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Mechanism of Drug Absorption from Micellar Solution. II.[1] Effect of Polysorbate 80 on the Absorption of Micelle-free Drugs

Akemi Kaneda, Kayoko Nishimura, Shozo Muranishi, and Hitoshi Sezaki

Faculty of Pharmaceutical Sciences, Kyoto University[2]

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Effect of Polysorbate 80 on the intestinal absorption of nine micelle-free drugs was examined in the rat. Regardless of the ionic nature of the compounds, two levels of the effect were observed, namely an absorption enhancing effect at relatively low concentration range and a small inhibitory effect at higher concentrations of the surfactant.

Pretreatment of the gut with the surfactant, mucosal uptake, and other absorption experiments revealed that the surfactant adsorbed onto the mucosal surface exerted an absorption modifying effect. Absorption promoting and inhibiting effects may be operative at the same time within the concentration range of the surfactant investigated and the observed net absorption modifying effect dependent on the relative magnitude of each.

Absorption enhancing effect was dependent on the surfactant-mucosal surface contact time and increased steadily with the time after pretreatment with Polysorbate 80. Absorption studies on sulfisoxazole from the different segments of the intestine provided evidence that the enhancing effect of Polysorbate 80 could be explained by the degree of saturation of the surfactant on the mucosal surface regardless of the regions of the intestine.

The role of surfactants in the intestinal absorption of various drugs has been studied extensively.[3–6] Much of the difficulty in interpreting some of these studies has been due to the different effects which surfactant can exert.

The previous report in this series[1] concerned the effect of Polysorbate 80 on the absorption of vitamin A alcohol and acetate from the solution recirculating through the rat large intestine.

The absorption of the vitamins, largely in the surfactant micellar phase, followed apparent biphasic zero-order kinetics. It was thereby noticed that in the initial rapid disappearance phase, Polysorbate 80 played an unique role in favoring the sorption of the micelle-entrapped vitamins on the intestinal membrane.

The present study was initiated to characterize the influence of Polysorbate 80 at the other extreme; effect on the intestinal absorption of water-soluble, poorly absorbed micelle-free compounds.

Experimental

Materials——Polysorbate 80 (Tokyo Kasei Kogyo Co., Ltd.); all other chemicals used in the study were reagent grade and used as such without further purification.

Drug Solutions——The drugs were dissolved in an isotonic buffer ($Na_2HPO_4 \cdot 12H_2O$ 4.4 mM–KH_2PO_4 1.3 mM) and the solutions were adjusted to pH 7.4. For sulfadimethoxine and quinine hydrochloride, drug solutions containing Polysorbate 80 were made isotonic and were buffered with ($Na_2HPO_4 \cdot 12H_2O$ 22.2 mM–KH_2PO_4 11.1 mM, pH 8.0) and ($Na_2HPO_4 \cdot 12H_2O$ 31.2 mM–KH_2PO_4 2.0 mM, pH 7.0) respectively. The drug solutions used for the experiments were prepared so that each ml yielded the following initial con-

1) Part I: K. Kakemi, H. Sezaki, S. Muranishi, and A. Kaneda, *Chem. Pharm. Bull.* (Tokyo), **18**, 1563 (1970).
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3) M. Gibaldi and S. Feldman, *J. Pharm. Sci.*, **59**, 579 (1970).
4) C.J. Kreutler and W.W. Davis, *J. Pharm. Sci.*, **60**, 1835 (1971).
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centrations: sulfisoxazole (0.1 mM), sulfadimethoxine (0.1 mM), metoclopramide (0.1 mM), salicylic acid (5 mM), isonicotinic acid (0.1 mM), quinine hydrochloride (1 mM), lactosyl isonicotinic acid hydrazone (0.5 mM), inulin (2 mM), and α-D-xylose (10 mM).

Dialysis Studies——Equilibrium dialysis was carried out at 37° using Visking cellulose tubings (8/32′). The outside compartment was filled with 5 ml of a buffer solution and the inside compartment filled with 3 ml of a drug solution containing Polysorbate 80. After equilibration, the free ligand concentration in the surfactant-free compartment was measured and the distribution constant (K_m) was calculated by the method of Kakemi, *et al.*[7]

Absorption Studies——Several experimental techniques were used to assess absorption. Male Wistar rats weighing about 180 to 200 g were used throughout the experiments.

