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Role of Intestinal Mucus in the Absorption of Quinine and Water-soluble Dyes from the Rat Small Intestine

Junzo Nakamura, Katsuhiko Shima, Toshikiro Kimura, Shozo Muranishi, and Hitoshi Sezaki

Faculty of Pharmaceutical Sciences, Kyoto University[1]

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Role of intestinal mucus in the absorption of quinine and four water-soluble dyes, methylene blue, bromthymol blue, bromphenol blue, and phenol red, from the rat small intestine was investigated.

Immediately after pretreatment with pH 6.5 buffer solution for 10 min and recovery period of 15 min after the pretreatment, absorption experiments were carried out at pH 6.5 using the *in situ* perfusion technique and *in vitro* uptake method by the everted sacs. Considering from the results of determination of protein and neutral sugar, it seems likely that mucus was secreted during the recovery period of 15 min after the pretreatment with pH 6.5 buffer solution. All drugs examined bound to mucus prepared from intestinal washings after the recovery period of 15 min.

It is suggested that the mucosal surface of the small intestine, mucus layer, has an important role in the absorptive process of quinine and four water-soluble dyes.

Keywords——rat small intestine; intestinal absorption; intestinal mucus; methylene blue; bromthymol blue; bromphenol blue; phenol red; quinine; *in situ* perfusion; *in vitro* uptake by everted sac

Goblet cells of the small intestine and the colon secrete a viscous glycoprotein mucus product which overlies the epithelial surface. Although knowledge of the components of the mucus film is limited, it is generally accepted that the film functions as a physical barrier against mechanical and chemical injury. Saggers and Lawson reported the penetration of antibiotics through hog gastric mucin *in vitro*.[2] Braybrooks and Barry have examined the effect of mucin preparation upon the absorption of tetracycline, phenylbutazone, and warfarin in an attempt to elucidate the possible role of the mucus lining.[3,4]

In our previous papers,[5,6] the mechanism of absorption of four water-soluble dyes, methylene blue (MB), bromthymol blue (BTB), bromphenol blue (BPB), and phenol red (PR), highly ionized compounds of very low lipoid solubilities at physiological pH range of the small intestine, from the rat small intestine was investigated. In addition, it was suggested[7] that the mucosal surface of the small intestine plays an important role in the absorption process of an ion pair complex, quinine and BTB. The present investigation was undertaken to clarify the role of mucus in the absorption of quinine, as a model of lipophilic compound, and the four water-soluble dyes from the rat small intestine.

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Experimental

Materials——Quinine hydrochloride, MB, BTB, BPB, and PR were of reagent grade and used without further purification. All other reagents used were of the finest grade available.

Preparation of Drug Solution——The isotonic buffer solution of pH 6.5 was prepared from 0.123 M Na_2HPO_4 and 0.163 M NaH_2PO_4.

Analytical Methods——The same spectrophotometric methods were used as described previously.[5,7]

Procedure of Absorption Experiments——Absorption experiments were carried out at pH 6.5 using *in situ* perfusion technique and *in vitro* uptake by the everted sacs,[5,7] except that the everted sacs were placed in a centrifuge tube containing 10 ml (MB, BTB, BPB, and PR) or 20 ml (quinine) of an isotonic buffer solution.

Role of intestinal mucus in the absorption of drugs were examined under three conditions as follows. No pretreatment: Immediately after washing out the intestinal contents with 50 ml of pH 6.5 buffer solution, absorption experiments were carried out. Buffer pretreatment: Intestinal contents were washed out as described above and the small intestine was perfused with 40 ml of buffer solution for 10 min. At the end of perfusion period, the small intestinal lumen was washed with 60 ml of pH 6.5 buffer solution. Immediately after the pretreatment, the absorption experiments were carried out. Recovery period of 15 min: Intestinal contents were washed out and the small intestine was pretreated as described above. Then animals were left intact for 15 min. Immediately after this recovery period, the absorption experiments were carried out. These procedures are summarized in Chart 1. Results were compared statistically using a Student's t-test.

