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Effect of Bile Salts on the Gastrointestinal Absorption of Drugs. IV. Site of Intestinal Absorption of Sodium Taurocholate and Its Consequence on Drug Absorption in the Rat[1,2]

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The site specificity in the absorption of sodium taurocholate (STC), one of the most common bile salts, and its consequence on drug absorption was investigated in the rat using the *in situ* perfusion technique. The absorption of STC by the jejunum was negligible both below and above the critical micellar concentration and it was absorbed solely by the ileum. Marked but variable change in absorbability of drugs was observed depending upon the physicochemical nature and the absorptive characteristics of drugs. The extent of the bile salt enhancement of the absorption of sulfaguanidine was more predominant in the ileum than in the jejunum, while the inhibitory effect of STC on the absorption of imipramine was less remarkable in the ileum. Possible mechanisms of these site specificity was discussed.

Knowledge of the nature of the gastrointestinal barrier and their permeation by drugs is a great help in understanding the pharmacological activity of most of drugs. Recent studies in this laboratory have shown that conjugated trihydroxy bile salts, sodium taurocholate (STC) and sodium glycocholate,[4] and their deconjugated form, sodium cholate,[1] considerably influence the absorption of a variety of water-soluble drugs in many ways in the perfused rat small intestine.

The present experiments were designed to clarify the site specificity in the intestinal absorption of STC, one of the most common bile salts, and its consequence on the absorption of water-soluble drugs.

Experimental

Materials——STC and sodium taurodeoxycholate (STDC) were synthesized by the method of Norman[5] and were chromatographically pure.[6] Imipramine hydrochloride and 2-allyloxy-4-chloro-N-(2-diethylaminoethyl) benzamide hydrochloride (ACDB) were kindly supplied by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan. All other drugs used in these experiments were of analytical grade.

Preparation of Drug Solutions——The composition of isotonic buffer solution used as the medium was NaH_2PO_4–Na_2HPO_4 and all experiments were carried out at pH 6.5.

Analytical Methods——i) Sulfonamides: Sulfonamides were estimated colorimetrically as described previously.[4a]

ii) Sulfanilic Acid: Analytical procedure for sulfanilic acid was the same as that for sulfonamides, which has been described previously.[4a]

iii) ACDB: The analytical procedure for ACDB has been previously reported.[4a]

iv) Imipramine Hydrochloride: A sample solution (0.4 μmole) was alkalinized with one ml of 3N NaOH and extracted with 7 ml of *n*-heptane by the addition of sodium chloride. An aliquot of the organic phase was then shaken with acid media and the optical density of the latter phase was determined spectrophotometrically at 251 mμ.

1) Part III: T. Kimura, K. Inui, and H. Sezaki, *Yakuzaigaku*, **31**, 167 (1971).
2) Presented to the 91st Annual Meeting of the Pharmaceutical Society of Japan, April 1971, Fukuoka.
3) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*
4) *a*) K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and M. Murakami, *Chem. Pharm. Bull.* (Tokyo), **18**, 275 (1970); *b*) K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and A. Okita, *ibid.*, **18**, 1034 (1970).
5) A. Norman, *Arkiv Kemi*, **8**, 331 (1955).
6) A.F. Hofmann, *J. Lipid Res.*, **3**, 127 (1962).

v) Quinine Hydrochloride: The analytical procedure for quinine was the same as that for imipramine, except that organic solvent used was ethylene chloride and the absorption was measured at 251 mμ.

vi) N^1,N^1-Anhydro-bis-(β-hydroxyethyl) Biguanide Hydrochloride (ABOB): ABOB (0.4 μmole) was extracted with isopentyl alcohol by the addition of 3N NaOH and sodium chloride. An aliquot of the organic phase was then shaken with pH 6.5 phosphate buffer, and the optical density of the latter phase was determined spectrophotometrically at 236 mμ.

vii) Bile Salts: The analytical procedure for bile salts were a modification of that of Eriksson and Sjövall.[7] Four ml of 81% sulfuric acid was added to one ml of sample solution (0.2 μmole) and mixed thoroughly. The tubes were heated for 15 min at 60° in a water bath, then cooled in tap water, and allowed to stand for 15 min at room temperature before the measurements. STC was measured at 320 mμ, STDC at 308 mμ. No change was observed in the blank value before and after the intestinal perfusion. In the case of simultaneous perfusion of quinine and a bile salt, quinine was first removed by extraction and the residue was assayed for the bile salt as described above.

