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# Effect of Pharmaceutical Adjuvants on the Rectal Permeability to Drugs. IV. Effect of Pharmaceutical Adjuvants on the Rectal Permeability to Macromolecular Compounds in the Rat

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The effect of adjuvants such as sodium deoxycholate (SDC), sodium lauryl sulfate (SLS), disodium ethylenediaminetetraacetate (EDTA) and tetracycline hydrochloride (TC) on the permeability to water-soluble compounds with various molecular weights through the rat rectal membrane was evaluated by using an *in situ* technique. Creatinine, $^{14}$C-inulin, $^{125}$I-insulin, $^{125}$I-polyvinylpyrrolidone and $^{125}$I-albumin were selected as marker drugs. Significant increases in the permeability of the rectal membrane to the marker drugs were observed in the cases of SDC and SLS. On the other hand, the effect of EDTA, and especially TC, became smaller as the molecular weight of the marker drugs increased. The order of effectiveness of adjuvants on the permeability was SDC > SLS > EDTA > TC.

**Keywords**——rat rectum permeability; surfactant; chelating agent; water-soluble compound; high molecular weight drug; *in situ* perfusion

We have examined the effects of sodium deoxycholate (SDC), sodium lauryl sulfate (SLS), and disodium ethylenediaminetetraacetate (EDTA) on the permeability of the rectal membrane and found that these adjuvants increased the rectal permeability to sulfaguanidine[1] and sulfanilic acid.[2] Histological changes in the rectal tissue were shown to be the cause of the increased permeability.[1] Whether such an increase in the permeability is specific for low molecular weight drugs with limited absorbability (such as sulfanilic acid and sulfaguanidine) or whether it also occurs for drugs with limited absorbability due to high molecular weight is uncertain.

The objective of this investigation was to clarify the nature of the altered permeability of the rectal membrane to drugs when the rectal tissue is histologically affected by a concomitantly administered drug. Compounds of various molecular weights with limited absorbability were used as marker drugs in the present experiments in the presence of chemicals which may induce changes in the permeability of rectal membrane on contact with rectal tissue.

## Experimental

**Materials**——The adjuvants used were SDC (reagent grade, Wako Pure Chemicals Co.), EDTA (reagent grade, Wako Pure Chemicals Co.), SLS (J.P. grade) and TC (J.P. grade). The marker drugs used were creatinine (M.W. = 113, reagent grade, Tokyo Kasei Kogyo Co.), $^{125}$I-insulin (M.W. = 6000, Dainabot RI Lab.), $^{14}$C-inulin (M.W. = 5500), $^{125}$I-polyvinylpyrrolidone (PVP, M.W. = 35000), and $^{125}$I-albumin (M.W. = 69000, the Radiochemical Centre Amersham). They were used after further purification through a Sephadex G-25 column, except for creatinine.

**Absorption Experiment**——Male Wistar rats weighing 200 to 250 g were fasted overnight and treated as described previously.[1] The perfusion apparatus was connected to the rectum, and saline solution containing one of the adjuvants at a concentration of 5 mM (25 mM in the case of EDTA) and a marker drug was perfused at a flow rate of 20 ml/15 min. The concentrations used were creatinine 3 mg/ml, $^{14}$C-inulin, $^{125}$I-labeled insulin, PVP, and albumin 0.1 $\mu$Ci/ml. Blood samples (0.4 ml) were taken every 30 min and the concentrations of the marker drug in blood were determined. The ratios of the concentration of the marker drug in blood to the initial concentration in the perfusate

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were calculated. As a control run, saline solution containing the marker drug alone was perfused similarly.

