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Further Studies on the Mechanism of the Absorption of Ion Pair Complex from the Rat Small Intestine

Junzo Nakamura, Noriyuki Muranushi, Toshikiro Kimura, Shozo Muranishi, and Hitoshi Sezaki

Faculty of Pharmaceutical Sciences, Kyoto University[1]

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Absorption properties of an ion pair complex, quinine and bromthymol blue (BTB), were investigated by the rat small intestine.

Although the disappearance of quinine from the *in situ* recirculated solution was enhanced in the presence of BTB and the converse was not applied for the anionic component, the enhancement of uptake by the everted intestine was observed by both components. By rinsing the everted intestine at the end of an incubation period, no effect of quinine on the tissue accumulation of BTB was found. Effect of counter ions on the binding to mucosal homogenates, brush borders, and bovine serum albumin (BSA) was hardly observed at all. Pretreatment of the intestine with pH 6.5 phosphate buffer for 10 min almost completely diminished the enhancing effect of BTB on the disappearance of quinine from the *in situ* recirculated solution. However, 15 min was enough after the pretreatment to regain such an enhancing effect of the anionic component.

It is suggested that the mucosal surface of the small intestine plays an important role in the absorptive process of an ion pair complex.

Keywords——rat small intestine; intestinal absorption; ion pair; quinine; bromthymol blue; *in situ* perfusion; everted sac; brush border; mucosal homogenate

In the previous reports from this laboratory,[2,3] it was demonstrated that the absorption of amines in the presence of various anionic agents was enhanced at all sites examined and kinetically the uptake by the mucosal membrane was increased in the rectum and the small intestine. Furthermore, the enhancement in the absorption was observed not by the anionic but by the cationic component of an ion pair complex. The present investigation was undertaken to gain further insight into the possible mechanisms which can be operative under various conditions of the absorption of ion pair complex with particular emphasis on the role of the mucosal surface of the small intestine. Quinine and bromthymol blue(BTB) which bear positive and negative charge respectively at physiological pH range of the small intestine were chosen as a model system due to their ionogenic nature, stability, ease of assay, and absorptive properties.

1) Location: *Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto.*
2) K. Kakemi, H. Sezaki, S. Muranishi, and Y. Tsujimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 1641 (1969).
3) E. Suzuki, M. Tsukigi, S. Muranishi, H. Sezaki, and K. Kakemi, *J. Pharm. Pharmacol.*, **24**, 138 (1972).

Experimental

Materials——Quinine hydrochloride and BTB were of reagent grade and used without further purification. All other reagents used in these experiments were of the finest grade available.

Preparation of Drug Solutions——Drugs were dissolved in isotonic buffer solution of NaH_2PO_4–Na_2HPO_4 for pH 6.5.

Apparent Partition Coefficient——Apparent partition coefficient was determined by the method described in the previous report from this laboratory.[3]

Analytical Methods——Spectrophotometric determination was applied to all drugs investigated.

Quinine in Perfused Solution: One ml of 3N NaOH and 6 ml of ethylene dichloride were added to 3 ml sample solution. The mixture was shaken for 20 min and then centrifuged for 10 min at 2500 rpm. After seperation of aqueous phase 5 ml of the organic phase was added to 5 ml of 1N HCl. The mixture was shaken for 10 min and then centrifuged for 10 min at 2500 rpm. The optical density of the aqueous phase was determined at 251 nm.

BTB in Perfused Solution: Four ml sample solution was alkalinized with 3 ml of 3N NaOH and determined spectrophotometrically at 617 nm.

BTB in Tissue: The small intestine was homogenized in three-fold its wet weight of distilled water. A mixture of 5 ml of the homogenate and 5 ml acetone was shaken for 15 min and then centrifuged for 10 min at 2500 rpm. A 3 ml aliquot of the supernatant was alkalinized with 3 ml of 1N NaOH and determined spectrophotometrically at 621 nm.

Counter ion did not affect the assay of quinine and BTB.

Procedure of Absorption Experiments——The procedure of *in situ* absorption experiment from the rat small intestine was the same as reported in the previous paper.[4] Pretreatment was carried out as follows. The pH 6.5 buffer solution, 0.9% NaCl, and 1 mM disodium ethylenediaminetetraacetate (EDTA-2Na) were perfused for 10 min at the rate of 5 ml/min in the rat small intestine. At the end of perfusion period, the small intestinal lumen was washed with 60 ml of pH 6.5 buffer solution as completely as possible, and then the drug solution was perfused for 10 min.

