.1 ml./hr. Therefore, the effect of the physiological surfactant on the biological membrane is consistent with the previously reported data (19, 20). Furthermore, Levine *et al.* (21) reported a progressive loss of structural integrity of everted rat intestinal sacs after eversion and incubation in oxygenated buffer. It is possible that the effect of sodium taurodeoxycholate on the permeability of the everted rat intestine to polar water-soluble drugs may be due to an acceleration by the surfactant of the loss of structural integrity of the preparation. This remains to be confirmed.

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