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Factors Influencing Absorption and Excretion of Drugs. VI.[1] Effect of Sodium Cholate on *in Situ* Rat Intestinal Absorption of Buformin

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The effect of sodium cholate on the absorption of strongly basic drug, buformin, was studied in the *in situ* rat small intestinal loops. In cases of either jejunum or ileum, the absorption of buformin significantly increased above the critical micelle concentration of sodium cholate. Since sodium cholate had little effect on the physico-chemical property such as the apparent partition coefficient of buformin, it became evident that the enhancement of buformin absorption was not caused by the intraluminal effect of the bile salt. The absorption of buformin was also enhanced in the jejunal and ileal loops pretreated with sodium cholate. On the absorptive surface, sodium cholate did not increase the affinity of buformin to the intestinal mucosal surface. In addition, the release of membrane components such as protein, total phosphorus, and phospholipid from the intestine was accelerated to greater extent by sodium cholate. From these results, it is suggested that sodium cholate alters the composition of the *in situ* rat intestinal membrane by producing a release of membrane components from the intestinal tissues, resulting in an increase in the permeability of the small intestine to buformin.

Keywords——buformin; absorption; rat intestine; sodium cholate; partitioning

Bile salts are the most important physiologic surface-active agents influencing the intestinal absorption of nutrients and drugs. It has been widely known[3] that bile salts play an important role in the intestinal absorption of lipophylic compounds such as lipids and lipid-soluble vitamin. On the other hand, recently, some investigators have reported the influence of bile salts on the intestinal absorption of poorly lipid-soluble substances. For example, Mayersohn, *et al.*[4] reported that sodium deoxycholate enhanced the oral absorption of riboflavin and flavin mononucleotide. Feldman and Gibaldi[5,6] showed that sodium taurodeoxycholate markedly increased the permeability of everted rat intestine to salicylate ion. Feldman, *et al.*[7] reported that sodium deoxycholate enhanced the gastrointestinal absorption of phenol red in rats. In addition, Kimura, *et al.*[8] and Gaginella, *et al.*[9] have investigated the effects of bile salts on the absorption of N′,N′-anhydro-bis-(β-hydroxyethyl) biguanide, quinine, and isopropamide iodide, being positively charged at physiologic pH, in rat intestine. However, no detailed study has been done on the effects of bile salts on the gastrointestinal absorption of strongly basic, relatively unabsorbable drugs.

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The purpose of the present study was to determine the effects of an unconjugated bile salt, sodium cholate, on the absorption of strongly basic drug, buformin as an oral blood sugar-lowering agent, in an *in situ* rat intestine.

Experimental

Materials and Equipment——Buformin hydrochloride was prepared according to the method of Shapiro, *et al.*,[10] mp 177—178°. Sodium cholate was purchased from Nakarai Chemicals, Ltd., Kyoto, Japan. Other chemicals were of reagent grade.

A Shimadzu QV-50 spectrophotometer was utilized.

Preparation of Sample Solutions——The isotonic phosphate buffer solution used as the medium in an *in situ* experiment consisted of 77 mM NaCl and 83 mM NaH_2PO_4, and the pH of the solution was adjusted to pH 6.0 with 2N NaOH solution. The initial concentration of buformin was 1000 μg/ml.

Test Animals——Male Wistar Rats weighing 175—250 g were used. The rats were fasted about 20 hr with drinking water *ad libitum* prior to the experiment. They were housed in cages having wide mesh floors to prevent coprophagy.

Absorption Studies from *in Situ* Rat Intestinal Loops——The rats were anesthetized with urethane and a midline incision was made. The small intestine was located and washed with physiologic saline warmed at 37°. The jejunal or ileal loop, 5 cm long, was formed. The jejunal loop was approximately 15 cm from the pylorus and the ileal one approximately 2.5 cm upward the ileo-cecal junction. One-half milliliter of buformin solution in the presence or absence of sodium cholate was injected into the loop, through a ligature, by means of a syringe and blunt needle. As the needle was removed, the ligature was completed. After 2 hr, the animal was sacrificed and the loop was excised. The loop containing buformin was homogenized in 4 ml of 1/15M phosphate buffer, pH 7.2. The homogenizer was washed with 3.5 ml of the buffer and then with ethanol. The homogenate and subsequent washings were added to a 25 ml volumetric flask and brought to volume with ethanol. The entire homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was decanted and filtered into a 100 ml volumetric flask. The precipitate was washed with 70% ethanol, the washings added to the filtrate and the filtrate brought to volume with 70% ethanol. The determination of buformin in the filtrate was carried out by the procedure reported previously.[11]