Binding to Mucosal Homogenates, Brush Borders, and Bovine Serum Albumin (BSA)——The binding to mucosal homogenates and brush borders were measured as reported in the previous paper,[4] except that mucosal homogenates was prepared by homogenizing in twenty-fold its wet weight of pH 6.5 buffer solution. Equilibrium dialysis method was adopted to estimate the binding to BSA (0.05% BSA, crystallized and lyophilized, Sigma Chemical Co.).

***In Vitro* Uptake by the Everted Sacs of the Small Intestine**——The procedure was the same as reported in the previous paper.[4] The drug solution was saturated with gas (95% O_2, 5% CO_2). The amount disappeared from the incubation medium was calculated by the difference in concentration of drug between the initial and the final solution. Determination of the accumulation of BTB in the intestinal tissue was carried out as follows. At the end of an incubation period, the intestine was rinsed in sequence in each of three separate beakers containing 50 ml of pH 6.5 buffer solution. After rinsing, the accumulation of BTB in the intestinal tissue was determined spectrophotometrically as mentioned elsewhere.

Results

Effect of Counter Ion on the Apparent Partition Coefficient of Quinine and BTB at 37°

The formation of ion pair complex was examined with the partition behavior of quinine and BTB in the presence of each other. Apparent partition coefficients of quinine and BTB to chloroform/water and benzene/water at pH 6.5 are summarized in Table I. As shown in Table I, the apparent partition coefficients of quinine and BTB were increased in the presence of counter ion, which indicates possible formation of lipoid-soluble ion pair complex.

Effect of Counter Ion on the Intestinal Transfer of Quinine and BTB

Absorption experiments from the rat small intestine were carried out at pH 6.5 using the *in situ* perfusion technique and results are summarized in Table II and Fig. 1. Effect of BTB on the disappearance of quinine from the lumen is shown in Table II. The disappearance of quinine from the lumen was significantly enhanced in the presence of BTB in 10, 15, 30, and 60 min. However, no effect of quinine on the disappearance from the

4) J. Nakamura, Y. Yoshizaki, M. Yasuhara, T. Kimura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 683 (1976).

lumen and the tissue accumulation of BTB were found in 10, 15, and 60 min as shown in Fig. 1.

Binding to Mucosal Homogenates, Brush Borders, and BSA

Binding to mucosal homogenates, brush borders, and BSA was examined *in vitro*. BSA was selected as a model of membrane protein. As shown in Table III, effect of counter ion

TABLE I. Effect of Counter Ion on the Apparent Partition Coefficient of Quinine and BTB at 37°

Drug	Organic solvent	PC
Quinine	chloroform	10.9
Quinine (+BTB)	chloroform	64.2
Quinine	benzene	0.89
Quinine (+BTB)	benzene	10.5
BTB	chloroform	0.14
BTB (+quinine)	chloroform	13.7
BTB	benzene	0.05
BTB (+quinine)	benzene	14.1

PC: apparent partition coefficient of quinine and BTB at pH 6.5, 37°
Concentration of drugs: Quinine (0.05 mM), BTB (0.05 mM)

TABLE II. Effect of BTB on the Intestinal Transfer of Quinine

	Disappearance from lumen (%)			
	10	15	30	60 min
Control	6.0±1.3(6)	9.5±2.0(8)	17.7±2.5 (6)	25.4±3.3(7)
+BTB	9.2±1.8(5)	13.2±2.4(9)	22.1±3.9(10)	30.5±4.2(5)
Level of significance	$p<0.01$	$p<0.01$	$p<0.05$	$p<0.05$

Concentration of drugs: Quinine (0.05 mM), BTB (0.05 mM), pH 6.5, *in situ* perfusion (flow rate 5 ml/min, perfusion volume 40 ml, 37°)
Numbers in parentheses represent number of experiments.
Results are expressed as the mean±S.D.
Results were compared statistically using a Student's t-test.