**Exsorption Experiment**——Male Wistar rats weighing 200 to 250 g were fasted overnight and treated as described previously.[1] The perfusion apparatus was connected to the rectum of the rat, and saline solution containing one of the adjuvants at a concentration of 5 mM (25 mM in the case of EDTA) was perfused through the rectal lumen at a flow rate of 20 ml/15 min. A marker drug was injected into the jugular vein simultaneously with the start of perfusion. The marker drugs were given in the following doses; creatinine 50 mg, $^{14}$C-inulin, $^{125}$I-labeled insulin, PVP, and albumin 5 $\mu$Ci. The perfusate was sampled every 15 min thereafter and blood samples (0.4 ml) were taken at the midpoint of each interval. Concentrations of the marker drug in the perfusate and blood were determined and the apparent rectal clearance values were calculated.[1]

**Assay Procedure**——Creatinine: Creatinine was determined spectrophotometrically after conversion into creatinine picrate through the Jaffe reaction in alkaline media.[2]

$^{14}$C-Inulin: Blood samples were dried and subjected to combustion (automatic sample combustion system, model ASC-111). The $^{14}CO_2$ produced was trapped in ethanolamine solution. Aquasol II® solution (New England Nuclear) was added to an aliquot of the perfusate containing $^{14}$C-inulin. Radioactivities were measured in a liquid scintillation counter (Aloka, LSC-651) and the amount of $^{14}$C-inulin in the samples was calculated. $^{125}$I-Labeled insulin, PVP, and albumin: The amount of these markers in blood and perfusate was measured in a well-type gamma counter (Packard automatic scintillation spectrometer, model 5110).

## Results and Discussion

For each marker drug, the area under the blood/perfusate concentration ratio–time curve for 180 min ($AUCR_{180}$) and the apparent rectal clearance value averaged over 90 min ($ARC_{90}$) were determined. The ratios of these values to those obtained from control experiments were calculated and changes in the bidirectional permeability of the rectal membranes (rectal lumen $\rightleftharpoons$ blood) were evaluated.

The $AUCR_{180}$ values and $ARC_{90}$ values of the marker drugs and the ratios of these values to those obtained in the control experiments are summarized in Tables I and II. Since albumin and PVP were not absorbed and albumin was not excreted into the rectal lumen in the control experiments, the ratios could not be determined.

In order to examine the effect of each adjuvant on the elimination rate constant of each marker drug from blood, the apparent elimination rate constant of the marker drug from blood was determined when the adjuvant solution was perfused. No significant change in the rate constant was observed in the presence of the adjuvants used. Therefore an increase in the blood concentration of a marker drug observed upon perfusion of an adjuvant solution was assumed to be caused not by suppression of elimination from the blood but by increased absorption.

To confirm that $^{125}$I-labeled insulin and albumin were actually absorbed, plasma fractions of the blood samples were added to trichloroacetic acid and centrifuged. Radioactivities in the supernatant layer and the precipitate were determined. Most of the activity was found in the precipitated fraction.

Changes in the rectal permeability to creatinine (M.W. = 113) in the presence of the adjuvants were similar to those obtained for sulfanilic acid and sulfaguanidine.[1,2] The permeability to inulin (M.W. = 5500) and insulin (M.W. = 6000), which are known to be less absorbable due to their large molecular weights, was also markedly increased in the presence of the adjuvants. These results are shown in Figs. 1 and 2. When SDC or SLS was added, a significant increase in apparent rectal clearance of the marker drugs was observed shortly after the perfusion was started. When EDTA or TC was added, on the other hand, the absorption rates of the marker drugs increased gradually with time. This indicates that different adjuvants have different effects on the rectal tissue. Similar results have been observed for a low molecular weight drug, sulfaguanidine.[1]

Although it has been reported that rectal absorption of insulin or heparin was enhanced by various kinds of surfactants,[3-7] it was found that EDTA and TC, which are chelating agents, also enhanced the absorption of these drugs. In the cases of PVP (M.W. = 35000) and

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TABLE I. Effect of Adjuvants on *AUCR* of Marker Drugs in the Rat