A. Recirculation Technique: Intestinal transfer rates of the drugs except sulfadimethoxine and isonicotinic acid were determined by the method of Kakemi and the procedures have been described in previous communication.[1] The following briefly summarizes the notes any departure from the previous method. Initial volumes of the recirculating solution for the entire small intestine, the upper 45 cm small intestine, and the large intestine were 40, 30, and 25 ml respectively. The upper 45 cm small intestine was measured from the upper portion of the duodenum by running along the thread. The entire recirculation time was 75 minutes and phenol red was used as a volume change indicator.

In the case of pretreatment with Polysorbate 80, an isotonic buffer solution containing the surfactant only was recirculated for thirty minutes prior to a regular run. After the pretreatment, the entire recirculating solution containing the surfactant was withdrawn as completely as possible with the aid of a hypodermic syringe followed by a usual absorption experiment.

B. *In Situ* Single Loop Technique: This technique was applied for the absorption studies of sulfadimethoxine and isonicotinic acid since the absorption of these two drugs from the large intestinal lumen under recirculating condition was very small.

With the rat under pentobarbital anesthesia, the large intestine from the upper colon to the anus was exposed and both ligatures were cannulated with polyvinyl tubings. The intestine was flushed with normal saline until the washings became clear. Two ml of drug solution per 200 g body weight was injected into the loop from the proximal end. The ligature was secured and the incision closed. After 75 minutes, the solution in the loop was withdrawn as completely as possible, and the loop was flushed further with normal saline. All the washings were combined with the initial solution and made up to 50 ml with normal saline. From the difference of the amount of the drug between the initial solution and the combined effluent, amount absorbed was calculated.

C. Single Perfusion Technique: Details of the procedure has been described previously.[1]

***In Vitro* Drug Uptake Experiment**——The large intestine was removed under pentobarbital anesthesia and flushed with cold normal saline. The upper large intestine was everted on a glass rod and sacs (5 cm long) were prepared. No solution was injected into the serosal side of the sacs. Each sac was immersed in 6 ml of drug solution and after 15 minutes incubation at 37°, amount of the drug uptake was determined.

Assay Procedures——The following procedures were found to give reproducible results over the required concentration range in the presence of Polysorbate 80. Sulfisoxazole, sulfadimethoxine, and metoclopramide hydrochloride: Analyzed colorimetrically for diazotizable amines. Salicylic acid: Four ml of 0.1% $Fe(NO_3)_3$ solution in 0.07 N HNO_3 was added to 2 ml sample and the color developed was estimated spectrophotometrically at 530 nm. Isonicotinic acid: Determined by the method of Nielsch, *et al.*[8] Quinine hydrochloride: One ml of 3 N NaOH and 7 ml of ethylene dichloride were added to 2 ml of sample and the mixture was shaken for 20 minutes. After separation, 5 ml of organic phase was shaken for 10 minutes with 5 ml of 1N HCl and the extinction of organic phase was determined spectrophotometrically at 251 nm. Lactosyl isonicotinic acid hydrazone: Determined by the method of Kakemi, *et al.*[9] Inulin: Determined by the method of Galli.[10] Five ml of conc. HCl and 1 ml of 0.3 mg% of thiobarbituric acid were added to 1 ml of sample. The mixture was boiled for 20 minutes in a water bath. After cooling, the solution was measured spectrophotometrically at 434 nm. α-D-Xylose: Determined by the method of Duncan.[11] Polysorbate 80: Determined by the method as previously described.[1]

Result and Discussion

The effect of Polysorbate 80 on the rate of absorption of various drugs from the rat large intestine as a function of its concentration is illustrated in Fig. 1, 2, and 3. Drugs shown in

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9) K. Kakemi, T. Arita, H. Sezaki, and N. Takasugi, *Chem. Pharm. Bull.* (Tokyo), **13**, 551 (1965).
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11) I.J. Duncan, *Ind. Eng. Chem., Anal. Ed.*, **15**, 162 (1943).

