Effect of Systemic Anaphylaxis on the Absorption of Salicylic Acid from the Rat Small Intestine

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Abstract □ Rats were immunized by intraperitoneal injection of ovalbumin (egg albumin) emulsified in Freund's incomplete adjuvant, and then the effect of an intravenous challenge with ovalbumin on salicylic acid absorption from the small intestine was examined by means of an *in situ* recirculation technique. The disappearance of salicylic acid from the luminal solution was significantly decreased in rats treated with ovalbumin compared with control groups treated with saline. The decreased absorption of salicylic acid in ovalbumin-immunized rats was related to the anti-ovalbumin antibody responses examined by passive cutaneous anaphylactic reactions. On the other hand, the decreased absorption of salicylic acid was not found in ovalbumin-immunized rats challenged intravenously with bovine γ-globulin. Similar results were also noted in rats immunized *via* the footpads with ovalbumin. However, no significant change was observed in the intestinal absorption of salicylic acid in normal (nonimmunized) rats challenged intravenously with ovalbumin. Furthermore, intestinal absorption of sulfadimethoxine and sulfanilamide was significantly decreased during systemic anaphylaxis, whereas no change was observed in the absorption of sulfisoxazole, quinine, sulfanilic acid, and phenolsulfonphthalein. This suggests that the intestinal absorption of rapidly absorbed drugs, including salicylic acid, is more sensitive to systemic anaphylaxis than that of poorly absorbed drugs.

Keyphrases □ Salicylic acid—intestinal absorption, effect of anaphylaxis, rats □ Absorption, intestinal—salicylic acid, effect of anaphylaxis, rats □ Anaphylaxis—effect on intestinal absorption of salicylic acid, rats

In a previous paper, it was reported that the intestinal absorption of salicylic acid was decreased by the intravenous challenge with bovine γ-globulin in rats immunized intraperitoneally with the same antigen, and it was suggested that systemic anaphylaxis influenced the intestinal absorption of drugs (1). The present report concerns the effect of intravenous challenge with ovalbumin on the intestinal absorption of salicylic acid in rats immunized parenterally with ovalbumin. Ovalbumin was chosen on the grounds that (*a*) ovalbumin induces a good humoral and cellular response in the guinea pig, as demonstrated by Audibert *et al.* (2), (*b*) ovalbumin is widely used for IgE antibodies (3, 4), and (*c*) ovalbumin is one of the most potent immunogens in rats (5–8). To prove the immunogenicity, the anti-ovalbumin antibody was examined by the passive cutaneous anaphylaxis technique (9–14), and the intestinal absorption of various drugs was investigated to clarify the mechanisms by which the absorption of salicylic acid was decreased during systemic anaphylaxis.

EXPERIMENTAL

Materials—Ovalbumin[1] (crystallized and lyophylized) and bovine γ-globulin[1] (Cohn fraction II) were used without further purification. Salicylic acid[2], sulfadimethoxine[2], sulfanilamide[2], sulfisoxazole[2], quinine[2], sulfanilic acid[2], phenolsulfonphthalein[2], and all reagents[2] used in these experiments were reagent grade.

The isotonic buffer solution (pH 6.5) was prepared from 0.123 M Na_2HPO_4 and 0.163 M NaH_2PO_4. Salicylic acid was dissolved in this buffer solution at a concentration of 1 mM for absorption experiments. All other drugs were dissolved in the same buffer solution at a concentration of 0.1 mM.

Immunization—Male Wistar albino rats, 150–200 g, were used in these studies and were maintained on a diet free of ovalbumin and bovine γ-globulin. For intraperitoneal immunization, 1 mg of ovalbumin dissolved in 0.25 mL of saline (0.9% w/v) was emulsified with an equal volume of Freund's incomplete adjuvant[3] and was injected into the peritoneal cavity under light ether anesthesia. For footpad immunization, 0.5 mL of ovalbumin dissolved in 0.05 mL of saline was emulsified with an equal volume of Freund's incomplete adjuvant and was injected into both the right and left footpads under light ether anesthesia. The same dose of antigen with adjuvant was administered at 10-d intervals thereafter. Animals were immunized 1–3 times, and absorption studies were carried out 1 week after the last immunization.