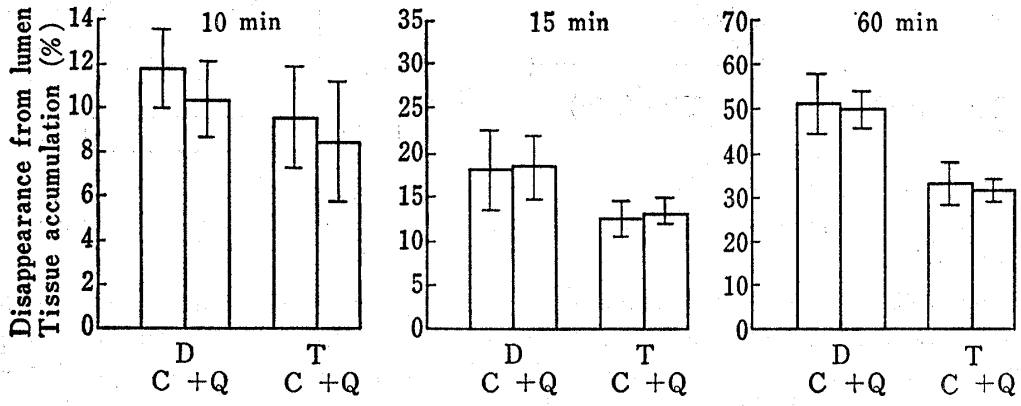

Fig. 1. Effect of Quinine on the Intestinal Transfer of BTB

C: control +Q: +quinine D: disappearance from lumen T: tissue accumulation
Concentration of drugs: Quinine (0.05 mM), BTB (0.05 mM), pH 6.5, *in situ* perfusion (flow rate 5 ml/min, perfusion volume 40 ml, 37°)
Each column represents the mean value of at least five experiments.
Vertical bars indicate ±S.D.

Table III. Binding to Mucosal Homogenates, Brush Borders, and BSA

Drug	Binding (%)		
	Mucosal homogenates	Brush borders	BSA
Quinine	40.7±1.6(3)	7.3±1.2(4)	1.9±1.1(4)
Quinine (+BTB)	38.9±3.1(4)	7.1±1.3(3)	2.9±0.5(3)
BTB	89.8±0.8(4)	47.5±4.8(5)	53.6±0.4(4)
BTB (+quinine)	89.2±0.3(4)	49.2±3.9(5)	54.7±0.5(4)

Numbers in parentheses represent number of experiments.
Results are expressed as the mean±S.D.

on the binding of quinine and BTB to mucosal homogenates, brush borders, and BSA were hardly observed at all.

Effect of Counter Ion on the Uptake by Everted Sacs of Quinine and BTB

In order to examine the uptake by intestinal mucosa, uptake by the everted sacs was carried out at pH 6.5. Everted sacs of 30 cm from the proximal end of duodenum and 30 cm from the distal end of ileum were used. As shown in Fig. 2, disappearance of quinine and BTB from the incubation medium were enhanced in the presence of counter ion in 5 and 10 min both jejunum and ileum. It is of interest to note that the disappearance of BTB from the incubation medium was enhanced in the presence of quinine despite the fact that no effect of quinine on the disappearance from lumen and the tissue accumulation of BTB was

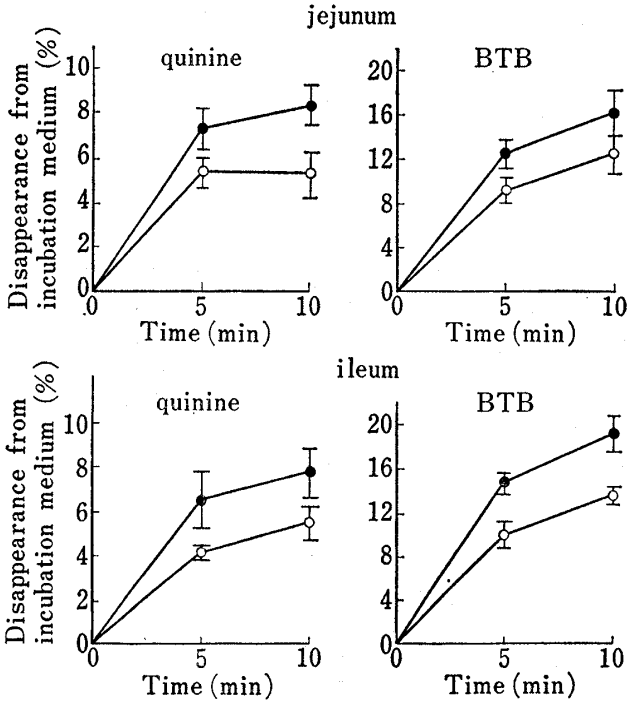

Fig. 2. Effect of Counter Ion on the Uptake by Everted Sacs of Quinine and BTB
○: control ●: +counter ion

