Physiologic Surface-Active Agents and Drug Absorption III: Effect of Bile Salt on Drug Absorption in Goldfish

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Abstract □ Sodium taurodeoxycholate significantly increases the uptake of nonionized 4-aminoantipyrine in goldfish. Studies of drug uptake as a function of bile salt concentration suggest that sodium taurodeoxycholate exerts an essentially all-or-none effect which is consistent with a mechanism involving rapid adsorption of the bile salt on the external membranes of the fish and consequent alteration of membrane permeability when a minimum "surface" concentration is reached. Evidence is also presented to demonstrate that the adsorption of 4-aminoantipyrine, in the presence and absence of sodium taurodeoxycholate, involves a passive process.

Keyphrases □ Absorption, drug—physiologic surfactant effect □ Taurodeoxycholate, Na—nonionized 4-aminoantipyrine absorption □ Goldfish—4-aminoantipyrine absorption □ Transfer, passive—4-aminoantipyrine

The influence of surfactants on biologic membranes and drug absorption has been extensively studied (1). Bile salts, normally present in the gastrointestinal tract, share many of the physical-chemical and biological properties of commercial surfactants. Accordingly, many studies have demonstrated that bile salts affect the integrity of various biologic membranes and thereby enhance drug absorption (2–4).

The use of goldfish as a test animal for assessing absorption phenomena has been proposed by Levy and Gucinski (5). Studies have shown that polysorbate 80, in concentrations below the critical micelle concentration, significantly decreases the time required for secobarbital and pentobarbital-induced death in the goldfish (6). This effect was attributed to membrane alteration by the surfactant resulting in more rapid uptake of the barbiturates, and a decrease in the time required to reach a lethal concentration in the fish. Similar effects on ethanol and pentobarbital-induced overturn time were observed upon exposure of goldfish to sodium taurodeoxycholate (7). However since sodium taurodeoxycholate is toxic to goldfish the possibility exists that the bile salt may produce some pharmacologic effect that is inadequate to promote overturn but makes the fish more susceptible to the pharmacologic action of ethanol and pentobarbital. Hence it was desirable to investigate more directly the influence of sodium taurodeoxycholate on membrane permeability. This was accomplished in the present study by determining the effect of sodium taurodeoxycholate on the uptake of 4-aminoantipyrine by the goldfish.

EXPERIMENTAL

Goldfish, *Carassius auratus*, common variety, weighing about 3–6 g. were used as the test animal. All fish in a given experiment were from the same lot. Two different lots of fish were used during the study. Sodium taurodeoxycholate (Maybridge Research Chemicals) and 4-aminoantipyrine (Eastman Organic Chemicals) were used. The drug or drug and bile salt were dissolved in water and the solutions were adjusted to pH 6.1, with either 0.01 *N* HCl or NaOH, so that 4-aminoantipyrine (pKa = 4.1) could be studied in the nonionized form. After each experiment the pH of the solution was again measured and found to be 6.1 ± 0.1.

Absorption of 4-aminoantipyrine in goldfish was determined according to Levy and Miller (8) except for the following modifications. Four fish were placed simultaneously in 1 l. of test solution for 15 min., then rinsed for 5 sec. in distilled water and stored in individual containers in a freezer until assayed (within 24 hr).

The method used for the determination of 4-aminoantipyrine in solution and in fish tissues was the colorimetric method of Brun (9) as modified by Levy and Miller (8). Blank values were obtained from tissues of fish which had been immersed in water or water with varying concentrations of sodium taurodeoxycholate for 15 min., rinsed, and frozen until assayed. There was no difference in the blank values of tissues from fish exposed to water with bile salt or water alone. Therefore, an average blank of 20 mcg. 4-aminoantipyrine equivalent/g. fish tissue was used to correct all analytical data.

Drug recovery determinations were carried out by immersing fish in water or water with 10 m*M* sodium taurodeoxycholate for 15 min., rinsing, and then homogenizing the fish with known amounts of drug and assaying as described above. Recovery data are reported in Table I. Sodium taurodeoxycholate pretreatment did not affect drug recovery and the average recovery (42 fish) was 103%. A further assessment of total drug recovery involved immersing four fish in 20 ml. of a 10-mg. % 4-aminoantipyrine solution (adjusted to pH 6.1) for 1 hr., rinsing, and assaying both the fish and the solution for drug content. A quantity representing 99.6% of the drug lost from the solution was recovered in the goldfish. Accordingly, the recovery of 4-aminoantipyrine from goldfish tissues was considered to be complete and the apparent drug uptake values were simply corrected for blank values.

