Table II—Inhibition of DNA, RNA, Protein, and Lipid Synthesis by Quinine (Q) and Quinidine (QD)

		Concentration, moles/l. × 10^4 1.3	2.5	3.8
		Mean % Inhibition ± *SE*[a]		
DNA synthesis	Q	34 ± 3.5	69 ± 6.1	79 ± 2.6
	QD	32 ± 5.7	75 ± 2.1	78 ± 3.2
RNA synthesis	Q	33 ± 0.5	55 ± 2.4	62 ± 1.9
	QD	36 ± 2.9	56 ± 2.1	61 ± 1.9
Protein synthesis	Q	51 ± 3.9	60 ± 0.2	73 ± 1.4
	QD	50 ± 3.4	63 ± 3.8	71 ± 1.1
Lipid synthesis	Q	8 ± 1.8	14 ± 3.3	27 ± 4.1
	QD	7 ± 2.3	15 ± 1.8	35 ± 3.7

[a] Each value from four determinations. In no instance is p less than 0.2 for any pair of values.

same. The difference in antifibrillatory action of the drugs may be due in part to differences in chemical structure which, in turn, may reflect cardiac receptor specificity.

REFERENCES

(1) J. V. Taggart, D. P. Earle, Jr., R. W. Berliner, C. G. Zubrod, W. J. Welch, N. B. Wise, E. F. Schroeder, I. M. London, and J. A. Shannon, *J. Clin. Invest.*, **27,** 80(1948).

(2) K. F. Wenckelbach, *J. Amer. Med. Ass.*, **81,** 472(1923).

(3) O. H. Scherbaum and E. Zeuthen, *Exp. Cell Res.*, **6,** 221 (1954).

(4) K. A. Conklin, S. C. Chou, and S. Ramanathan, *Pharmacology*, **2,** 247(1969).

(5) J. E. Byfield and O. H. Scherbaum, *Anal. Biochem.*, **17,** 434(1966).

(6) J. E. Byfield, H. Henze, and O. H. Scherbaum, *Life Sci.*, **6,** 1099(1967).

(7) S. C. Chou and S. Ramanathan, *ibid.*, **7,** 1953(1968).

(8) J. E. Byfield and O. H. Scherbaum, *Science*, **156,** 1504 (1967).

(9) E. Uyeki, E. Geiling, and K. Dubois, *Arch. Int. Pharmacodyn. Ther.*, **97,** 191(1954).

(10) J. Webb, P. Saunders, and K. Nakamura, *J. Pharmacol. Exp. Ther.*, **101,** 287(1951).

(11) L. Crevasse and J. C. Shipp, *Amer. Heart J.*, **68,** 667(1964).

(12) M. E. Hess and N. Hangaard, *Circ. Res.*, **6,** 256(1958).

(13) J. W. Moulder, *J. Infec. Dis.*, **85,** 195(1949).

(14) E. Haas, *J. Biol. Chem.*, **155,** 321(1944).

(15) J. D. Fulton, *Ann. Trop. Med. Parasitol.*, **32,** 77(1938).

(16) K. A. Conklin, S. C. Chou, and S. Ramanathan, *Fed. Proc.*, **28,** 361(1969).

(17) S. C. Chou, S. Ramanathan, and W. C. Cutting, *Pharmacology*, **1,** 60(1969).

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Physiologic Surface-Active Agents and Drug Absorption VII: Effect of Sodium Deoxycholate on Phenol Red Absorption in the Rat

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Abstract □ The influence of sodium deoxycholate on the absorption of phenol red in the rat was studied using three techniques to assess absorption—*viz.*, urinary excretion data after oral administration to intact animals, loss of drug from *in situ* intestinal loops, and transfer of drug across the isolated everted intestine. Each of the methods provided evidence that the bile salt markedly enhances the absorption of phenol red by altering the permeability of the intestinal membranes. In the intact rat, these effects appear to be reversible.

Keyphrases □ Phenol red absorption, rat—sodium deoxycholate effect □ Sodium deoxycholate—effect on phenol red absorption, rat □ Colorimetric analysis—phenol red

The role of bile salts in the intestinal solubilization and absorption of fats and fat-soluble vitamins has been studied extensively (1–3). Recently, it has been reported that bile salts may also enhance the intestinal absorption of poorly lipid-soluble substances. Mayersohn *et al.* (4) reported a 1.5- to 1.8-fold increase in the urinary recovery of riboflavin in man when sodium deoxycholate was administered 0.5 hr. prior to the oral ingestion of the vitamin. The results suggest that the bile salt enhancement of riboflavin absorption may be due to changes in the permeability of the gastrointestinal membranes to the transport of the vitamin. Feldman and Gibaldi (5, 6) have shown that relatively low micellar concentrations of sodium taurodeoxycholate markedly increase the permeability of everted rat intestine to salicylate ion.