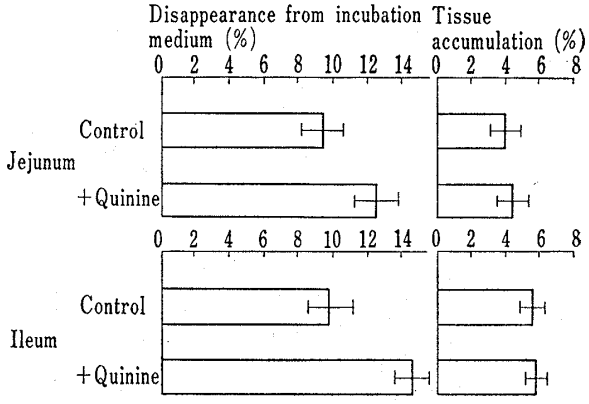

Fig. 3. Effect of Quinine on the Uptake by Everted Sacs of BTB for 5 min

observed in the case of *in situ* perfusion. To clarify this discrepancy, further experiment was carried out. At the end of an incubation period, the intestine was rinsed in sequence in each of three seperate beakers containing 50 ml of pH 6.5 buffer solution. After rinsing, the accumulation of BTB in the intestinal tissue was determined. The results are shown in Fig. 3. Although the disappearance of BTB from incubation medium was enhanced in the presence of quinine, no effect of quinine on the tissue accumulation of BTB was found after rinsing the everted sacs. From these results, it seems that BTB could approach the mucosal surface as an ion pair complex.

Effect of Pretreatment on the Intestinal Transfer and the Uptake by Everted Sacs of Quinine

Pretreatment was carried out in order to elucidate the mechanism of the absorption of ion pair complex in more detail. Effect of pretreatment on the disappearance from lumen of quinine for 10 min is shown in Table IV. After pretreatment with pH 6.5 buffer solution,

Table IV. Effect of Pretreatment on the Intestinal Transfer of Quinine for 10 min

Condition	Disappearance from lumen (%)
No pretreatment	6.0±1.3(6)
No pretreatment (+BTB)	9.2±1.8(5)
Buffer pretreatment	9.3±1.5(6)
Buffer pretreatment (+BTB)	8.7±1.3(6)
0.9% NaCl pretreatment	8.9±1.6(6)
0.9% NaCl pretreatment (+BTB)	9.3±0.9(6)
1 mM EDTA pretreatment	8.1±0.8(4)
1 mM EDTA pretreatment (+BTB)	8.1±0.6(5)
Recovery period of 15 min after buffer pretreatment	6.8±0.7(4)
Recovery period of 15 min after buffer pretreatment (+BTB)	9.2±1.0(7)
0.05 mM BTB pretreatment	9.7±0.7(7)

Numbers in parentheses represent number of experiments.
Results are expressed as the mean±S.D.
In the case of the recovery experiments, animals were left intact for 15 min after washing with pH 6.5 buffer solution.

0.9% NaCl, or 1 mM EDTA for 10 min, effect of BTB on the disappearance from lumen of quinine disappeared. However, the recovery period of 15 min after pretreatment with pH 6.5 buffer solution was enough to resume the enhancement of disappearance from lumen of quinine to the control level in the presence of BTB. The percentage disappearance of quinine from the lumen after pretreatment with 0.05 mM BTB was 9.7±0.7%, which indicate that BTB would not alter the membrane permeability. Furthermore, effect of pretreatment on the uptake by the everted sacs of quinine for 5 min was examined. The results are shown in Table V. After pretreatment with pH 6.5 buffer solution for 10 min, no effect of BTB on the disappearance from incubation medium of quinine was observed. On the contrary, the recovery period of 15 min after pretreatment with pH 6.5 buffer solution was

Table V. Effect of Pretreatment on the Uptake by Everted Sacs of Quinine for 5 min

Condition	Disappearance from incubation medium (%)	
	Jejunum	Ileum
No pretreatment	5.4±0.7(5)	4.1±0.4(5)
No pretreatment (+BTB)	7.3±0.9(7)	6.5±1.3(7)
Buffer pretreatment	8.5±1.8(7)	6.3±1.3(7)
Buffer pretreatment (+BTB)	7.8±1.7(6)	7.3±1.8(5)
Recovery period of 15 min after buffer pretreatment	5.5±0.5(4)	4.4±0.6(5)
Recovery period of 15 min after buffer pretreatment (+BTB)	9.7±1.8(4)	8.0±1.1(4)

Numbers in parentheses represent number of experiments.
Results are expressed as the mean±S.D.

enough to resume the enhancement of disappearance from incubation medium of quinine to the control level in the presence of BTB.