Pretreatment with Sodium Cholate in Absorption Experiment from *in Situ* Rat Intestinal Loop——In order to clarify the mechanism of the effect of sodium cholate on buformin absorption, the intestinal loops were pretreated with sodium cholate solution. Two milliliters of the buffer solution of sodium cholate at the concentration of 5—20 mM was perfused for 30 min in the rat jejunum, the first 10 cm portion of the intestine approximately 15 cm distal to the pylorus, or the ileum, 10 cm portion upward the ileo-cecal junction. The intestine was washed well with physiologic saline to remove the bile salt as completely as possible. The jejunal or ileal loop, 5 cm long, was formed as described above, and then buformin solution was injected into the loop. In control experiment, the intestine was pretreated with the buffer solution.

Determination of Partition Coefficients——Partition coefficients were determined by the procedure reported in the paper from this laboratory.[12]

Determination of Buformin Content in *in Situ* Rat Small Intestinal Tissue——The determination of buformin contents in the jejunum and ileum was carried out by the procedure reported previously.[11]

Measurement of Membrane Components released from *in Situ* Rat Small Intestine——The release of the membrane components such as phosphorus, phospholipid, and protein from the *in situ* rat jejunal and ileal membranes was determined according to the procedure reported in the paper from this laboratory.[11]

Determination of Critical Micelle Concentration of Sodium Cholate——The critical micelle concentration (CMC) of sodium cholate was determined according to the method of Corrin, *et al.*[13] using toluidine blue O.

Result and Discussion

Effect of Sodium Cholate on Absorption of Buformin from *in Situ* Rat Small Intestine

The absorption of buformin from the *in situ* rat jejunal and ileal loops was examined in the absence or presence of sodium cholate, and the obtained results are represented in Fig. 1. The ileal absorption of buformin scarcely changed at concentrations of sodium cholate below 10 mM but considerably increased at concentrations of the bile salt exceeding 10 mM. On

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the other hand, the absorption of buformin from the jejunal loop was enhanced at concentrations of sodium cholate exceeding 7.5 mM. Dietschy, *et al.*[14] and Schiff, *et al.*[15] have reported that the transport mechanism of sodium cholate includes passive absorption, which takes place in all regions of the intestine, and active absorption, which occurs only in the ileum, and that sodium cholate is absorbed more rapidly in rat ileum than in the jejunum. Thus, it is suggested that although the initial concentration of sodiun cholate enhancing the absorption of buformin is different between the jejunum and ileum, the actual concentration of the bile salt influencing the absorption of the drug is approximately similar in both intestinal regions, and that the drug absorption is enhanced at concentrations of sodium cholate above the CMC, which is 7.5 mM in the phosphate buffer solution containing 1000 μg/ml of the drug.

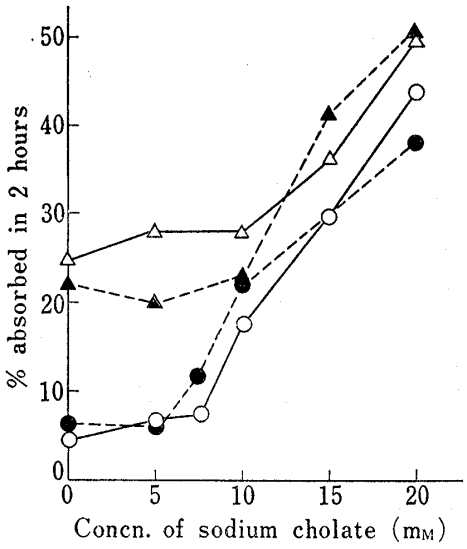

Fig. 1. Effect of Sodium Cholate on Absorption of Buformin from Rat Intestinal Loops

Each value is expressed as the mean of 3 to 7 animals.
—○—: jejunum, —△—: ileum } with sodium cholate
---●---: jejunum, ---▲---: ileum } pretreated with sodium cholate

Table I. Apparent Partition Coefficients of Buformin in the Presence of Various Concentration of Sodium Cholate

Concn. of sodium cholate (mM)	Partition coefficient[a]	
	Chloroform pH 6.0	*n*-Octanol pH 6.0
0	0.011	0.001
1	0.035	0.001
5	0.043	0.016
10	0.050	0.051
20	0.042	0.130

The initial concentration of buformin in the phosphate buffer was 1000 μg/ml.
a) values between chloroform or *n*-octanol and the phosphate buffer at pH 6.0, 37°

In order to clarify the mechanism of the enhancement of the intestinal absorption of buformin by sodium cholate, further study was performed by dividing into the following components: (a) the effect of sodium cholate on the partition coefficient of the drug, (b) the absorption of the drug from the intestinal loops pretreated with sodium cholate, (c) the effect of sodium cholate on the accumulation of the drug in the intestinal tissues, and (d) the effect of sodium cholate on the release of membrane components from the intestine.

