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Effect of Bile Salts on the Gastrointestinal Absorption of Drugs. II. Mechanism of the Enhancement of the Intestinal Absorption of Sulfaguanidine by Bile Salts[1,2]

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The mechanism of the enhancement of the intestinal absorption of sulfaguanidine by bile salts, sodium taurocholate and sodium glycocholate, together with the poor absorbability of the drug itself was investigated, using the *in situ* single loop preparations. It was found that the cause of the poor absorbability of sulfaguanidine could be attributed neither to its dimer formation nor to the iceberg formation in aqueous solution. Since bile salts did not affect its physico-chemical properties such as apparent partition coefficient and diffusion constant, and did not form micellar complex with the sulfa drug, it became evident that the absorption enhancement was not caused by their intraluminal effect. On the absorptive surface, bile salts did not increase the affinity of the sulfa drug to the intestinal mucosal surface. The exsorption study demonstrated that such enhancement was caused by the direct action of bile salts to the structure of the absorptive surface. This effect was reversible and could not be explained only by the calcium depletion.

In the previous report of this series, we have shown that bile salts affect the intestinal absorption of various drugs in many different ways in rats.[4] Absorption of sulfanilamide was not affected while absorption of sulfaguanidine and phenol red, poorly absorbable drugs, were enhanced by bile salts above their critical micellar concentration. On the other hand, absorption of 2-allyloxy-4-chloro-N-(2-diethylaminoethyl) benzamide hydrochloride was inhibited by bile salts above the critical micellar concentration.

There are some papers concerning with the increase of membrane permeability by bile salts. Davenport has shown that sodium taurocholate increased the permeability of canine gastric mucosa.[5] Recently, Feldman and Gibaldi reported that the transfer rates of salicylate and salicylamide across the everted rat intestine were increased by the alteration of membrane structure, increase in membrane permeability, by sodium taurodeoxycholate (STDC).[6] Nightingale, *et al.* also reported that STDC singificantly increased the uptake of 4-aminoantipyrine in goldfish by alteration of membrane permeability.[7] However, there are few reports concerning with the effect of bile salts on the absorption enhancement of so-called poorly absorbable drugs.

In this report, the mechanism of the enhancement of the intesitnal absorption of sulfaguanidine by bile salts, sodium taurocholate and sodium glycocholate, was investigated together with the poor absorbability of the drug itself.

1) This paper forms Part XXXXIII of "Absorption and Excretion of Drugs," Preceding paper, Part XXXXII: K. Kakemi, H. Sezaki, R. Konishi, T. kimura, and M. Murakami, *Chem. Pharm. Bull.* (Tokyo), **18**, 275 (1970).
2) Presented to the 19th Kinki Branch Meeting of Pharmaceutical Society of Japan, Osaka, October, 1969.
3) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*
4) K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and M. Murakami, *Chem. Pharm. Bull.* (Tokyo), **18**, 275 (1970).
5) H.W. Davenport, *Proc. Soc. Exptl. Biol. Med.*, **125**, 670 (1967).
6) S. Feldman and M. Gibaldi, *J. Pharm. Sci.*, **58**, 425 (1969); *idem, ibid.*, **58**, 967 (1969).
7) C.H. Nightingale, R.J. Wynn, and M. Gibaldi, *J. Pharm. Sic.*, **58**, 1005 (1969); M. Gibaldi and C.H. Nightingale, *ibid.*, **57**, 1354 (1968).

Experimental

Materials——Sodium taurocholate and sodium glycocholate were synthesized by the method of Norman,[8] and were chromatographically pure. All other drugs used in these experiments were analytical grade.

Procedure of Absorption Experiments——Male Wistar rats weighing 150—220 g were used in all experiments. The degree of intestinal absorption was measured using *in situ* single loop preparations. With the rat under pentobarbital anesthesia, the intestine was exposed. The loop used was whole small intestine, from the pylorus to the ileo-cecal junction, and both ligatures were cannulated with polyvinyl tubings. No major blood vessels were occluded by these ties. The bile duct was ligated in all experiments. Physiologic saline solution was injected into the loop until washings became clear. Five ml of drug solution per 150 g body weight was injected into the loop from the proximal end. The ligature was secured and the incision closed. After one hour, the solution in the loop was withdrawn as completely as possible, and washed with physiologic saline. The washings were combined with the initial sample and made up to 50 ml with physiologic saline. From the difference of the drug in amount between the stock solution and the combined effluent, the eliminated amount of the drug from the stock solution was calculated.