Effect of Pretreatment on the Intestinal Transfer and the Uptake by Everted Sacs of BTB

Effect of pretreatment on the intestinal trasfer of BTB for 10 min are shown in Table VI. In contrast to quinine, the disappearance from lumen of BTB was enhanced after pretreatment with pH 6.5 buffer solution or 0.9% NaCl. In the case of pretreatment with pH 6.5 buffer solution, tissue accumulation of BTB was enhanced in the presence of quinine, but no enhancement was found after pretreatment with 0.9% NaCl. No effect of quinine on the disappearance from lumen, tissue accumulation, and net absorption of BTB was found after pretreatment with 1 mM EDTA. By the recovery period of 15 min after pretreatment with pH 6.5 buffer solution, no effect of quinine on the disappearance from lumen, tissue accumulation, and net absorption of BTB were found. In addition, effect of pretreatment on the uptake by everted sacs of BTB for 5 min was examined both jejunum and ileum as shown in Table VII. After pretreatment with pH 6.5 buffer solution, the disappearance of BTB from incubation medium in the presence of quinine was enhanced. Similarly, no effect of quinine on the disappearance of BTB from incubation medium was observed by the recovery period of 15 min after pretreatment with pH 6.5 buffer solution.

TABLE VI. Effect of Pretreatment on the Intestinal Transfer of BTB for 10 min

Condition	Disappearance from lumen (%)	Tissue accumulation (%)	Net absorption (%)
No pretreatment	11.8±1.8(7)	9.6±2.3(7)	2.3±1.7(7)
No pretreatment (+Q)	10.4±1.7(5)	8.5±2.7(5)	2.1±2.2(5)
Buffer pretreatment	17.1±1.2(5)	13.9±0.4(5)	3.2±1.3(5)
Buffer pretreatment (+Q)	22.3±1.8(5)	19.6±3.9(5)	2.7±2.8(5)
0.9% NaCl pretreatment	20.3±2.4(7)	14.2±2.7(7)	6.2±2.5(7)
0.9% NaCl pretreatment (+Q)	26.2±2.6(7)	16.8±1.0(4)	9.3±3.1(4)
1 mM EDTA pretreatment	17.6±2.3(6)	14.4±1.8(6)	3.3±1.0(6)
1 mM EDTA pretreatment (+Q)	17.0±3.8(6)	12.5±2.7(6)	4.7±2.5(6)
Recovery period of 15 min after buffer pretreatment	21.6±2.0(4)	16.7±2.2(4)	5.0±1.5(4)
Recovery period of 15 min after buffer pretreatment (+Q)	22.7±2.4(6)	17.9±1.9(6)	4.8±3.6(6)

+Q: +quinine
Numbers in parentheses represent number of experiments.
Results are expressed as the mean±S.D.

TABLE VII. Effect of Pretreatment on the Uptake by Everted Sacs of BTB for 5 min

Condition	Disappearance from incubation medium (%)	
	Jejunum	Ileum
No pretreatment	9.4±1.2(8)	9.9±1.3(10)
No pretreatment (+Q)	12.5±1.3(9)	14.6±1.0 (9)
Buffer pretreatment	10.1±1.2(4)	10.4±1.0 (8)
Buffer pretreatment (+Q)	14.2±1.6(8)	16.7±1.2 (6)
Recovery period of 15 min after buffer pretreatment	12.6±0.5(4)	11.8±0.4 (5)
Recovery period of 15 min after buffer pretreatment (+Q)	12.3±2.6(4)	12.9±2.3 (4)

Numbers in parentheses represent number of experiments.
Results are expressed as the mean±S.D.

Discussion

It has been reported that absorption enhancement by the ion pair formation was obvious in the stomach as well as in the rectum, and not so remarkable in the small intestine. Levy and Matsuzawa[5] have shown that the apparent lipoid-water partition coefficient of certain water-soluble acidic dyes (BTB, methyl orange, and eosin blue) and some of their lipoid-soluble complexes do not reflect the intestinal absorption characteristics of the complexes. In the present study, the disappearance of quinine from the small intestinal lumen was significantly enhanced in the presence of BTB, whereas no effect of quinine on the disappearance from the lumen and the tissue accumulation of BTB was found. Kakemi, *et al.*[2] have shown that certain organic anions, such as lauryl sulfate and saccharinate could enhance the gastrointestinal absorption of otherwise poorly absorbable pharmaceutical amines, and that the enhancement in the absorption was not by the anionic but by the cationic component of ion pair complex. Results shown in Table II and Fig. 1 are consistent with those of the previous findings.

