IMMUNOLOGICAL CONTROL OF DRUG ABSORPTION FROM THE GASTROINTESTINAL TRACT: EFFECT OF LOCAL ANAPHYLAXIS ON THE INTESTINAL ABSORPTION OF LOW MOLECULAR WEIGHT DRUGS IN THE RAT

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Intestinal absorption of various drugs was examined by means of in situ recirculation technique during local anaphylaxis. The antibody was determined by passive cutaneous anaphylaxis technique in rats immunized once or three times. The optimal condition of local anaphylaxis was determined by the leakage of Evans Blue. The most significant increase in leaks of the dye was observed by the intraluminal challenge with 400 mg of ovalbumin for 10 min in ovalbumin-immunized rats, and this condition was chosen as the optimal condition of local anaphylaxis.

Under this condition, intestinal absorption of caffeine, phenylbutazone, and bromthymol blue (BTB) significantly decreased in ovalbumin-immunized rats compared with the control, whereas no significant effect was noted in the intestinal absorption of salicylic acid, quinine, pralidoxime iodide (2-PAM), tetracycline, and phenol red. In normal rats, no significant decrease was obtained in the intestinal absorption of caffeine, phenylbutazone, and BTB. On the other hand, the decreased absorption of BTB was not found in ovalbumin-immunized rats by the intraluminal challenge with bovine θ-globulin. Furthermore, there was no significant change in the decreased absorption of BTB between rats immunized once and three times. The most effective condition for decreased BTB absorption was observed by the intraluminal challenge with 200 mg of ovalbumin for 10 min in ovalbumin-immunized rats, which almost correlated with the data of Evans Blue leakage.

From these observations, it appears that the mucosal immune responses affect the intestinal absorption of low molecular weight drugs.

Keywords — intestinal absorption; immunization; anaphylaxis; ovalbumin; passive cutaneous anaphylaxis; bromthymol blue; Evans Blue; mucosal permeability; mucus

INTRODUCTION

The principal role of the gastrointestinal tract is that of digestion and absorption of nutrients. The epithelial surface of the gastrointestinal tract represents an extensive surface area exposed to a intraluminal environment containing a variety of foreign substances such as food antigens, microorganisms, and toxins. The old theory that the gastrointestinal tract is an impenetrable barrier to foreign antigens is no longer held valid and experimental and clinical studies suggest that intact proteins present within the gastrointestinal tract penetrate the mucosal barrier under physiologic and pathogenic conditions.¹⁻⁵ The penetration of foreign proteins into the circulation may in turn evoke allergic or inflammatory reactions manifested as a disease state.

Recently, Walker et al. reported that oral and parenteral immunization with the antigenic macromolecule interferes specifically with the intestinal absorption of macromolecular anti-
gen6-10) and several immunologic and non-immunologic defenses help to control and regulate the uptake of intestinal antigens. These include intestinal antibodies, intestinal flora, peristalsis, the gastric barrier, mucus release from goblet cells, hepatic filtration and proteolysis. In addition, many attempts have been made to clarify the intestinal transport of macromolecular antigens during local immune responses.11-16) For example, Bloch et al. reported that the alteration in vascular and mucosal permeability which accompanies intestinal anaphylaxis was reflected by increased retention of 125 I-labelled rat serum albumin in gut wall segments.14)

On the other hand, many low molecular weight organic chemical compounds such as penicillins and cephalosporins have the ability to combine with macromolecules in the body and induce allergic reactions. However, few studies were done to investigate the effect of immune system on the intestinal uptake and transport of low molecular weight organic compounds. Previous studies in our laboratory have shown that intestinal absorption of salicylic acid was decreased by the intravenous challenge with bovine γ-globulin or ovalbumin in rats immunized with the same antigen and systemic anaphylaxis interfered with the intestinal absorption of low molecular weight compounds.17-20)

The present study concerns the effect of local immune responses on the intestinal absorption of low molecular weight compounds and the possible role of intestinal immune responses.