RESULTS

The uptake of drug in goldfish immersed in 250 mg. % 4-aminoantipyrine with varying concentrations of sodium taurodeoxycholate is reported in Table II. Bile salt concentrations of 1 and 3 m*M* significantly increased the absorption of 4-aminoantipyrine. A plot of 4-aminoantipyrine uptake *versus* log sodium taurodeoxycholate concentration is shown in Fig. 1. The curve follows a typical dose-response pattern and it is evident from the large change in slope over a relatively small concentration range (*viz.*, 0.5 to 1 m*M*) that the effects of sodium taurodeoxycholate on drug uptake represent a practically "all-or-none" phenomenon. Further characterization of the curve at higher bile salt concentrations could not be

Table I—Recovery of 4-Aminoantipyrine from Goldfish Tissue

Control[a]		Sodium Taurodeoxycholate[b]	
Amount of Drug Added per Fish, mg.[c]	Recovery, %	Amount of Drug Added per Fish, mg.[c]	Recovery, %
0.3 (5)	113	0.4 (4)	104
0.5 (2)	98	0.7 (3)	113
0.6 (4)	104	1.0 (4)	108
0.9 (4)	97	1.5 (4)	98
1.0 (4)	97	2.5 (4)	101
2.0 (4)	100		

[a] Fish immersed in distilled water at pH 6.1 for 15 min. prior to homogenization. [b] Fish immersed in 10 m*M* bile salt solution (adjusted to pH 6.1) for 15 min. prior to homogenization. [c] Number of individual fish used per drug level is noted in parentheses.

Table II—Effect of Sodium Taurodeoxycholate on 4-Aminoantipyrine Uptake in Goldfish Immersed for 15 min. in 250 mg. % Drug Solution at pH 6.1

Sodium Taurodeoxycholate Concn., m*M*.	No. of Fish	Mean Drug Uptake ±1 *SD*, mcg./g. fish
Control	7	222 ± 26
0.1	8	175 ± 55
0.5	11	208 ± 22
1.0	12	340 ± 73[a]
3.0	8	351 ± 44[a]

[a] Results significantly different from control and from uptake at 0.1 and 0.5 m*M* bile salt, $p < 0.01$ (Student's *t* test).

obtained due to the toxicity of sodium taurodeoxycholate to the fish. Concentrations of 3 m*M* bile salt caused an obvious decrease in fish activity and occasional overturn. Higher concentrations produced overturn or death within 15 min.

Drug uptake, in a second lot of fish, as a function of 4-aminoantipyrine concentration is shown in Table III and the lower portion of Fig. 2. A linear relationship is observed between uptake and drug concentration which may be described by the least-squares regression equation: uptake (mcg./g. fish) = 0.74 × drug concentration (mg. %) + 10.6, with a correlation coefficient of 0.983. Drug uptake in fish immersed in drug solution with 1 m*M* sodium taurodeoxycholate was enhanced at all drug levels. Differences were statistically significant at 250, 350, and 600 mg. % 4-aminoantipyrine (see Table III). A linear relationship was also observed between uptake and drug concentration in the presence of bile salt, as shown in the upper portion of Fig. 2. These data were fitted by the least-squares regression equation: uptake (mcg./g. fish) = 0.86 × drug concentration (mg. %) + 2, with a correlation coefficient of 0.988.

DISCUSSION

In the present study the minimum concentration range of sodium taurodeoxycholate required to elicit a change in membrane permeability was 0.5 to 1 m*M* (Fig. 1). Above this level, enhancement of drug uptake was independent of bile salt concentration over a threefold range. Levy and Anello (10) have demonstrated that the drug transfer rate-limiting barrier in the case of 4-aminoantipyrine is the external membranes (skin and gills) of the fish. Hence, sodium taurodeoxycholate seems to have a direct effect on the permeability characteristics of these membranes. The mechanism appears to involve a rapid adsorption of sodium taurodeoxycholate on the external membranes of the fish and consequent alteration of membrane permeability (7). However, it would appear that a minimum surface concentration of bile salt is required to elicit disruption of the membrane and further adsorption of bile salt molecules is of no consequence. An analogous mechanism has been proposed to explain the lytic effects of quaternary ammonium compounds on bacterial membranes (11). In each case it would seem that a minimum fraction of the surface must be covered with adsorbate molecules before membrane alteration occurs. Also in each case the membrane effects are simply a function of monomer concentration. Sodium taurodeoxycholate exists only as the monomer over the concentration range and experimental conditions used in the present study (12).

