

**Effect of Bile Salts on the Gastrointestinal Absorption of Drugs. I.<sup>1,2)</sup>**KIICHIRO KAKEMI, HITOSHI SEZAKI, RYOJI KONISHI,  
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(Received July 28, 1969)

The effects of bile salts, sodium taurocholate and sodium glycocholate, on drug absorption from the rat small intestine were investigated using the *in situ* perfusion technique. There are three likely mechanisms by which bile salts can affect drug absorption from the rat small intestine; first, the loss of thermodynamic activity of a drug due to the formation of the micellar complex; second, the local concentration build-up effect such as an accumulation on the absorptive surface; third, the direct effect on the permeability characteristics of the intestinal mucosa. The absorption of sulfanilamide was not affected, but the absorption of sulfaguanidine and phenol red, poorly absorbable drugs, were enhanced by bile salts above their critical micellar concentration. On the other hand, the absorption of 2-allyloxy-4-chloro-N-(2-diethylaminoethyl)benzamide hydrochloride (A.C.D.B.) was inhibited by bile salts above the critical micellar concentration. Possible mechanisms of these effects are discussed.

It has been recognized that bile salts play an important role in the intestinal absorption of lipids<sup>4)</sup> and other nutrients,<sup>5)</sup> and that they themselves are absorbed by an active transport process which is located only in the ileum.<sup>6)</sup> However, there are few reports concerning the effect of bile salts on drug absorption from the intestine and the effect of drugs on the ileal active transport of bile salts.<sup>7)</sup>

Bates, *et al.*<sup>8)</sup> have shown that bile salts increased the solubility and the rate of dissolution of some poorly water-soluble drugs, such as griseofulvin, hexestrol and glutethimide. So it will be possible that bile salts increase the dissolution rate-limited drug absorption. On the other hand, Gibaldi and Nightingale<sup>9)</sup> have recently shown that sodium taurodeoxycholate potentiated the pharmacologic effects of ethanol and pentobarbital, and the absorption of 4-aminoantipyrine in goldfish. This suggests that these physiologic surfactants may have some effects on drug absorption from solution by alteration of membrane permeability. Such mechanisms, however, have not been elucidated, although it may be interpreted in terms of the interactions between drug and bile salts as well as the direct effect on membrane surface.

In this report, the effect of bile salts, sodium taurocholate and sodium glycocholate, on the intestinal absorption of some drugs from solution was systematically investigated in the rat. Drugs used in this study were 2-allyloxy-4-chloro-N-(2-diethylaminoethyl)benzamide hydro-

- 1) This paper forms Part XXXXII of "Absorption and Excretion of Drugs," Preceding paper, Part XXXXI: K. Kakemi, H. Sezaki, and T. Kondo, *Chem. Pharm. Bull.* (Tokyo), **17**, 1864 (1969).
- 2) Presented to the 88th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1968.
- 3) Location: *Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto.*
- 4) A.F. Hofmann and B. Borgström, *Federation Proc.*, **21**, 43 (1962); J.R. Senior, *J. Lipid Res.*, **5**, 495 (1964); W.J. Simmonds, *Am. J. Clin. Nutr.*, **22**, 266 (1969); *etc.*
- 5) A.J. Quick and G.E. Collentine, *Am. J. Physiol.*, **164**, 716 (1951); J.A. Olson, *Am. J. Clin. Nutr.*, **9**, 1 (1961); *etc.*
- 6) M.R. Playoust, L. Lack, and I.M. Weiner, *Am. J. Physiol.*, **208**, 363 (1965); J.M. Dietschy, *J. Lipid Res.*, **9**, 297 (1968); *etc.*
- 7) L. Lack and I.M. Weiner, *J. Pharmacol. Exptl. Therap.*, **139**, 248 (1963).
- 8) T.R. Bates, M. Gibaldi, and J.L. Kanig, *J. Pharm. Sci.*, **55**, 191 (1966); *idem, ibid.*, **55**, 901 (1966); *idem, Nature*, **210**, 1331 (1966).
- 9) M. Gibaldi and C.H. Nightingale, *J. Pharm. Sci.*, **57**, 1354 (1968).