In early reports from this laboratory,[6] it has been pointed out that intestinal absorption of barbituric acid derivatives as well as many other anionic and cationic drugs are related not to their lipoid/water partition coefficient but rather to their binding tendency to the intestinal mucosal homogenates. Also, it was suggested in the previous reports[4,7] that the binding to the mucosa, especially to the brush borders(microvilli), is important as the first step in the absorptive process of four water-soluble dyes, methylene blue, BTB, bromphenol blue, and phenol red. However, as shown in Table III, effect of counter ion on the binding of quinine and BTB to mucosal homogenates, brush borders, and BSA were hardly observed at all. It seems reasonable to assume that quinine and BTB are not bound to mucosal homogenates, brush borders, and BSA as an intact ion pair and that the intestinal membrane surface has a dissociating effect on ion pair complex.

Effect of counter ion was examined further by everted sac technique. As shown in Fig. 2, disappearance of quinine and BTB from the incubation medium was enhanced in the presence of counter ion in 5 and 10 min both jejunum and ileum. Since no effect of quinine on the tissue accumulation of BTB was found after rinsing the everted sacs as shown in Fig. 3, it is probable that BTB could approach the mucosal surface as an ion pair.

Pretreatment was carried out in order to clarify the role of the mucosal surface on the absorption of ion pair complex in more detail. As shown in Table IV, enhancing effect of BTB on the disappearance of quinine from lumen disappeared after pretreatment with pH 6.5 buffer solution. On the contrary the disappearance of BTB from lumen was enhanced by the pretreatment with pH 6.5 buffer solution. However, within 15 min after the pretreatment, enhancing tendency of BTB in the disappearance of quinine from the lumen was recovered.

Similar results were obtained in the case of uptake by the everted sacs as shown in Tables V and VII. After pretreatment with pH 6.5 buffer solution for 10 min, effect of BTB on the disappearance from incubation medium of quinine was not observed. Again, the recovery period of 15 min after pretreatment was enough to resume the enhancing effect of BTB. On the other hand, in the presence of quinine, the disappearance of BTB from incubation medium was enhanced after the pretreatment. However, such effect of quinine on BTB becomes unrecognizable 15 min after the pretreatment. These findings are compati-

5) G. Levy and T. Matsuzawa, *J. Pharm. Sci.*, **54**, 1003 (1965).
6) K. Kakemi, T. Arita, R. Hori, R. Konishi, and K. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 248 (1969); K. Kakemi, T. Arita, R. Hori, R. Konishi, K. Nishimura, H. Matsui, and T. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 255 (1969).
7) J. Nakamura, Y. Yoshizaki, M. Yasuhara, T. Kimura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 691 (1976).

ble with the ones observed in the *in situ* experiments. It is considered that the mucosal surface of the small intestine would be altered by the pretreatment with pH 6.5 buffer solution and be recovered after kept standing for 15 min. In an early report,[8] prolonged *in vitro* and *in vivo* exposure of intact absorptive cells to potent mucolytic and proteolytic substances failed to remove the surface coat so long as integrity of the epithelial cells is maintained. Accordingly, it seems probable that the surface coat(glycocalyx) could not be removed by the pretreatment with pH 6.5 buffer solution. It is reasonable to assume, but dose not prove, that the mucus on the mucosal surface of the small intestine would be removed by the pretreatment with pH 6.5 buffer solution and is secreted again during the recovery period of 15 min. Recently Walker, *et al.*[9] have shown the importance of the surface of the gut in the intestinal uptake of macromolecules. In these experiments, the gut sacs from immunized and control animals were rinsed in saline after 3 hr of incubation. More radioactivity was present in the first rinse fluid of gut sacs from immunized rats than in the rinse fluid of controls, suggesting that in immunized rats antigen was loosely attached to the intestinal surface, probably as antigen-antibody complexes.

The mucosal surface of the small intestine, perhaps the mucus from the goblet cell, has an important role in the absorptive process of ion pair complex.

8) S. Ito, *J. Cell. Biol.*, **27**, 475 (1965).
9) W.A. Walker, M. Wu, K.J. Isselbacher, and K.J. Bloch, *J. Immunol.*, **115**, 854 (1975).