Antiserum and Passive Cutaneous Anaphylaxis—Blood obtained by heart puncture 10 days 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 method used was similar to that described by Goose and Blair (9). The titer of the antisera was estimated by serial dilution from 1:1 to 1:32. After light intraperitoneal pentobarbital anesthesia, the hair was removed from the back of the rat with an electric clipper. Diluted anti-ovalbumin sera (0.1 mL) was 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 rat small intestine was the same as that reported previously (15). Briefly, animals were anesthetized with intraperitoneal pentobarbital, and the small intestine was cannulated for *in situ* recirculation. The entire length of the small intestine, from the pylorus to the ileocecal junction, was used for the absorption studies. The bile duct was ligated in all experiments. Ovalbumin dissolved in 0.25 mL of saline was administered into a right femoral vein for 5 min. Immediately thereafter, 40 mL of a drug solution kept at 37°C was recirculated through the intestine at a rate of 5 mL/min using a peristaltic pump. 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. Results were expressed as the mean ± *SD*. Statistical analyses were performed using the Student's *t* test.

Analytical Methods—*Salicylic Acid*—Three milliliters of sample solution was acidified with 0.1 mL of concentrated HCl and extracted with 7 mL of chloroform. An aliquot of the organic phase was then shaken with 0.1 M NaOH, and the optical density of the aqueous phase was determined spectrophotometrically at 295 nm.

Aromatic Amines—All the aromatic amines were diazotized, coupled with 2-diethylaminoethyl-1-naphthylamine, and then were extracted with isoamyl alcohol after the addition of sodium chloride. The optical density of the organic layer was determined at each absorption maximum, 552–560 nm (16).

Quinine—Three milliliters of a sample solution was alkalinized with 1 ml of 3 M NaOH and extracted with 6 ml of ethylene dichloride. An

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Nakarai Chemical Co., Japan.
[3] Difco Laboratories, Detroit, Mich.

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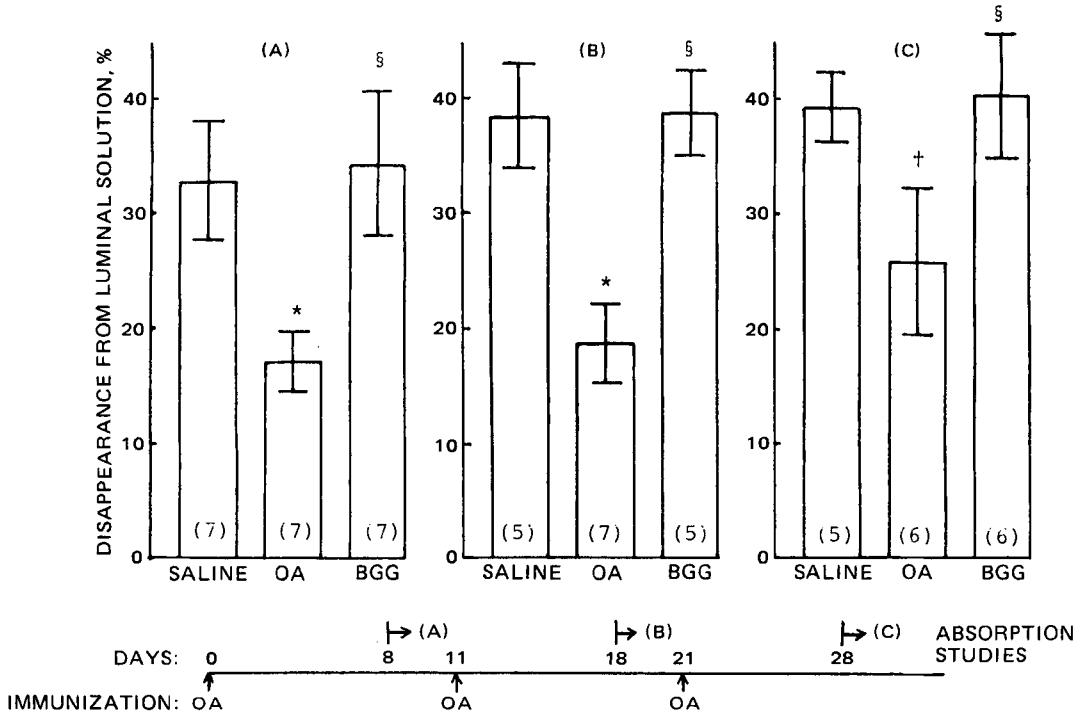