Preparation of Drug Solution——The composition of isotonic buffer solution used as the medium were citric acid–Na_2HPO_4 for pH 4.5, NaH_2PO_4–Na_2HPO_4 for pH 6.5, and $NaHCO_3$ for pH 8.3.

Analytical Method——The analytical procedure for sulfaguanidine has been previously reported.[4]

Apparent Partition Coefficient——Apparent partition coefficients were determined as previously described in the report from this laboratory.[9]

Diffusion Constant——Diffusion constants were determined by the method of Nakagaki, *et al.*[10]

Binding Tendency of Sulfaguanidine to the Intestinal Mucosa——The binding of sulfaguanidine was determined by the method of Meyer and Guttman.[11] This method was employed because of its rapidity, simplicity, and good reproducibility for comparative purposes. The experimental system was the same as they described, and other conditions were set arbitrarily. The preparation of the intestinal mucosa was as follows. Rats were anesthetized with sodium pentobarbital. The entire small intestine was removed and immediately irrigated with cold physiologic saline. The intestine was then everted, and the mucosa was removed with a glass slide. The mucosa was weighed, homogenized, and the concentration was made up to 25 percent (wet weight). Seven ml of the homogenates were used in the experiments.

Procedure of Exsorption Experiments——In order to clarify whether the effect on the sulfaguanidine absorption was caused by the interaction between sulfaguanidine and bile salts in the perfusion solution, intraluminal interaction, or by some actions of bile salts to the mucosal membrane, exsorption study was carried out. The procedure of the operation was the same as *in situ* perfusion method for the intestinal absorption study described in the papers from this laboratory.[12] The bile duct was ligated in all experiments. Sulfa drug, 5 mg/0.5 ml in 30% N,N′,-dimethylacetamide solution, was administered intravenously to the rat from the femoral vein, and the small intestine was perfused with pH 6.5 buffer at the rate of 1 ml/min. The perfusion solution was collected every five minutes, and the exsorption rates were calculated from the amount of drug in the solution. In some experiments, to investigate the effect of bile salts, the small intestine was perfused first with pH 6.5 buffer for 30 min, and with 20 mM sodium taurocholate (pH 6.5 buffer solution) for the next one hour, followed by perfusion with the pH 6.5 buffer again.

Results and Discussion

The exact nature of poor absorbability of sulfaguanidine has not been elucidated to date since the pioneer work by Rose and Spinks.[13] Since there had been several reports about the ionic state of sulfaguanidine at neutral pH, we further investigated it.

Rose and Spinks,[13] and Brodie, *et al.*[14] claimed that sulfaguanidine is almost completely undissociated at neutral pH. On the other hand, Matsuura[15] and Wilson[16] claimed that it is a strong base and is highly ionized at neutral pH. In Fig. 1 is shown the pH-dependency of

8) A. Norman, *Arkiv Kemi*, **8**, 331 (1955).
9) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1534 (1967).
10) M. Nakagaki, N. Koga, and S. Iwata, *Yakugaku Zasshi*, **82**, 1134 (1962).
11) M.C. Meyer and D.E. Guttman, *J. Pharm. Sci.*, **57**, 1627 (1968).
12) 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).
13) F.L. Rose and A. Spinks, *Brit. J. Pharmacol.*, **2**, 65 (1947).
14) B.B. Brodie, H. Kurz, and L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **130**, 20 (1960).
15) H. Matsuura, *Shikoku Acta Medica*, **15**, 94 (1959).
16) T.H. Wilson, "Intestinal Absorption," W.B. Saunders Company, London, 1962, p. 247.

the apparent partition coefficient of sulfaguanidine to isopentyl alcohol at 37°, which is approximately similar to the result of Scholtan.[17] It is evident that sulfaguanidine is in unionized form at pH 6.5. This was supported further by the results of ultraviolet absorption spectra analysis and electrophoresis.

