Effect of Surfactants on Absorption through Membranes III: Effects of Dioctyl Sodium Sulfosuccinate and Poloxalene on Absorption of a Poorly Absorbable Drug, Phenolsulfonphthalein, in Rats

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Abstract □ The influence of dioctyl sodium sulfosuccinate and poloxalene on the GI absorption of phenolsulfonphthalein in the rat was studied. Urinary excretion data after oral administration of the drug to intact rats and loss of the drug from the whole small intestine as a loop were both utilized to assess the effect of the surfactants on absorption. Dioctyl sodium sulfosuccinate markedly increased the absorption of the drug, and the extent was dependent on the surfactant concentration. Maximum effect was observed at the reported ED_{50}, in rats, of the surfactant as a fecal softener. The mechanism responsible for absorption enhancement seems to be an alteration of the permeability of the intestinal membrane. Micellar complexation between the drug and the surfactant resulted in a lesser increase in absorption at the higher surfactant concentrations. Poloxalene did not increase drug absorption, but higher concentrations caused a decrease in absorption due to micellar entrapments of the drug molecules. The influence of dioctyl sodium sulfosuccinate on the peritoneal absorption of the drug was also investigated. Lower doses of the surfactant increased absorption of the drug by altering the membrane permeability. Higher doses decreased absorption due to unavailability of the drug molecules entrapped in the micelles.

Keyphrases □ Surfactants—effects of dioctyl sodium sulfosuccinate and poloxalene on absorption of phenolsulfonphthalein in rat □ Dioctyl sodium sulfosuccinate—effect on absorption of phenolsulfonphthalein in rat, urinary excretion and *in situ* intestinal loop data □ Poloxalene—effect on absorption of phenolsulfonphthalein in rat, urinary excretion and *in situ* intestinal loop data □ Absorption—effects of dioctyl sodium sulfosuccinate and poloxalene, rats

Dioctyl sodium sulfosuccinate USP (I) and poloxalene[1] are two surfactants used medicinally as fecal softeners. It was suspected that the presence of I in combination with oxyphenisatin acetate, a nonabsorbable peristaltic stimulant, resulted in promotion of intestinal absorption of oxyphenisatin and, therefore, caused jaundice in several patients (1, 2). A systematic study of the effect of these surfactants on the absorption of various drugs was undertaken. A previous report (3) showed that both surfactants enhanced the absorption of pentobarbital, a readily absorbable drug, through the goldfish membrane. Dioctyl sodium sulfosuccinate was also found to change the rate of absorption of pentobarbital from the rat intestine (4).

The purpose of the present investigation was to study the effect of both surfactants on the GI absorption of a poorly absorbable drug, phenolsulfonphthalein (phenol red), in the rat.

Although Lish and Weikel (5) reported that I enhances the absorption of phenolsulfonphthalein from the rat colon while poloxalene does not, these investigators used only one concentration of the surfactants. Since the action of surfactants on drug absorption is known to be concentration dependent, and since more recent reports (6) have shown that phenolsulfonphthalein, like most other drugs, is absorbed mainly from the upper GI tract, a more detailed study of the effect of various concentrations of the surfactants on the absorption of phenolsulfonphthalein from the intact rat and from *in situ* intestinal loops was deemed appropriate. The effect of I on the peritoneal absorption of phenolsulfonphthalein was also examined.

EXPERIMENTAL

Poloxalene, dioctyl sodium sulfosuccinate[2], and phenolsulfonphthalein[3] were all used as received. All other chemicals were either USP or reagent grade.

Absorption Studies in Intact Rats—Male Sprague–Dawley rats, 200–380 g, were starved for 18–20 hr with water allowed *ad libitum*. The experiments were performed in a crossover fashion with each rat serving as its own control. About half the number of animals received a control dose of 2 ml of phenolsulfonphthalein solution (0.75 mg/ml) in distilled water by gastric intubation. The other half received an equal volume of the same solution in distilled water containing one concentration of the surfactants studied. After a recovery period of 3 days, the procedure was reversed.

Urine was collected for 24 hr, and the amount of phenolsulfonphthalein excreted was determined spectrophotometrically at 560 nm following the method of Feldman and Gibaldi (7). The percent of the drug absorbed was calculated by means of a standard curve.