Procedure of Absorption Experiments——Male Wistar rats weighing 150—200 g were used in all experiments. The procedure of absorption experiments from rat jejunum and ileum was the same as those reported in the papers from this laboratory.[4a,8] For experiments on the jejunum, the first 20 cm of the intestine beyond the ligament of Treiz was used. Ileal segments were cannulated at the ileocecal junction and 20 cm proximally. Twenty ml of drug solution was perfused in each segment at the rate of approximately 5 ml/min. After one hour, the perfused solution in the intestine was withdrawn as completely as possible, the intestinal lumen was flushed 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.

Preliminary experiments with imipramine using a conventional apparatus revealed that extreme adsorption of this drug to silicone tubings took place. Therefore, in the experiments for imipramine absorption, a teflon cannula was used for the cannulation of the intestine since this material does not adsorb imipramine.

In the studies on gastric absorption, the *in situ* ligation method of Schanker, *et al.*[9] was adopted. Percentage absorbed was calculated from the difference of the amount of a drug at the initial and final states.

Result

1. Absorption of STC and STDC

Absorption of STC and STDC by rat jejunum and ileum was examined using *in situ* perfusion method; the results are given in Table I. STC was absorbed mainly by the ileum and its absorption by the jejunum was negligible both below and above the critical micellar

Table I. Absorption of STC and STDC by Rat Jejunum and Ileum

Bile salt	Concentration (mM)	% absorbed in one hour		
		Stomach	Jejunum	Ileum
STC	1	—	2.4±0.1	62.9±7.5
STC	20	1.2±1.1	0.1±4.5	25.4±2.1
STDC	20	—	0.6±1.6	6.4±0.4

Results are expressed as the mean ±S.D. in at least 4 animals.

concentration (CMC, 2.9 mM for STC). STDC was also absorbed only by the ileum, but the ileal absorption was lower than that of STC.

The ileal absorption of STC in one hour is plotted as a function of the initial concentration in Fig. 1. As is evident from the figure, the absorptive process is saturable and a double reciprocal plot of the same data gives the values of K_m and V_{max} 20 mM and 12.5 μmoles·

7) S. Eriksson and J. Sjövall, *Arkiv Kemi*, **8**, 311 (1955).
8) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1883 (1967).
9) L.S. Schanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957).

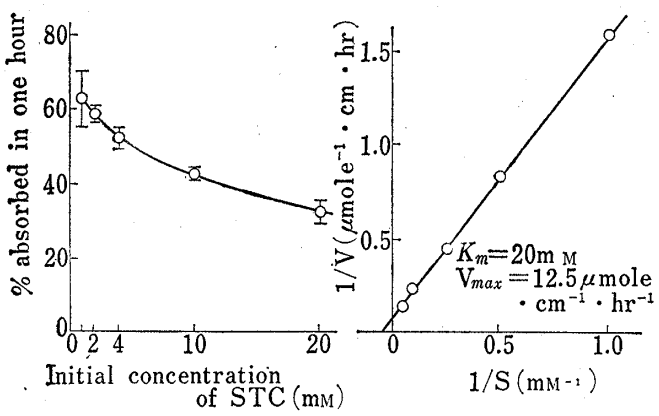

Fig. 1. Left: Ileal Absoption of STC in One Hour as a Function of the Initial Concntration Right: Double Reciprocal Plot of the Same Data.

Results are expressed as the mean±S.D. in at least 4 animals.

$cm^{-1} \cdot hr^{-1}$, respectively. These values are extremely greater than the *in vitro* values of Playoust and Isselbacher[10] and of Dietschy, *et al.*[11]

The time course of the ileal STC absorption is illustrated in Fig. 2. Absorption follows apparent first order kinetics at concentrations below the CMC and the slopes at 1 mM and 2 mM give the same values. On the other hand, the curves are bent upward above the CMC.

Fig. 3 shows the concentration dependency of the ileal STC absorption rate given as the initial velocity (percentage absorbed in 20 min). It is evident from this figure that the absorption of STC is inhibited above the CMC.