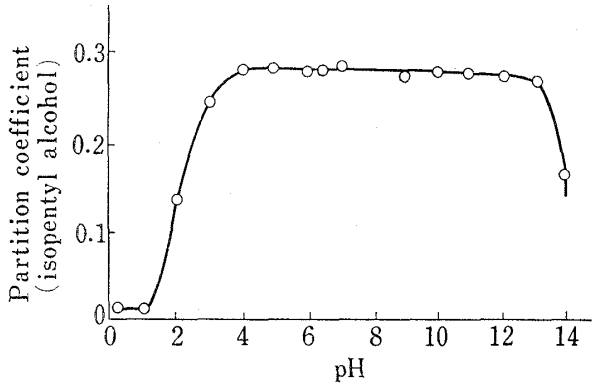

Fig. 1. pH-Profile of Apparent Partition Coefficient of Sulfaguanidine

Concentration of sulfaguanidine=0.1 mM

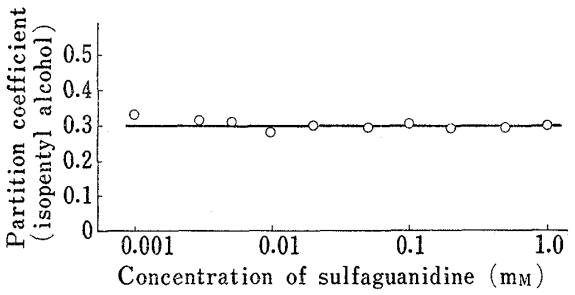

Fig. 2. Concentration Dependency of Apparent Partition Coefficient of Sulfaguanidine at pH-6.5

Hogben, *et al.* noted that sulfaguanidine, unionized at pH 7.2, is poorly absorbable because of its low lipid-solubility.[18] In addition, Rose and Spinks considered that the increased molecular weight resulting from dimer formation would probably lead to slower absorption.[13] As shown in Fig. 2, the apparent partition coefficient of sulfaguanidine were independent of the concentration at pH 6.5. And its ultraviolet absorption spectra analysis and diffusion constant were also independent of the concentration at pH 6.5. From these results, it is evident that sulfaguanidine does not form dimer in aqueous solution. Other cause of the slow absorbability might be attributed to the hydration or iceberg formation. But, as is evident from Table I, sodium tetraphenylborate, structure breaking anion of water, did not affect the apparent partition coefficient of sulfaguanidine. This suggests that sulfaguanidine does not hydrate in the buffer solution.

With the above in mind, it became desirable to determine the effect of bile salts on the absorption on sulfaguanidine. For this purpose, it is convenient to devide the effect of bile salts on the absorption of sulfaguanidine into two stages: luminal phase and absorptive surface.

Table I. Apparent Partition Coefficient of Sulfaguanidine to Isopentyl Alcohol at pH 6.5

Adjuvant[a]	Partition coefficient
None	0.280
Sod. taurocholate	0.275
Sod. glycocholate	0.271
Sod. tetraphenylborate	0.281
Sod. lauryl sulfate	0.292

a) concentration=20 mM

Table II. Effect of Bile Salts on Diffusion Constant of Sulfaguanidine at pH 6.5, 37°

Bile salt[a]	$D \times 10^6$ (cm^2/sec)
None	9.46
Sod. taurocholate	8.40
Sod. glycocholate	9.11

a) concentration=20 mM

17) W. Scholtan, *Arzneimittel-Forsch.*, **18**, 505 (1968).
18) C.A.M. Hogben, D.J. Tocco, B.B. Brodie, and L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **125**, 275 (1959).