Figure 1—*Effect of intravenous challenge with ovalbumin(OA) or bovine γ-globulin (BGG) on the intestinal absorption of salicylic acid in 15 min in rats immunized intraperitoneally once (A), twice (B), or three times (C) with ovalbumin. Ovalbumin or bovine γ-globulin (0.5 mg) dissolved in 0.25 mL of saline was administered immediately before the start of intestinal recirculation. Numbers in parentheses represent the number of experiments; vertical bars indicate ±SD. Key: (*) p < 0.001; (†) p < 0.005; (§) not significantly different, compared with the appropriate control.*

aliquot of the organic phase was shaken with acidic media, and the optical density of the latter phase was determined spectrophotometrically at 251 nm (17).

Phenolsulfonphthalein—One milliliter of sample solution was alkalinized with 4 ml of 1 M NaOH and determined spectrophotometrically at 560 nm.

RESULTS

The absorption of salicylic acid from the small intestine was examined at pH 6.5 in ovalbumin-immunized rats using an *in situ* recirculation technique. The protocol of the immunization with ovalbumin and the absorption studies are shown in each figure and table.

The results of absorption studies in rats immunized once, twice, or three times with ovalbumin are summarized in Fig. 1. The disappearance of salicylic acid from the luminal solution in 15 min was significantly decreased in rats challenged intravenously with ovalbumin compared with control rats treated with saline. On the other hand, decreased absorption of the drug by the intravenous challenge with bovine γ-globulin was not observed in ovalbumin-immunized rats. These results indicate the remarkable specificity of antigen-antibody reactions.

In this study, the passive cutaneous anaphylaxis method was employed for the identification of the anti-ovalbumin antibodies. The results of the anaphylaxis experiment are shown in Table I. There is a good relationship between the decrease of salicylic acid absorption and passive cutaneous anaphylactic reactions. To examine the duration of the action, absorption studies and anaphylactic reactions were carried out 1, 5, 10, and 16 weeks after the third immunization. The results are shown in Fig. 2 and Table II, respectively. The reduction of salicylic acid absorption by the intravenous challenge with ovalbumin was observed for 10 weeks, but did not last for 16 weeks after the last immunization. Similarly, the sera withdrawn from rats 1, 5, and 10 weeks after the last immunization were able to sensitize rat skin for passive cutaneous anaphylaxis, but anaphylactic reactions were negative for the serum withdrawn from rats at 16 weeks, with one exception. Thus, the decreased salicylic acid absorption agrees with the passive cutaneous anaphylactic reactions.

Figure 3 shows the effect of intravenous administration of ovalbumin on salicylic acid absorption from the small intestine in nonimmunized rats. The decreased absorption of the drug by the intravenous challenge

Table I—Passive Cutaneous Anaphylactic Reactions in Rats Immunized Intraperitoneally Once, Twice, or Three Times with Ovalbumin [a]

No. of Immunizations	Antiserum Dilution 1:1	1:2	1:4	1:8	1:16	1:32
1	2.4	0.9	0.5	0.1	0	0
2	2.0	1.1	0.5	0.1	0	0
3	2.2	1.1	0.5	0.3	0	0

[a] The diameter of the blue discoloration was scored 0–4 as described in *Experimental*. Data represent the mean of 8–10 experiments.