chloride (A.C.D.B.), phenol red, sulfanilamide, and sulfaguanidine. The drugs were selected on the ground that (1) their rate and extent of the absorption from the rat intestine had been well investigated in this laboratory, (2) two of them were in the category of so-called poorly absorbable drugs, a highly favorable characteristics as absorption enhancement marker in this study, (3) specialized transport mechanism had been revealed in one of the drugs, (4) their physico-chemical properties such as ionic nature at physiologic pH were different, and (5) their ready availability and well-documented assay procedures.

### Experimental

**Materials**—Sodium taurocholate and sodium glycocholate were obtained from Sigma Chemical Co., and A.C.D.B. was obtained from Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, and used without further purification. All other drugs used in these experiments were of analytical grade.

**Procedure of Absorption Experiments**—Male Wistar rats weighing 150–200 g were used in all experiments. The procedure of absorption experiments from rat small intestine was the same as those reported in the papers from this laboratory<sup>10)</sup> except that rats were anesthetized lightly with ether, treated, and subjected to drug absorption experiment as usual manner. Animals were awake throughout the subsequent one hour experimental period. The bile duct was ligated in all experiments. Forty ml of drug solution was perfused at the rate of approximately 5 ml/min. After one hour, the perfused solution in the small intestine was withdrawn as completely as possible, and washed with physiologic saline. The washings were combined to the perfusion solution and made up to 100 ml with physiologic saline. From the difference of drugs in amount between the initial perfusion solution and the combined effluent, the eliminated amount of the drug from the perfusion solution was calculated.

**Preparation of Drug Solution**—Phenol red was dissolved in physiologic saline. The composition of isotonic buffer solutions used as the medium for other drugs was  $\text{NaH}_2\text{PO}_4\text{--Na}_2\text{HPO}_4$ .

**Analytical Methods**—i) A.C.D.B.: The sample solution was alkalized with 1 ml of 1N NaOH, and extracted with 5 ml of benzene by the addition of 0.5 g of sodium chloride. The organic phase was separated and determined spectrophotometrically at 294  $m\mu$ .

ii) Phenol Red: The extraction method with isopentyl alcohol by the addition of 1N HCl and sodium chloride was used to minimize the contamination of intestinal contents. An aliquot of the organic phase was then shaken with alkaline media, and the optical density of the latter phase was determined spectrophotometrically at 550  $m\mu$ .

iii) Sulfanilamide and Sulfaguanidine: Sulfonamides were diazotized, coupled with 2-diethylamino-ethyl-1-naphthylamine, and the colored material was extracted with isopentyl alcohol by the addition of sodium chloride. The organic phase was determined spectrophotometrically at 550  $m\mu$ .

**Apparent Partition Coefficients**—Apparent partition coefficients were determined as previously described in the reports from this laboratory.<sup>11)</sup>

**Measurement of Micellar Interactions**—Interactions between drugs and bile salts were determined using a molecular sieve technique of Ashworth and Heard.<sup>12)</sup>

**Pretreatment in Absorption Experiments**—In order to clarify whether the effects on the drug absorption were caused by the interactions between drugs and bile salts in the perfusion solution, intraluminal interaction, or by some actions of bile salts to the mucosal membrane, pretreatment with the solution of bile salts was carried out. The buffer solution of bile salts at the concentration of 40 mM was perfused for 30 min in the rat small intestine, and the small intestine was washed well with physiologic saline to remove the bile salts as completely as possible, and then the solution of a drug only was perfused for one hour. In control experiments, pretreatments were done with physiologic saline.