Luminal Phase

In this section we wish to investigate the effect of bile salts on some physico-chemical properties of sulfaguanidine. In Table I are shown the effect of bile salts on its apparent partition coefficient to isopentyl alcohol at pH 6.5. What is evident from the table is that both sodium taurocholate and sodium glycocholate do not affect at all, and the other typical synthetic anionic surfactant, sodium lauryl sulfate (SLS), too . As shown in Table II, bile salts do not affect its diffusion constant at pH 6.5. In addition, our previous report has shown that sulfaguanidine does not form micellar complex with bile salts.[4] From these results, it can be considered that there is no effect of bile salts on the absorption of sulfaguanidine in the luminal phase, as far as we investigated.

Absorptive Surface

In Fig. 3 and Fig. 4 were shown the intestinal absorption of sulfaguanidine affected by bile salts at various pH's. As is evident from both figures, bile salts enhanced the absorption of sulfaguanidine at any pH, and it should be noted that the more pronounced effect was observed

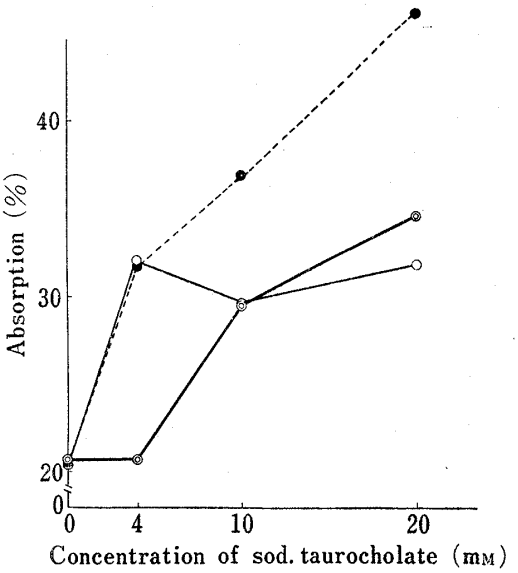

Fig. 3. Effect of Sod. Taurocholate on the Intestinal Absorption of Sulfaguanidine at Various pH's

----●----: pH 8.3
—◎—: pH 6.5
—○—: pH 4.5

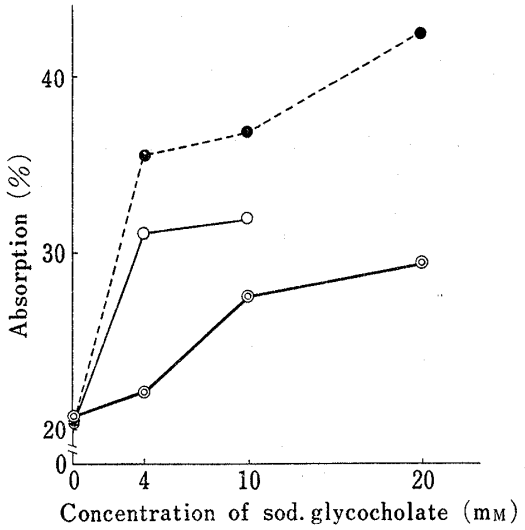

Fig. 4. Effect of Sod. Glycocholate on the Intestinal Absorption of Sulfaguanidine at Various pH's

Symbols are the same as in Fig. 3.
At pH 4.5, no data is available at 20 mM sodium glycocholate because of its insolubility.

at pH 8.3. In addition, these enhancement effects appear to have saturable tendency at any pH, which is identical to our previous findings at pH 6.5.[4] SLS also enhanced the absorption of sulfaguanidine more strongly. Fig. 5 shows the time course of the intestinal absorption of sulfaguanidine with or without 20 mM bile salts. It appears that any of them follows apparent first order kinetics.