Table II—Passive Cutaneous Anaphylactic Reactions in Rats Immunized Intraperitoneally Three Times with Ovalbumin [a]

Group [b]	Antiserum Dilution 1:1	1:2	1:4	1:8	1:16	1:32
A	2.8	1.7	0.9	0.6	0.4	0.4
B	1.2	0.3	0	0	0	0
C	0.4	0.1	0	0	0	0
D	0.25	0	0	0	0	0

[a] The diameter of the blue discoloration was scored 0–4 as described in *Experimental*. Data represent the mean of 8–10 experiments. [b] Passive cutaneous anaphylaxis experiments were carried out 1 week (A), 5 weeks (B), 10 weeks (C), and 16 weeks (D) after the last immunization.

Table III—Effect of Intravenous Challenge with Ovalbumin on the Intestinal Absorption of Various Drugs in 15 min in Rats Immunized Intraperitoneally with Ovalbumin [a]

Drug	Challenge: Saline, % absorbed	Challenge: Ovalbumin, % absorbed
Salicylic acid	38.5 ± 4.6 (5)	18.7 ± 3.4 (7) [b]
Sulfadimethoxine	28.7 ± 2.6 (6)	20.6 ± 2.5 (6) [b]
Sulfanilamide	17.8 ± 2.4 (5)	11.6 ± 1.2 (6) [b]
Sulfisoxazole	12.9 ± 1.5 (5)	11.3 ± 0.9 (5)
Quinine	11.3 ± 0.9 (6)	9.9 ± 1.3 (6)
Sulfanilic acid	4.3 ± 0.6 (5)	3.6 ± 0.8 (5)
Phenolsulfonphthalein	1.7 ± 0.6 (6)	1.7 ± 0.2 (6)

[a] 0.5 mg of ovalbumin dissolved in 0.25 mL of saline was administered immediately before the start of intestinal recirculation. Results are expressed as the mean ± *SD* followed by the number of experiments in parentheses. [b] $p < 0.001$, compared with the appropriate control.