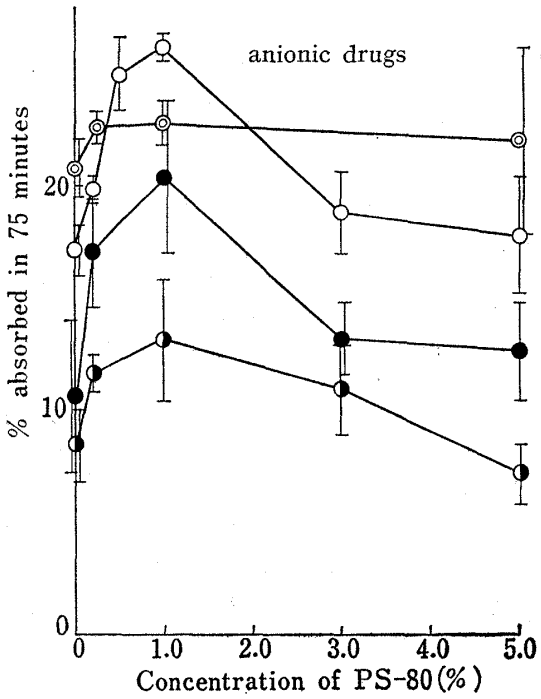

Fig. 1. Effect of Polysorbate 80 (PS-80) Concentration on the Absorption of Anionic Drugs. Absorption from the Solution Recirculating through the Rat Large Intestine (○ ◑), and from the Solution in the Rat Large Intestinal Loop. (◎ ●).

○: salicylic acid ◑: sulfisoxazole
◎: isonicotinic acid ●: sulfadimethoxine

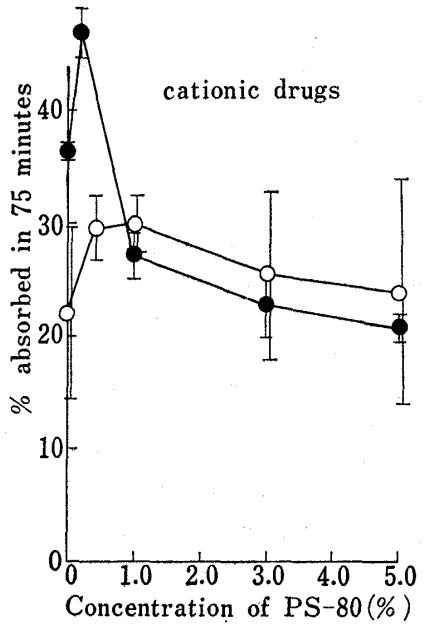

Fig. 2. Effect of Polysorbate 80 (PS-80) Concentration on the Absorption of Cationic Drugs from the Solution recirculating through the Rat Large Intestine.

●: quinine-HCl
○: metoclopramide-HCl

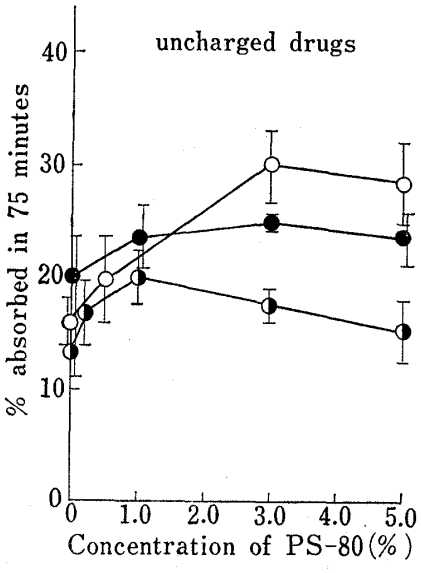

Fig. 3. Effect of Polysorbate 80 (PS-80) Concentration on the Absorption of Uncharged Drugs from the Solution recirculating through the Rat Large Intestine.

◑: xylose, ○: lactose-INAH, ●: inulin

Table I. Drugs and Their Physicochemical Properties

	Drug	pK_a	K_m[a)]
Anionic drug	sulfisoxazole	5.1	0.05
	isonicotinic acid	4.9	0
	sulfadimethoxine	6.7	0.10
	salicylic acid	3.0	0.03
Cationic drug	quinine-HCl	8.4	0
	metoclopramide-HCl	9.0	0
Uncharged drug	α-D-xylose	—	0
	lactose-INAH	—	0
	inulin	—	—

a) Apparent distribution constant, K_m, was obtained by the equation $M=K_mFS$, where M is the concentration of drug in micelles (mcg/ml), F is the concentration of free drug in water (mcg/ml), and S is the concentration of the surfactant (g. %).[7]