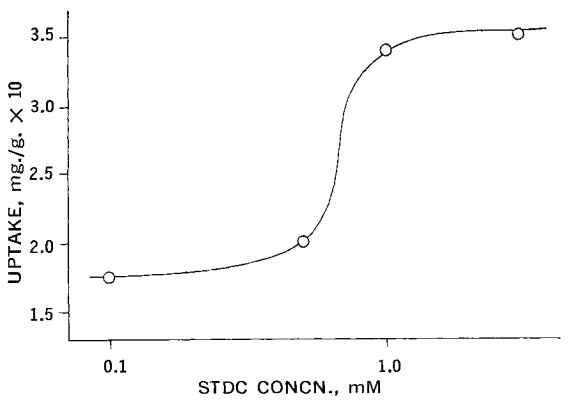

Figure 1—*Log dose-response type plot showing the uptake of 4-aminoantipyrine in goldfish immersed for 15 min. in 250 mg.% drug solution at pH 6.1 as a function of sodium taurodeoxycholate concentration (log scale).*

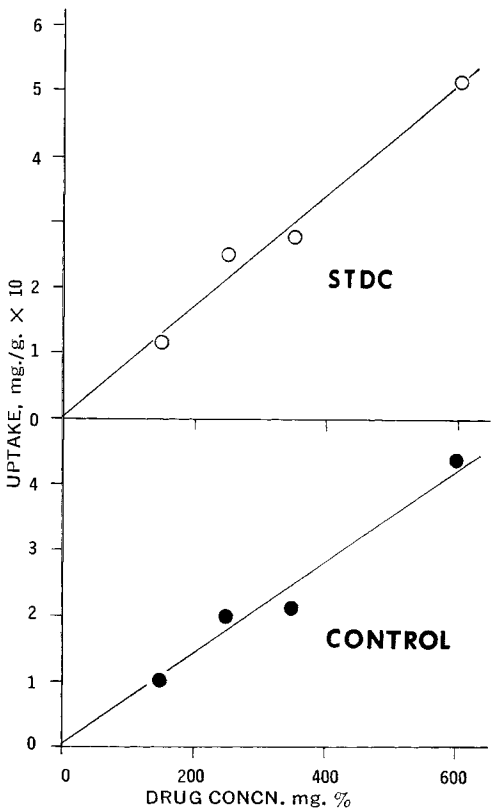

Figure 2—*Uptake of 4-aminoantipyrine in goldfish immersed for 15 min. in drug solution at pH 6.1 as a function of drug concentration in the presence of 1 m*M *sodium taurodeoxycholate (upper plot) and in the absence of bile salt (lower plot).*

Quantitative changes in the dose-response plot shown in Fig. 1 are anticipated from lot-to-lot of fish due to differences in size as well as susceptibility of the fish to surfactant. Earlier studies with a particular lot of fish showed that 0.1 m*M* sodium taurodeoxycholate significantly enhanced the uptake of 4-aminoantipyrine (7). These findings are in contrast to those of the present study where concentrations of up to 0.5 m*M* bile salt were without effect on drug uptake. Also of interest however is the fact that the earlier lot of goldfish were significantly more sensitive to the bile salt and concentrations of 0.5 m*M* sodium taurodeoxycholate produced death within minutes after immersion. This enhanced susceptibility may be related to differences in the fraction of biologic surface required to be covered before membrane alteration occurs.

Further evidence that the transfer of 4-aminoantipyrine is a passive process and that sodium taurodeoxycholate has a direct

Table III—Effect of Sodium Taurodeoxycholate (1 m*M*) on 4-Aminoantipyrine Uptake in Goldfish Immersed for 15 min. in Drug Solution at pH 6.1

4-Aminoantipyrine Concn., mg. %	Control: No. of Fish	Control: Mean Drug Uptake ± 1 *SD*, mcg./g. fish	Sodium Taurodeoxycholate: No. of Fish	Sodium Taurodeoxycholate: Mean Drug Uptake ± 1 *SD*, mcg./g. fish
150	12	102 ± 18	12	113 ± 24
250	8	196 ± 40	7	248 ± 30[a]
350	8	208 ± 30	4	275 ± 27[a]
600	12	439 ± 84	12	517 ± 54[a]