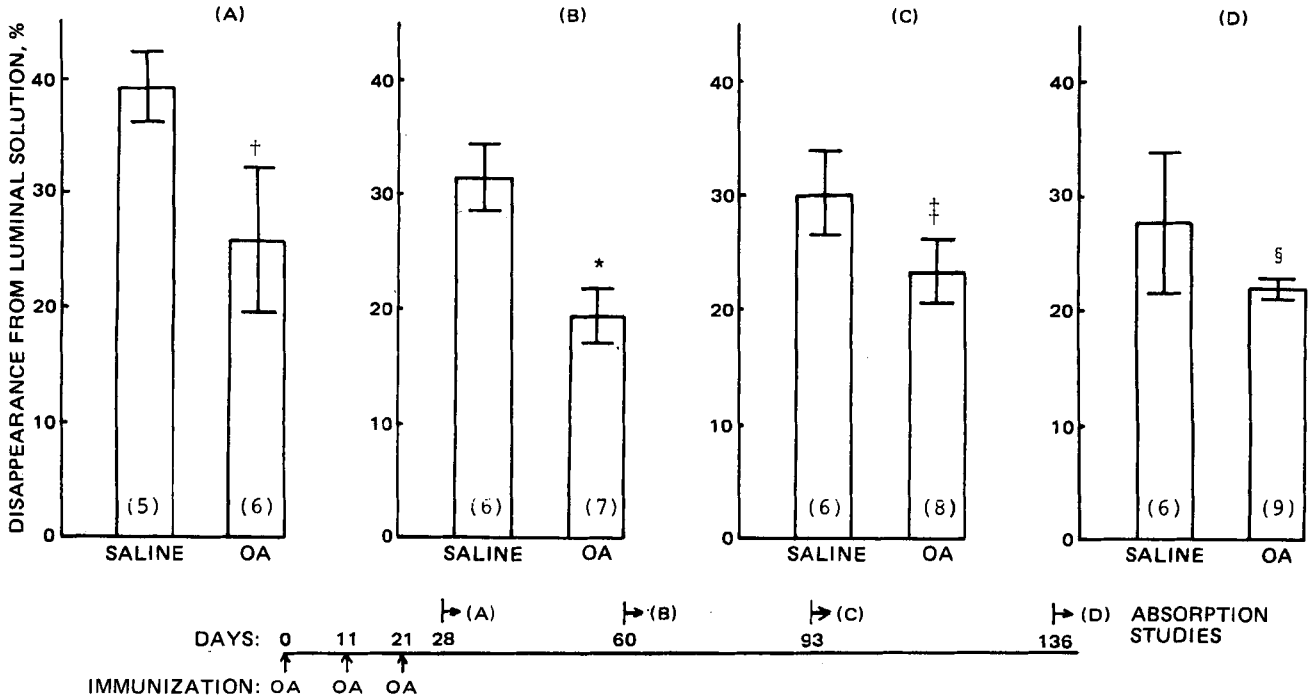

Figure 2—*Effect of intravenous challenge with ovalbumin(OA) on the intestinal absorption of salicylic acid in 15 min in rats immunized intraperitoneally three times with ovalbumin. Absorption studies were carried out 1 week (A), 5 weeks (B), 10 weeks (C), and 16 weeks (D) after the last immunization. Ovalbumin (0.5 mg) dissolved in 0.25 mL of saline was administered immediately before the start of intestinal recirculation. Numbers in parentheses represent the number of experiments, vertical bars indicate* ± SD. *Key: (*)* $p < 0.001$; *(†)* $p < 0.005$; *(‡)* $p < 0.01$; *(§) not significantly different, compared with the appropriate control.*

failed to be found in normal rats. From the results described above, it is reasonable to consider that systemic anaphylaxis results in the decrease of salicylic acid absorption. This result is consistent with previous data that salicylic acid absorption was decreased by the intravenous challenge with bovine γ-globulin in rats immunized with the same antigen. To elucidate the mechanism by which systemic anaphylaxis decreased salicylic acid absorption, the intestinal absorption of drugs with various absorbability was examined (Table III). Sulfadimethoxine is a rapidly absorbed drug like salicylic acid. Sulfanilamide, sulfisoxazole, and quinine are moderately absorbable drugs. Sulfanilic acid and phenolsulfonphthalein are poorly absorbed. As is evident from the table, the absorption of sulfadimethoxine and sulfanilamide was decreased by the intravenous challenge with ovalbumin; no significant change was observed in the intestinal absorption of sulfisoxazole, quinine, sulfanilic acid, and phenolsulfonphthalein.

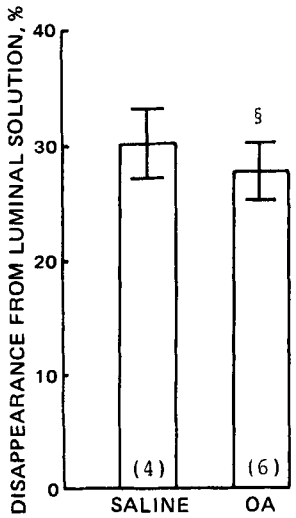

Figure 3—*Effect of intravenous challenge with ovalbumin (OA) on the intestinal absorption of salicylic acid in 15 min in normal (nonimmunized) rats. Ovalbumin (0.5 mg) dissolved in 0.25 mL of saline was administered immediately before the start of intestinal recirculation. Numbers in parentheses represent the number of experiments; vertical bars indicate* ± SD. *Key: (§) not significantly different compared with the control.*

To examine the effect of the route of immunization with the antigen, ovalbumin was injected into the footpads of rats twice and three times. As shown in Fig. 4, the intestinal absorption of salicylic acid was significantly decreased in rats challenged intravenously with ovalbumin compared with the control (challenged with saline). However, there was no effect of the intravenous challenge with the different antigen, bovine γ-globulin on salicylic acid absorption. These results agree well with the data shown in Fig. 1. In this case, the passive cutaneous anaphylaxis experiment can again be used to prove the existence of the anti-ovalbumin antibody in rats immunized *via* the footpads (Table IV). The data in the table provide definitive evidence for the existence of anti-ovalbumin antibodies in rats immunized *via* the footpads, as in rats immunized intraperitoneally.

