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Effect of Pharmaceutical Adjuvants on the Rectal Permeability of Drugs. III. Effect of Repeated Administration and Recovery of the Permeability

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Aqueous solutions of sodium deoxycholate, sodium lauryl sulfate, disodium ethylenediaminetetraacetate, and polyethylene glycol 400 were repeatedly perfused in the rectal lumen of rats. Changes in the permeability and the recovery of the altered histology of the rectal membrane were examined. An experimental apparatus which allowed perfusion of the adjuvant solutions in the rectal lumen without surgical operation was used.

It was found that the epithelial cells affected by the adjuvants returned to the normal state within 2 h after the pretreatment. However, the goblet cells did not show complete recovery even at 24 h after the pretreatment, and the permeability of the membrane was still higher than the control value at that time. The permeability of the membrane on repeated treatment with the adjuvants was not increased as much as on the first treatment.

Keywords—permeability change; rectal absorption; formulation adjuvant; sulfanilic acid; creatinine

The authors have reported previously that sodium deoxycholate (SDC), sodium lauryl sulfate (SLS), disodium ethylenediaminetetraacetate (EDTA), and polyethylene glycol 400 (PEG) induce morphological changes in the rectal tissue and increase the permeability of the rectal membrane.¹⁾ When these agents are added to drug formulations, the rectal tissue may be exposed to them repeatedly after each administration. Therefore, from the standpoint of safety of drug formulations, it is very important to investigate the possible changes in morphology and permeability of the rectal membrane when the rectal tissue is repeatedly exposed to such adjuvants. There have been few reports concerning this point. Only Gracey *et al.*²⁾ have described the effect of repeated oral administration of SDC on the histology of the small intestine and the recovery from the induced changes. Details of the mechanisms are still unclear.

For the present investigation, we devised an apparatus for rectal perfusion with which sample solutions could be perfused through the rectal lumen without surgical operation, and changes in the histology and permeability of the rectal tissue and recovery from such changes were examined when the rectal tissue was repeatedly exposed to solutions containing adjuvants.

Experimental

Materials—Sodium deoxycholate (SDC), sodium lauryl sulfate (SLS), disodium ethylenediaminetetraacetate (EDTA), and polyethylene glycol 400 (PEG) were used as adjuvants. Sulfanilic acid and creatinine were used as marker drugs to determine inward (rectal lumen to blood) and outward (blood to rectal lumen) permeabilities, respectively.

Perfusion Experiments—In order to perfuse sample solutions through the rectal lumen without surgical operation, a newly designed adaptor, shown in Fig. 1, was used. Male Wistar rats weighing about 250 g were fasted for 24 h, anesthetized by means of pentobarbital injection and fixed on a plate at an angle of 37° so that the head of