The purpose of the present investigation was to determine the effects of an unconjugated bile salt, sodium deoxycholate (SDC), on the absorption of a water-soluble, poorly absorbed compound, phenol red, in the rat using several experimental techniques to assess absorption.

EXPERIMENTAL

Absorption Studies in Intact Rats—Male Sprague-Dawley rats weighing between 200 and 300 g. were used in all experiments. The animals were fasted 24 hr. (with water allowed *ad libitum*) prior to gastric intubation of 1.5 ml. of a 1-mg./ml. phenol red solution in distilled water containing 100 m*M* sodium deoxycholate (SDC).

Table I—Total Urinary Excretion of Phenol Red in Individual Rats after Oral Administration of a 1.5-mg. Dose with and without 150 μmole Sodium Deoxycholate (SDC)

Rat	Control, % of Dose	SDC, % of Dose
1	4.9	8.0
2	3.2	9.7
3	2.9	11.7
4	4.6	10.9
5	2.7	6.3
Mean ±*SD*	3.7 ± 1.0	9.3 ± 2.2

A solution of 1 mg./ml. phenol red served as the control. The studies were performed in a crossover fashion with each animal serving as his own control. Following intubation, the animal was placed in a restraining cage. Urine was collected quantitatively every hour for 8 hr. and then at convenient intervals until no measurable levels of phenol red could be detected in the urine (16–24 hr.). Urination was induced by causing the rats to inhale ether vapors for a few seconds. The collected urine was assayed colorimetrically for phenol red using methods described previously (7). The total amount of phenol red excreted in each urine sample was determined by means of standard curves. In a second set of experiments, the test animals were injected intraperitoneally with 1.5 ml. of a 1-mg./ml. phenol red solution. Immediately following the injection, 1.5 ml. of 100 m*M* SDC was administered by gastric intubation.

The same animals were used in control studies in which the phenol red injection was followed by gastric intubation of 1.5 ml. distilled water in place of the bile salt. Urine was collected and assayed as described previously.

Absorption Studies from *In Situ* Intestinal Loops—The method of Levine *et al.* (8) was utilized to study the *in situ* absorption of phenol red from the rat small intestine. Fasted Sprague-Dawley rats weighing between 195 and 240 g. were used for this portion of the study. The animals were anesthetized with ether and maintained under light anesthesia during the surgical procedures. A midline incision was made, the small intestine was located, and two loops, each 5.08 cm. (2 in.) long, were formed. The first loop was approximately 15.24 cm. (6 in.) from the pylorus with 2.54 cm. (1 in.) of intestine separating the two consecutive loops. One-half milliliter of a 1-mg./ml. phenol red solution in the presence or absence of SDC was injected into a loop, through a ligature, by means of a syringe and blunt needle. In experiments utilizing 100 m*M* SDC, the loops in any animal contained either phenol red or phenol red plus the bile salt. The solutions were placed in the intestinal loops in a crossover fashion. The midline incision was then closed and the animals were allowed to recover. The animals were sacrificed after 3 hr. and the loops were excised, homogenized,[1] and assayed for phenol red content according to Levine (9). The percent of phenol red absorbed from each loop was calculated after correcting for assay recovery based on the amount of phenol red recovered from loops after 3 hr. *in vitro* incubation experiments in pH 7.2 buffer at 37°.

Drug Transfer in Isolated Everted Rat Intestine—Two consecutive everted rat small intestine segments, each 10 cm. in length, were prepared by methods previously described (5). Each segment was suspended in 80 ml. of mucosal solution consisting of modified Krebs-Henseleit buffer at pH 7.4 containing 0.2 mg./ml. phenol red and, in bile salt experiments, 100 m*M* SDC. Since both phenol red and SDC are negatively charged at pH 7.4, little or no interaction was expected between the two compounds. 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 oxygen–carbon dioxide (95:5) and maintained at 37 ± 0.1° by means of a water bath. The serosal solution consisted of 2 ml. of modified Krebs-Henseleit buffer, pH 7.4. The experimental procedure for sampling of the serosal solution has been discussed previously (5). Each experiment was run over a 2-hr. period, and the consecutive segments were placed in either the phenol red control buffer or phenol red–SDC–buffer in a crossover fashion.

[1] Eberbach homogenizer, Eberbach Corp.

The serosal samples were diluted appropriately with distilled water, alkalinized with 3 *N* NaOH, and assayed for phenol red spectrophotometrically at 560 mμ. Neither SDC nor "blank" fluid interfered with the assay procedure.