MATERIALS AND METHODS

Materials — Ovalbumin, bovine γ-globulin, phenylbutazone, tetracycline and pralidoxime iodide were purchased from Sigma Chemical Co., St. Louis, Mo. Freund’s incomplete adjuvant was obtained from Difco Laboratories, Detroit, Mich. Evans Blue was obtained from Merck, Darmstadt. Caffeine, bromthymol blue (BTB), quinine, phenol red, and all other reagents used in these experiments were reagent grade obtained from Nakarai Chemical Co., Japan.

Preparation of Drug Solution — The isotonic buffer solution of pH 6.5 was prepared with 0.123 m Na₂HPO₄ and 0.163 m NaH₂PO₄. Salicylic acid, caffeine, and 2-PAM were dissolved in this buffer solution at a concentration of 1 mm for absorption studies.

Animals and Immunization — Male Wistar albino rats ranging from 150—200 g were used in these studies. The rats, which had been fed on a diet free of ovalbumin and bovine γ-globulin, were immunized according to the following schedule. One milligram of ovalbumin or bovine γ-globulin dissolved in 0.25 ml of saline (0.9% w/v) was emulsified with an equal volume of incomplete Freund’s adjuvant and was injected intraperitoneally to rats under light ether anesthesia. Animals were immunized one to three times and absorption studies were carried out 10 d after the final immunization.

Antiserum and Passive Cutaneous Anaphylaxis Reactions — Blood obtained by heart puncture 10 d after the last immunization was allowed to clot at room temperature, stored overnight at 4 °C and centrifuged for 10 min at 3000 rpm. The passive cutaneous anaphylaxis (PCA) method used was similar to that described by Goose and Blair.21) The titer of the antiserum was estimated by serial dilution from 1:1 to 1:32. After light pentobarbital anesthesia, the hair was removed from the back of the rat with an electric clipper. Diluted anti-ovalbumin sera (0.1 ml) were injected intracutaneously at six skin sites. Seventy two hours after the intracutaneous injection, 10 mg of antigen dissolved in 1 ml of saline containing 0.5% (w/v) Evans Blue dye was administered intravenously. The animals were killed 30 min after the challenge, and the skin was excised. The diameters of the blue discolorations were measured and the wheals were scored as 0 for < 5 mm, 1 for 5—10 mm, 2 for 10—15 mm, 3 for 15—20 mm, and 4 for > 20 mm.

Absorption Studies — The procedure of the in situ absorption study in the small intestine of rats was the same as that reported previously.17—20) Briefly, each animal was anesthetized with intraperitoneal pentobarbital injection and
the small intestine was cannulated for in situ recirculation. The entire length of the small intestine, from the pylorus to the ileo-cecal junction, was used for the absorption studies. The bile duct was ligated in all experiments. Ovalbumin dissolved in pH 6.5 phosphate buffer solution was recirculated through the intestine at a rate of 5 ml/min using a peristaltic pump. After the antigen pretreatment, 40 ml of a drug solution kept at 37 °C was recirculated through the intestine in the same manner. At the end of an experimental period, the perfused solution in the small intestine was withdrawn as completely as possible, and the intestinal lumen was washed with pH 6.5 buffer solution. The washings were combined with the perfused solution and made up to 100 ml with pH 6.5 buffer solution. The amount absorbed by the lumen was calculated as the difference between the amount of the drug in the initial and the final solutions.

Exsorption Studies — The exsorption of Evans Blue into the gut lumen during local anaphylaxis was studied by a modified method described in previous reports from this laboratory.22–25) The operation was the same as the absorption studies. After the intravenous administration of Evans Blue (1% w/v, 0.5 ml), ovalbumin in 40 ml of pH 6.5 buffer solution was recirculated through the intestine at a rate of 5 ml/min using a peristaltic pump. Then, the small intestine was recirculated with pH 6.5 buffer solution in the same manner. At the end of recirculation, the amount of the dye in the perfusate was determined spectrophotometrically.

Analytical Methods — Spectrophotometric determination was made of all the drug investigated.

Salicylic Acid: Three ml of sample solution was acidified with 0.1 ml of 35% HCl and extracted with 7 ml of chloroform. An aliquot of the organic phase was then shaken with 0.1 N NaOH and the optical density of the aqueous phase was determined spectrophotometrically at 295 nm.