Effect of Sodium Cholate on Partition Coefficient of Buformin

In order to make clear whether the enhancing effect of sodium cholate on buformin absorption was caused by the formation of buformin-sodium cholate complex having better affinity for lipoidal membrane or not, we investigated partition studies. As can be seen from Table I, the partition coefficient of buformin between chloroform or *n*-octanol and the phosphate buffer at pH 6.0 was only slightly increased by sodium cholate. Thus, the result indicates that the increase in lipid solubility of buformin by sodium cholate plays a minor role in the

14) J.M. Dietschy, H.S. Salomon, and M.D. Siperstein, *J. Clin. Invest.*, **45**, 832 (1966).
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enhancement of the intestinal absorption of the drug. Concequently, it is suggested that the enhancement of the intestinal absorption of buformin by sodium cholate is not caused by the effect of the bile salt on the physico-chemical property of the drug in the intraluminal phase.

Absorption of Buformin in Small Intestine pretreated with Sodium Cholate

The absorption of buformin was determined in the *in situ* jejunal and ileal loops pretreated with various concentrations of sodium cholate. As shown in Fig. 1, the pattern of change in buformin absorption in the loops pretreated with sodium cholate was almost the same as that in the presence of the bile salt. Namely, the absorption of buformin increased in the jejunum pretreated with sodium cholate solutions at concentrations above 7.5 mM and in the ileum exceeding 10 mM. Some investigators[6,7,16–18] have reported that the enhancing effect of various surfactants on drug absorption is attributed to their direct action on the biological membrane. Accordingly, it is suggested that the enhancement of buformin absorption by sodium cholate is due to the effect of the bile salt on the permeability of the intestinal membrane. In addition, the above result demonstrates that a modification of the permeation characteristics of the intestinal mucosa occurs above the CMC of sodium cholate.

Effect of Sodium Cholate on Accumulation of Buformin in Intestinal Tissues

The previous report[11] from our laboratory demonstrated that approximately half the amount of buformin disappeared from the *in situ* rat intestinal lumens remained in the intestinal tissues, and that the drug was bound to extremely less extent to the mucosa. In order to clarify the effect of sodium cholate on the intestinal mucosa, the behaviour of buformin in the jejunum and ileum was investigated in the presence of sodium cholate. As shown in Table II, in cases of either jejunum or ileum, the disappearance of buformin from the lumens was markedly promoted by the presence of 20 mM sodium cholate, but the accumulation of the drug in the intestinal tissues was not affected by the bile salt. Furthermore, the effect of sodium cholate on the time course of the accumulation of buformin in the jejunal tissue was determined. As indicated in Table III, in either absence or presence of sodium cholate, buformin was accumulated relatively rapidly in the intestinal tissues. These results suggest that sodium cholate does not increase the affinity of buformin to the intestinal mucosal surface.

Table II. Effect of Sodium Cholate on Absorption of Buformin in 2 Hours from the *in Situ* Rat Jejunm and Ileum

		% disappeared from lumen	Content (%) in tissue	% absorbed
Control	jejunum	15.7±1.1	11.7±1.9	4.1±1.8
	ileum	21.0±1.1	9.8±1.7	11.2±2.7
20 mM sodium cholate	jejunum	56.5±2.1	10.0±0.8	46.5±2.3[a]
	ileum	61.7±2.0	8.5±0.4	53.2±2.3[b]

The initial concentration of buformin was 1000 μg/ml.
The values represent the mean ± standard deviation for 3 to 5 animals.
statistical difference from control values:
a) $p < 0.005$, *b*) $p < 0.025$

16) K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and M. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **18**, 275 (1970).
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Table III. Effect of Sodium Cholate on Time Course of Buformin Absorption from the *in Situ* Rat Jejunum

	Perfusion time (min)	% disappeared from lumen	Content (%) in tissue	% absorbed
Control	30	8.4±1.9	8.4±0.7	0
	60	13.9±1.9	10.8±0.5	3.1±1.5
	120	15.7±1.1	11.7±1.9	4.1±1.8
20 mM sodium cholate	30	34.0±3.4	10.1±1.5	23.9±4.9
	60	39.6±6.6	13.8±1.9	25.8±8.2
	120	56.5±2.1	10.0±0.8	46.5±2.3

The initial concentration of buformin was 1000 μg/ml.
The values represent the mean±standard deviation for 3 to 5 animals.