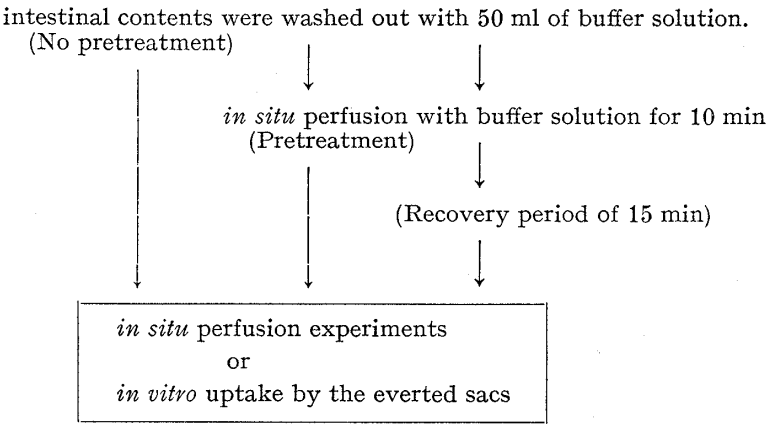

Chart 1. Experimental Procedures

Binding to Intestinal Mucus——The intestinal mucus was prepared as follows. Forty ml of pH 6.5 buffer solution was 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, and then animals were left intact for 15 min. At the end of the recovery period, the small intestinal lumen was washed with 40 ml of pH 6.5 buffer solution. The washings were centrifuged at 2° at 10000 rpm for 30 min to remove particulate material. The supernatant was concentrated by ultrafiltration using Toyo UP-20 (Toyo Roshi Co.,) ultrafiltration membrane (average mol. wt. cut off 20000). Equilibrium dialysis method was adopted to estimate the binding as described in the previous papers.[5,6]

Results

Alteration of the mucosal surface of the small intestine during the recovery period of 15 min after pretreatment with pH 6.5 buffer solution was examined by determining the protein and sugar contents in intestinal washing with 40 ml of pH 6.5 buffer solution. As is evident from Table I, protein and neutral sugar were found in intestinal washings. This result confirmed that intestinal mucus was secreted during the recovery period of 15 min.

In Fig. 1 are shown the results of perfusion experiments and uptake by the everted sacs of quinine. In the case of *in situ* perfusion experiments, the intestinal transfer was examined by the disappearance from lumen. The intestinal transfer of quinine was enhanced after pretreatment with pH 6.5 buffer solution for 10 min ($p<0.01$). However, the recovery

Table I. Effect of Recovery Period on Protein and Sugar Components on Intestinal Surface

	Immediate washings	After 15 min
Protein (μg/ml)	38.6±3.9(4)	134.2±31.8(4)
Neutral sugar (μg/ml)	3.6±1.2(4)	7.4± 2.5(4)

Protein was determined by the method of Lowry, *et al.* using bovine serum albumin as standard.[8]
Neutral sugar was determined with anthrone with D-glucose as standard.[9]

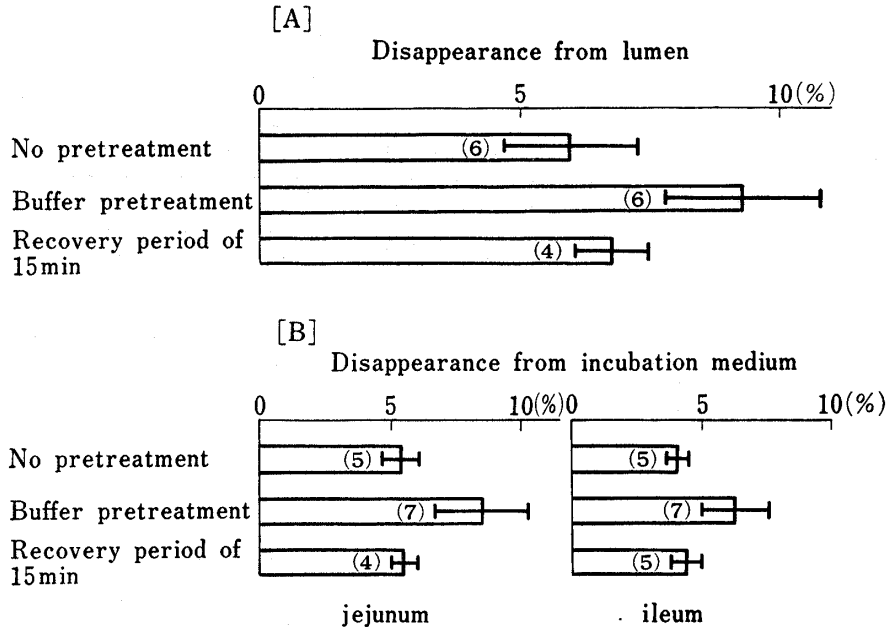

Fig. 1. Effect of Pretreatment on the Intestinal Transfer for 10 min [A] and Uptake by Everted Sacs for 5 min [B] of Quinine