[a] Significantly different from control values, $p < 0.05$ (Student's *t* test).

effect on the absorbing membranes is shown in Table II and Fig. 2. Levy and Miller have shown that under conditions analogous to those used in the present study (*viz.*, drug concentration in the external medium is very much higher than in the fish tissue and the volume of the external medium is sufficiently large so that drug concentration in this medium is essentially constant throughout the experiment) the absorption of 4-aminoantipyrine follows apparent zero-order kinetics where the first-order absorption rate constant (k_a) may be calculated from the relationship k_a = absorption rate/drug concentration in the external medium. Assuming a passive absorption process, it follows that the absorption rate or uptake of drug per fixed interval of time is directly proportional to drug concentration in the fluids bathing the fish. Evidence of this relationship is shown in the lower portion of Fig. 2. The mean absorption rate constant calculated from these data is 4.9×10^{-3} min.$^{-1}$ which is in excellent agreement with previously obtained values values (8).

The effect of 1 m*M* sodium taurodeoxycholate on the absorption of 4-aminoantipyrine by goldfish immersed in solutions at various drug concentrations is shown in Table III and the upper plot of Fig. 2. Again a linear relationship is noted between drug uptake and 4-aminoantipyrine concentration in the external medium which is suggestive of a passive drug transport process even in the presence of the surfactant. These data provide further evidence that the bile salt acts *via* a direct effect on the permeability characteristics of the external membranes rather than by interacting with drug since the degree of enhancement of 4-aminoantipyrine absorption is independent of drug concentration in the external medium.

The maximum effect of sodium taurodeoxycholate on 4-aminoantipyrine uptake was observed in the bile salt dose-response study (Table II, Fig. 1). Concentrations of 1 and 3 m*M* bile salt resulted in a 1.5 to 1.6-fold increase in drug uptake. A similar degree of enhancement of 4-aminoantipyrine uptake in goldfish was observed in the presence of 0.01% polysorbate 80 (10). The effect of 1 m*M* sodium taurodeoxycholate on the second lot of fish, as noted in Table III, resulted in only a 1.2-fold increase in drug uptake. These findings illustrate the considerable lot-to-lot variations frequently observed in studies utilizing goldfish (8). The differences between lots with respect to the degree of bile salt effect probably reflect differences in membrane susceptibility, as discussed above. It is likely that in the second lot of fish the dose-response curve shown in Fig. 1 is shifted to the right so that a 1 m*M* bile salt concentration does not produce a maximal effect.

The present results provide direct evidence of the influence of sodium taurodeoxycholate on the external membranes of the goldfish and further, support the use of pharmacologic effect endpoints to assess the absorption rate of drugs in goldfish (5, 10, 13).

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Comparative Chemical and Toxicological Evaluation of Residual Ethylene Oxide in Sterilized Plastics

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Abstract □ The use of various gases for the sterilization of nondisposable and disposable plastic medical devices has become firmly established in the past decade and a half. However, the subject of sterilant residues remaining in plastic devices following an ethylene oxide gas sterilization has only recently been investigated. This study correlates ethylene oxide residues as determined by gas chromatography to the ability of these residues in polyolefins to elicit toxicological responses in several biological systems. A comparison of the results from a quantitative hemolysis test, rabbit intramuscular implantations with subsequent pathological investigation, cell culture responses, a series of fish tests, and intradermal irritation studies, to the ethylene oxide desorption data, revealed that within 24 hr. after sterilization, no significant toxicities were produced by ethylene oxide-sterilized polyolefins. The concentrations of the gas had decreased from a high of 3.20 mg./g. of plastic to as little as 0.20 mg./g. of plastic after aeration at room temperature for 24 hr.

Keyphrases □ Plastics, sterilized—ethylene oxide (EtO) residual □ Ethylene oxide residual, plastics—toxicity □ Guppy—toxicity determination, EtO residual □ Cell cultures—toxicity determination, EtO residual □ Intradermal irritation—toxicity determination, EtO residual □ Erythrocyte hemolysis—toxicity determination, EtO residual □ GLC—analysis

The toxicology of ethylene oxide (EtO) in both the liquid and gaseous phases has been well demonstrated in man and other animals (1–4). In addition, it has been shown that this epoxide is an effective sterilant for materials which cannot be sterilized by conventional high temperature methods (5). Recently, several reported studies have indicated that residual ethylene oxide in plastic medical devices may be implicated in toxicities