Caffeine: One ml of sample solution was extracted with 6 ml of chloroform. The optical density of the organic phase was determined spectrophotometrically at 276 nm.

Phenylbutazone: Three ml of the sample solution was acidified with 0.5 ml of 3 N HCl and extracted with 20 ml of n-heptane. An aliquot of the organic phase was then shaken with 2.5 N NaOH and the optical density of the aqueous phase was determined spectrophotometrically at 265 nm.

BTB: Two ml of sample solution was alkalinized with 5 ml of 1 N NaOH and the optical density was determined spectrophotometrically at 617 nm.

Quinine: Three ml of a sample solution was alkalinized with 1 ml of 3 N NaOH and extracted with 6 ml of ethylene dichloride. An aliquot of the organic phase was then shaken with acidic media, and the optical density of the latter phase was determined spectrophotometrically at 251 nm.

2-PAM: One-half ml of sample solution was acidified with 4 ml of 0.1 N HCl and shaken with 4 ml of water-saturated isoamylalcohol. An aliquot of the aqueous phase was then alkalinized with 5 ml of 0.2 N NaOH and the optical density was determined spectrophotometrically at 336 nm.

Phenol Red: One ml of sample solution was alkalinized with 4 ml of 1 N NaOH and the optical density was determined spectrophotometrically at 560 nm.

Tetracycline: Five ml of sample solution was acidified with 1 ml of 5 N HCl and boiled for 3 min. After cooling in water, the optical density was determined spectrophotometrically at 435 nm.

Evans Blue: After recirculation, the perfusate was centrifuged for 10 min at 2500 rpm and the optical density of the supernatant fluid was determined spectrophotometrically at 606 nm.

Statistical Analyses — Results were expressed as the mean ± standard deviation (S.D.). Statistical analyses were performed using Student's t-test.
RESULTS

Passive Cutaneous Anaphylactic Reactions

The passive cutaneous anaphylaxis method was employed for the identification of the anti-ovalbumin antibodies in ovalbumin-immunized rats. The average PCA titers calculated from at least 7 rats are shown in Table I. As shown in Table I, the reactions were positive in the region of 1:1 to 1:8 in sera of rats immunized once, and the scores were also positive in rats immunized three times with ovalbumin (1:1 – 1:16).

The Leakage of Evans Blue in the Perfusate

The leakage of Evans Blue in rats having different doses of intraluminal challenge with ovalbumin was examined by means of exsorption studies. As shown in Table II, a significant increase in leaks of the dye was observed by the intraluminal challenge with 200 and 400 mg of ovalbumin for 15 min compared to the control value. The amount of leakage of the dye at 40 mg of the antigen did not increase significantly. The intraluminal exposure to 400 mg of ovalbumin was more effective on the leakage of the dye than that with 200 mg of ovalbumin.

Table III shows the leakage of Evans Blue in rats having different pretreatment time of intraluminal challenge with ovalbumin in ovalbumin-immunized rats. A significant increase in leaks of the dye was obtained by the intraluminal challenge with 400 mg of ovalbumin for 10 min in ovalbumin-immunized rats, and this was chosen as the optimal condition of local anaphylaxis. The intraluminal challenge with 400 mg of antigen for 15 min, and 30 min was not as effective as that for 10 min.

Intestinal Absorption of Drugs during Local Anaphylaxis

Under the above optical condition of local

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**TABLE I. Passive Cutaneous Anaphylactic Reaction in Rats Immunized Intraperitoneally Once (× 1) or Three Times (× 3) with Ovalbumin**

<table>
<thead>
<tr>
<th>No. of immunization</th>
<th>1:1</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>1:32</th>
</tr>
</thead>
<tbody>
<tr>
<td>×1</td>
<td>1.8</td>
<td>1.5</td>
<td>0.5</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>×3</td>
<td>2.0</td>
<td>1.4</td>
<td>0.7</td>
<td>0.5</td>
<td>0.2</td>
<td>0</td>
</tr>
</tbody>
</table>

The diameter of the blue discoloration was scored 0 – 4 as described in Materials and Methods. Data represent the mean of 7 – 10 experiments.