Concentration of quinine: 0.05 mM.
Horizontal bars indicate ±S.D.
Numbers in parentheses represent number of experiments.

period of 15 min after the pretreatment was enough to restore to the level of no pretreatment. The uptake by the everted sacs was examined by the disappearance from the incubation medium. After pretreatment with pH 6.5 buffer solution for 10 min, the enhancement of uptake by the everted sacs of quinine was observed ($p<0.01$). Similarly, the recovery period of 15 min after the pretreatment was enough as well to restore to the level of no pretreatment.

MB is a cationic dye which was well absorbed as the percentage disappearance from lumen and net absorption for 60 min values was 61.5±3.1 and 46.0±2.9%, respectively.[5] The results of MB is shown in Fig. 2. The intestinal transfer of MB was slightly enhanced after pretreatment with pH 6.5 buffer solution. In contrast to quinine, it is of interest to note that the intestinal transfer of MB was further enhanced after the recovery period of 15 min ($p<0.01$). However, no effect of pretreatment with pH 6.5 buffer solution and recovery period of 15 min on the uptake by the everted sacs of MB was observed. Probably, it seems that the uptake by the everted sacs of MB was already saturated.

BTB, BPB, and PR are anionic dyes. In BTB, the most part of the amount disappeared from lumen was found in the intestinal mucosa.[5] As shown in Fig. 3, the intestinal transfer of BTB was significantly enhanced after pretreatment with pH 6.5 buffer solution ($p<0.02$).

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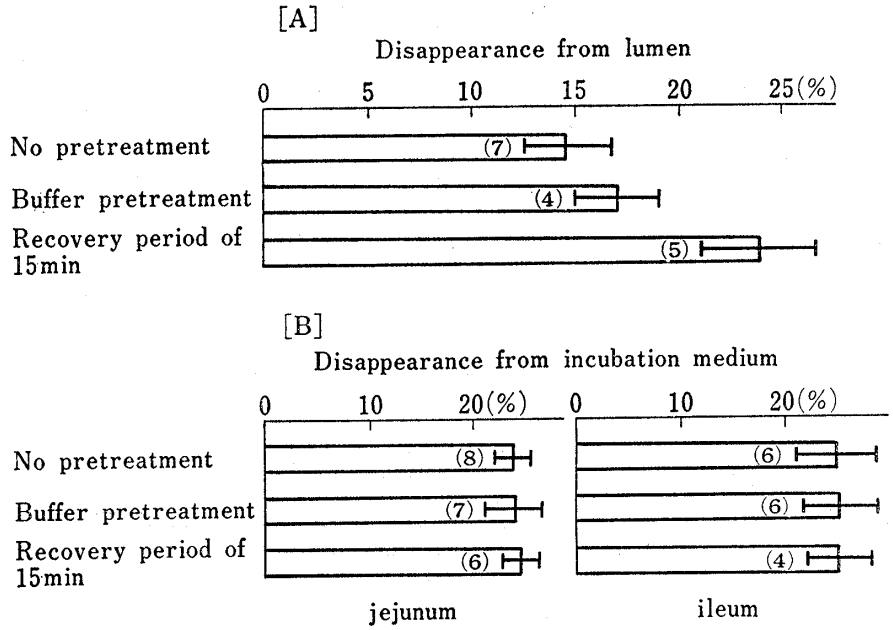

Fig. 2. Effect of Pretreatment on the Intestinal Transfer for 10 min [A] and Uptake by Everted Sacs for 5 min [B] of MB

Concentration of MB: 0.1 mM.
Horizontal bars indicate ±S.D.
Numbers in parentheses represent number of experiments.