DISCUSSION

There has been considerable interest recently in the immunological consideration of the GI tract. A previous paper reported the decreased absorption of salicylic acid following intravenous challenge with bovine γ-globulin in rats immunized intraperitoneally with this antigen (1). In the case of bovine γ-globulin, however, the decreased absorption of the drug by the antigen challenge was observed in rats immunized twice and three times, but not in rats immunized only once. Furthermore, the effect was maintained for at least 5 weeks after the third immunization, but disappeared within 10 weeks.

In the present study, the effect of intravenous challenge with ovalbumin on the intestinal absorption of salicylic acid was investigated in rats parenterally immunized with the same antigen. As shown in Figs. 1 and

Table IV—Passive Cutaneous Anaphylactic Reactions in Rats Immunized in the Footpads Once, Twice, or Three Times with Ovalbumin [a]

No. of Immunizations	Antiserum Dilution					
	1:1	1:2	1:4	1:8	1:16	1:32
1	2.3	0.9	0.5	0.1	0	0
2	4.0	4.0	4.0	4.0	2.6	1.2
3	3.7	3.2	2.9	2.1	2.0	2.0

[a] The diameter of the blue discoloration was scored 0–4 as described in *Experimental*. Data represent the mean of 10 experiments.

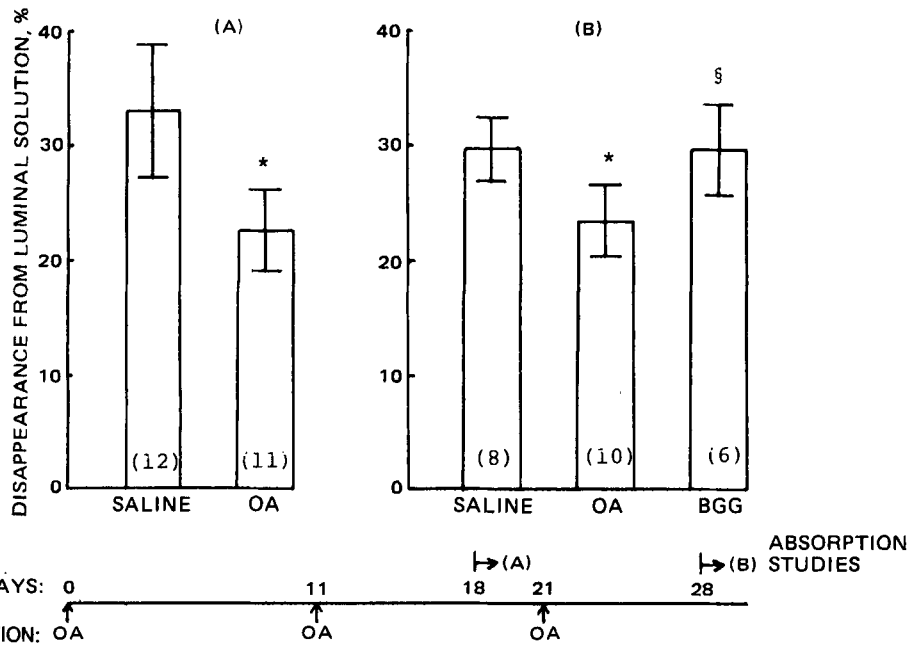

Figure 4—*Effect of intravenous challenge with ovalbumin (OA) on the intestinal absorption of salicylic acid in 15 min in rats immunized in the footpads twice (A) or three times (B) with ovalbumin. Ovalbumin or bovine γ-globulin (BGG) (0.5 mg) dissolved in 0.25 mL of saline was administered immediately before the start of intestinal recirculation. Numbers in parentheses represent the number of experiments; vertical bars indicate* ± SD. *Key: (§) not significantly different compared with the control.*

4, the intestinal absorption of salicylic acid was significantly decreased by the intravenous challenge with ovalbumin in ovalbumin-immunized rats. It seems likely that these results are not dependent on the route of immunization (intraperitoneal or intrafootpad). Figure 4 also shows that intravenous challenge with bovine γ-globulin failed to decrease salicylic acid absorption in ovalbumin-immunized rats. This indicates the specificity of the antigen–antibody reactions.