| | Isotonic saline | | SDC | | | SLS | | | EDTA | | | TC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *AUCR*[a] | ±S.E. | *AUCR* | ±S.E. | Ratio[b] | *AUCR* | ±S.E. | Ratio | *AUCR* | ±S.E. | Ratio | *AUCR* | ±S.E. | Ratio |
| Creatinine | 6.3 | 1.1 | 262.5 | 21.2 | 41.7 | 233.7 | 18.8 | 37.2 | 164.1 | 11.1 | 26.0 | 174.3 | 16.3 | 27.7 |
| Inulin | 2.4 | 0.5 | 105.6 | 5.7 | 44.0 | 93.5 | 8.2 | 39.1 | 30.5 | 4.1 | 12.7 | 12.9 | 1.4 | 5.4 |
| Insulin | 2.5 | 0.4 | 117.6 | 5.6 | 46.1 | 133.2 | 5.8 | 52.2 | 44.6 | 2.3 | 17.5 | 8.2 | 0.5 | 7.1 |
| PVP | | | 30.8 | 2.6 | | 26.1 | 2.5 | | 15.5 | 2.8 | | 4.8 | 0.6 | |
| Albumin | | | 30.6 | 3.1 | | 25.7 | 2.2 | | 9.6 | 1.3 | | | | |

*a*) Area under the blood/perfusate concentration ratio–time curve, $\times 10^{-3} \cdot 180$ min.
*b*) The ratio was calculated as the *AUCR* of marker drug with adjuvant/the *AUCR* of marker drug without adjuvant.

TABLE II. Effect of Adjuvants on the Apparent Rectal Clearance of Marker Drugs in the Rat

| | Isotonic saline | | SDC | | | SLS | | | EDTA | | | TC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *ARC*[a] | ±S.E. | *ARC* | ±S.E. | Ratio[b] | *ARC* | ±S.E. | Ratio | *ARC* | ±S.E. | Ratio | *ARC* | ±S.E. | Ratio |
| Creatinine | 1.1 | 0.1 | 47.4 | 5.8 | 43.1 | 55.8 | 7.9 | 50.7 | 29.8 | 3.2 | 27.1 | 15.2 | 1.6 | 13.8 |
| Inulin | 0.9 | 0.2 | 55.1 | 6.2 | 59.9 | 37.2 | 6.7 | 40.4 | 8.2 | 0.8 | 8.9 | 2.3 | 0.5 | 2.5 |
| Insulin | 1.6 | 0.2 | 54.4 | 6.8 | 34.8 | 48.9 | 7.9 | 31.4 | 13.4 | 1.0 | 8.6 | 9.6 | 1.0 | 6.2 |
| PVP | 0.2 | 0.02 | 8.0 | 0.5 | 40.0 | 6.9 | 1.3 | 34.3 | 0.7 | 0.1 | 3.3 | 0.2 | 0.03 | 0.8 |
| Albumin | | | 10.0 | 1.5 | | 7.2 | 1.2 | | 0.8 | 0.1 | | | | |

*a*) Apparent rectal clearance averaged over 90 min, $10^{-3} \cdot$ ml/min.
*b*) The ratio was calculated as the *ARC* of marker drug with adjuvant/the *ARC* of marker drug without adjuvant.



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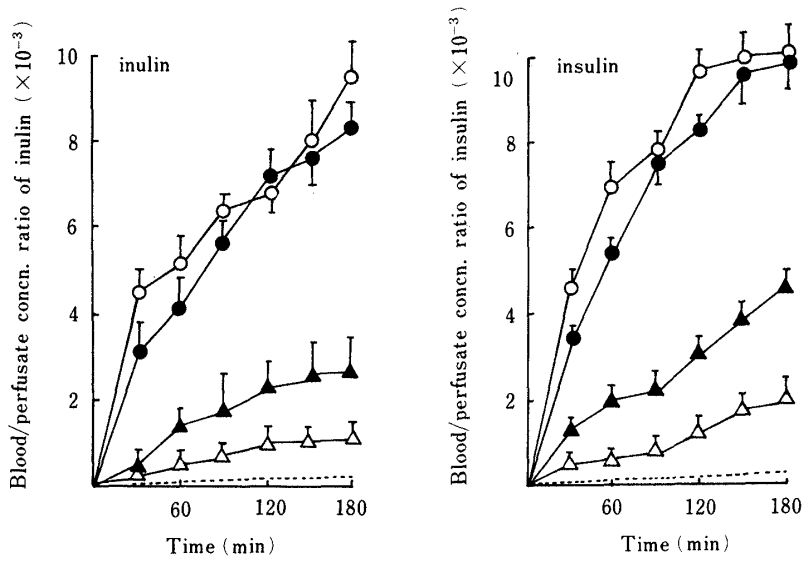


Fig. 1. Effect of Adjuvants on the Absorption of Inulin and Insulin

----, control; —●—, sodium deoxycholate; —○—, sodium lauryl sulfate; —▲—, EDTA; —△—, tetracycline hydrochloride.
Each point represents the mean ± S.E. of four experiments.