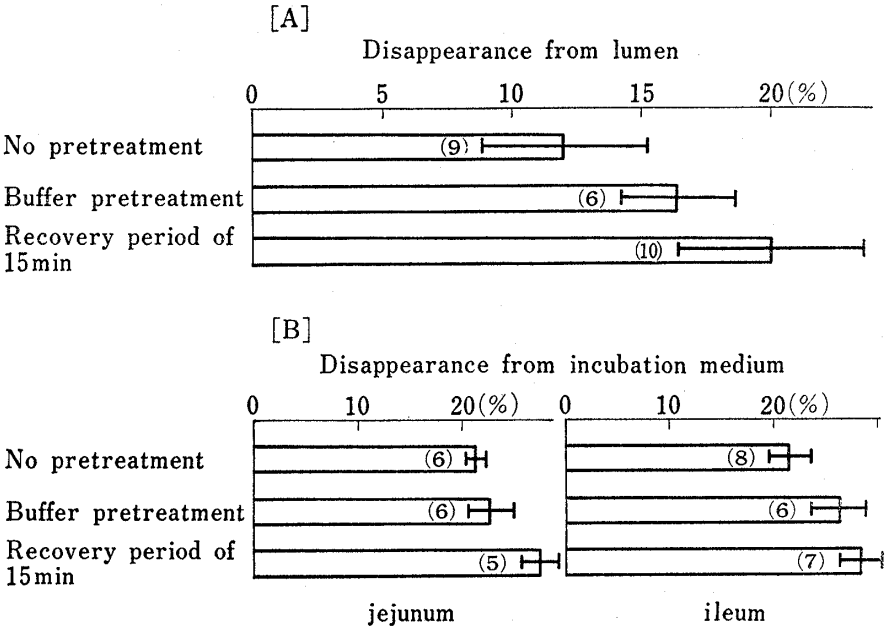

Fig. 3. Effect of Pretreatment on the Intestinal Transfer for 10 min [A] and Uptake by Everted Sacs for 5 min [B] of BTB

Concentration of BTB: 0.1 mM.
Horizontal bars indicate ±S.D.
Numbers in parentheses represent number of experiments.

By the recovery period of 15 min after the pretreatment, further enhancement of the intestinal transfer of BTB was found ($p<0.05$). In the case of the uptake by everted sacs (jejunum), no effect was found after the pretreatment. However, the enhancement of the uptake by the everted sacs of BTB was observed after the recovery period of 15 min ($p<0.01$). In ileal

everted sacs, the uptake was enhanced after the pretreatment ($p<0.01$). By the recovery period of 15 min, no further enhancement was observed.

BPB and PR were hardly absorbed at all. However, larger accumulation of BPB in the intestinal tissue was noted than PR.[5] In Fig. 4 are shown the results of BPB. The *in situ*

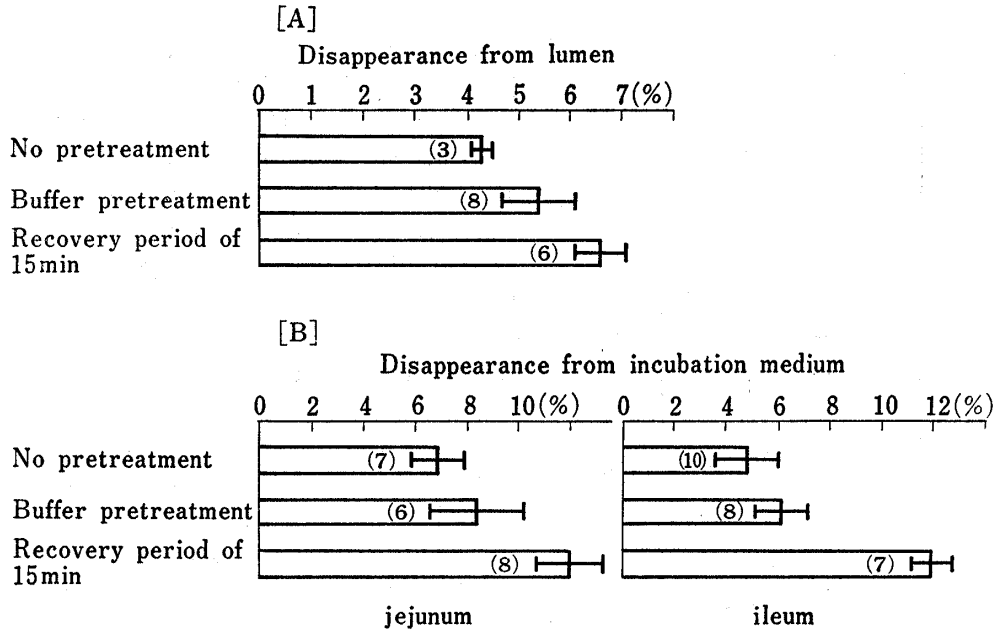

Fig. 4. Effect of Pretreatment on the Intestinal Transfer for 10 min [A] and Uptake by Everted Sacs for 5 min [B] of BPB

Concentration of BPB: 0.1 mM.
Horizontal bars indicate ±S.D.
Numbers in parentheses represent number of experiments.