**Binding Tendency of Phenol Red to the Intestinal Mucosa**—In some experiments for the absorption of phenol red, following procedure was used to determine the amount bound to the mucosal surface. After the one hour perfusion, the small intestine was washed with forty ml of physiologic saline, isolated, and homogenized. The homogenates were combined to the perfusion solution and the washings, and made up to 100 ml with physiologic saline. From the difference of percentage remained of phenol red between this method and Absorption Experiments described above, amount of phenol red bound to the intestinal mucosa was calculated.

10) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1883 (1967); K. Kakemi, T. Arita, and S. Muranishi, *ibid.*, **13**, 862 (1965).

11) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1534 (1967).

12) R.W. Ashworth and D.D. Heard, *J. Pharm. Pharmacol.*, **18**, Suppl., 98S (1966).

## Results and Discussion

It has been shown that many of the compounds influence on bile secretory mechanisms. Treatment of animals with barbiturates, for instance, has a pronounced effect on the rate of bile flow.<sup>13)</sup> Since the ultimate goal of our investigation is to assess the influence of hepatic function on the various biopharmaceutical parameters, anesthetic drugs affecting on the hepatic disposition were avoided as in the usual unanesthetized biliary secretory investigations.<sup>14)</sup> It was shown that there wasn't any significant change in the absorption characteristics of the drug tested in the absence of bile salts.

The effects of sodium taurocholate and sodium glycocholate on the intestinal absorption of four kinds of drugs are summarized in Table I. It is of interest to note that the bile salts, main components of bile, demonstrated varying effect on the intestinal absorption of drugs.

TABLE I. Effect of Bile Salts on Drug Absorption from Rat Small Intestine<sup>a)</sup>

Drug	Absorption	
	Sod. taurocholate <sup>b)</sup>	Sod. glycocholate <sup>b)</sup>
A.C.D.B. <sup>c)</sup>	66	46
Phenol red <sup>d)</sup>	278	298
Sulfanilamide <sup>e)</sup>	98	98
Sulfaguanidine <sup>e)</sup>	351	367

a) pH 6.5. All values are expressed as the percentages of the control experiments.  
 b) 40.0 mM    c) 1.0 mM    d) 0.085 mM (80 µg/ml)    e) 0.1 mM

There are three likely mechanisms by which bile salts can affect drug absorption from the rat small intestine. First, the micellar complex formed may have an absorption rate constant

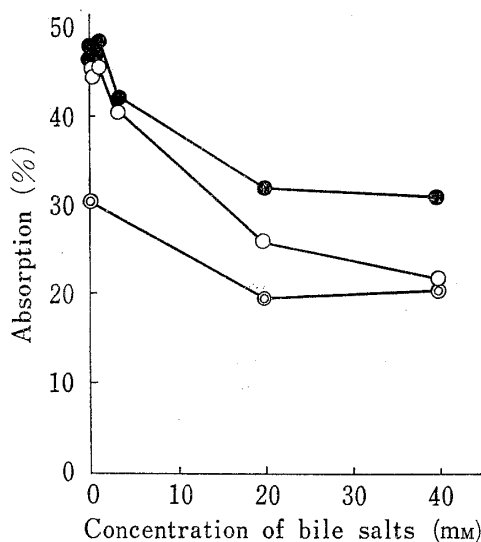


Fig. 1. Effect of Bile Salts on the Intestinal Absorption of A.C.D.B. in Rats

concentration of A.C.D.B. = 1.0 mM  
 —●—: with sod. taurocholate (pH 6.5)  
 —○—: with sod. glycocholate (pH 6.5)  
 —⊖—: with sod. taurocholate (pH 5.0)