A significant decrease in the intestinal absorption of salicylic acid was noted by an ovalbumin challenge in rats immunized only once with ovalbumin (Fig. 1). This result is consistent with the passive cutaneous anaphylaxis data shown in Table I, but differs from our previous experiments where there was no significant change in the intestinal absorption of the drug in rats immunized only once with bovine γ-globulin. Furthermore, as shown in Fig. 2, intestinal absorption of salicylic acid was significantly decreased more than 10 weeks after the last immunization by the challenge with ovalbumin. In the case of bovine γ-globulin, the decreased absorption of salicylic acid was observed within 5 weeks and had disappeared within 10 weeks after the last immunization. From the results described above, it is reasonable to consider that ovalbumin is a more potent antigen than bovine γ-globulin in rats under these conditions.

The mammalian GI system contributes to the total immune response of the body. In both qualitative and quantitative terms, this contribution is no better defined than that of other organs. In addition, systemic anaphylaxis has been accompanied by changes in vascular and mucosal permeability in the rat. These changes are reflected in an enhanced retention of labeled serum albumin in intestinal tissues and by the increased concentration of such proteins in intestinal fluids. Bloch *et al.* suggested that during intestinal anaphylaxis, there is an increased movement of protein from the lumen into the systemic circulation (18, 19). Similarly, Thomas and Parrot could show that the systemic immune response, as well as the local immune answer, can change the normal protein absorption (20).

In contrast to the well-studied change of macromolecule transport during intestinal or systemic anaphylaxis (21–25), few studies have investigated the immunological consideration on the intestinal transport of low molecular weight organic compounds. Since, from the standpoint of biopharmaceutics, it might be worthy to examine the absorption of such compounds during intestinal or systemic anaphylaxis, the intestinal absorption of several low molecular weight drugs was examined in immunized rats by means of an *in situ* recirculation technique. As is evident from Table III, intestinal absorption of sulfadimethoxine and sulfanilamide was significantly decreased by the intravenous challenge with ovalbumin. There was, however, no significant change in the intestinal absorption of sulfisoxazole, quinine, sulfanilic acid, and phenolsulfonphthalein in rats challenged with ovalbumin. These findings indicate that poorly absorbed drugs are less sensitive to systemic anaphylaxis than well-absorbed drugs. As the absorption of poorly absorbed drugs was not increased by systemic anaphylaxis, it is suggested that progressive loss of structural integrity of the epithelium did not occur during systemic anaphylaxis.

The mechanism by which the intestinal absorption of salicylic acid was decreased during systemic anaphylaxis is not completely defined at present. In this case, various mechanisms may be considered. One of the most important mechanisms is the alteration of the mesenteric blood flow during anaphylaxis. It is well known that the absorption rates of rapidly absorbed drugs are controlled by the mesenteric blood flow. Beubler and Lêmbech showed that the absorption rate of salicylic acid was affected by the intestinal blood flow (26). Therefore, changes in the vascular system during systemic anaphylaxis might influence the absorption rate of the drug. Another possibility is that the release of histamine, serotonin, and other vasoactive substances induced by systemic anaphylaxis may contribute to the decreased absorption of salicylic acid. These chemical mediators play an important role in increased capillary permeability, vasodilation, and smooth muscle contraction. Moreover, Lake *et al.* have shown the enhanced release of goblet cell mucus during intestinal anaphylaxis (27, 28). This discharge of goblet cell mucus may also represent a barrier against the intestinal absorption of salicylic acid. Additional studies are needed to further clarify this observation.