In order to make clear whether the effects were caused by some action of bile salts to the absorptive surface or not, we first investigated the effects of bile salts on the binding of sulfaguanidine to the intestinal mucosa. As shown

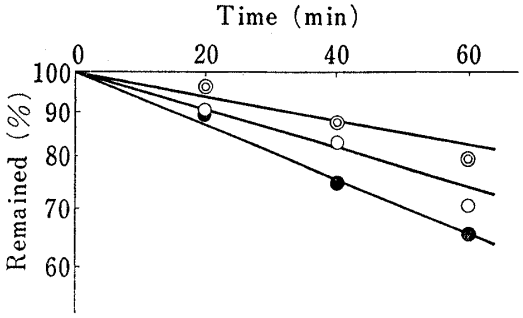

Fig. 5. Time Course of the Intestinal Absorption of Sulfaguanidine at pH 6.5

—◎—: none
—●—: with 20 mM sod. taurocholate
—○—: with 20 mM sod. glycocholate

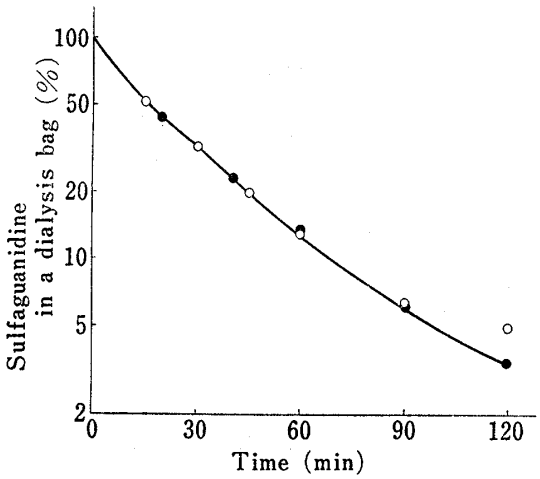

Fig. 6. Effect of Sod. Taurocholate on the Binding of Sulfaguanidine to the Intestinal Mucosa

—●—: control
—○—: with 20 mM sod. taurocholate

in Fig. 6, the decrease of sulfaguanidine in a dialysis bag was not affected by the presence of 20 mM sodium taurocholate. Namely, sodium taurocholate did not affect the binding of sulfaguanidine to the intestinal mucosa. Essentially the same pattern was observed with sodium glycocholate. In the following, it shall be considered that the effect is caused by the direct action of bile salts to the structure of mucosal membrane.

Webling and Holdsworth have demonstrated that bile salts and SLS form calcium complexes,[19] and Tidball has shown that calcium depletion increase the permeability of the intestinal epithelium.[20] In order to clarify whether the enhanced absorption of sulfaguanidine was caused by the depletion of calcium by bile salts or not, we investigated the effect of simultaneous treatment with bile salts and disodium ethylenediamine tetraacetic acid (EDTA) of the intestinal absorption of sulfaguanidine. Feldman and Gibaldi have demonstrated that EDTA and STDC alter membrane permeability by different mechanisms.[6] In contrast, as shown in Table III, it appears that both bile salts and EDTA enhanced the absorption of sulfaguanidine by an apparent common mechanism at pH 6.5. In the case of simultaneous treatment, the degree of the enhancement was similar to the case of bile salt or EDTA alone. The different results are probably caused by the different experimental condition or by using the different kind of bile salts. On the other hand, the effect of simultaneous treatment with bile salts

Table III. Effect of Simultaneous Treatment with Bile Salt and EDTA on the Intestinal Absorption of Sulfaguanidine at pH 6.5

	Absorption (%)		
	Control	EDTA 1 mM	EDTA 10 mM
None	20.8	28.6	28.1
Sod. taurocholate	33.3	30.3	—
Sod. glycocholate	29.4	26.4	—

concentration of bile salt=20 mM

Table IV. Effect of Simultaneous Treatment with Bile Salt and EGTA on the Intestinal Absorption of Sulfaguanidine at pH 8.3

	Absorption (%)		
	Control	EGTA 1 mM	EGTA 10 mM
None	20.6	37.3	36.1
Sod. taurocholate	46.1	45.5	—
Sod. glycocholate	42.4	45.3	—

concentration of bile salt=20 mM

19) D.D'A. Webling and E.S. Holdsworth, *Biochem. J.*, **100**, 652 (1966).
20) C.S. Tidball, *Am. J. Physiol.*, **206**, 243 (1964).

and ethylene glycol bis(β-aminoethyl ether)-N,N′-tetraacetic acid (EGTA), which formed a chelate complex with calcium and not with magnesium, was investigated. EGTA did not affect the absorption of sulfaguanidine at pH 6.5, well below the level above which chelation with calcium ion would be expected. Table IV shows the result at pH 8.3. The simultaneous treatment resulted in a similar enhancement to that observed with bile salt alone, and in a further enhancement compared to that observed with EGTA alone. This suggests that the enhancement of the absorption of sulfaguanidine cannot be explained only by the calcium depletion at pH 8.3.

