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Effect of Pharmaceutical Adjuvants on the Rectal Permeability of Drugs. II. Effect of Tween-Type Surfactants on the Permeability of Drugs in the Rat Rectum

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The effect of Tween-type surfactants on the permeability of the rectal membrane was investigated by determining the absorption and apparent rectal clearance of marker drugs, using the *in situ* perfusion technique in rats. The tested substances were polysorbates 85, 80, 60, 40, 21, and 20, and polyoxyethylene sorbitan 20.

When these surfactants were added in aqueous solutions, they did not significantly affect the permeability. On the other hand, these surfactants affected the histological nature of the rectal tissue and enhanced the permeability when they were added in oil.

Keywords——rectal absorption; apparent absorption rate; apparent rectal clearance; effect of Tween-type surfactant; sulfanilic acid; creatinine; emulsion; oily vehicle; rat

In our previous report,[1] we demonstrated that the permeability of rat rectal membrane to drugs was increased by some excipients in the formulations and that the histological changes in rectal tissues caused by the excipients were related to the increased permeability.

Among the excipients investigated, polysorbate 80 (Tween 80) showed little effect on the rectal tissue, and the permeability of the rectal membrane to drugs was not increased either. On the other hand, many investigations[2-8] have been reported on the enhancement of absorption of drugs with limited absorbability from suppositories using oily vehicles by the addition of nonionic surfactants. However, the cause of the enhancement has not yet been fully explained.

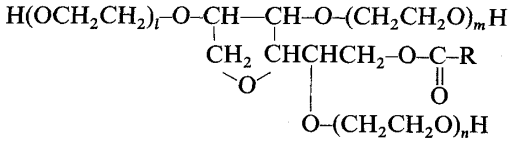
In our previous work, the effect of Tween 80 on the permeability of rectal membrane was evaluated in an aqueous medium. The possibility remains, therefore, that the surfactant may affect the histological nature of the rectum and, hence, the permeability of the rectal membrane, when the surfactant is added in an oily vehicle or some types of emulsion are used. Therefore, the effect of Tween-type surfactants on the permeability of the rectal membrane was further investigated.

Experimental

Materials——The surfactants used were polysorbates 85, 80, 60, 40, 21, and 20 (Tweens 85, 80, 60, 40, 21, and 20, respectively, Tokyo Kasei Kogyo Co., Japan) and polyoxyethylene sorbitan 20 (Sorbox 20, Nikko Chemicals Co., Japan). Table I shows the chemical structures and HLB values of the surfactants. Olive oil was of J.P. grade and miglyol oil was from Dynamit Nobel Chemicals, Co., West Germany. Sulfanilic acid and creatinine used as marker drugs were of reagent grade.

***In Situ* Perfusion Experiments in Rats**——Male Wistar rats weighing 180 to 250 g were fasted overnight and treated as described previously.[1] A perfusion apparatus was connected to the rectum of the rat, and isotonic phosphate buffer solution at pH 7.4, miglyol oil, olive oil, or each of these media containing one of the surfactants at a concentration of 5% (w/v) was perfused at a rate of 20 ml/15 min for 60 min. Then a saline solution was perfused for 5 min to wash out the remaining medium. Isotonic phosphate buffer solution containing sulfanilic acid at a concentration of 3 mg/ml was perfused, and then creatinine solution (50 mg/0.5 ml) was injected into the jugular vein.

TABLE I. Structural Diagrams of the Tween-Type Surfactants and Sorbox-20



Polyoxyethylene sorbitan esters		R	HLB
Tween	Polyoxyethylene ($l+m+n$)		
20	20	Monolaurate	16.7
40	20	Monopalmitate	15.6
60	20	Monooleate	15.0
80	20	Monostearate	14.9
21	4	Monolaurate	13.3
85	20	Trioleate	11.0
Sorbox 20	20	—	—

Samples of blood and the perfusion media were taken every 15 min after the injection, and the amounts of sulfanilic acid[9] and creatinine[10] in the blood and creatinine in the perfused media were determined. The values obtained from the experiments in which isotonic phosphate buffer solution without any surfactant was perfused were used as control values.