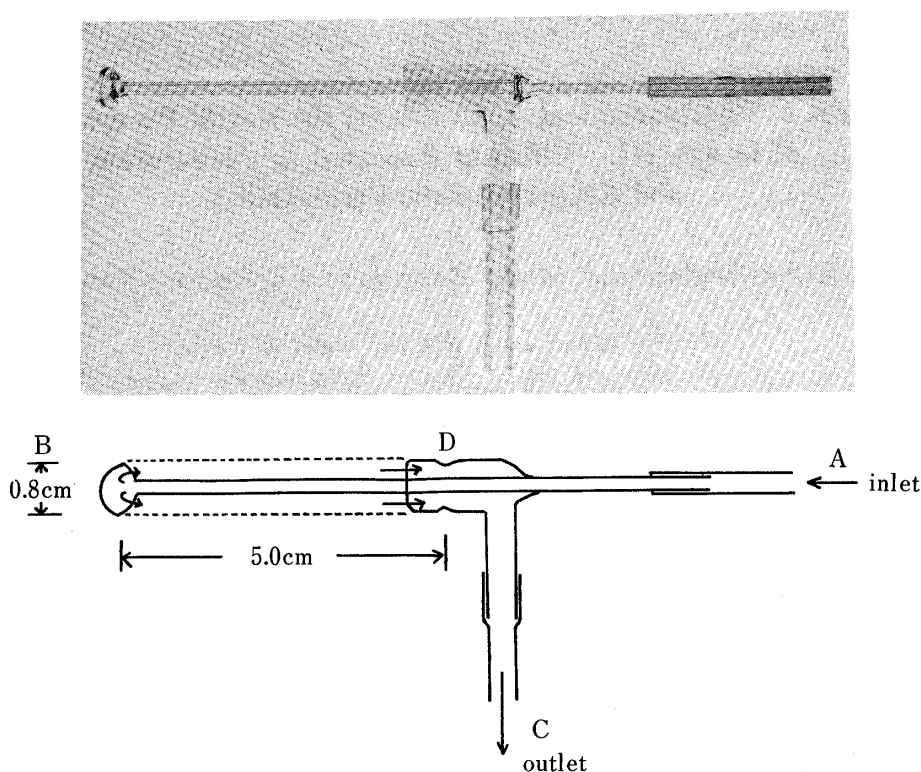


Fig. 1. Experimental Arrangement for Determination of the Absorption Rate of Sulfanilic Acid and Apparent Rectal Clearance of Creatinine from the Rat Rectal Lumen without Surgery

the rat was in a higher position.

The portion (B—D) of the adaptor was inserted through the anus of the rat and the depression (D) was adjusted to the anus. The other part of the adaptor was fixed to the tail of the rat. In this setup, the diameter of the part B is about 8 mm, which is almost the same as that of the rectal lumen, so this part serves as a plug and there are small pores around it. Therefore the introduced sample solution flows from A to B, then passes into the rectal lumen (B to D) and emerges through an outlet C for collection.

Pretreatment with Adjuvants—After insertion of the adaptor into the rat, isotonic phosphate buffer solution at pH 7.4 containing various concentrations of an adjuvant was introduced into the apparatus at a flow rate of 20 ml/15 min for 60 min. These pretreated rats were kept in cages until used.

Determination of Rectal Permeability—Immediately (0 h), or at 2 h, and 24 h after the pretreatment, isotonic phosphate buffer solution containing sulfanilic acid as a marker drug at a concentration of 3 mg/ml was perfused through the apparatus. In the repeated treatment experiments with adjuvants, the solution corresponding to that used at the pretreatment but containing sulfanilic acid at a concentration of 3 mg/ml was perfused through the apparatus at 2 and 24 h after the pretreatment. After a 7.5 min perfusion, 0.5 ml of saline solution containing creatinine at a concentration of 50 mg/0.5 ml was injected into the jugular vein. Sample solutions were collected from the outlet C every 15 min thereafter and blood samples were taken at the midpoint of each interval. Concentrations of sulfanilic acid³⁾ and creatinine⁴⁾ in the blood samples and that of creatinine in the perfused solutions were determined. The area under the blood concentration–time curve up to 90 min (AUC_{90}) for sulfanilic acid and the apparent rectal clearance (ARC) of creatinine were then calculated.

The values obtained from the experiments in which isotonic phosphate buffer solution without any adjuvant was perfused immediately, and at 2 and 24 h after the pretreatment were used as control values.

Preparation of Rectal Tissue Samples for Optical Microscopic Observation—After the perfusion experiment, the rectum was removed and washed with cold saline solution at 4 °C. Tissue samples were prepared in a usual manner after fixation with formalin and stained with hematoxylin–eosin solution.

Results and Discussion

Changes in permeability of the rectal membrane after adjuvant pretreatment were

TABLE I. Histological Study of Effect of Adjuvants on the Structural Integrity of the Rectal Membrane

	Time (h)	Presence of epithelial cells	Presence of goblet cells	Edema of lamina propria ^{a)}	Change in mucous membrane ^{a)}
Control		100 (%)	100 (%)	—	—
Sodium deoxycholate 5 mM		20	0	+	+
	2	95	10	—	—
	24	100	40	—	—
Sodium lauryl sulfate 5 mM		60	20	—	+
	2	100	30	—	+
	24	100	50	—	+
EDTA 25 mM		60	80	—	—
	2	100	80	—	—
	24	100	85	—	—
Polyethylene glycol 50%		50	20	+	+
	2	100	50	+	—
	24	100	95	+	—

a) Key: — = normal; + = slight.