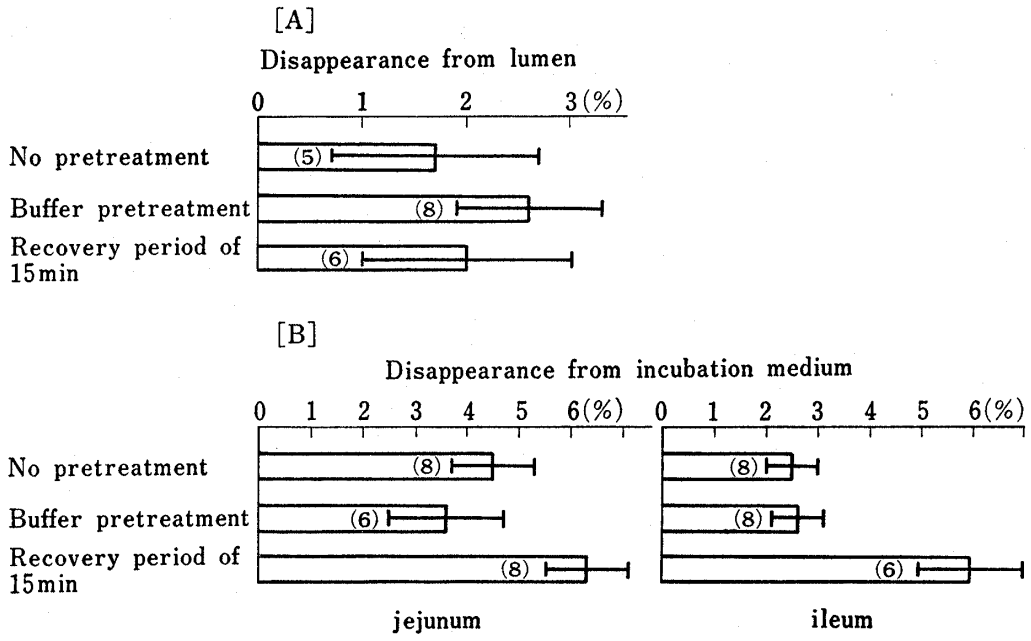

Fig. 5. Effect of Pretreatment on the Intestinal Transfer for 10 min [A] and Uptake by Everted Sacs for 5 min [B] of PR

Concentration of PR: 0.1 mM.
Horizontal bars indicate ±S.D.
Numbers in parentheses represent number of experiments.

intestinal transfer of BPB was significantly enhanced by the pretreatment ($p<0.05$). Moreover, the enhancement was observed after the recovery period of 15 min ($p<0.01$) in the similar manner as MB and BTB. After pretreatment with pH 6.5 buffer solution, the uptake of BPB by everted sacs was slightly enhanced. Furthermore, the recovery period of 15 min was enough to enhance the uptake of BPB ($p<0.001$). In contrast to BPB, no effect of pretreatment and recovery period of 15 min on the intestinal transfer of PR was found in Fig. 5. However, it is of interest to note that the *in vitro* uptake of PR was significantly enhanced after the recovery period of 15 min ($p<0.001$). From these results, it seems that PR, loosely bound to the intestinal surface, was washed out with 60 ml of pH 6.5 buffer solution at the end of perfusion period.

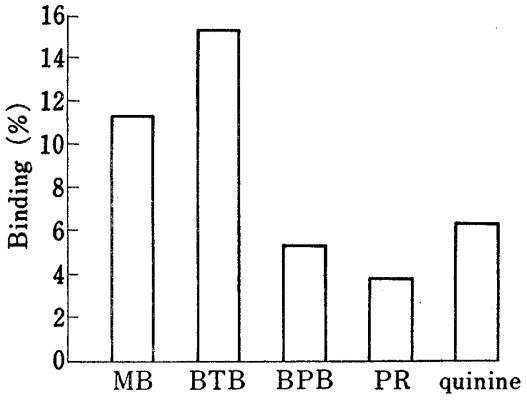

Fig. 6. Binding to Intestinal Mucus

Drugs were dissolved in pH 6.5 buffer solution at the concentration of 0.1 mM.
Concentration of intestinal mucus=379.0 μg protein/ml.