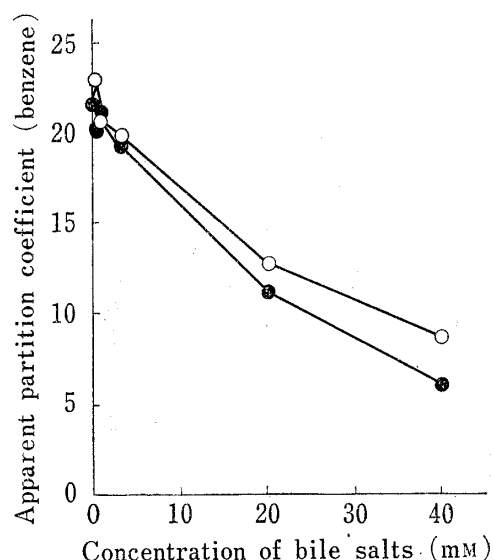


Fig. 2. Effect of Bile Salts on the Apparent Partition Coefficients of A.C.D.B.

concentration of A.C.D.B. = 1.0 mM, pH 6.5  
 —○—: with sod. taurocholate  
 —●—: with sod. glycocholate

13) C.D. Klassen and G.L. Plaa, *J. Pharmacol. Exptl. Therap.*, **161**, 361 (1968).

14) H.O. Wheeler and O.L. Ramos, *J. Clin. Invest.*, **39**, 161 (1960); J.M. Dietschy, *ibid.*, **47**, 286 (1968).

different from that of free drug itself due to the loss of thermodynamic activity of a drug in the medium. Second, bile salts may affect the absorption rate of a drug by a local concentration build-up effect such as an accumulation on the absorptive surface, and third, and is worthy of note, it may have a direct effect on the permeability characteristics of the intestinal mucosa thereby enhancing the absorption of a poorly absorbable drug.

i) A.C.D.B.—A.C.D.B. is the salt of a weak base ( $pK_a$  7.9). It possesses relatively high apparent partition coefficients even in the pH range below its  $pK_a$  and the ionic form is fairly well absorbed from the rat small intestine.<sup>15)</sup> As shown in Fig. 1, the absorption of A.C.D.B. at pH 6.5 was inhibited by bile salts above their critical micellar concentrations. This inhibitory effect was also observed at pH 5.0. In Fig. 2 are shown the effect of bile salts on the apparent partition coefficient to benzene at 37° and pH 6.5. This depression of the partition coefficient by bile salts was parallel to the tendency of the inhibition of the intestinal absorption of A.C.D.B. Similar depression of the apparent partition coefficient was observed at pH 5.0. In Fig. 3 are shown the micellar interactions between drugs and bile salts. A.C.D.B.

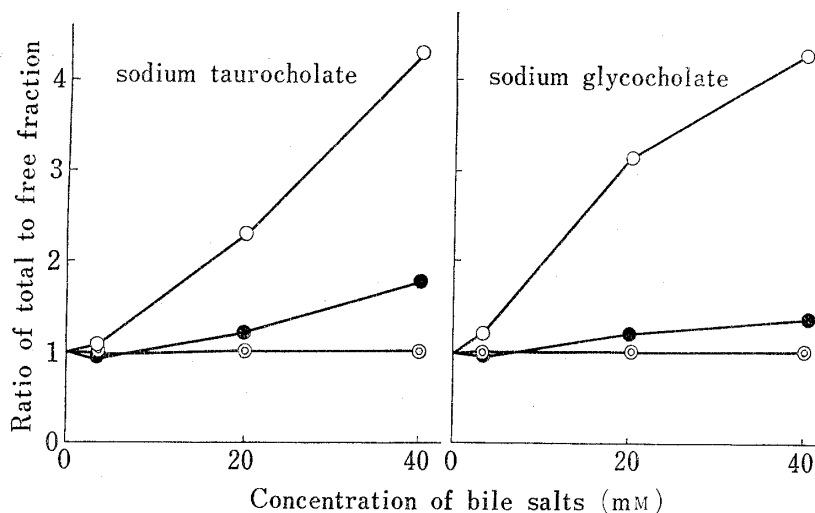


Fig. 3. Micellar Interactions between Drugs and Bile Salts

Drug concentrations are the same as in Table I. pH 6.5.