In a second set of experiments, the test animals were injected intraperitoneally in a crossover fashion with 0.5 ml of phenolsulfonphthalein solution (0.75 mg/ml) in distilled water or an equal volume of the same solution in distilled water containing one concentration of dioctyl sodium sulfosuccinate. The amount of the drug excreted in the urine was determined as already described, and the percent absorbed in the presence of various concentrations of the surfactant was calculated.

Absorption Studies from *In Situ* Intestinal Loops—Fasted Sprague–Dawley rats, 200–300 g, were used. The degree of intestinal absorption was measured using *in situ* single loop preparations (8, 9). The loop used was whole small intestine. The animals were anesthetized with ether, and a midline incision was made to expose the small intestine. Ligatures were placed about 1 cm distal to the pyloric junction and 1 cm proximal to the ileocecal junction. Care was taken not to occlude major blood vessels. Physiological saline was injected into the loop until the washings became clear.

The rats were kept under light anesthesia for 20–25 min, before closing the ileocecal end, to allow for absorption of any residual solution. Five milliliters of phenolsulfonphthalein solution (0.75 mg/ml) in distilled water or in various solutions of the surfactants was introduced into the intestines through the pyloric ligature by means of a syringe and a blunt needle. The midline incision was then closed and the animals were allowed to recover. The animals

[1] Ethylene oxide–propylene oxide polymer of average molecular weight 8350, obtained as Pluronic F-68, Wyandotte Chemicals Corp., Wyandotte, Mich.

[2] Aerosol O.T. 100%, Sargent-Welch Scientific Co.

[3] Analytical grade, J. T. Baker.

Table I—Total Urinary Excretion of Phenolsulfonphthalein after Oral Administration of 1.5-mg Dose with and without Dioctyl Sodium Sulfosuccinate[a]

Surfactant Dose, mg	Dose Absorbed, % ± *SD*
Control	4.8 ± 0.7
10	8.4 ± 0.9
Control	5.4 ± 1.0
20	11.1 ± 3.0
Control	4.5 ± 0.5
30	11.0 ± 1.7
Control	4.1 ± 0.7
40	8.2 ± 1.7

[a] Values represent mean of nine animals.

were sacrificed after 3 hr; the whole small intestine was removed, washed with saline to remove any blood adhering to its surface, homogenized, and assayed for drug content according to Lish and Weikel (5).

The percent of phenolsulfonphthalein absorbed was calculated by measuring the difference between the amount introduced into the loop and the amount recovered from the intestinal homogenate.

Solubility Studies—Equilibrium solubility was employed to determine the extent and the nature of possible interactions between the drug and the surfactants. Excess amounts of powdered phenolsulfonphthalein were placed in 25-ml ampuls with 10-ml solutions containing various surfactant concentrations. The ampuls were sealed and then rotated in a constant-temperature water bath at 37 ± 0.1°. After equilibrium was achieved, an aliquot was filtered through 0.45-μm filters[4]; the amount of the drug in solution (adjusted to pH 10 with sodium hydroxide) was determined spectrophotometrically at 560 nm.

Partition Studies—Solutions of phenolsulfonphthalein (0.05%) were prepared in a phosphate buffer (0.01 *M*, pH 6.0) or in the same buffer containing various concentrations of I. Ten milliliters of these solutions was added to an equal volume of *n*-octanol, previously saturated with the buffer solution, in a 50-ml erlenmeyer flask. The contents were equilibrated at 37 ± 0.1° for 2 days. After separation by centrifugation (at 37°), an aliquot of the aqueous phase was assayed spectrophotometrically for its drug content. The partition coefficient was calculated by difference. The organic phase, *n*-octanol, was chosen on the basis of its favorable nonpolar lipid character (10, 11). The pH of the solutions simulates the average pH of the small intestine.

