Physiologic Surface-Active Agents and Drug Absorption I: Effect of Sodium Taurodeoxycholate on Salicylate Transfer Across the Everted Rat Intestine

STUART FELDMAN* and MILO GIBALDI

Abstract □ Sodium taurodeoxycholate (STDC) increases the permeability of the everted intestine to salicylate. There appear to be two levels of effects; a small effect (30 to 50 percent increase in salicylate-transfer rates) at concentrations of STDC below or about the CMC and a more pronounced effect (100 to 125 percent increase in transfer rates) at concentrations of STDC above the CMC. Each level of effect is significantly different from control values and from each other but there is no significant concentration dependence within a given level. There is evidence that the site of the bile salt effect is more accessible from the serosal side of everted intestinal preparations than from the mucosal side.

Keyphrases □ Surfactants, physiologic—drug absorption effect □ Salicylate transfer—Na taurodeoxycholate effect □ Everted rat intestine—experimental technique □ Serosal, mucosal response—Na taurodeoxycholate □ Colorimetric analysis—spectrophotometer

For several years, work in this laboratory has been concerned with the role of physiologic surface-active agents in the gastrointestinal absorption of drugs. More recently, the authors have considered the possible influence of physiologic surfactants, *viz.* bile salts, on membrane permeability. During the course of preliminary studies of the effects of orally administered sodium deoxycholate and sodium taurodeoxycholate on gastric emptying and intestinal transit in the rat (1, 2), it was noted that these bile salts produced a marked increase in the permeability of the gastrointestinal membranes. Davenport (3) had reported similar effects in the canine gastric pouch exposed to sodium taurocholate. Studies in goldfish (4) also showed increased permeability in that sodium taurodeoxycholate was found to potentiate the pharmacologic effects of pentobarbital and ethanol and to enhance the absorption of 4-aminoantipyrine.

At this juncture it was decided to use the everted rat intestine which permits greater control of variables and greater experimental flexibility to elucidate the physical-chemical mechanism of the observed effects. This particular membrane has been used with marked success in elucidating the influence of complex formation on drug absorption (5–7). The advantages offered by this system are manifest in the references cited. The present report is concerned with effects of sodium taurodeoxycholate (STDC) on the transfer of salicylate ion across the everted rat intestine.

EXPERIMENTAL

Intestinal-Transfer Rate Measurements—The procedure developed by Crane and Wilson (8) was used with the modifications described by Reuning and Levy (5). Male Sprague-Dawley rats[1] weighing 190 to 250 g. (fasted 24 hr.) were anesthetized with ether. After a midline abdominal incision, the entire small intestine was removed and the rat was sacrificed. The intestine was immediately rinsed with several portions of cold normal saline and cut 15 cm. from the pylorus. The 15-cm. portion was discarded and the intestine was everted on a glass rod. Two segments from the proximal end, each 10 cm. in length (when stretched by an 8-g. weight) were obtained. The two consecutive segments were designated first and second intestinal segments, respectively. The proximal end of each segment was attached to a cannula and the distal end tied and attached to an 8-g. weight. An equal number of first and second intestinal segments were used in each phase of the study. Each segment was suspended in 80 ml. of mucosal solution which consisted of modified Krebs-phosphate buffer (KPB) (9), without calcium or magnesium, at pH 6.0. In addition, the mucosal solution contained 2.0 mg./ml. sodium salicylate and various concentrations of STDC. A pH of 6.0 was chosen so that salicylic acid with a pKa of 3 would be essentially 100% ionized and the transfer of salicylate ion could be studied. Since both salicylate and STDC are negatively charged at pH 6, little or no interaction between the two was expected. The pH of the solutions were checked before and after each experiment and did not vary by more than 0.1 pH unit. All solutions were adjusted to 150 m*M* Na^+ by the addition of sodium chloride. The mucosal solution was oxygenated continuously by a mixture of 95% oxygen–5% carbon dioxide at a rate of approximately 1 to 2 bubbles/sec. from a tube with an inside diameter of 1.6 mm. The entire system was maintained at 37 ± 0.1°.