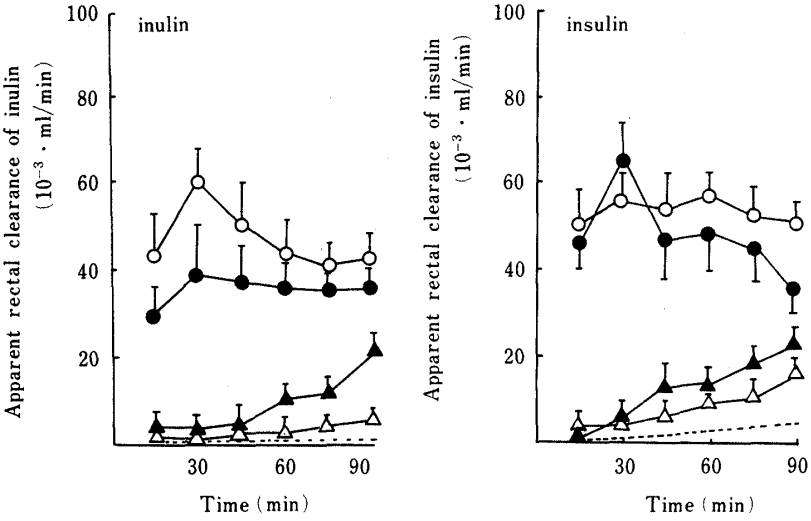


Fig. 2. Effect of Adjuvants on the Apparent Rectal Clearance of Inulin and Insulin

----, control; —●—, sodium deoxycholate; —○—, sodium lauryl sulfate; —▲—, EDTA; —△—, tetracycline hydrochloride.
Each point represents the mean ± S.E. of four experiments.

albumin (M.W. = 69000), although they did not permeate through the rectal membrane in the control experiment, they did permeate in the presence of the adjuvants. Absorption of these macromolecular compounds through the small intestine has already been reported to be increased by adjuvants.[8]

In our previous work,[1] we showed that hemoglobin and other proteins appeared in the rectal lumen when SDC or SLS was perfused. One possible mechanism for the excretion of high molecular weight albumin and PVP into the rectal lumen is concomitant efflux of these macromolecules with blood or body fluid, not permeation through the rectal tissue. However, albumin and PVP were actually absorbed from rectal lumen, possibly against the efflux of body fluid. We therefore concluded that albumin and PVP did permeate through the rectal tissues. Albumin and PVP were not absorbed from the rectal lumen in control experiments,

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and in the presence of the adjuvants the amounts absorbed were significantly smaller than that of creatinine. In addition, among the adjuvants, EDTA and TC exhibited much smaller effects on the absorption of the marker drugs with high molecular weight. Albumin was scarcely absorbed even in the presence of EDTA or TC. TC may bind with divalent metal ions and also with proteins, so the effect of TC on the histology of rectal tissue may be somewhat different from that of EDTA.

Unless there is a special transport mechanism,[9] absorption of a macromolecular compound which is not absorbed under normal conditions requires increased permeability of the tissue due to some histological change. The effect of surfactants such as SDC or SLS and chelating agents on the nature of membrane surfaces has been reported previously.[1] In the present investigations too, histological changes in the rectal tissues were observed after perfusion of the adjuvant solutions.

## Conclusion

It was found that enhancement of the permeability of rectal membrane by surfactants (SDC and SLS) or chelating agents (EDTA and TC) resulted in the increased absorption of both macromolecular drugs and low molecular drugs which are poorly absorbable under normal conditions. This increased permeation was observed in the outward (blood to rectal lumen) process as well as the inward (rectal lumen to blood) process.

Significant increase in the permeability of the rectal membrane to marker drugs was observed in the cases of SDC and SLS. On the other hand, the effect of EDTA, and especially TC, became smaller as the molecular weight of the marker drug was increased.

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