RESULTS

Absorption in Intact Rats—Table I shows the urinary recovery of phenol red, expressed as percent of dose, after oral administration of 1.5 ml. of a 1-mg./ml. phenol red solution in the presence or absence of 150 μmole SDC. The results show that coadministration of the bile salt with phenol red results in an approximately threefold increase in the urinary recovery of phenol red. Differences are significant at the 99.5% level of confidence as determined by Student's *t* test (10). A representative plot of the excretion rate of phenol red *versus* time for both experimental situations is presented in Fig. 1.

Studies in which phenol red was injected intraperitoneally and SDC given orally indicate that the bile salt has no influence on the urinary recovery of phenol red. The mean percent of phenol red recovered in the urine after oral administration of the bile salt was 41.2 ± 11.3% (mean ±*SD*, four rats), essentially identical to the control value of 42.4 ±11.7% (mean ±*SD*, four rats).

This finding rules out an effect of the bile salt on the net distribution and elimination patterns of phenol red and suggests that the increase in urinary recovery of phenol red is due to increased gastrointestinal absorption of the compound in the presence of the bile salt.

Absorption from Intestinal Loops—It was of interest to consider the absorption of phenol red from a well-defined section of the rat small intestine. The intestinal loop preparation was well suited for these experiments. Utilization of this technique made it possible to study the absorption of phenol red in the presence and absence of the bile salt without the complicating problems of enterohepatic circulation and intestinal transit. Also, it was possible by using this technique to control the initial concentration of bile salt present at a specific site within the intestinal lumen.

The results obtained from the intestinal loop experiments are presented in Table II. The percent of phenol red absorbed in control studies was 5.6%. This value is in good agreement with the value of 7% reported by Levine (9). In the presence of 10 m*M* sodium deoxycholate, the mean percent absorbed was 24.3%. A still larger increase in phenol red absorption was observed in the presence of 100 m*M* SDC (69.3%).

Phenol Red Transfer across Everted Rat Intestine—The effect of SDC on the intestinal transfer of phenol red is noted in Table III. The results are consistent with the findings of the *in vivo* and *in*

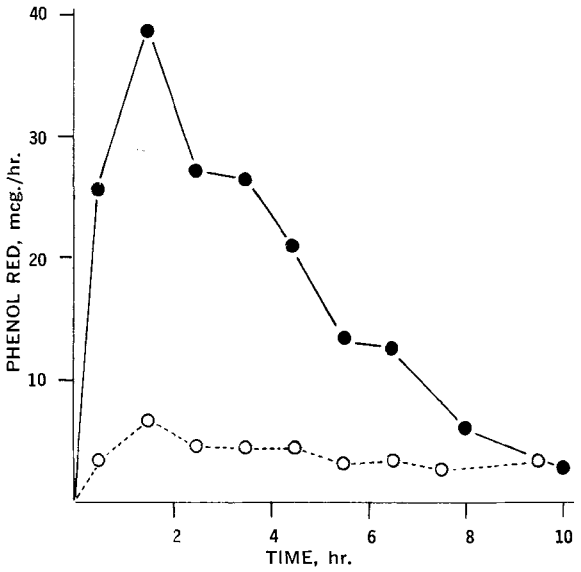

Figure 1—*Urinary excretion rates of phenol red after oral administration with and without sodium deoxycholate (SDC). Key:* ●, *SDC; and* ○, *control.*

Table II—Effect of Sodium Deoxycholate (SDC) on the Absorption of Phenol Red from Rat Intestinal Loops

Solution	No. of Loops	Absorbed ±*SD*, %
Control	3	5.6 ± 1.2
10 m*M* SDC	6	24.3 ± 15.1
100 m*M* SDC	3	69.3 ± 3.5

situ studies. The bile salt produced a 75% increase in the steady-state transfer rate of the drug across the isolated tissue preparation.

DISCUSSION

Each of the three methods used to assess absorption of phenol red in the rat provided evidence that sodium deoxycholate at concentration exceeding the critical micelle concentration markedly alters the permeability of the gastrointestinal membranes to this poorly absorbed compound. It is of particular interest to note that the *in vitro* technique, *i.e.*, the everted intestinal preparation, provided an excellent indication of *in vivo* absorption phenomenon with respect to the bile salt effect. Until recently, the isolated everted intestine technique has been used almost exclusively for studying active accumulation and transport of various nutrients. The present study indicates that this *in vitro* technique may also have considerable value in studying drug absorption which usually involves passive rather than active processes.