Effect of Sodium Cholate on Release of Membrane Components from Small Intestine

The results described in Tables II and III suggest that the cause of the enhancement of buformin absorption is caused by a direct action of sodium cholate on the intestinal mucosa. The alteration of the intestinal tissues in the presence of sodium cholate was investigated by determining the release of the components such as phosphorus, phospholipid, and protein from the *in situ* jejunal and ileal membranes. The results are summarized in Table IV. The data presented in Table V show the ratio of the total amount of membrane component released during a 2 hr exposure to sodium cholate solution compared to the amount released in the saline. The release of protein and total phosphorus from the mucosa markedly increased at concentrations of sodium cholate above 5 mM in the jejunum and above 10 mM in the ileum. Such a difference in the apparent concentration of sodium cholate influencing the intestinal mucosa would be attributed to a difference in the absorption rate of sodium cholate in both intestinal regions as described above. The release of phospholipid significantly increased at concentrations of sodium cholate above 10 mM in either jejunal or ileal region, and this enhancement of phospholipid release would be based upon a relatively strong solubilizing effect of salt on the membrane component.

Table IV. Protein, Total Phosphorus, and Phospholipid Phosphorus released in 2 Hours from the *in Situ* Rat Jejunum and Ileum in the Presence of Various Concentration of Sodium Cholate

Concn. of sodium cholate (mM)		Protein (mg)	Total phosphorus (μg)	Phospholipid phosphorus (μg)
0	jejunum	3.29±0.88	47.7± 8.2	21.7± 7.7
	ileum	1.83±1.25	131.8±47.0	45.5± 5.3
5	jejunum	4.12±2.21	78.5±21.4	17.0± 4.6
	ileum	3.75±2.37	107.6±17.7	35.3±14.0
10	jejunum	19.55±5.02	313.0±81.8	87.1± 2.2
	ileum	4.05±0.62	177.3±17.4	86.9±10.9
20	jejunum	14.97±0.47	624.2±33.1	230.7±38.6
	ileum	15.81±3.13	603.4±90.1	235.8±37.9

The values represent the mean±standard deviation for 3 animals.

Thus, the release of membrane components from the intestine was enhanced to greater extent by sodium cholate. The profile of enhancing effect of sodium cholate on the release of the intestinal membrane components is in fair agreement with that of the bile salt on the intestinal absorption of buformin. In addition, from findings on the influence of sodium

Table V. Ratio of Amount of Membrane Component released at 2 Hours after Exposure to Various Concentrations of Sodium Cholate

Concn. of sodium cholate (mM)		Ratio (sodium cholate/saline)		
		Protein	Total phosphorus	Phospholipid phosphorus
5	jejunum	1.25[a]	1.65[a]	1.00[a]
	ileum	2.05[a]	1.00[a]	1.00[a]
10	jejunum	5.94[b]	6.56[c]	4.01[d]
	ileum	2.21[e]	1.35[a]	1.91[c]
20	jejunum	4.55[d]	13.09[d]	10.63[e]
	ileum	8.64[c]	4.58[e]	5.18[b]

statistical difference from the value in the saline:
a) not significant, $p > 0.05$, *b*) $p < 0.01$, *c*) $p < 0.025$, *d*) $p < 0.0005$, *e*) $p < 0.005$

cholate and sodium taurocholate upon the rat intestinal mucosa, Nadai, *et al.*[19] suggested that the histological change of the intestinal tissue caused by the bile salts would be the primary cause of the enhancement in the absorption of drugs which penetrate the intestinal membrane very slowly.

From the findings described above, it is suggested that sodium cholate alters the composition of the *in situ* rat intestinal membrane by producing a release of membrane components from the intestinal tissue, resulting in an increase in the permeability of the intestine to buformin.

19) T. Nadai, M. Kume, A. Tatematsu, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 543 (1975).