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Effect of Microcapsule Core–Wall Ratio and Aggregate Size on the Properties of Tableted Microcapsules

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Abstract □ Microcapsules containing sodium phenobarbital cores and ethylcellulose walls have been tableted. The thickness of the tablets, the breaking strength, and the dissolution characteristics were studied and found to be affected by the microcapsule core–wall ratio and the size of the microcapsule aggregates.

Keyphrases □ Microcapsules—sodium phenobarbital and ethylcellulose, tablets, effect of core–wall ratio and aggregate size on dissolution □ Dissolution—tablets composed of microcapsules, sodium phenobarbital and ethylcellulose, effect of core–wall ratio and aggregate size □ Sustained-release formulations—tableted microcapsules, sodium phenobarbital and ethylcellulose, effect of core–wall ratio and aggregate size on dissolution

Microcapsules consist of a thin wall which can enclose a solid or liquid core material. One of the many important reasons for microencapsulating medicaments is to achieve sustained release. Good sustained release has been achieved by microencapsulating poorly water-soluble medicaments such as aspirin and phenobarbital (1). With very water-soluble substances such as sodium phenobarbital, the rate of release is slowed by microencapsulation, being controlled partly by the wall thickness. However, no satisfactory sustained release has been achieved with water-soluble substances (1–3). Tableting of microcapsules has been shown to slow the release significantly and provide a sustained- or prolonged-action release (4–6). The microcapsules which have been tableted appear to be mainly those with ethylcellulose walls prepared using a modification of the method described by Fanger *et al.* (7); in most cases, this technique has produced aggregates (2, 5, 7). This work studies the effect of the aggregate size and the core–wall ratio on the properties of the prepared tablets.

EXPERIMENTAL

Materials—Phenobarbital sodium[1] (99.4% pure), ethylcellulose[1] (viscosity 5% w/w solution in 80:20 toluene–ethanol mixture: 14.13 cP; degree of substitution: 2.50; ethoxy content: 47.5%), and cyclohexane[2] (99.5% pure, bp 80–81°C, fp 5.95°C; wt/mL at 20°C: 0.776) were purchased commercially.

Preparation of Microcapsules—The method used was a modification of an original technique by Fanger *et al.* (7) as further modified by Agyilirah and Nixon (5). This method involves deposition of polymeric wall-forming material onto dispersed particles of core by cooling below a critical liquid–liquid phase separation temperature. A typical example of microcapsule preparation was as follows. Ten grams of sodium phenobarbital and 5 g of ethylcellulose were dispersed in 500 mL of cyclohexane. The stainless steel stirrer was adjusted to the middle of the dispersion to obtain uniform stirring and a speed of 500 rpm was used. The temperature was raised slowly to 80°C over a period of 1 h after which it was allowed to reflux for 30 min. While continuing the stirring, the temperature was allowed to decline at a controlled rate. The ethylcellulose separated, first as a liquid, which was deposited round the core particles, and when the temperature had reached 25°C the stirring was stopped so that the microcapsules could be filtered and dried.

Preparation of Tablets—The tablets were made by compressing 250-mg quantities of the dried microcapsules. The die was fitted onto a 9.5-mm flat lower punch, and 250 mg of microcapsules was placed in it. The upper punch was carefully placed in position, making sure no microcapsules were lost. The punch and die arrangement was placed under the compression head, which was lowered onto the upper punch until the required compression pressure was attained. A compression pressure of 315 kg/cm^2 was maintained for 1 min and then quickly removed. Tablets were prepared from microcapsules of core–wall ratios 4:1, 1:1, 1:2, and 1:4 as well as from 2:1 core–wall ratio microcapsules sieved into sizes of 215, 302.5, 427.5, 605, and 855 μm using British Standard sieves.

Determination of Tablet Thickness and Breaking Strength—The thickness of the tablets was determined by means of a micrometer screw gauge. For each tablet five different measurements were taken at five

[1] B.D.H. Chemicals Ltd., Poole, England.
[2] Fisons Scientific Apparatus, Loughborough, Leicestershire, England.

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