**TABLE II. Effect of the Challenging Dose of the Antigen on the Leakage of Evans Blue in Ovalbumin-Immunized Rats**

<table>
<thead>
<tr>
<th>OA (mg)</th>
<th>Amount of Evans Blue (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.7 ± 3.9 (5)</td>
</tr>
<tr>
<td>40</td>
<td>24.0 ± 19.8 (6) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td>27.3 ± 8.9 (6) &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>400</td>
<td>39.8 ± 19.2 (5) &lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Animals were immunized with ovalbumin once and the leakage of Evans Blue was examined by means of in situ exsorption experiments in rats challenged intraluminally with ovalbumin for 15 min. Evans Blue (1%) in saline was administered intravenously before the antigen challenge and the small intestine was perfused with pH 6.5 buffer solution for 60 min at the rate of 5 ml/min. Results are expressed as the mean ± S.D. with the number of experiments in parentheses. <sup>a</sup> Not significantly different, <sup>b</sup> p < 0.01, <sup>c</sup> p < 0.02, compared with the control. OA = ovalbumin.
anaphylaxis, intestinal absorption of various drugs was examined by means of in situ recirculation technique. As demonstrated in Table IV, intestinal absorption of caffeine, phenylbutazone, and BTB by the intraluminal antigen challenge was significantly decreased compared to buffer treatment as the control, whereas no significant effect was noted on the intestinal absorption of salicylic acid, quinine, 2-PAM, phenol red and tetracycline by the intraluminal antigen exposure.

**Table III.** Effect of the Challenging Time of the Antigen on the Leakage of Evans Blue in Ovalbumin-Immunized Rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Amount of Evans Blue (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.7 ± 3.9 (5)</td>
</tr>
<tr>
<td>5</td>
<td>49.7 ± 26.5 (7)(^a)</td>
</tr>
<tr>
<td>10</td>
<td>54.3 ± 33.2 (6)(^b)</td>
</tr>
<tr>
<td>15</td>
<td>39.8 ± 19.2 (5)(^c)</td>
</tr>
<tr>
<td>30</td>
<td>37.0 ± 24.0 (6)(^c)</td>
</tr>
</tbody>
</table>

*Animals were immunized with ovalbumin once and the leakage of Evans Blue was examined by means of in situ exsorption experiments in rats challenged intraluminally with 400 mg of ovalbumin. Evans Blue (1%) in saline was administered intravenously before the antigen challenge and the small intestine was perfused with pH 6.5 buffer solution for 60 min at the rate of 5 ml/min. Results are expressed as the mean ± S.D. with the number of experiments in parentheses. \(^a\) p<0.01, \(^b\) p<0.02, \(^c\) p<0.05, compared with the control.*

**Table IV.** Intestinal Absorption of Drugs during Local Anaphylaxis

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Buffer</th>
<th>Absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OA</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>72.9±4.3 (6)</td>
<td>69.2±3.6 (6)(^a)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>64.8±3.4 (7)</td>
<td>59.6±4.6 (7)(^b)</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>62.8±4.0 (8)</td>
<td>48.5±13.2 (8)(^c)</td>
</tr>
<tr>
<td>BTB</td>
<td>58.9±6.2 (8)</td>
<td>51.1±6.3 (10)(^d)</td>
</tr>
<tr>
<td>Quinine</td>
<td>28.7±1.8 (8)</td>
<td>26.7±5.3 (10)(^a)</td>
</tr>
<tr>
<td>2-PAM</td>
<td>26.0±2.0 (6)</td>
<td>23.1±3.1 (6)(^a)</td>
</tr>
<tr>
<td>Phenol red</td>
<td>7.2±1.6 (6)</td>
<td>7.5±1.5 (6)(^a)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4.8±1.9 (6)</td>
<td>6.1±1.8 (7)(^a)</td>
</tr>
</tbody>
</table>