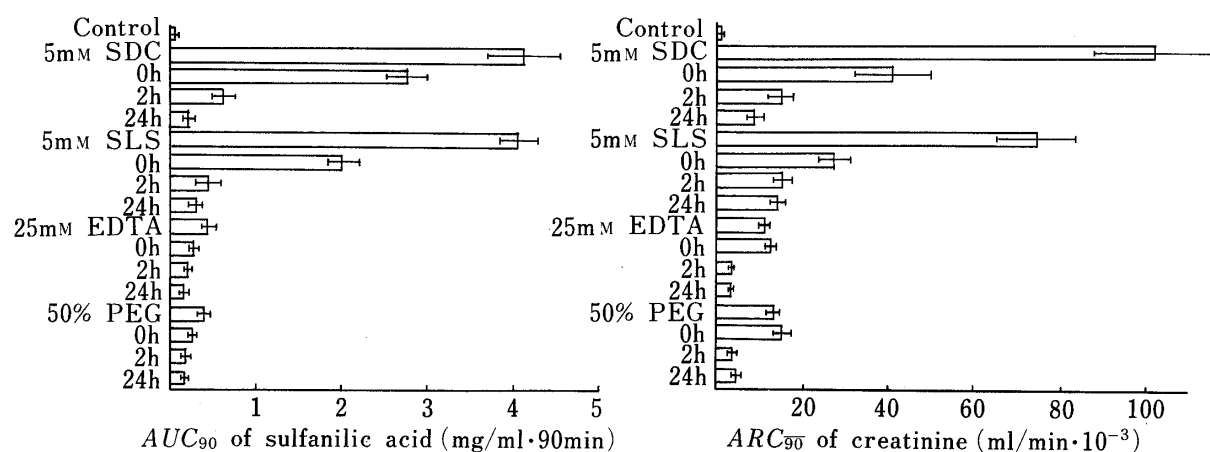


Fig. 2. Effect of Treatment with Adjuvants on the Rectal Permeability

Control, perfused with isotonic phosphate buffer; 0h, immediately after adjuvant pretreatment; 2h, 2h after adjuvant pretreatment; 24h, 24h after adjuvant pretreatment.

Each point represents the mean value of 3 to 5 rats with the standard error shown as a bar.

evaluated in terms of AUC_{90} values of sulfanilic acid and ARC values of creatinine. The AUC_{90} values of sulfanilic acid and average ARC values over 90 min (ARC_{90}) of creatinine determined at the time of pretreatment, and immediately (0h) and at 2 and 24h after the pretreatment are shown in Fig. 2. In the control recovery experiments, AUC_{90} values of sulfanilic acid and ARC_{90} values of creatinine at the time of treatment with isotonic phosphate buffer containing sulfanilic acid, and immediately and at 2 and 24h after isotonic phosphate buffer showed no significant difference. In the presence of adjuvant, AUC_{90} values of sulfanilic acid and ARC_{90} values of creatinine were both increased, indicating that the permeability of the rectal membrane was enhanced. However, these values decreased rapidly as the adjuvants were withdrawn from contact with the rectal membrane. The AUC_{90} values of sulfanilic acid decreased to 1/6, 1/9, 1/2, and 1/3 of the original values at 2h after the withdrawal of the adjuvant in the cases of SDC, SLS, EDTA, and PEG, respectively. The