After introduction of the everted intestine to the mucosal solution, 2 ml. of KPB, pH 6.0 (serosal solution) was placed in the intestinal segment. In a number of experiments the serosal solution also contained varying concentrations of STDC. The serosal solution was withdrawn at frequent intervals during the experiment and the serosa rinsed with 2 ml. of KPB or KPB containing STDC. The rinse was combined with the initial sample for analysis Another 2 ml. of solution was then placed in the intestine to serve as serosal solution for the next time interval. Each experiment was run over a 2-hr. period.

In certain experiments, the intestinal segments were incubated for 1 hr. in either KPB or KPB containing 10 m*M* STDC. After incubation, the segments were rinsed with KPB (prewarmed to 37°) and placed in fresh KPB solution containing 2.0 mg./ml. sodium salicylate. Samples were withdrawn over a 1-hr. period as described above.

The large mucosal volume and the maintenance of sink conditions (*i.e.*, salicylate concentration in the serosal solution never exceeded 15% of mucosal solution concentration), by frequent sampling and replacement of serosal solution, ensured an essentially constant concentration gradient. In no instance was more than 6% of the total amount of salicylate initially present in the mucosal solution transferred to the serosal side during the course of the experiment.

Assay Procedure—The serosal samples were acidified to pH 1 with 6 *N* HCl and sufficient 0.1 *N* HCl was added to bring the total volume to 5 ml. Five milliliters of Trinder's reagent (10) was then added and the solution filtered.[2] The filtered solution was analyzed in a spectrophotometer[3] at 540 mμ. The amount of salicylate in each sample was calculated by means of standard curves. Neither STDC nor blank serosal fluid interfered with the assay procedure.

Data Evaluation—Repeated determination of transfer rate constants under various experimental conditions revealed no

[1] Blue Spruce Farms, Altamont, N. Y.

[2] Millipore filter, 0.45μ.

[3] Hitachi-Perkin-Elmer, model 139.

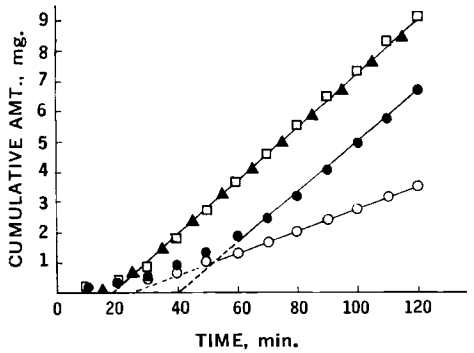

Figure 1—*Effect of micellar concentrations of STDC on salicylate transfer across the everted intestine of the rat. Plot shows cumulative amount transferred as a function of time. Key: ○, control; ●, 10 mM STDC; □, 50 mM STDC; △, 100 mM STDC.*

significant differences between the first and second segment. Accordingly, no distinction was made, with respect to segment, in evaluating and reporting the data. Statistical analysis was made using the Student's *t* test on both unpaired and paired (control *versus* test condition in the two intestinal segments of a single rat) data (11).

RESULTS

Effects of STDC Concentrations Above the CMC—The critical micelle concentration (CMC) of STDC, at 20 to 25° and under conditions of pH and ionic strength similar to those of the present study, is about 2 to 4 m*M* (12). The CMC at 37° is probably somewhat higher. Figure 1 shows a cumulative plot of the amount of salicylate transferred (mucosal to serosal) *versus* time from the KPB buffer and from mucosal solutions containing 10, 50, and 100 m*M* STDC.

The rates of salicylate transfer were obtained from a least-squares fit of the linear (or apparent steady state) region of the cumulative plots. This region was discerned in each case by plotting the rate of salicylate transfer *versus* time. Typical plots are shown in Fig. 2. The plateau indicates attainment of steady-state conditions with respect to the membrane. The steady-state transfer rates determined in this manner are reported in Table I.