*Animals were immunized with ovalbumin once and intestinal absorption of drugs during local anaphylaxis was examined by means of in situ recirculation technique. Four hundred milligrams of ovalbumin in pH 6.5 buffer solution was recirculated through the intestine for 10 min before the start of intestinal drug recirculation. Results are expressed as the mean ± S.D. with the number of experiments in parentheses. \(^a\) Not significantly different, \(^b\) p<0.05, \(^c\) p<0.02, \(^d\) p<0.01, compared with each control. OA = ovalbumin.*
challenge with an unrelated antigen, bovine γ-globulin, in ovalbumin-immunized rats is illustrated in Fig. 2. The results show no significant change on the absorption of BTB by the intraluminal challenge with bovine γ-globulin in ovalbumin-immunized rats.

**Intestinal Absorption of BTB in Rats Immunized with Ovalbumin Once or Three Times**

Fig. 3 shows the effect of a number of immunizations on the intestinal absorption of BTB. A significant decrease in BTB absorption was observed by the intraluminal challenge with ovalbumin in rats immunized with the antigen once or three times. There was no significant difference between the two groups.

**Intestinal Absorption of BTB after an Intraluminal Challenges with Varied Doses of Ovalbumin**

The effect of the challenging dose of the antigen on the intestinal absorption of BTB is shown in Table V. Animals were challenged intraluminally with 4, 40, 200 and 400 mg of the antigen. A significant decrease in BTB absorption was observed in rats challenged with 40, 200 and 400 mg of ovalbumin, whereas no significant change was noted in rats challenged with 4 mg of the antigen. A maximum response was obtained in rats challenged with 200 mg of ovalbumin.

**Intestinal Absorption of BTB after Intraluminal Challenges with Ovalbumin for Varied Durations of Exposure**

The effect of the length of challenging time of the antigen on the intestinal absorption of BTB was also examined in ovalbumin-immunized rats and the results are presented in Table VI. Animals were challenged with 400 mg of ovalbumin for 5, 10, 15, 30 and 60 min. The decreased absorption of BTB was noted in rats challenged with ovalbumin for 10, 15 and 30 min. However, no significant change was observed by the intraluminal challenge with ovalbumin for 5 and 60 min. The most effective condition for decreased BTB absorption was observed by the intraluminal challenge with 200

![Graph showing absorption percentages](image)

**FIG. 1. Intestinal Absorption of Drugs in Normal Rats**

Intestinal absorption of caffeine, phenylbutazone, and BTB was examined by means of in situ recirculation technique in normal rats. Four hundred milligrams of ovalbumin in pH 6.5 buffer solution was recirculated through the intestine for 10 min before the start of intestinal recirculation. Vertical bars indicate ± S.D. a) Not significantly different, compared with each control. OA = ovalbumin.

**FIG. 2. Effect of Intraluminal Challenge with Bovine γ-Globulin on the Intestinal Absorption of BTB in Ovalbumin-Immunized Rats**

Animals were immunized with ovalbumin once and intestinal absorption of BTB was examined by means of in situ recirculation technique. Four hundred milligrams of ovalbumin or bovine γ-globulin in pH 6.5 buffer solution was recirculated through the intestine for 10 min before the start of intestinal drug recirculation. Vertical bars indicate ± S.D. a) p < 0.05, b) not significantly different, compared with the control. OA = ovalbumin, BGG = bovine γ-globulin.
mg of ovalbumin for 10 min in ovalbumin-immunized rats.

**The Time Course of Intestinal Absorption of BTB**

The time course of intestinal absorption of BTB was also examined by means of in situ recirculation technique (Fig. 4). Intraluminal exposure to the specific antigen in immunized rats led to a significant reduction in BTB absorption for 30—60 min compared with the control.

**DISCUSSION**

This study shows that important alterations in caffeine, phenylbutazone, and BTB absorption occur during local anaphylaxis in the small intestine of the rat. Intestinal challenge with a specific antigen in sensitized animals led to a significant inhibition in caffeine, phenylbutazone, and BTB absorption. The intestinal response was shown to be specific for the sensitizing antigens because there was no significant effect on the BTB absorption in ovalbumin-immunized rats challenged with an unrelated antigen, bovine \( \gamma \)-globulin. Furthermore, intestinal antigen challenge in normal rats had no effect on the absorption of these drugs, which is reasonable since local immune responses result in the decreased absorption of BTB in the small intestine of the rat. The results which showed no significant changes in the decreased absorption of BTB between rats immunized once and rats immunized three times suggested that this immune response may be independent of the immunization time. These results are also supported by the presence of anti-ovalbumin antibody titers examined by the PCA technique.