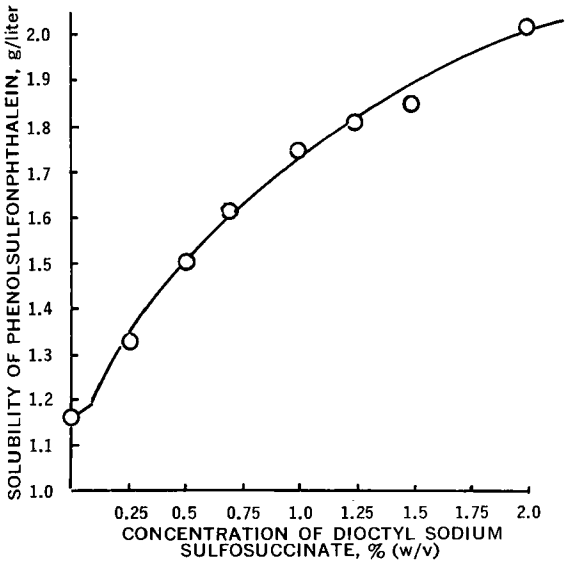

Figure 1—*Effect of dioctyl sodium sulfosuccinate on the equilibrium solubility of phenolsulfonphthalein at 37°.*

[4] Millipore.

Table II—Effect of Dioctyl Sodium Sulfosuccinate on the Absorption of Phenolsulfonphthalein from Rat Intestinal Loops[a]

Surfactant Concentration, % (w/v)	Dose Absorbed, % ± *SD*
Control	4.5 ± 0.4
0.25	17.5 ± 5.8
0.5	54.0 ± 20.0
1.0	73.6 ± 16.1
1.5	50.7 ± 11.4

[a] Values represent mean of at least six loops.

RESULTS AND DISCUSSION

Phenolsulfonphthalein is a poorly absorbable, weak acid (pKa 7.9). The poor absorption of the drug from the small intestine is attributed to the low lipid–water partition coefficient of the unionized form, while the poor absorption from the stomach can be attributed to the opening of the sulfolactone ring at low pH (≤2) and the generation of the benzenesulfonic acid group that becomes ionized at the stomach pH (11). Intestinal perfusion studies in rats have shown that phenolsulfonphthalein is equally absorbed in the proximal and distal regions of the small intestine (12). At the concentration employed in this study (0.75 mg/ml), phenolsulfonphthalein absorption from the rat intestinal tract occurs mainly by passive diffusion (13).

Effect of Dioctyl Sodium Sulfosuccinate on GI Absorption of Phenolsulfonphthalein—Table I shows the urinary recovery of phenolsulfonphthalein as the percent of dose after oral administration of 2 ml of a 0.75-mg/ml solution in the presence or absence of various concentrations of I. The percent of the drug absorbed in control animals was, on the average, about 5% of the dose administered. All doses of the surfactant significantly increased drug absorption. Differences between absorption from solutions containing I and control solutions are significant at $p < 0.001$ as determined by the Student *t* test for paired data.

The effect of I on the absorption of phenolsulfonphthalein from the intact rat was dependent on the amount of the surfactant administered. Maximum effect was observed at the dose level of 20–30 mg, corresponding to a 1–1.5% surfactant concentration in the administered solution. The dose that caused the maximum increase in drug absorption was approximately equal to 100 mg/kg, the ED_{50} reported for the fecal-hydrating effect of the surfactant (14).

The enhancement of phenolsulfonphthalein absorption in the intact rat due to coadministration of the surfactant could be the result of one or more mechanisms. For example, dioctyl sodium sulfosuccinate was reported to retard gastric emptying when administered intraduodenally (15). Such a delay in the GI transit rate could conceivably result in increased absorption of phenolsulfonphthalein when the two drugs are administered together. Other possible mechanisms include a possible interaction between the surfactant and the drug and/or a direct effect of the surfactant on the GI membrane with a resultant change in drug absorption.

Therefore, it was of interest to consider the absorption of the drug from a well-defined segment of the GI tract. The whole intestine as a loop is well suited for this purpose. This technique allows the study of the surfactant effect without the complicating problem of gastric emptying and permits a better control of the concentration of the surfactant at the absorption site. In addition, it was of interest to determine if *in situ* techniques correlate with absorption studies from intact animals.