The results show a greater than twofold increase i n the rate of salicylate transfer in the presence of micellar concentrations of STDC ($p < 0.001$). There were no significant differences between the transfer rates observed at different STDC concentrations above the CMC. However, there was a marked concentration effect on the apparent lag time (*i.e.*, the intercept on the *x*-axis as determined by extrapolation of the linear portion of the curve, as shown in Fig. 1). The lag time was reduced from about 37 min., in mucosal solutions containing 10 m*M* STDC, to about 20 min., in mucosal solutions containing 50 and 100 m*M* STDC.

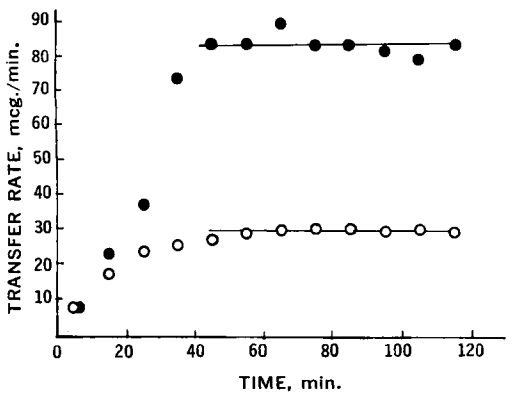

Figure 2—*Rate of salicylate transfer across the everted intestine of the rat. Key: ○, control; ●, 50 mM STDC. Solid lines denote steady state.*

Table I—Effect of STDC on Transfer Rate of Salicylate Across the Rat Small Intestine at pH 6.0

	No. of Intestinal Segments	Transfer Rate ±*SD*, mcg./min.	Lag Time ±*SD*, min.
Control	19	40 ± 5	22 ± 4
STDC, mucosal (m*M*)			
100	2	90 (87, 93)	19 (18, 20)
50	4	89 ± 10	21 ± 5
10	11	87 ± 10	37 ± 5
5	6	57 ± 9	33 ± 8
1	7	48 ± 7	18 ± 2
Incubation			
Control	4	40 ± 4	10 ± 2
STDC, 10 m*M*	4	85 ± 7	5 ± 2
STDC, mucosal-serosal, m*M*			
10	6	80 ± 12	15 ± 3
5	2	59 (59, 59)	9 (7, 11)
1	6	53 ± 5	13 ± 2

Effects of STDC Concentrations Below or About the CMC—Mucosal solutions containing 1 and 5 m*M* STDC produced considerably smaller changes in membrane permeability than those observed with solutions containing micellar concentrations of STDC. Differences between the effects at micellar and premicellar concentrations were significant at the 99.9% level of confidence. Typical plots are shown in Fig. 3 and the results are summarized in Table I. Statistical evaluation of the data suggests that the effect of 1 m*M* STDC on salicylate transfer is equivocal. Unpaired comparison of the steady-state rates with and without 1 m*M* STDC indicates significance at the 95% level of confidence, but paired comparison (four pairs) demonstrates no significant difference. The difference in rates between control solutions and solutions containing 5 m*M* STDC were highly significant based on either unpaired or paired analysis.

Effects of STDC Incubation on Salicylate Transfer—Figure 4 shows a cumulative plot of salicylate transfer after a one-hour incubation of the intestinal segments in drug-free mucosal solutions containing 10 m*M* STDC or KPB alone (control). The mean data are summarized in Table I. Salicylate transfer rates after incubation in 10 m*M* STDC were about equal to the rates observed when 10 m*M* STDC was initially present with the drug. In addition, transfer rates from control solutions were identical in both experimental procedures. Incubation resulted in a considerable reduction in lag time. This was evident in both control and STDC-treated segments.