—○—: A.C.D.B.      —●—: Phenol red  
—⊙—: sulfaguanidine and/or sulfanilamide

formed micellar complex whose fraction increased as the concentration of bile salts increased. At pH 5.0, where this compound is almost completely ionized, same result was obtained. This does not rule out the possibility of the formation of ion-pair complex (non-micellar in nature) between A.C.D.B. and bile salts which bear a negative charge at this pH. However, the above results could be interpreted in terms of the loss of thermodynamic activity of

TABLE II. Effect of Pretreatment with Bile Salts on Drug Absorption<sup>a)</sup>

Drug	Absorption	
	Sod. taurocholate <sup>b)</sup>	Sod. glycocholate <sup>b)</sup>
A.C.D.B. <sup>b)</sup>	93	94
Phenol red <sup>b)</sup>	300	300
Sulfaguanidine <sup>b)</sup>	92	97

a) pH 6.5. All values are expressed as the percentages of the control experiments.

b) Drug concentrations are the same as in Table I.

15) K. Kakemi, H. Sezaki, and S. Horiuchi, *Yakuzaigaku*, 27, 229 (1967).

A.C.D.B. due to the formation of the micellar complex. This was supported by the results shown in Table II, that the absorption of this compound was not affected by the pretreatment with bile salts.

ii) Phenol Red—As shown in Fig. 4, the intestinal absorption of phenol red, strong acid, was increased by the addition of bile salts at the concentrations above the critical micellar

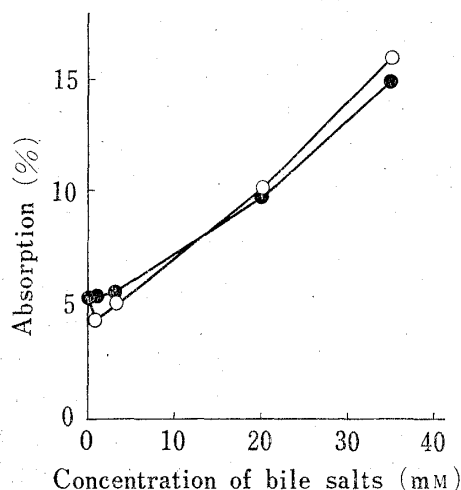


Fig. 4. Effect of Bile Salts on the Intestinal Absorption of Phenol Red in Rats

concentration of phenol red = 0.085 mM (30  $\mu\text{g/ml}$ )

Symbols are the same as in Fig. 2.

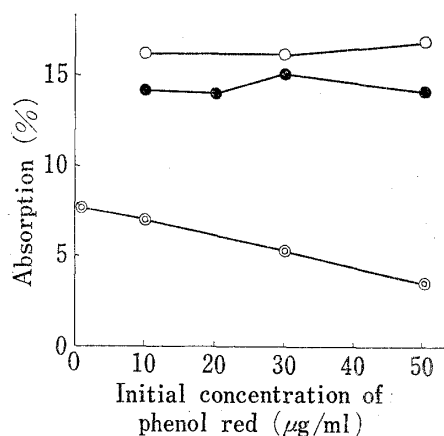


Fig. 5. Effect of Bile Salts on the Concentration Dependency of Phenol Red Absorption

—○—: control  
—○—: with 40 mM sod. glycocholate  
—●—: with 40 mM sod. taurocholate

concentration. Recently Kunze and Vogt<sup>16)</sup> reported that phenol red was absorbed not only by simple diffusion but by an active transport mechanism of low capacity. Lowest curve of Fig. 5 seems to support their view and the percentage of intestinal absorption of phenol red is dependent on the initial concentrations. With the above in mind, it became desirable to determine the effect of bile salts on this specialized transport of phenol red at the bile salts concentration of 40 mM. The results are shown in Fig. 5. Both sodium taurocholate and sodium glycocholate increased the intestinal absorption of phenol red. Saturation of the absorption, however, was not seen at the concentration examined. In addition, the effect of bile salts on the binding tendency of phenol red to the intestinal mucosa was investigated, and increase of the binding fraction of phenol red to the intestinal mucosa was observed by bile salts. Apparent partition coefficient of this compound was very small and any effect of the bile salts on the partition characteristics was hardly observed. On the other hand, as shown in Fig. 3, this compound interacted with bile salts a little. These observations, together with the results that the absorption of phenol red being increased by pretreatment with bile salts (Table II) supports the view that the absorption enhancing effect could be attributed to the direct action of bile salts to the mucosal membrane. It is likely that bile salts are adsorbed