The results obtained from intestinal loop experiments are presented in Table II. A significant increase in the absorption of phenolsulfonphthalein in the presence of the surfactant was observed. Differences between absorption from solutions containing I and control solutions were significant at $p < 0.001$ as determined by the Student *t* test for unpaired data. The intestinal loop experiments also indicated a dose-dependent effect of the surfactant on the intestinal absorption of phenolsulfonphthalein. Although there were quantitative differences in the extent of absorption between intestinal loops and intact rat experiments, the activity of the surfactant followed more or less the same pattern. These quantitative

Table III—Apparent Partition Coefficients for Phenolsulfonphthalein (0.05%) in the Presence of Various Concentrations of Dioctyl Sodium Sulfosuccinate

Surfactant Concentration, % (w/v)	Apparent Partition Coefficient (*n*-Octanol–Water)
Control	0.09
0.01	0.08
0.1	0.08
0.25	0.07
0.5	0.08
1.0	0.07
1.5	0.08

differences are to be expected in view of the differences in the effective concentration of both the drug and the surfactant at the absorption site and the differences in techniques. Nevertheless, the intestinal loop experiments proved to be in good qualitative correlation with intact animal absorption studies.

The results of the absorption studies from intestinal loops suggest that other factors besides delay in gastric emptying are mainly responsible for absorption enhancement. A significant change in the ability of the drug to permeate the GI membrane may result from an interaction between phenolsulfonphthalein and the surfactant to form a complex with a higher affinity for the membrane. The magnitude of interaction of the drug with the surfactant was determined by equilibrium solubility studies. Figure 1 shows that the solubility of the drug increases above the critical micelle concentration (CMC) of the surfactant, indicating micellar complexation.

The possibility that a complex of the drug and the surfactant has better affinity for lipoidal membranes was investigated by partition studies (Table III). Both premicellar and postmicellar concentrations of the surfactant did not increase the partitioning of phenolsulfonphthalein between *n*-octanol and water.

On the basis of the absorption, solubility, and partition studies, it is suggested that the enhancement of phenolsulfonphthalein absorption represents the sum of two main effects: modification of the permeability of the membrane and micellar complexation of the drug. The membrane effect of the surfactant is believed to increase absorption, while the micellar entrapment of the drug molecules usually decreases absorption. The results of the absorption studies indicate that the membrane effect is predominant. However, at higher concentrations of the surfactant (higher doses), the micellar entrapment results in a lesser increase in absorption. Several reports (4, 8, 16–20) attributed the absorption enhancement effect of various surfactants to a direct effect on the biological membrane.

The absorption studies in intact rats indicated that any effect of the surfactant on membrane permeability was reversible. Urinary excretion data obtained from control studies in rats, 3 days after each had received the surfactant, were essentially identical to those obtained from control studies in rats with no previous exposure to the surfactant.

Table IV—Total Urinary Excretion of Phenolsulfonphthalein after Intraperitoneal Administration of 0.375-mg Dose with and without Dioctyl Sodium Sulfosuccinate[a]

Surfactant Dose, mg	Dose Absorbed, % ± *SD*
Control	60.4 ± 6.8
0.05	68.8 ± 3.4
Control	51.8 ± 7.2
0.25	60.9 ± 4.6
Control	50.4 ± 4.0
0.5	55.6 ± 3.4
Control	49.0 ± 6.8
2.5	40.2 ± 8.6
Control	56.0 ± 4.5
5.0	35.4 ± 6.7
Control	57.0 ± 4.9
7.5	29.1 ± 6.1

[a] Values represent mean of five animals.

Table V—Total Urinary Excretion of Phenolsulfonphthalein after Oral Administration of 1.5-mg Dose with and without Poloxalene[a]

Surfactant Dose, mg	Dose Absorbed, % ± *SD*
Control	5.6 ± 1.6
10	5.6 ± 1.3
Control	4.5 ± 0.9
30	4.3 ± 0.8
Control	5.3 ± 1.2
50	5.3 ± 0.6
Control	5.0 ± 1.0
100	3.7 ± 1.0

[a] Values represent mean of at least five animals.

Effect of Dioctyl Sodium Sulfosuccinate on Peritoneal Absorption of Phenolsulfonphthalein—In view of the finding that the surfactant could change the permeability of the GI membrane, it was of interest to determine whether it has a similar effect on the peritoneal membrane. The results are shown in Table IV. The concomitant administration of the surfactant with the drug resulted in a small but significant ($p < 0.01$ as determined by the Student *t* test for paired data) increase in the drug absorption up to a 0.5-mg dose (corresponding to a 0.1% surfactant concentration). Above this dose, the drug absorption was significantly decreased. The effect of the surfactant on the peritoneal absorption of the drug was qualitatively similar to its effect on the GI absorption. The extent in increase in absorption, however, was less in the case of peritoneal absorption, and a decrease in absorption was observed at higher surfactant concentrations.