Effects of STDC in Both Mucosal and Serosal Solutions—The rates of salicylate transfer from mucosal solutions, containing 1, 5, and 10 m*M* STDC, to serosal solutions containing equal concentrations of STDC are listed in Table I. Figure 5 shows salicylate transfer when both mucosal and serosal solutions contain 10 m*M* STDC and when STDC is present initially only in the mucosal solution. The data in Fig. 5 typify those observed at lower STDC concentrations. At each concentration studied there was no significant difference in rate of salicylate transfer when the effects of mucosal STDC were compared to those of mucosal-serosal STDC. However, as shown in Fig. 5 and as observed at each concentration studied (see Table I), STDC in the serosal solution, produced a marked decrease in the apparent lag time. Also of

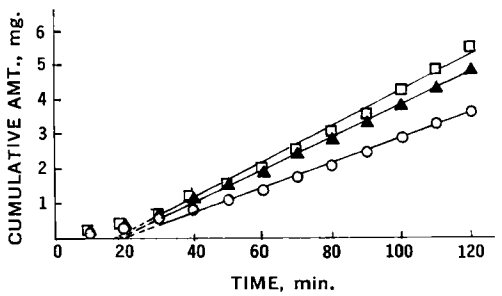

Figure 3—*Effect of STDC concentrations, below or about the CMC, on salicylate transfer across the everted intestine of the rat. Key: ○, control; △, 1 mM STDC; □, 5 mM STDC.*

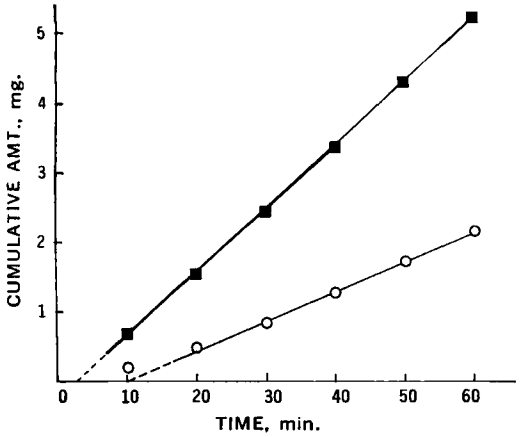

Figure 4—*Effect of incubation on salicylate transfer across the everted intestine of the rat. Prior to the determination of transfer rate, the intestine was incubated for 1 hr. in control mucosal solution* (○) *or mucosal solution containing 10 m*M *STDC* (●).

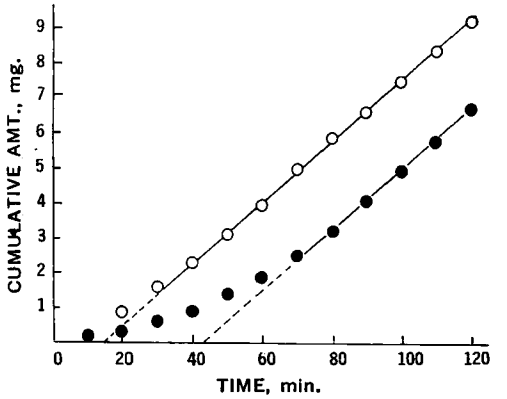

Figure 5—*Effect of STDC in mucosal and serosal solutions on salicylate transfer across the everted intestine of the rat. Key:* ○, *10 m*M *STDC, mucosal-serosal;* ●, *10 m*M *STDC, mucosal solution alone.*

interest is the fact that 1 m*M* STDC in both mucosal and serosal solutions results in salicylate transfer rates which are significantly different from control levels on the basis of either paired (four pairs) or unpaired analysis.