In the previous report,[5] it was suggested that the binding to the mucosa, especially to the brush border (microvilli), is important as the first step in the absorptive process of four water-soluble dyes, MB, BTB, BPB, and PR. Binding to intestinal mucus prepared from intestinal washing after the recovery period of 15 min was examined *in vitro*. As shown in Fig. 6, all drugs examined bound to intestinal mucus.

Discussion

The mucus film, synthesized and secreted by the mucosal epithelial cells of the gut, must permit the secretion and absorption of many molecules through the gastrointestinal mucosa. It may simply slow the rate of drug passage or it may loosely bind and concentrate the drug at the membrane surface which may increase the passive diffusional rate. Saggers and Lawson reported[2] that the larger antibiotic molecules diffused more rapidly through 15% hog mucin than the smaller antibiotic molecules. It seems possible that some form of gel filtration was taking place in the mucus, the smaller antibiotic molecules becoming trapped in pores in the cross-linked mucopolysaccharides and the larger molecules being unable to pass into the pores in the mucin and so diffusing round the outside of the mucus plug. Braybrooks and Barry have shown[3,4] that the bioavailability of tetracycline in the presence and absence of a porcine gastric mucin dispersion (1% w/v) has been studied by *in vivo* perfusion of the rat samll intestine, the rat everted gut, a diffusion cell technique, and the Sartorius Absorption Simulator Apparatus. In the presence of the mucin preparation, an approximate 50% reduction in the numerical values of the parameters used to measure the tetracycline movement across the membrane was found. From these results, it seems reasonable to assume that intestinal mucus on the membrane surface could be disadvantageous to the absorption of drugs across the mucosal membrane.

In order to examine the effect of intestinal mucus, not in the lumen but on the mucosal surface, on the intestinal absorption of quinine and four water-soluble dyes, new method was employed as described elsewhere. As is evident from Table I, the amount of mucus on the intestinal mucosa increased by the recovery period of 15 min, compared with the amount of mucus after the pretreatment. It seems that mucus would be secreted during the recovery period of 15 min. As can be seen from Fig. 1, the intestinal transfer and the uptake by everted sacs of quinine was decreased after the recovery period of 15 min. Consequently, intestinal mucus could be disadvantageous to the absorption of quinine. On the other hand, the

intestinal transfer of MB, BTB, and BPB was enhanced after the recovery period of 15 min as shown in Fig. 2, 3, and 4. Similarly, the enhancement of the uptake by the everted sacs of BTB, BPB, and PR was observed after the recovery period of 15 min as shown in Fig. 3, 4, and 5. From these results, intestinal mucus could be advantageous to the absorption of water-soluble dyes. Considering from the enhancement of intestinal transfer of quinine, MB, BTB, and BPB by the pretreatment, it seems that the mucosal surface of the small intestine would be altered by the pretreatment, perhaps mucus would be removed by the pretreatment. By the recovery period of 15 min, the intestinal transfer and the uptake by the everted sacs of quinine restored to the level of no pretreatment. But, no restoration of the intestinal transfer of MB, BTB, and BPB was found. This observation is not explained by the mucus layer on the mucosal surface. Consequently, it may be possible that the structure and components of intestinal mucus after the recovery period of 15 min were different from ones of no pretreatment. In the case of no pretreatment, gastric mucus, bile, and pancreatic juice may loosely bind, which may be removed by the pretreatment with pH 6.5 buffer solution. Forstner and Forstner[10,11] have shown the effect of $CaCl_2$ on the physical properties of the mucin using purified rat intestinal goblet cell mucin. These findings strongly suggested that the mucin became smaller and more dense as calcium was added, a process most probably achieved by loss of intramolecular water. Martin, *et al.* reported[12] that sodium deoxycholate (SDC), sodium taurodeoxycholate (STDC), and sodium glycocholate (SGC) produced a breakdown in mucus structure. The order of effectiveness in producing structural breakdown can be given as SDC>STDC>SGC. Considering from the enhancement of the absorption of a poorly absorbable drug in the presence of bile salts,[13] it seems that three-dimensional architecture of mucus is important in the absorptive process of the drug. In the previous report,[7] it was suggested that the mucosal surface of the small intestine plays an important role in the absorptive process of an ion pair complex, quinine and BTB. From the foregoing results, it seems reasonable to assume that the binding to intestinal mucus is important in the process of absorption of four water-soluble dyes, MB, BTB, BPB, and PR, from the rat small intestine. A higher concentration of a dye at the membrane surface could be advantageous to the absorption across the mucosal membrane.

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