Measurement of Osmotic Pressure——Osmotic pressures of phosphate buffer solutions each containing one of the following surfactants at a concentration of 5 or 10% (w/v) were measured with an osmometer (advanced osmometer, model 3L; Advance Instrument Co., Massachusetts). Surfactants used were Tweens 80, 40 and 20, and Sorbox 20.

Preparation of Rectal Tissue Samples for Optical Microscopy——Immediately after the perfusion experiments, rectal tissues were surgically excised and washed with cold saline solution. The excised tissues were then fixed with 10% formaldehyde and cut into slices. The slices were stained with haematoxylin–eosin solution.

Preparation of Emulsions——An oil and the phosphate buffer solution were mixed at different ratios and Tween 20 was added to each mixture at a level of 5% (w/v). The mixtures thus prepared were homogenized in a homogenizer (type 18/10, Ultra-Turrax, Janke & Kunkel Co., West Germany).

Results

When the rectal lumen was perfused with olive oil or miglyol oil in the absence of surfactants, absorption of sulfanilic acid and apparent rectal clearance (ARC) of creatinine were not significantly different from control values obtained when isotonic phosphate buffer solutions were used in the pretreatment. This indicates that olive oil or miglyol oil alone does not affect the permeability of the rectal membrane to the marker drugs.

The rectum was pretreated with isotonic phosphate buffer solution containing a surfactant at a level of 5% (w/v), and the rectal absorption of sulfanilic acid was determined. The results are shown in Table II in terms of AUC_{90} (area under the blood concentration-time curve up to 90 min) and the profiles are shown in Fig. 1(A). In comparison with control values, 1.4- to 3.4-fold increases in AUC_{90} values were observed when a medium containing a surfactant was perfused beforehand. In the case where polyoxyethylene sorbitan 20 (Sorbox 20), which does not possess a lipophilic moiety in its molecule, was present in the medium, AUC_{90} of sulfanilic acid was 2.7 times greater than the control value. The increase in AUC_{90} in the presence of surfactants, relative to control values, was statistically significant ($p<0.05$) while differences among the surfactants were not significant ($p>0.05$).

Averaged ARC values of creatinine in 90 min (ARC_{90}) are summarized in Table III. Except for Tweens 20 and 40, these surfactants in isotonic phosphate buffer did not affect

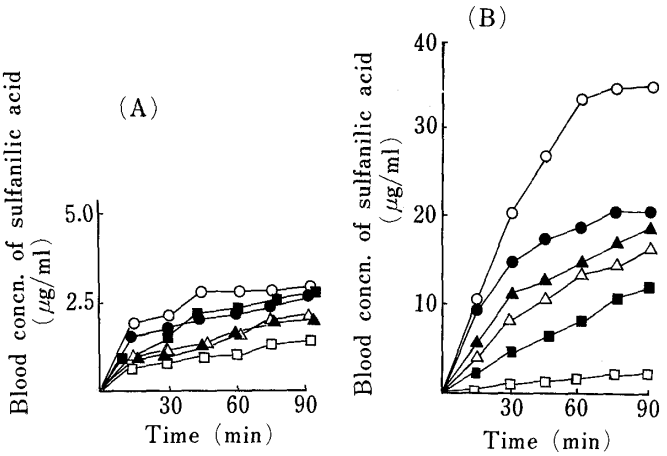


Fig. 1. Effect of Tween-Type Surfactants on the Absorption of Sulfanilic Acid (A) in Phosphate Buffer Solution and (B) in Miglyol Oil Solution

—□—, control; —○—, Tween 20; —●—, Tween 40; —△—, Tween 60; —▲—, Tween 80; —■—, Sorbox 20. Concentration of surfactant = 5% (w/v).