DISCUSSION

The preceding data confirm earlier observations (1–4) of the effects of bile salts on membrane permeability. These effects have now been demonstrated with a number of different biologic membranes. The present study suggests that the modification of membrane permeability by bile salts is a very complex process, at least in the everted intestinal preparation. The site of action of the bile salts, with respect to increasing transfer rate, appears to be somewhat beyond the mucosal surface. For example, placing 5 and 10 m*M* STDC on both sides of the intestinal membrane results in salicylate-transfer rates identical to those observed when STDC is initially present only in the mucosal solution. However, STDC in the serosal solution results in a significant decrease in the apparent lag time when the two situations are compared. This finding suggests that the site which bile salts affect in modifying membrane permeability is more accessible from the serosal side of the everted intestinal preparation than from the mucosal side. A number of observations made in the present study support this conclusion. Among these is the fact that in paired segments of the intestine, 1 m*M* STDC in the mucosal solution had no significant effect on salicylate transfer. However, in similar experiments where 1 m*M* STDC was present in both serosal and mucosal solutions, a small but highly significant effect on transfer rate was noted. It is also of interest to point out that the abrupt increase in salicylate transfer in the presence of mucosal STDC appeared to coincide with the appearance of bile salt in the serosal solution as judged by foaming.

The concept that there may be different permeability coefficients for mucosal to serosal and serosal to mucosal transfer, respectively, has recently been considered by Benet (13). Newey *et al.* (14) have noted that the inhibitory effects of ouabain on intestinal active transport of amino acids are evident when ouabain is placed in the serosal solution but not when it is placed on the mucosal side of the intestine. This difference has been explained in terms of a relatively impermeable mucosal barrier to ouabain transfer. Leaf (15) has shown that the addition of antidiuretic hormone to the serosal side of a toad bladder preparation greatly increased the rate of entry of ^{14}C-urea from the mucosal medium while the rate of entry from the serosal medium was unaffected. Moreover, the antidiuretic hormone has to be presented to the serosal surface of the bladder in order to be effective. The results of the present investigation suggest the possible utility of bile salts as a probe to study the asymmetric permeability properties of the everted intestinal membrane.

Studies in which the everted intestinal preparation was first incubated in STDC and then exposed to drug solution show that the bile salt need not be present simultaneously with drug to enhance salicylate transfer rates. The results also indicate that the effects of 10 m*M* STDC are not easily reversed. This conclusion must be qualified in that although the incubated intestinal segment was rinsed thoroughly to remove adhering STDC, there was probably some bile salt remaining in the tissue upon transfer to the drug solution.

The data in Table I suggest an unusual relationship between permeability (as judged by salicylate transfer rates) and bile salt concentration. This relationship is depicted in Fig. 6 where transfer rate is plotted as a function of log STDC concentration in the mucosal solution. There appear to be two levels of effect: one at 1 and 5 m*M* and a second at 10, 50 and 100 m*M* STDC. Each level of effect is significantly different from control values and from each other but there is no significant concentration dependence within a given level. This suggests that the bile salt effect is mediated *via* two different mechanisms. One mechanism appears to be operative at concentrations of STDC below or about the CMC and results in small increases in salicylate transfer rates. A second mechanism, which results in large increases in the permeability of the intestinal membrane, appears to be operative at STDC concentrations above the CMC.

The possibility that monomeric and micellar species of bile salt affect the biologic membrane in a significantly different manner is supported by recent studies on bile salt transfer in isolated jejunal loops of the rat. Dietschy (16) reports that at concentrations below the CMC the rate of passive transfer of sodium taurocholate increased linearly with increasing mucosal concentrations of bile salt.[4] According to Dietschy (16), "since the concentration of