Table I were chosen for the present communication due to their ionogenic nature, negligible micellar complexation tendency, and large intestinal absorptive characteristics. Regardless of the ionic nature of the drugs at the pH of the recirculating solution, there appears to be two levels of the surfactant effect on the absorption of drugs from the intestinal lumen; absorption enhancing effect at relatively low concentration range and a small inhibiting effect at higher concentrations. The latter effect was also observed in the absorption of micelle-entrapped vitamin A from the rat large intestine.[1]

In the previous report,[1] it was postulated that the initial rapid disappearance of vitamin A from the perfusate was mainly due to the surfactant mediated sorption of the drug to the absorptive membrane. However, in the single perfusion experiments on sulfisoxazole and sulfadimethoxine (Fig. 4), no such significant disappearance in the first one minute was observed. Slight increase observed in sulfadimethoxine might be attributed to the absorption characteristics of the drug from aqueous solution in which initial sorption onto the membrane is comparatively larger than the other water-soluble drugs.[12] Also typical time courses of the disappearance of sulfisoxazole shown in Fig. 5 fail to demonstrate biphasic pattern and the rapid disappearance tendency in the first 15 minutes of absorption which were observed in the case of micelle-entrapped vitamin A acetate. As is evident from Fig. 5, disappearance of sulfisoxazole from the *in situ* intestinal lumen with or without the surfactant was found to be monoexponential.

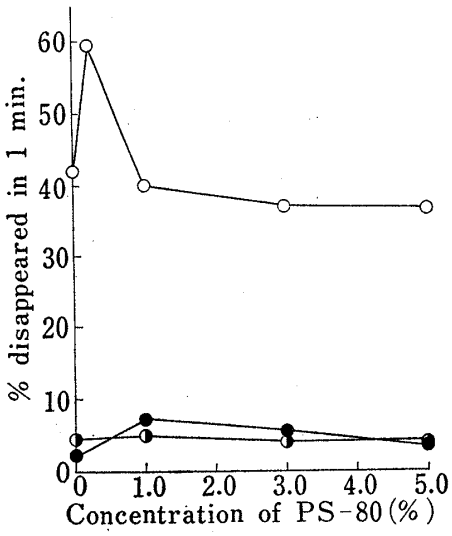

Fig. 4. Disappearance of various Drugs from the Large Intestinal Lumen by One-Minute Single Perfusion

○: vitamin A acetate
●: sulfadimethoxine
◑: sulfisoxazole

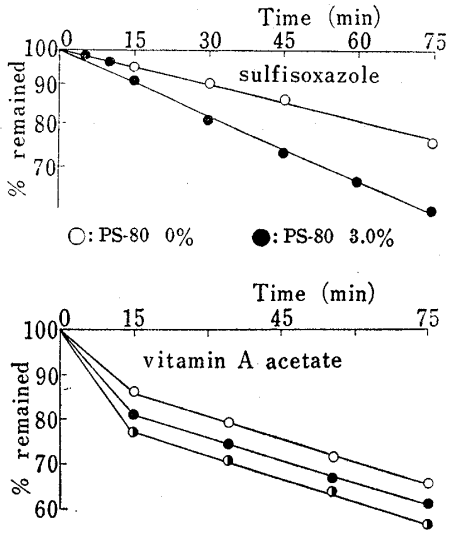

Fig. 5. Comparison of the Time Courses of Absorption

upper: absorption of sulfisoxazole from the rat small intestins
lower: absorption of vitamin A acetate from the rat large intestine[1]

Since the drugs used in the present study are largely free from micellar interaction with the surfactant (Table I), they are distributed mainly in the aqueous non-micellar phase. Therefore, concentration of the drugs in such phase may be built up with the increase of the surfactant concentration in solution, which might be resulted in apparent absorption enhancing effect. However, theoretical curve, calculated on the basis of the micellar volume fraction reported by Yamada, *et al.*,[13] failed to explain such enhancing effect for the absorption (Fig. 6).

12) Unpublished data.
13) H. Yamada and R. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), 13, 1279 (1965).

Since the drug in micelle phase is unavailable for absorption, the effective concentration of the drug is less than the apparent one, and a decreased absorption rate might be expected. However, as shown in Fig. 6, absorption inhibiting effect at higher concentration range of Polysorbate 80 cannot be explained by such weak micellar complexation of sulfisoxazole and Polysorbate 80 alone.