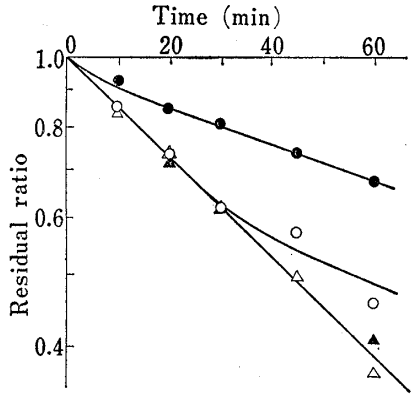

Fig. 2. Time Course of the Ileal Absorption of STC

Each point represents the mean in at least 4 animals.
●: 20 mM ○: 4 mM ▲: 2 mM △: 1 mM

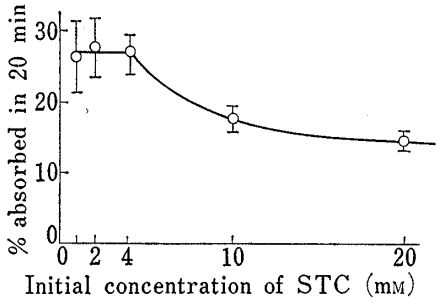

Fig. 3. Concentration Dependency of the Ileal STC Absorption Rate Given as the Initial Velocity (percentage abosrbed in 20 min)

Results are expressed as the mean ±S.D. in at least 4 animals.

TABLE II. Effect of STC on Drug Absorption by Rat Jejunum and Ileum

Drug	% absorbed in one hour			
	Jejunum		Ileum	
	None	STC	None	STC
Sulfaguanidine	4.0±1.0	8.6±2.4	3.8±1.3	10.9±2.4
Sulfapyridine	35.0±3.5	41.2±2.9	47.1±2.5	45.1±7.9
ABOB	4.2±1.2	8.9±1.1	1.5±1.0	9.4±1.0
ACDB	37.3±4.7	27.9±3.1	41.4±2.6	35.1±3.9
Imipramine	39.8±4.2	19.9±1.8	45.5±1.9	37.7±1.6
Quinine	23.3±2.7	18.6±1.8	28.6±3.0	27.2±3.2
Sulfanilic acid	2.7±1.9	9.5±1.0	2.2±0.3	9.7±1.7
Sulfisoxazole	29.4±2.6	26.0±3.9	25.6±2.5	31.2±4.2

concentration of STC=20 mM.
Results are expressed as the mean ±S.D. in at least 4 animals.

10) M.R. Playoust and K.J. Isselbacher, *J. Clin. Invest.*, **43**, 467 (1964).
11) J.M. Dietschy, H.S. Salomon, and M.D. Siperstein, *J. Clin. Invest.*, **45**, 832 (1966).

2. Effect of STC on Drug Absorption by Jejunum and Ileum

Effect of STC on the absorption of drugs by rat jejunum and ileum is summarized in Table II. The bile salt enhanced the absorption of three poorly-absorbable drugs, sulfaguanidine, sulfanilic acid and ABOB, whose ionic nature at physiologic pH was different, and the extent of absorption enhancement was similar in the jejunum and the ileum. On the other hand, STC decreased the absorption of three basic drugs, ACDB, imipramine and quinine.

In order to elucidate the site specificity of the effects of bile salts on drug absorption, effect of STC on the absorption of the three drugs, sulfaguanidine, imipramine and quinine, was furhter investigated.

i) Sulfaguanidine——Effect of bile salts on the absorption of sulfaguanidine is shown in Table III. As described above, the enhancement of the absorption of sulfaguanidine by 20 mM

Table III. Effect of Bile Salts on the Absorption of Sulfaguanidine by Rat Stomach, Jejunum and Ileum

	% absorbed in one hour		
	Stomach	Jejunum	Ileum
None	3.5±1.4	4.0±1.0	3.8±1.3
20 mM STC	5.2±1.1	8.6±2.4	10.9±2.4
40 mM STC	—	9.6±0.8	13.1±0.5
20 mM STDC	—	14.1±1.0	22.5±3.4
20 mM SLS	15.0±7.4	22.2±2.4	24.7±4.9

Results are expressed as the mean ±S.D. in at least 4 animals.

STC (initial concentration) was the same degree both in the jejunum and in the ileum. The enhancement effect of the jejunal absorption of sulfaguanidine almost ceased progressing at the concentration of STC above 20 mM, while the ileal one was further increased by STC at the concentration of 40 mM. STDC enhanced the absorption of sulfaguanidine in the ileum more predominantly than in the jejunum. In contrast, the enhancement effect on the absorption of sulfaguanidine by sodium lauryl sulfate (SLS), a synthetic anionic surfactant, was similar in the jejunum and the ileum.

ii) Imipramine——Table IV shows the inhibitory effect of bile salts on the absorption of imipramine in various intestinal segments. Percentage inhibition are indicated in paren-

Table IV. Effect of Bile Salts on the Absorption of Imipramine by Rat Stomach, Jejunum and Ileum