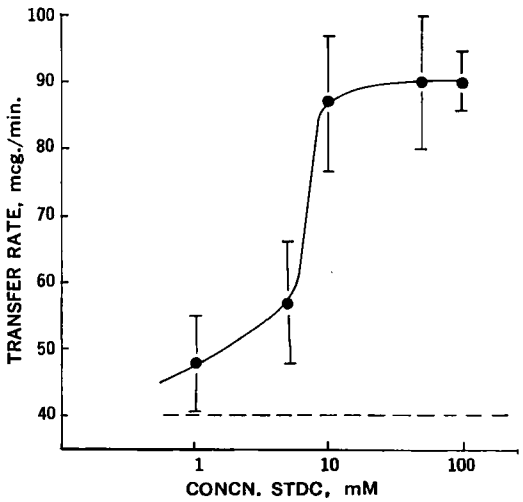

Figure 6—*Effect of mucosal concentrations of STDC (log scale) on mean steady-state transfer rates of salicylate across the everted intestine of the rat. Bars denote ±1 SD. Dashed line indicates mean control value. Number of experiments listed in Table I.*

[4] It should be noted that bile salt transfer in proximal portions of the intestine is a passive process (16).

monomer becomes almost constant as the total concentration of bile acid is raised to and beyond the CMC, the rate of passive diffusion similarly should reach a limiting value at this same point if bile acid monomer were the only species diffusing passively across the bowel wall." In fact, transfer rate actually increased more rapidly with increasing concentrations of sodium taurocholate above the CMC. Micellar taurocholate moves across the intestinal membrane twice as fast as the monomeric form. Based on the authors' findings one need not conclude that micellar transport of taurocholate proceeds at a faster rate than monomer transport or indeed that it occurs at all. It is possible that micellar concentrations of bile salt in contact with the mucosa significantly enhance the permeability of the membrane toward the monomeric species.

The nature of the mechanisms by which bile salts exert their effects on the biologic membrane is not clear at present. Studies in the literature and in this laboratory suggest certain working hypotheses which may be explored. The low level, premicellar, effects of STDC may be associated with depletion of membrane calcium. Webling *et al.* (17) have demonstrated a weak interaction between bile salts and calcium, and the effects of calcium depletion on membrane permeability have been shown by Tidball (18) with ethylenediamine tetraacetic acid (EDTA). Unpublished data from this laboratory indicate that the effects of EDTA on salicylate transfer across the everted intestine are of the same order as those observed with low concentrations of STDC. The ability of bile salts to solubilize phospholipids (19), which are components of the cell membrane, and actually restructure the membrane offers a possible explanation for the high level effect of STDC. These possibilities are being actively explored.

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Regulation of Dissolution Rate by Pellet Geometry

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Abstract □ The influence of surface configuration on the dissolution behavior of pellets suspended in a turbulent flow field has been investigated. The rate of dissolution of extruded cellulose acetate hydrogen phthalate pellets was found to be a complex function of surface geometry. The study was undertaken to determine the *in vitro* sustained release characteristics of pellets of a form such that their loss of effective surface area during dissolution is minimized. Factors involved in the design of such pellets are discussed and data are presented which indicate the dissolution characteristics of test forms.

Keyphrases □ Dissolution rate—pellet geometry effect □ Extrusion process—pellet formation □ Pellet surface area—dissolution effect □ Colorimetric analysis—spectrophotometer

Processes occurring at the solid-liquid or solid-gas interface of multiphase systems can often be rate-controlled effectively by regulating the solid surface area available. This would imply that, by maintaining a constant surface area, the rate of a surface-controlled process could also be held constant. A number of workers have observed the absorption of subcutaneous pellet implants of certain steroids to be proportional to exposed surface (1–3), and that absorption occurs at a reasonably constant rate if surface area is maintained by repeated implantations. This principle can be applied to processes in which the solid phase is consumed and not replenished by forming the solid into a shape such that loss of mass will not result in a loss of surface area. In the simplest case, the participation of a given element of surface in the overall process is independent of its topography and of its general location with regard to the other elements of surface in the system. Smokeless powders and solid rocket fuels are frequently produced in a form designed to maintain a reasonably constant surface area during combustion and represent an outstanding example of this principle.

In contrast to this mechanism, loss of mass from a surface by dissolution is not, in general, a simple function of surface area alone, but must be considered as