The optimal condition for local anaphylaxis was determined by measuring the intraluminal

![Graph showing absorption percentages]

**FIG. 3. Effect of Intraluminal Challenge on the Intestinal Absorption of BTB in Immunized Rats**

Intestinal absorption of BTB was examined by means of in situ recirculation technique in ovalbumin-immunized rats once (×1) or three times (×3). Four hundred milligrams of ovalbumin in pH 6.5 buffer solution was recirculated through the intestine for 10 min before the start of intestinal drug recirculation. Vertical bars indicate ± S.D. a) \( p < 0.01 \), compared with each control. OA = ovalbumin.

**TABLE V. Effect of the Challenging Dose of the Antigen on the Intestinal Absorption of BTB in Ovalbumin-Immunized Rats**

<table>
<thead>
<tr>
<th>OA (mg)</th>
<th>Absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>58.9±6.2 (8)</td>
</tr>
<tr>
<td>4</td>
<td>61.4±5.4 (6) a)</td>
</tr>
<tr>
<td>40</td>
<td>50.0±4.2 (6) b)</td>
</tr>
<tr>
<td>200</td>
<td>49.1±6.3 (10) c)</td>
</tr>
<tr>
<td>400</td>
<td>51.1±5.3 (11) c)</td>
</tr>
</tbody>
</table>

Animals were immunized with ovalbumin once and intestinal absorption of BTB was examined by means of in situ recirculation technique. Ovalbumin in pH 6.5 buffer solution was recirculated through the intestine for 10 min before the start of intestinal drug recirculation. Results are expressed as the mean ± S.D. with the number of experiments in parentheses. a) Not significantly different, b) \( p < 0.02 \), c) \( p < 0.01 \), compared with the control. OA = ovalbumin.
TABLE VI. Effect of the Challenging Time of the Antigen on the Intestinal Absorption of BTB in Ovalbumin-Immunized Rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Buffer</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>59.7±5.7 (6)</td>
<td>53.7±6.3 (7)</td>
</tr>
<tr>
<td>10</td>
<td>58.9±6.2 (8)</td>
<td>51.1±5.3 (11)</td>
</tr>
<tr>
<td>15</td>
<td>57.9±3.7 (7)</td>
<td>50.6±6.6 (8)</td>
</tr>
<tr>
<td>30</td>
<td>59.1±4.3 (6)</td>
<td>51.1±5.9 (5)</td>
</tr>
<tr>
<td>60</td>
<td>62.1±4.5 (8)</td>
<td>60.3±6.4 (7)</td>
</tr>
</tbody>
</table>

Animals were immunized with ovalbumin once and intestinal absorption of BTB was examined by means of in situ recirculation technique. Four hundred milligrams of ovalbumin in pH 6.5 buffer solution was recirculated through the intestine before the start of intestinal drug recirculation. Results are expressed as the mean ± S.D. with the number of experiments in parentheses. a) Not significantly different, b) p<0.01, c) p<0.05, compared with the control. OA = ovalbumin.

FIG. 4. Time Course of BTB Absorption in Ovalbumin-Immunized Rats

Animals were immunized with ovalbumin once and intestinal absorption of BTB was examined by means of in situ recirculation technique for 15, 30, 45, and 60 min in ovalbumin-immunized rats. Four hundred milligrams of ovalbumin in pH 6.5 buffer solution (●) or pH 6.5 buffer solution (○) was recirculated through the intestine for 10 min before the start of intestinal drug recirculation. Vertical bars indicate ± S.D. a) Not significantly different, b) p<0.01, c) p<0.05, compared with the control.