	% absorbed in one hour		
	Stomach	Jejunum	Ileum
None	21.5±4.3	39.8±4.2	45.5±1.9
10 mM STC	—	32.5±2.2 (18.4)	—
20 mM STC	16.3±3.2 (24.2)	19.9±1.8 (50.0)	37.7±1.6 (17.1)
20 mM STDC	—	14.1±1.6 (64.6)	16.8±2.8 (63.1)
20 mM SLS	—	6.1±2.8 (84.7)	8.3±2.5 (81.8)

Results are expressed as the mean ±S.D. in at least 4 animals.
Percentage inhibition are indicated in parentheses.

theses. As described above, inhibitory effect of STC in the ileum at the concentration of 20 mM (initial concentration) is inferior to that in the jejunum and corresponds to that in

the jejunum at 10 mM. STDC, only slightly absorbed even by the ileum, inhibits the absorption of this drug in the ileum as the same degree as in the jejunum. The inhibitory effect of SLS is analogous to that of STDC.

iii) Quinine——As shown in Table V, the inhibitory tendency of STC on the absorption of quinine is similar but slightly inferior to the case of imipramine.

TABLE V. Effect of STC on the Absorption of Quinine by Rat Jejunum and Ileum

	% absorbed in one hour	
	Jejunum	Ileum
None	23.3±2.7	28.6±3.0
20 mM STC	18.6±1.8 (20.2)	27.2±3.2 (4.9)
20 mM SLS	13.4±2.1 (42.5)	14.4±2.4 (49.7)

Results are expressed as the mean ±S.D. in at least 4 animals.
Percentage inhibition are indicated in parentheses.

Discussion

1. Absorption of STC and STDC

It has been established that bile salts are mainly absorbed by an active transport process, located only in the ileum[10-12] and is dependent upon the concentration of Na^+ but not upon the presence of glucose.[10] Since the proximal small bowel lacks active absorptive sites, conjugated bile salts are absorbed only by relatively slow passive ionic diffusion at physiologic pH. Most of those studies, however, were carried out *in vitro* levels and at bile salts concentrations below their CMC and few studies concerning the ileal transport of bile salts above their CMC have been reported. As is evident from Table I, STC absorption by the jejunum is negligible whereas its ileal absorption is remarkable. These findings concerning the site of the bile salt transport are consistent with previous *in vitro*[10-12] and *in vivo* (in man) works.[13] In the present experiments, however, the ileal absorption of STC (20 mM) was not inhibited by metabolic inhibitors such as ouabain (1 mM and/or 5 mg/kg, *i.v.*), potassium cyanide (1 mM) and benzmalecene (5 mM), which does not agree with previous results of many *in vitro* experiments.[10,12b,c] The lower ileal absorption of STDC is consistent with the report of Schiff and Dietschy[14] that the V_{max} value is directly related to the number of hydroxyl groups on the bile acid molecule.

It is considered from Fig. 2 and 3 that the K_m value for the active transport of STC is much higher than the CMC, and that the decreased absorption at the higher concentrations and the bent curves of the time course are caused by the low affinity of the bile salt micelle to the carrier, in spite of the rapid adsorption of the micelle to the mucosal surface. Contribution of the self-inhibition at high concentrations can be ruled out as the pretreatment with 20 mM STC for 30 min did not affect the absorption of 4 mM STC.

2. Effect of STC on Drug Absorption by Jejunum and Ileum

i) Sulfaguanidine——In our previous report,[4b] it has been shown that the enhancement of the intestinal absorption of sulfaguanidine by STC is caused by the reversible direct action

12) *a*) L. Lack and I.M. Weiner, *Am. J. Physiol.*, **200**, 313 (1961); *b*) P.R. Holt, *ibid.*, **207**, 1 (1964); *c*) J.M. Dietschy, *J. Lipid Res.*, **9**, 297 (1968).
13) B. Borgström, G. Lundh, and A.F. Hofmann, *Gastroenterology*, **45**, 229 (1963); W.J. Simmonds, A.F. Hofmann, and E. Theodor, *J. Clin. Invest.*, **46**, 874 (1967).
14) E.R. Schiff and J.M. Dietschy, *J. Clin. Invest.*, **47**, 87a (1968).

of the bile salt to the structure of the absorptive surface, such as calcium depletion and breaking of intermolecular hydrogen bonds of membrane phospholipid. As shown in Table III, the enhancement of the absorption of this drug by 20 mM STC was similar in the jejunum and in the ileum. However, since the enhancement effect is dependent upon the concentration of STC below 20 mM,[4b] the effect should be more predominant in the ileum, where the bile salt is markedly absorbed. As a clue to elucidate this point, the effect of 40 mM STC on the absorption of sulfaguanidine was first investigated. Our previous report[4a] has shown that the enhancement effect in whole small intestine almost ceased progressing at the concentration of STC above 20 mM. As shown in Table III, the jejunal absorption tendency of sulfaguanidine was similar to that in whole small intestine and, nevertheless, the ileal one was further increased by STC at the concentration of 40 mM. In addition, STDC, whose ileal absorption is a little, enhanced the absorption of this drug in the ileum more predominantly than in the jejunum. Recently, using the *in vitro* method, Wall and Baker[15] reported that STC caused a marked drop in transmural resistance, especially in the ileum. It can be considered that there is some correlation between the above information and our results.