TABLE III. Effect of Bile Salts on the Intestinal Absorption of Sulfanilamide in Rats<sup>a)</sup>

Bile salt	Absorption (%)	Bile salt	Absorption (%)
None	44.5	40.0 mM sod. taurocholate	44.0
3.15 mM sod. taurocholate	42.3	40.0 mM sod. glycocholate	42.0

a) concentration of sulfanilamide = 0.1 mM, pH 6.5

16) H. Kunze and W. Vogt, *Arch. Exptl. Pathol. Pharmacol.*, **256**, 139 (1967); H. Kunze, *ibid.*, **259**, 260 (1968).

to the intestinal mucosa, and the concentration of phenol red, forming complex with the former, was elevated at the mucosal surface, thereby increasing the intestinal absorption of phenol red.

iii) Sulfanilamide and Sulfaguanidine—As shown in Table III, the intestinal absorption of sulfanilamide, unionized at pH 6.5, was hardly affected at all by bile salts. This agrees with the previous report from this laboratory that the intestinal absorption of sulfanilamide was not affected by various types of surface active agents up to the concentration of 5 mg/ml.<sup>17)</sup>

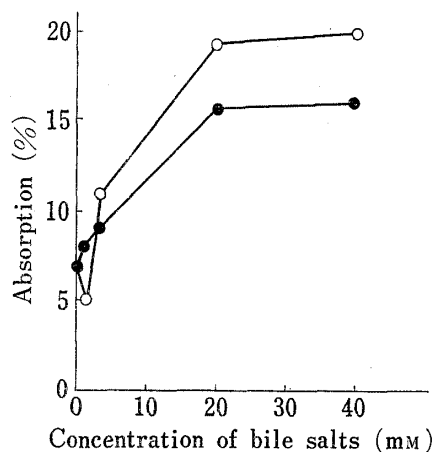


Fig. 6. Effect of Bile Salts on the Intestinal Absorption of Sulfaguanidine in Rats

concentration of sulfaguanidine = 0.1 mM,  
pH 6.5  
Symbols are the same as in Fig. 2.

Sulfaguanidine, another unionized drug tested, has been reported to be poorly absorbed from the gut.<sup>18)</sup> The nature of such poor absorbability of the drug has never been clarified. In Fig. 6 are shown the results of the intestinal absorption of sulfaguanidine, when 0.1 mM of the compound was perfused with various concentrations of bile salts. It can be seen that the intestinal absorption of sulfaguanidine was increased by both sodium taurocholate and sodium glycocholate at their concentrations above the critical micellar concentration, but that there was no significant increment from 20 mM to 40 mM. Since the drug does not form micellar complex with bile salts as can be seen from Fig. 3. It appears that the increased absorption of this compound in the presence of bile salts was probably due not to the intraluminal interaction nor the concentration effect on the absorptive surface but to the actions on the mucosal membrane in some way.

It is worthy of note that bile salts, normal constituents of the gastrointestinal tract, affect the intestinal absorption of various drugs in many ways. Such biopharmaceutical studies will significantly contribute to a greater understanding of the complex process of drug absorption and additional studies are in progress to further elucidate the observations presented in this communication.

17) K. Kakemi, T. Arita, H. Sezaki, and I. Sugimoto, *Yakugaku Zasshi*, **84**, 1210 (1964).

18) F.L. Rose and A. Spinks, *Brit. J. Pharmacol.*, **2**, 65 (1947).