TABLE II. Effect of Tween-Type Surfactants on AUC_{90} of Sulfanilic Acid

	HLB	Phosphate buffer	Miglyol oil	Olive oil
Control	—	61[a] ± 5	66[a] ± 8	78[a] ± 7
Tween 85	11.0	107 ± 13[b]	761 ± 56	929 ± 63
Tween 21	13.3	110 ± 9[b]	924 ± 85	787 ± 74
Tween 80	14.9	87 ± 4[b]	1072 ± 235	900 ± 87
Tween 60	15.0	94 ± 6[b]	1028 ± 94	900 ± 71
Tween 40	15.6	122 ± 11[b]	1346 ± 139	1160 ± 109
Tween 20	16.7	210 ± 14[b]	2220 ± 214	1831 ± 123
Sorbox	—	166 ± 12[b]	585 ± 49	—

a) Mean of five determinations ± S.E., μg/ml·90 min. Concentration of surfactant = 5% (w/v). The significance of differences between control and surfactants is indicated as follows: *b*) $p < 0.05$.

ARC_{90} of creatinine. Tweens 20 and 40 in the buffer solutions increased the ARC_{90} values of creatinine significantly when compared with the control value ($p < 0.05$). In these two cases, although ARC_{90} values were higher initially, they decreased with time to approach to the control value, as shown in Fig. 2(A).

The surfactants were added in miglyol oil and olive oil instead of isotonic phosphate buffer solution and the oil was perfused in the pretreatment. The effects of the surfactants on the absorption of sulfanilic acid and on the clearance of creatinine were evaluated. The results are shown in Tables II and III and in Figs. 1(B) and 2(B). Compared with the control values, 10- to 33-fold increases in AUC_{90} of sulfanilic acid and 10- to 24-fold increases in ARC_{90} values of creatinine were observed in the presence of the surfactants. In the presence of Sorbox 20, which is not a surfactant, a 9-fold increase in AUC_{90} of sulfanilic acid and a 10-fold increase in ARC_{90} of creatinine were obtained. Tween 20 increased the AUC_{90} of sulfanilic acid to a significantly higher degree ($p < 0.05$) than Tweens 21, 40, 60, 80, 85, and Sorbox 20. However, there was no significant difference among Tweens 21, 40, 60, 80, 85, and Sorbox 20 ($p > 0.05$).

Table IV gives the measured osmotic pressures of phosphate buffer solutions containing Tweens 20, 40, 80, and Sorbox 20, respectively. At a concentration of 5% (w/v), which was

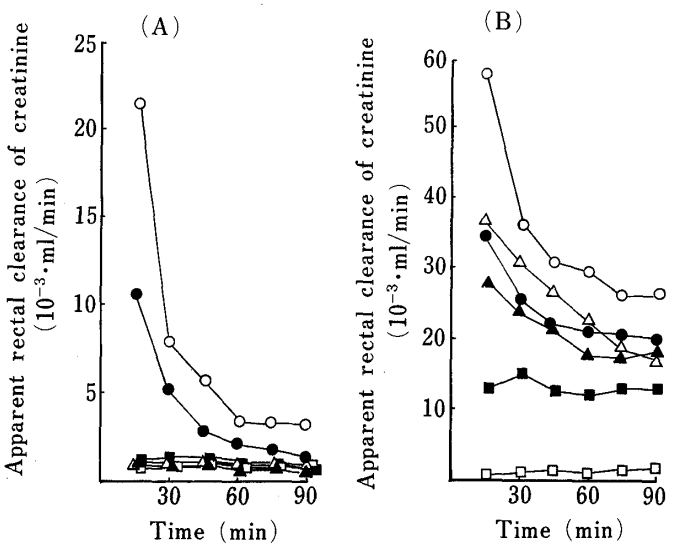


Fig. 2. Effect of Tween-Type Surfactants on Apparent Rectal Clearance of Creatinine (A) in Phosphate Buffer Solution and (B) in Miglyol Oil Solution

—□—, control; —○—, Tween 20; —●—, Tween 40; —△—, Tween 60; —▲—, Tween 80; —■—, Sorbox 20. Concentration of surfactant = 5% (w/v).