To confirm that the absorption of sulfaguanidine was enhanced by the direct action of bile salts to the mucosal membrane, the exsorption experiment was performed. The results are shown in Fig. 7. As the perfusion solution was changed to 20 mM sodium taurocholate, the exsorption rate of sulfaguanidine was markedly increased. In addition, as the bile salt solution was again changed to the simple buffer solution, the exsorption rate reduced to the control rate gradually. On the other hand, the exsorption rate of sulfanilamide, whose absorption was not affected by 40 mM bile salts, was also not affected by 20 mM sodium taurocholate. From the result of this experiment, it appears to be the strongest proof that the enhancement of the absorption of sulfaguanidine was caused by the direct action of bile salts to the mucosal membrane, and that the effect was reversible. The reversibility is supported by the result of our previous report that the pretreatment with bile salts did not enhance the intestinal absorption of sulfaguanidine.[4] The enhanced effect of SLS was irreversible by the result of the pretreatment study.

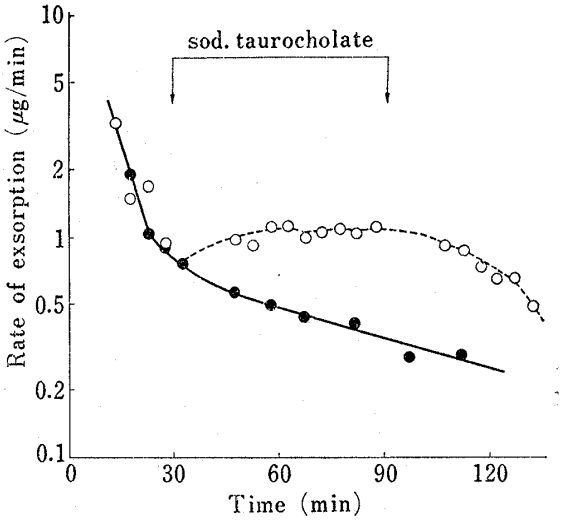

Fig. 7. Effect of Sod. Taurocholate on the Rate of Exsorption of Sulfaguanidine to Rat Small Intestine

—●—: control
—○—: with 20 mM sod. taurocholate

From the facts described above, we may conclude as follows; (1) the enhancenent of the intestinal absorption of sulfaguanidine by bile salts is not caused by the effect of bile salts on the physico-chemical properties of the sulfa drug in the intraluminal phase; (2) bile salts do not increase the affinity of sulfaguanidine to the intestinal mucosa; (3) the enhancemnet is caused by the direct action of bile salts to the structure of the absorptive surface; (4) the effect is reversible. The direct action could not be explained only by the calcium depletion. We must investigate another cause in this point. For instanse, it is considered that bile salts have some effects on the micellar organization of membrane phospholipids, where calcium ion may play the important role to maintain it, or they break intermolecular hydrogen bonds as well as disrupt the tight packing of hydrophobic region, and having done so, they stabilize the dissociated lipid in a much smaller charged aggregate.[21] It may be quite all right to consider that the enhanced effect by bile salts has the selectivity and the intestinal absorption of poorly-absorbable drugs, which are highly ionized drugs (phenol red),[4] have large molecular weights (heparin),[22] or have markedly low lipid-solubility (sulfaguanidine), are selectively enhanced by bile salts. A number of drugs and bile salts of varying physico-chemical properties must be tested before this hypothesis can be accepted.

21) A.F. Hofmann and D.M. Small, *Ann. Rev. Med.*, **18**, 333 (1967).
22) R.H. Engel and S.J. Riggi, *J. Pharm. Sci.*, **58**, 706 (1969).