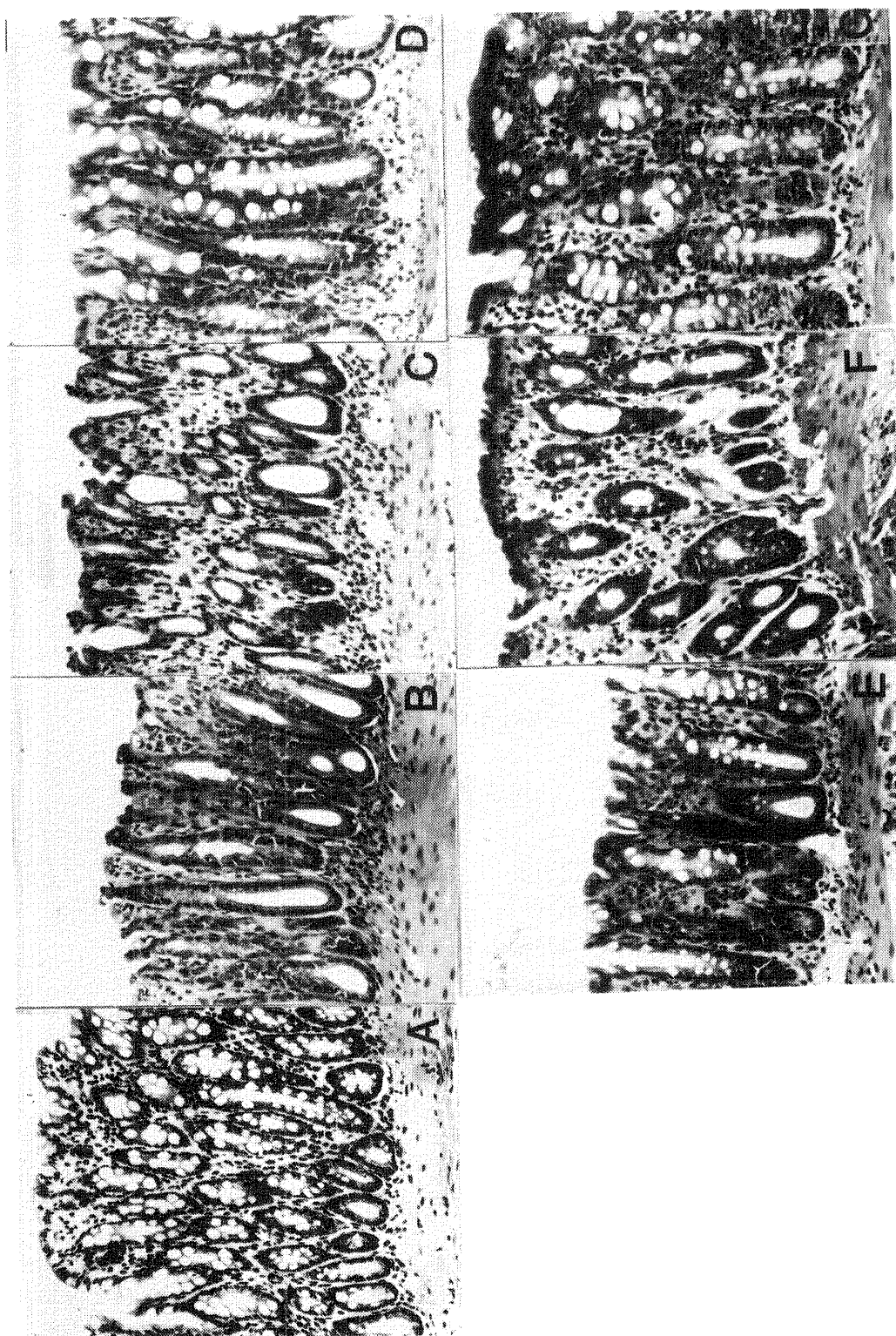


Fig. 3. Light Photomicrographs of the Rectal Lumen

A, perfused with isotonic phosphate buffer^{a)} (control solution); B, perfused with 5 mm SDC solution; C, perfused with control solution 2 h after 5 mm SDC pretreatment; D, perfused with control solution 24 h after 5 mm SDC pretreatment; E, perfused with 5 mm SLS solution; F, perfused with control solution 2 h after 5 mm SLS pretreatment; G, perfused with control solution 24 h after 5 mm SLS pretreatment.

a) No significant differences between the first and the second treatments with isotonic phosphate buffer solution are apparent.

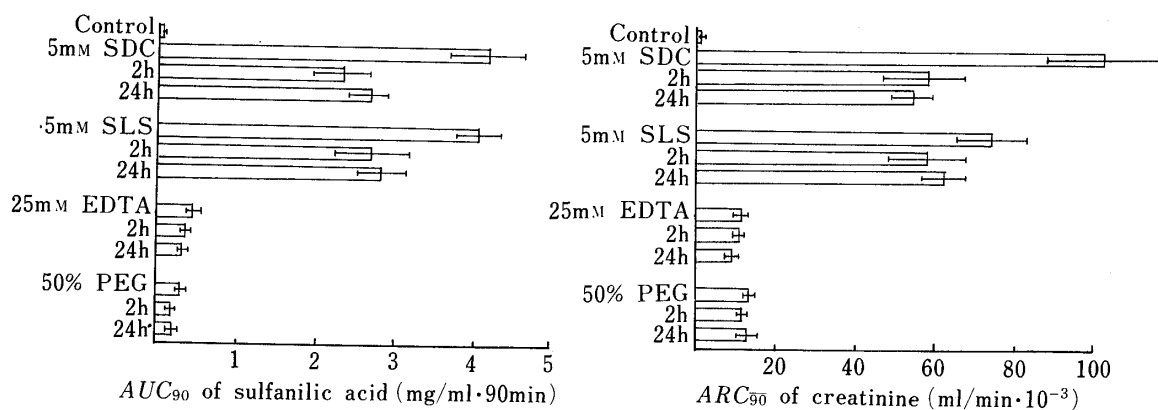


Fig. 4. Effect of Repeated Treatment with Adjuvants on the Rectal Permeability

Control, perfused with isotonic phosphate buffer; 2h, perfused with adjuvant 2h after adjuvant treatment; 24h, perfused with adjuvant 24h after adjuvant treatment.

Each point represents the mean value of 3 to 5 rats with the standard error shown as a bar.