TABLE III. Effect of Tween-Type Surfactants on Average Apparent Rectal Clearance of Creatinine

	HLB	Phosphate buffer	Miglyol oil	Olive oil
Control	—	$1.2\pm0.1^{a)}$	$1.2\pm0.1^{a)}$	$1.4\pm0.1^{a)}$
Tween 85	11.0	1.1 ± 0.1	21.6 ± 1.7	21.3 ± 2.5
Tween 21	13.3	1.1 ± 0.1	22.2 ± 2.1	19.6 ± 1.2
Tween 80	14.9	0.6 ± 0.1	17.5 ± 1.9	14.7 ± 2.9
Tween 60	15.0	0.8 ± 0.1	26.4 ± 3.1	21.4 ± 2.3
Tween 40	15.6	$4.1\pm1.4^{b)}$	23.9 ± 2.3	21.4 ± 2.3
Tween 20	16.7	$6.0\pm2.9^{b)}$	35.4 ± 5.1	28.8 ± 6.5
Sorbox	—	1.1 ± 0.1	13.1 ± 1.1	—

a) Mean of five determinations ± S.E., ml/min·10^{-3}. Concentration of surfactant = 5% (w/v). The significance of differences between control and surfactants is indicated as follows: *b*) $p<0.05$.

TABLE IV. Osmolality of Solutions Containing Surfactants and Sorbox 20 in Isotonic Phosphate Buffer

Conc.	Control	Tween 20	Tween 40	Tween 80	Sorbox 20
	286[a)]	—	—	—	—
5%	—	313	310	306	426
10%	—	348	338	333	614

a) mOsmols/kg.

employed in the perfusion experiments, Tween-type surfactants gave osmotic pressures of 306 to 313 mOsm while Sorbox 20 gave a higher value of 426 mOsm.

Emulsions containing Tween 20 (5% (w/v)) with different water-to-oil ratios were prepared and used in the perfusion experiment. After the pretreatment, AUC_{90} of sulfanilic

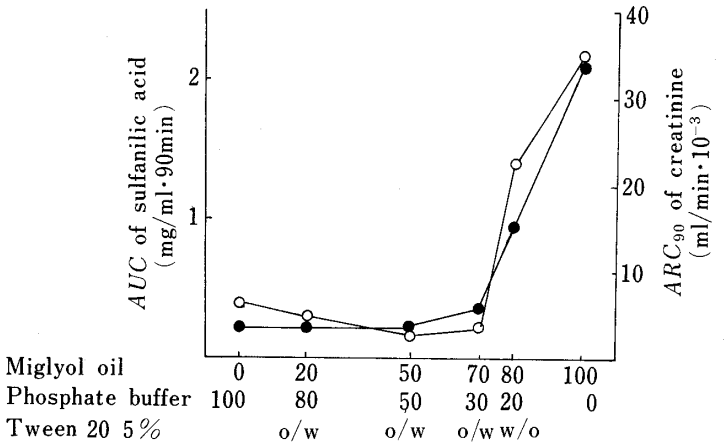


Fig. 3. Effect of Oil-to-Water Ratio of Emulsion on AUC_{90} of Sulfanilic Acid (●) and Average Apparent Rectal Clearance of Creatinine (○)

acid and ARC_{90} of creatinine were determined and the results are shown in Fig. 3. It was found that there is a critical water-to-oil ratio at which AUC_{90} of sulfanilic acid as well as ARC_{90} of creatinine changes remarkably and that the ratio lies between 30 : 70 and 20 : 80. The emulsions were examined and found to be of o/w type when the ratio was 30 : 70 and of w/o type when the ratio was 20 : 80.

Figures 4 and 5 show photomicrographs of rectal tissues prepared after each experiment. It was found that rectal tissues which exhibited significantly higher AUC_{90} of sulfanilic acid and ARC_{90} of creatinine showed remarkable histological changes.

Discussion

The difference in the effect of Tween-type surfactants on the permeability of the rectal membrane was examined when the surfactants were applied in aqueous media and oily vehicles. As stated in the experimental section, the rat rectum was pretreated with a perfusing medium containing a surfactant, then rectal absorption of a marker drug was determined by perfusing isotonic phosphate buffer solution containing the marker drug, and rectal clearance of intravenously injected marker drug was simultaneously determined. In this system, interaction between surfactants in the rectal lumen and intravenously injected creatinine can be eliminated. This is also the case with sulfanilic acid, if used, since washing with saline solution for 5 min removed most of the surfactants or oils from the rectal lumen.