The mechanism by which the surfactant changes the peritoneal absorption of the drug seems to be basically similar to the one observed in the GI absorption studies. At lower surfactant concentration (lower dose), there was a potentiation of absorption through a direct effect on the membrane. At higher concentrations, the drug was entrapped in the micelles and was not readily available so a decrease in absorption was observed. The effect of dioctyl sodium sulfosuccinate on the peritoneal membrane and the resultant change in permeability reported in this study are in agreement with the findings of Mattocks and Penzotti (21). These investigators reported that the same surfactant accelerated peritoneal dialysis of both urea and sodium phosphate, and they attributed its effect to direct action on the structure of the mesentary membrane by removal of phospholipids.

Effect of Poloxalene on GI Absorption of Phenolsulfonphthalein—The total urinary excretion of the drug in the presence and absence of the surfactant is shown in Table V. No increase in absorption was noticed with the lower doses of the surfactant, while a significant decrease ($p < 0.05$) was observed at higher doses. The results of the intestinal loop experiments are presented in Table VI. Again, the same pattern of surfactant activity was observed. There was no significant change at lower concentrations, but a significant decrease in absorption ($p < 0.001$) was observed at higher concentrations.

The failure of poloxalene to increase drug absorption from the GI tract of the rat is in contrast to the absorption-enhancing effect of similar concentrations of the surfactant of pentobarbital in the goldfish (3). This nonionic surfactant apparently can change the

Table VI—Effect of Poloxalene on the Absorption of Phenolsulfonphthalein from Rat Intestinal Loops[a]

Surfactant Concentration, % (w/v)	Dose Absorbed, % ± *SD*
Control	4.5 ± 0.4
1.0	3.9 ± 0.7
2.0	4.0 ± 1.0
3.0	1.4 ± 1.1

[a] Values represent mean of six loops.

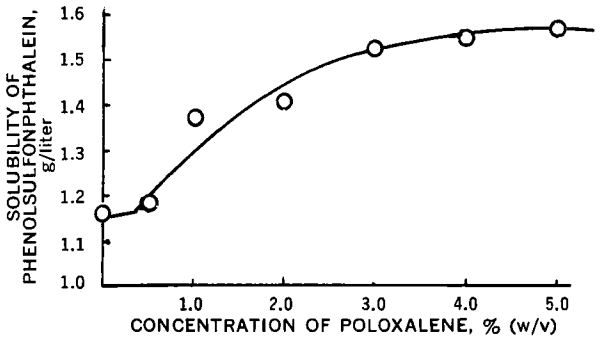

Figure 2—*Effect of poloxalene on the equilibrium solubility of phenolsulfonphthalein at 37°.*

permeability of the goldfish membrane but is unable to cause a significant change in the rat GI membrane. A similar nonionic surfactant, polysorbate 80, was also found to promote drug absorption in the goldfish but not in the rat (22). The lack of promotion of phenolsulfonphthalein absorption by poloxalene observed in this study is in agreement with the findings of Lish and Weikel (5) and the reported lower toxicity of nonionic surfactants toward biological membranes (23).

The decrease in absorption observed at higher concentrations of the surfactant is attributed to micellar entrapment of the drug molecules, as evidenced by the solubility curve shown in Fig. 2. It is suggested that the drug in the micellar phase is unavailable for absorption. Therefore, the effective concentration at the absorption site is less than the apparent concentration, resulting in a decrease in absorption. Higher concentrations of other nonionic surfactants were found by many investigators (24–27) to cause a similar decrease in drug absorption.

SUMMARY

Dioctyl sodium sulfosuccinate, an anionic medicinal surfactant, increased the absorption of phenolsulfonphthalein, a poorly absorbable drug, in the rat. Poloxalene, a nonionic medicinal surfactant, did not increase absorption of the drug; however, higher doses resulted in a decrease in absorption. The possibility that dioctyl sodium sulfosuccinate, a widely used fecal softener, causes a similar enhancement of drug absorption in humans is currently under investigation.

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