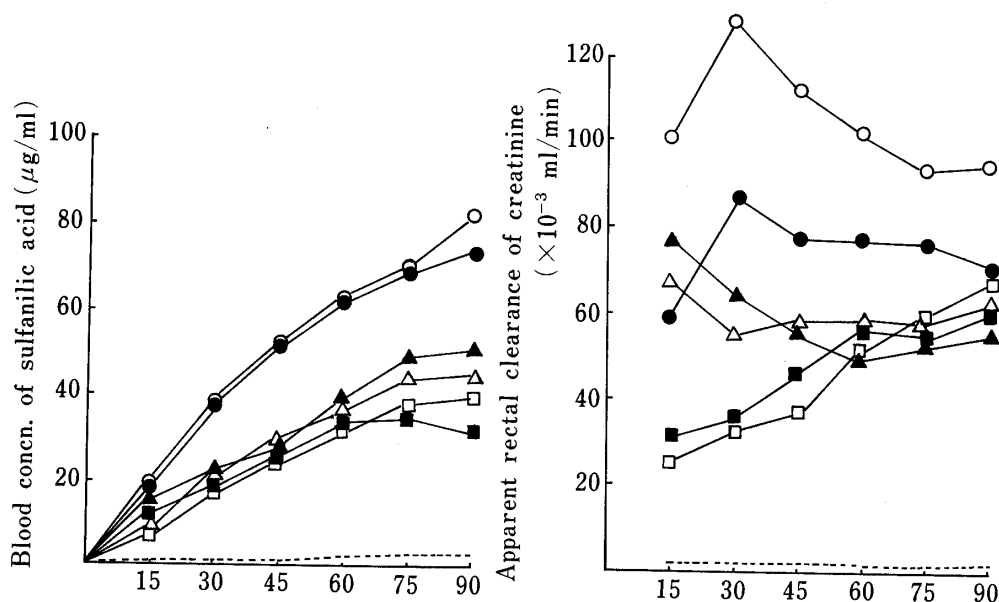


Fig. 5. Effect of Repeated Treatments with SDC and SLS on the Rectal Permeability

-----, control; —○—, perfused with 5 mM SDC solution; —△—, perfused with SDC solution 2h after SDC treatment; —□—, perfused with SDC solution 24h after SDC treatment; —●—, perfused with 5 mM SLS solution; —▲—, perfused with SLS solution 2h after SLS treatment; —■—, perfused with SLS solution 24h after SLS treatment.

ARC_{90} values of creatinine decreased more rapidly than the AUC_{90} values of sulfanilic acid. However, both AUC_{90} values and ARC_{90} values determined at 24 h after the withdrawal of the adjuvants were still higher than the control values.

Photomicrographs of tissue samples are shown in Fig. 3, and the histological evaluations are summarized in Table I. In parallel to the changes in permeabilities, the histological pictures indicated recovery from the damage. Although epithelial cells were almost normal after 2 h, mucous membranes were thinner than the control at that time and fewer goblet cells were observed even after 24 h. In the cases of SLS and SDC, there was a 60% deficiency of goblet cells. Although the increased permeability of the rectal membrane even after 24 h might have some relation to the deficiency of goblet cells, the mechanism still remains unclear.

Changes in the permeability when the rectal lumen was repeatedly exposed to the adjuvants are shown in Fig. 4 and the profiles for the cases of SLS and SDC are shown in Fig. 5. In all cases, the AUC_{90} values of sulfanilic acid and ARC_{90} values of creatinine were lower than those obtained after single exposure to the adjuvants. When the rectal lumen was again perfused with SLS and SDC 2 h after the first treatment, the obtained AUC_{90} values of sulfanilic acid were only 60% of those obtained on the first treatment. When the second treatment was given 24 h after the first treatment, the obtained AUC_{90} values were also about 60% of those obtained on the first treatment. As for the ARC_{90} values of creatinine, the effect of repeated treatment with the adjuvants was similar to that on AUC_{90} values. Thus, the second treatment did not have the same effect as the first treatment. Though the phenomenon may be related to incomplete recovery from the tissue damage induced by the first treatment, the exact mechanism still remains unknown.

Conclusion

In the present investigation, changes in histology and the recovery of altered permeability of the rectal membrane of rats treated with media containing formulation adjuvants were examined. The effect of repeated treatment with the adjuvants was also evaluated. It was found that the epithelial cells returned to the normal state in 2 h after the pretreatment. However, the goblet cells did not show complete recovery even at 24 h after the pretreatment. The permeability of the rectal membrane approached the control level at 24 h but was still higher than the control value at 24 h after the pretreatment. The permeability of the membrane after repeated treatment was not increased as much as on the first treatment.

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