Using the following equations, based on the proposed model,[1] changes in AUC_{90} of the marker drug and permeability of the marker drug through the rectal membrane into the blood were evaluated. In the experiment, the concentration of the marker drug in the perfusion medium was constant (3000 μg/ml) and the concentration of the marker drug in the blood was small enough to be neglected. Therefore the absorption of the marker drug can be approximated as a zero-order rate process. Assuming that the elimination process is of first-order, the concentration of the marker drug in blood at time t can be expressed by Eqs. (1) and (2). In apparent zero-order absorption, the apparent rate of absorption becomes the apparent rate constant of absorption.

$$\text{control: } C_0 \xrightarrow{k} C \xrightarrow{K_e} \qquad C=\frac{k}{VKe}(1-e^{-Ke\cdot t}) \tag{1}$$

$$\text{sample: } C_0 \xrightarrow{k_{add}} C_{add} \xrightarrow{K_e} C_{add}=\frac{k_{add}}{VKe}(1-e^{-Ke\cdot t}) \tag{2}$$

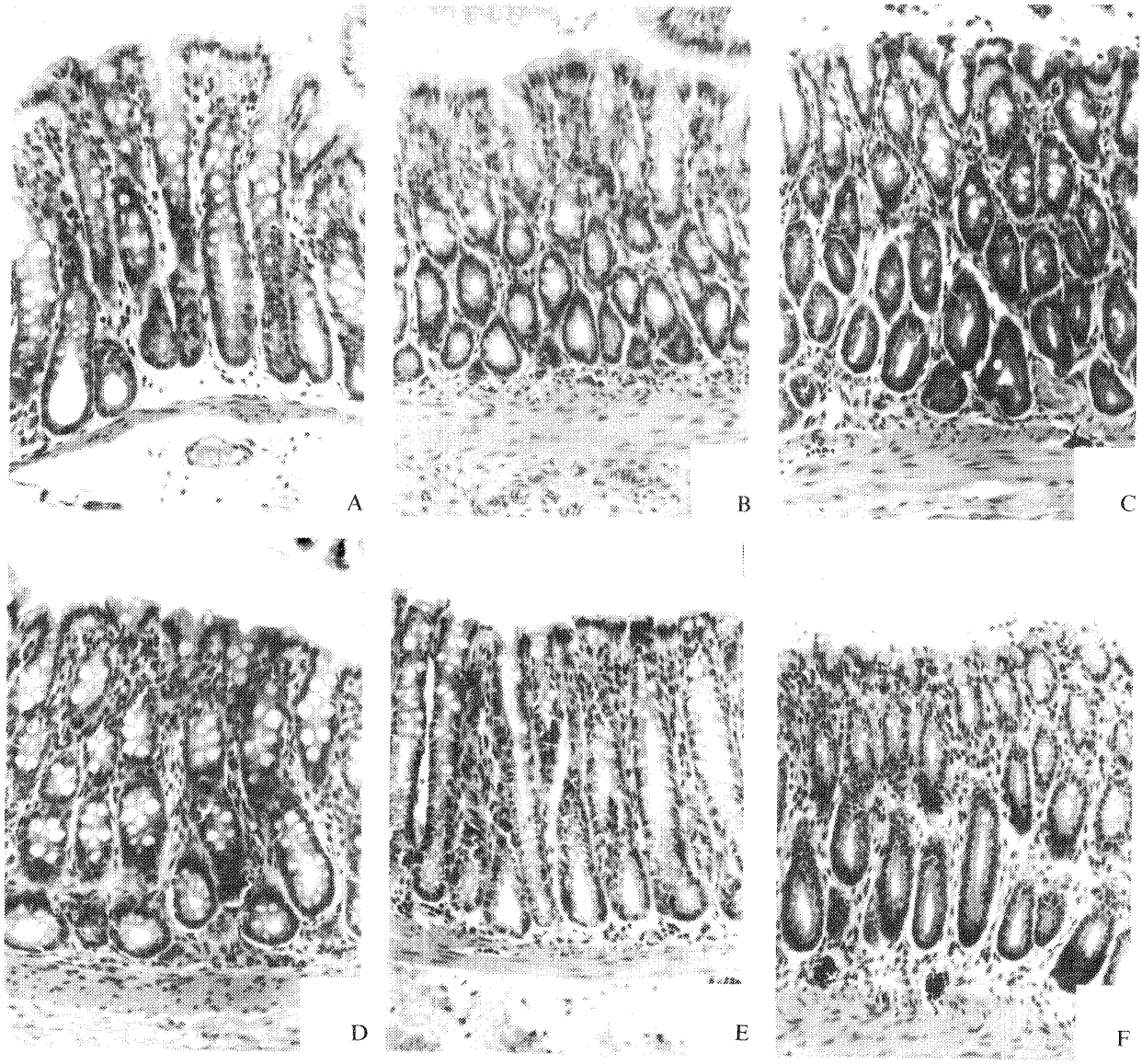


Fig. 4. Light Photomicrograph of Rectal Lumen

A: perfused with phosphate buffer for 60 min.
B: perfused with Tween 80 in phosphate buffer for 60 min.
C: perfused with Tween 80 in miglyol oil for 60 min.
D: perfused with miglyol oil for 60 min.
E: perfused with Tween 20 in phosphate buffer for 60 min.
F: perfused with Tween 20 in miglyol oil for 60 min.

In these equations, C_0 (μg/ml) = concentration of the marker drug (sulfanilic acid) in the perfusing solution (this was constant through the perfusion), C and C_{add} (μg/ml) = concentrations of the marker drug in blood at time t when the rectum was pretreated in the absence and presence of a surfactant, respectively, k and k_{add} (μg/min) = apparent rates of absorption when the rectum was pretreated in the absence and presence of a surfactant, respectively, V (ml) = volume of distribution of the drug, and Ke (min^{-1}) = elimination rate constant of the marker drug.

In this experiment, the elimination rate constant, Ke as well as apparent volume of distribution, V did not change when the rectum was pretreated with a medium containing a surfactant so that the terms $(1-e^{-Ke\cdot t})/VKe$ in Eqs. (1) and (2) should be equal at time t. Then the ratio of k_{add}/k can be expressed as C_{add}/C at time t. By determining C_{add} and C experimen-