Accumulation of Evans Blue dye. Because this dye has been shown to combine preferentially with plasma albumin, the measurement of the dye in the intestinal lumen largely represents the translocation of albumin molecules and reflect vascular and epithelial leakages of the protein. As shown in Tables II and III, intraluminal exposure to 400 mg of the antigen for 10 min in immunized rats was the most effective and this was chosen as the optimal condition for local anaphylaxis. In addition, the most effective condition for decreased BTB absorption was observed by the intraluminal challenge with 200 mg of ovalbumin for 10 min. From these results, it may be concluded that the decreased BTB absorption was dependent on the potency of the local immune responses, and these immunological responses occurred within minutes of intraluminal exposure and persisted even after the antigen withdrawal. However, no significant decrease in BTB absorption was noted by the intraluminal challenge with 400 mg of ovalbumin for 60 min. This result suggests that immunological responses may cease within 60 min of intraluminal exposure to the specific antigen.

The mechanism by which antigen-induced anaphylaxis produces intestinal dysfunction is not clearly demonstrated. In our previous reports, intestinal absorption of salicylic acid was significantly decreased by systemic
anaphylaxis and the decreased absorption of salicylic acid may be due to the reduced blood flow from the rat small intestine. However, the present study showed that no significant decrease in salicylic acid absorption was observed by local anaphylaxis. It is well known that intestinal absorption of salicylic acid, a readily absorbable drug, may be influenced by the mesenteric blood flow. From these observations, it seems likely that the reduced blood flow may not be responsible for the decreased absorption of drugs by local anaphylaxis.

Another possibility is that the changes in mucosal and vascular permeability by local anaphylaxis may result in the transport of these drugs. Bloch et al. demonstrated that the alteration in vascular and mucosal permeability which accompanies intestinal anaphylaxis was reflected by increased retention of $^{125}$I-labelled rat serum albumin in gut wall segments. More recently, Perdue et al. also reported that intestinal challenge with the specific antigen in sensitized animals led to the significant inhibition of Na+, K+, Cl− and water absorption. These alterations may play an important role in the decreased absorption of low molecular weight compounds in the same manner as the antigenic macromolecules. However, the present study shows that the absorption of phenol red, a poorly absorbable drug, was not affected by local anaphylaxis in the same manner as our previous finding during systemic anaphylaxis. On the other hand, previous reports from this laboratory indicate that gastric absorption of phenol red, examined by means of in situ loop technique, was significantly increased in ulcerated rats. Therefore, since the absorption of a poorly absorbable drug was not increased significantly by local anaphylaxis, it is suggested that progressive loss of structural integrity of the epithelium may not occur during local anaphylaxis.

The present study also indicates that local immune response did not result in the decreased intestinal absorption of 2-PAM, quinine, and tetracycline. Kakemi et al. reported that intestinal absorption of 2-PAM, which is one of the most famous quarternary ammonium compounds, became saturated when the concentration of the drug increased. Therefore, it was suggested that intestinal absorption of drugs which have specialized absorption mechanisms, might not be affected by local anaphylaxis.

Moreover, Lake et al. have shown the enhanced release of goblet cell mucus during intestinal anaphylaxis. Previous reports from this laboratory suggested that intestinal absorption of BTB might be influenced by the membrane components such as glycyoclyx and lipid and the mucosal surface of the small intestine, mucus layer. In addition, the disappearance of quinine from the in situ recirculated solution was enhanced in the presence of BTB by ion pair formation. Although the reason why intestinal absorption of BTB is significantly reduced by local anaphylaxis remains to be elucidated, these previous findings suggest that some intraluminal or membranous substances may interact with the BTB molecules. Phenylbutazone, and caffeine, which are readily absorbable drugs, are also influenced by local anaphylaxis. However, the reason for these phenomena has not been clearly demonstrated. Braybrook and Burry reported that the presence of mucin (1%) reduced the bioavailability of phenylbutazone from the rat small intestine in vivo and in vitro, which may be one of the most important mechanisms of the decreased phenylbutazone absorption. From these observations, it seems likely that this discharge of goblet cell mucus may also represent a barrier against the intestinal absorption of these drugs during local anaphylaxis.

REFERENCES