The fact that SLS enhanced the absorption of sulfaguanidine similarly in the jeiunum and in the ileum rules out the possibility that the site specificity of STC effect was caused by the morphological difference in the jejunum and the ileum.

In addition to those effects in the small intestine, the effect of STC on the absorption of sulfaguanidine by the stomach was investigated. It is well known that gastric ulcers can be induced in animals by feeding bile salts[16] and that bile salts impair the gastric mucosal barrier and allow hydrogen ions to diffuse into the stomach wall.[17] But as shown in Table III, the enhancement of the absorption of sulfaguanidine by STC in the stomach was very small.

ii) Imipramine and Quinine——The inhibition of the absorption of ACDB, imipramine and quinine could be interpreted in terms of the loss of thermodynamic activity of the drugs due to the formation of the micellar complexes with the bile salt (an intraluminal effect) from the results of following experiments; (1) a shift of their ultraviolet absorption spectra, (2) decrease of the apparent partition coefficient,[4a] and (3) the molecular sieve technique using Sephadex G-25.[18] This intraluminal effect was also supported by the results that the absorption of these compounds were hardly altered at all by the pretreatment of the intestine by the bile salt solutions. It is interesting to note that these inhibitory effect was more predominant in the jejunum than in the ileum. Even if the decrease of the intraluminal concentration of STC due to the ileal absorption of the bile salt were considered, inhibitory effect is not remarkable (Table IV). The reduced inhibitory effect of STC on the ileal absorption of these drugs would be due to the less stable micelles of the compound caused by a preferential absorption of the bile salt by this absorptive site. In contrast, such difference became very small in the case of STDC. This would be attributable to the poor absorbability by the ileum and, in addition, to the formation of much larger and stable micelles of the bile salt.[19]

The variation in the STC effect between the absorption of imipramine and that of quinine would mainly depend on the differences in the molecular structure of these compounds; namely, in contrast to quinine, an amphipathic compound like imipramine presumably converts the small compact micelle to a stable bulky mixed micelle.[20]

This effect was also reflected upon the absorption of STC. Table VI shows the absorption of STC in the presence of three drugs. As is evident from the table, imipramine inhibits the ileal absorption of STC. This would be caused by the reduction of the affinity for the

15) M.J. Wall and R.D. Baker, *Fed. Proc.*, **29**, 595 (1970).
16) D. Rywosch, *Arb. Pharmak. Inst. Dorpat.*, **2**, 102 (1888).
17) H.W. Davenport, *Gastroenterology*, **54**, 175 (1968).
18) R.W. Ashworth and D.D. Heard, *J. Pharm. Pharmacol.*, **18**, *Suppl.*, 98S (1966).
19) M.C. Carey and D.M. Small, *J. Colloid Interface Sci.*, **31**, 382 (1969).
20) M.C. Carey and D.M. Small, *Am. J. Med.*, **49**, 590 (1970).

Table VI. Absorption of STC by Rat Jejunum and Ileum in the Presence of Various Drugs

Drug	% absorbed in one hour	
	Jejunum	Ileum
None	0.1±4.5	25.4±2.1
Sulfaguanidine	1.6±2.8	25.4±4.7
Imipramine	4.5±1.8	16.5±3.3
Quinine	3.2±2.7	23.2±2.8

Concentration of STC=20 mM.
Results are expressed as the mean ±S.D. in at least 4 animals.

transport carrier of STC, due to the formation of bulky micelles. But further experiments will be required to explain it.

From the facts described above, we may conclude that the effect of bile salts on the gastrointestinal absorption of drugs is closely related to the site of the absorption of bile salts themselves; in the bile salt-absorptive site, the enhancement effect on the absorption of such a drug as sulfaguanidine due to the increment of the membrane permeability is high and, in contrast, the inhibitory effect on the absorption of such a drug as imipramine caused by the loss of thermodynamic activity of the drug due to the formation of the micellar complex, is low.