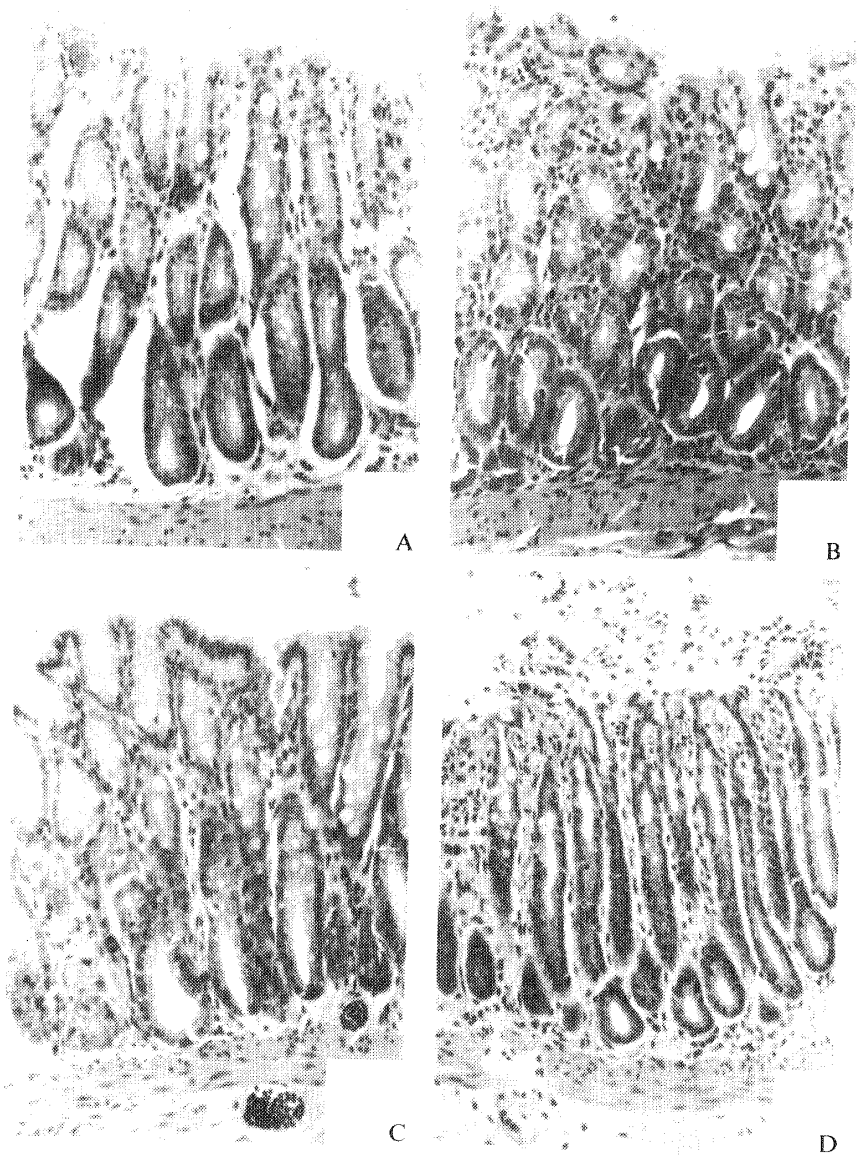


Fig. 5. Light Photomicrograph of Rectal Lumen

A: perfused with emulsion (w/o) for 60 min (water : oil = 2 : 8).
B: perfused with emulsion (o/w) for 60 min (water : oil = 3 : 7).
C: perfused with Sorbox 20 in phosphate buffer for 60 min.
D: perfused with Sorbox 20 in miglyol oil for 60 min.

tally, changes in k_{add} can be estimated. The values of ratio of absorption rate (RAR, k_{add}/k) are presented in Fig. 6, and profiles of the ratio of absorption rate were almost the same as those of ARC (Fig. 2). In this way, it was found that bidirectional permeations of the marker drugs (rectal lumen $\rightleftharpoons$ blood) were affected simultaneously.

The effect of the Tween-type surfactants in aqueous solutions on the permeability of the rectal membrane was rather small (Figs. 1(A) and 2(A)), as was reported previously.[1] Only 1.4- to 3-fold increases in AUC_{90} of sulfanilic acid were noted in the presence of the surfactants (Table II). Even in the case of Sorbox 20, which does not possess surface-active properties, a comparable increase in AUC_{90} was observed. Although the increases in AUC_{90} due to these agents were statistically significant ($p<0.05$) relative to the control value, there was no significant difference among the Tweens and Sorbox 20.

As for the apparent rectal clearance of creatinine, only phosphate buffer containing

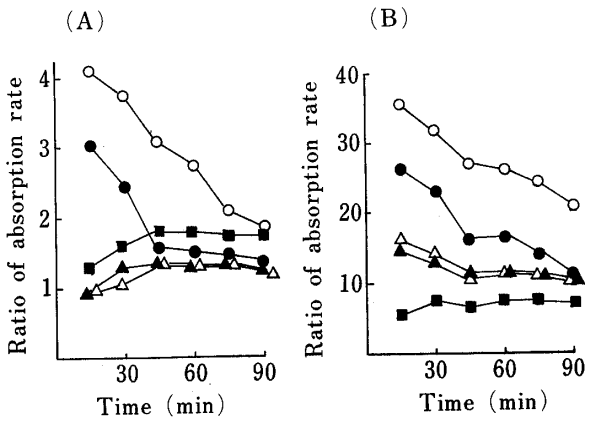


Fig. 6. Effect of Adjuvants on the Ratio of Apparent Absorption Rate Constant of Sulfanilic Acid (A) in Phosphate Buffer Solution, (B) in Miglyol Oil Solution

—○—, Tween 20; —●—, Tween 40; —△—, Tween 60; —▲—, Tween 80; —■—, Sorbox 20.