Care, of course, must be used in extrapolating the results of any *in vitro* model to predict results in the intact biologic system. Invariably, quantitative differences are noted and, in some instances, qualitative differences may be observed as well. The isolated everted intestine appears to be considerably more permeable to phenol red than the intact intestine. The clearance of phenol red from the mucosal solution, *i.e.*, the steady-state transfer rate divided by the mucosal concentration, is about 0.8 ml./hr. This value is of the same order of magnitude as the clearance of salicylate—*viz.*, 1.2 ml./hr. (5). However, the rate of intestinal absorption of salicylate is several orders of magnitude greater than that of phenol red. Chalfin *et al.* (11), Tidball *et al.* (12), and Baker *et al.* (13) previously found the small intestine to be considerably more permeable when everted than when not everted. Despite this significant qualitative shortcoming of the everted gut technique with respect to phenol red transfer, the effect of the bile salt on membrane permeability is as adequately demonstrated with this preparation as with the other techniques employed.

An important qualitative difference observed between the isolated and intact rat intestine is the ability to reverse the bile salt effect on membrane permeability. The authors have previously demonstrated that the effect of bile salts on the permeability of the everted intestine was not reversible (6). However, the results of *in vivo* absorption studies in rats suggest that this may not be the case in the intact intestine. Oral dosing studies carried out in a crossover fashion gave no indication that the effects of the orally administered bile salt were irreversible. Urinary excretion data obtained from control studies in two rats, 3 days after each had received SDC, were essentially identical to those obtained from control studies in rats who had no previous exposure to SDC.

The intestinal loop experiments summarized in Table II indicate a dose-dependent effect of the bile salt on the intestinal membranes. One-half milliliter of a 10 m*M* SDC solution resulted in a mean absorption rate that was four times greater than control values. The results were extremely variable from loop-to-loop as indicated by the very large coefficient of variation, *i.e.*, about 60%. This finding is in contrast to the twelvefold increase in phenol red absorption (coefficient of variation of 5%) produced by a 100 m*M* solution of SDC. The 5-μmole dose of SDC may be in the range of the minimum effective dose (MED) of deoxycholate needed to alter intestinal permeability while the 50-μmole dose is clearly above the MED.

Table III—Effect of 100 m*M* Sodium Deoxycholate (SDC) on the Steady-State Transfer Rate of Phenol Red across the Everted Rat Small Intestine—Mucosal Drug Concentration Maintained at 0.2 mg./ml.

Rat	Control, mcg./min.	SDC, mcg./min.
1	2.72	4.41
2	2.62	4.90
3	2.38	4.18
Mean ±*SD*	2.57 ± 0.17	4.50 ± 0.37

During the course of these studies, it was observed that there was an increase in the volume of fluid within the intestinal loop after the 3-hr. exposure to 100 m*M* SDC. This is consistent with the earlier observation of Feldman *et al.* (14) who reported an increase in fluid volume within the gastric pouch after exposure to 26 m*M* SDC. Despite the rather marked influx of fluid into the intestinal lumen, enhanced absorption of phenol red occurred in the presence of the bile salt. In control studies, little fluid remained within the intestinal lumen after the 3-hr. absorption period, indicating significant net water absorption in the absence of the bile salt.

The mechanism of action of the bile salt in altering the permeability of the intestinal tract to various drugs is still not certain. It has been suggested that the mechanism may involve an interaction of the bile salt micelle with membrane lipid, particularly phospholipid (15). This aspect of the problem is under further investigation.

REFERENCES

(1) A. M. Dawson, *Brit. Med. Bull.*, **23,** 247(1967).

(2) A. F. Hofmann, *Gastroenterology*, **50,** 56(1966).

(3) A. F. Hofmann, *Biochem. J.*, **89,** 57(1963).

(4) M. Mayersohn, S. Feldman, and M. Gibaldi, *J. Nutr.*, **98,** 288(1969).

(5) S. Feldman and M. Gibaldi, *J. Pharm. Sci.*, **58,** 425(1969).

(6) *Ibid.*, **58,** 967(1969).

(7) S. Feldman and M. Gibaldi, *Gastroenterology*, **54,** 918 (1968).

(8) R. M. Levine, M. R. Blair, and B. B. Clark, *J. Pharmacol. Exp. Ther.*, **114,** 78(1955).

(9) R. R. Levine, *ibid.*, **131,** 328(1961).

(10) G. W. Snedecor and W. G. Cochran, "Statistical Methods," 6th ed., Iowa State University Press, Ames, Iowa.

(11) D. Chalfin, I. L. Cooperstein, and C. A. M. Hogben, *Proc. Soc. Exp. Biol. Med.*, **99,** 746(1958).

(12) C. S. Tidball, T. R. Liebross, S. Thomas, and M. M. Cassidy, *Physiologist*, **10,** 325(1967).

(13) R. D. Baker, S. Watson, J. L. Long, and M. J. Wall, *Biochem. Biophys. Acta*, **173,** 192(1969).

(14) S. Feldman, R. J. Wynn, and M. Gibaldi, *J. Pharm. Sci.*, **57,** 1493(1968).

(15) S. Feldman and M. Gibaldi, *Proc. Soc. Exp. Biol. Med.*, **132,** 1031(1969).

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