Tween 20 or 40 showed statistically significant increases in ARC_{90} values of creatinine (Table III). Other agents tended to decrease ARC_{90} values, but not significantly.

Brayan *et al.*[11] examined the influence of polyethylene glycol 2000 on the digestive tract and found that transient morphological changes in the intestinal membrane were caused by the osmotic pressure of polyethylene glycol 2000 solution. Therefore we measured the osmotic pressures of solutions of Tweens 20 and 40, which caused significant increases in AUC_{90} of sulfanilic acid and ARC_{90} of creatinine, as well as Tween 80 and Sorbox 20 (Table IV). Hypertonicity was observed for Sorbox 20 at both concentrations, implying the involvement of osmotic pressure in the altered permeability of the rectal membrane. On the other hand, 5% solutions of Tweens 20, 40, and 80, which were used in the perfusion experiments, were almost isotonic. Therefore in these cases, interactions between the surfactant and the rectal membrane may be involved in the alteration of rectal permeability. However, even in the case of Tweens 20 and 40, the *ARC* values decreased with time (Fig. 2(A)) nearly to the control value at 90 min. In addition, no histological change was observed after the experiments (Fig. 4). This means that the change in the rectal permeability due to Tweens 20 and 40 is transient and reversible.

When Tween-type surfactants were added in oils, their effects on rectal permeability were much greater than those in aqueous solutions (Figs. 1(B) and 2(B)). As summarized in Tables II and III, 11- to 33-fold increases in AUC_{90} values and 10- to 24-fold increases in ARC_{90} values were observed when the rectum was pretreated with these surfactants dissolved in oils. Sorbox 20 also caused a 9-fold increase in AUC_{90} values as well as a 10-fold increase in ARC_{90} values.

When emulsions with various oil-to-water ratios were used as media for Tween 20, significant changes in rectal permeability occurred only when w/o type emulsions were applied. Therefore it became clear that Tween-type surfactants exhibit a significant effect on rectal permeability when added in oil or in w/o type emulsions.

During microscopic examination of rectal tissues after the experiment, histological changes were observed in the tissue samples which exhibited enhanced permeability for the drugs. Thus the histological changes were considered to be the cause of the enhancement of rectal permeability. The histological changes can be explained in the following way. When an oily solution of Sorbox 20 contacts the rectal tissue, a large amount of Sorbox 20 may dissolves in a small amount of aqueous phase present on the rectal membrane, causing a rapid increase in osmotic pressure, which may alter the morphology of the rectal membrane. As for Tween-type surfactants added in oil, they may also dissolve in a small amount of aqueous phase on the rectal membrane at relatively high concentrations. Some interactions between the surfactants and the rectal membrane may occur.[12] However, when the rectal lumen was pretreated with pure Tween 80, the resultant AUC_{90} of sulfanilic acid was 498.4±

36 μg/ml·90 min, which is roughly one-half of that obtained when Tween 80 was added in oil. Therefore the increased permeability of the rectal membrane cannot be attributable solely to direct interaction of Tweens and the rectal membrane.

Kitahara[13] reported that a small amount of water enhanced the affinity of the hydrophilic portion of nonionic surfactant when the surfactant was dissolved in oil. In our present investigation, Tween-type surfactants with higher HLB values influenced the rectal permeability more markedly. It can therefore be considered that a small amount of water on the rectal membrane enhanced the hydrophilicity of the hydrophilic portion of Tween-type surfactant and favored micelle formation. Then more water would participate in the formation of micelles, causing dehydration of the rectal tissue. The histological changes due to such dehydration might enhance the rectal permeability.

Conclusion

The effect of Tween-type surfactants on the permeability of the rectal membrane was investigated. When these surfactants were added in aqueous solutions, they did not have significant effects on the permeability. On the other hand, these surfactants affected the histological nature of the rectal tissue and enhanced the permeability when they were added in oil or w/o type emulsions.

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