In order to obtain additional information regarding the role of surfactant adsorbed on the permeability of the intestinal membrane, drug uptake experiment was carried out.

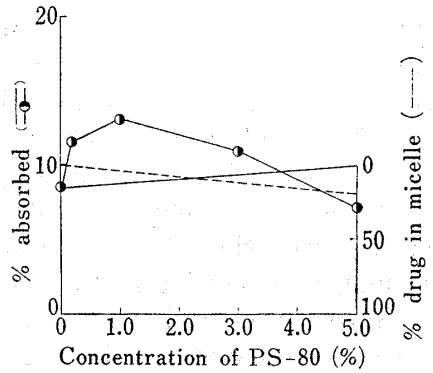

Fig. 6. Plot showing the Effect of Micellar Volume Fraction on the Absorption of Sulfisoxazole

—◑—: observed
——, ------: calculated

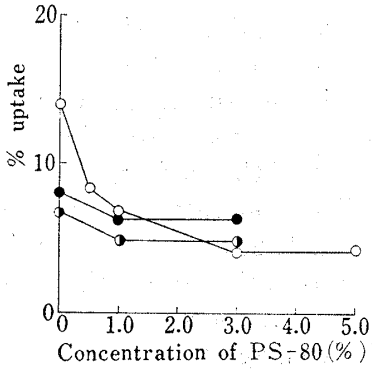

Fig. 7. Influence of the Concentration of Polysorbate 80 on the Uptake of Various Drugs by the Large Intestinal Mucosa

○: salicylic acid
●: quinine-HCl
◑: metoclopramide-HCl

Fig. 7 shows the uptake of salicylic acid, quinine hydrochloride, and metoclopramide hydrochloride with the large intestinal mucosa. As can be seen, it was found that percentage uptake of the drugs decreased when the surfactant concentration increased. It was also found in a separate experiment that percentage uptake into the everted sacs prepared from the intestine pretreated with Polysorbate 80 was almost equal to the one obtained from the non-pretreated rat, which suggests that Polysorbate 80 adsorbed onto the intestinal membrane may be one of the factors which contribute to the absorption inhibiting effect.

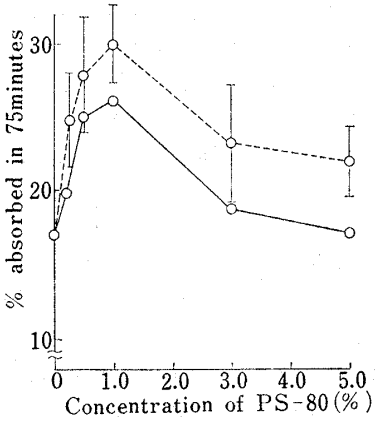

Fig. 8. Absorption of Salicylic Acid from the Rat Large Intestine

—○—: with Polysorbate 80 (same as Fig. 1)
---○---: pretreated for 10 minutes with 3.0% Polysorbate 80 solution

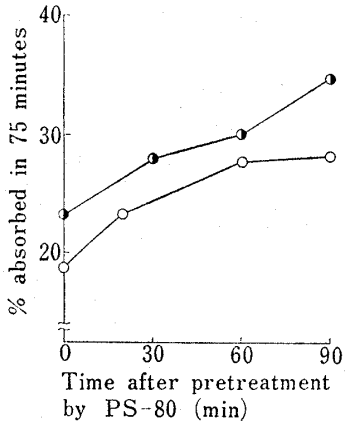

Fig. 9. Plot showing the Relationship between the Increase of Absorption and the Standing Time after Pretreatment with 3.0% Polysorbate 80 Solution

—◑—: absorption of sulfisoxazole fromthe rat small intestine
—○—: absorption of salcylic acid from the rat large intestine

Results of the *in situ* salicylic acid absorption experiment shown in Fig. 8 further support the view that the surfactant adsorbed onto the membrane exert an absorption modifying effect. The absorption enhancing or inhibiting pattern in non-pretreated rats was similar to the pretreated ones.

Experiments were also carried out to determine the influence of surfactant as a function of contact time. The intestine was recirculated with 3.0% solution of Polysorbate 80 for 10 minutes and the surfactant solution was withdrawn from the intestine. For that purpose, no solution was perfused through the intestine so as not to wash out any surfactant which had been adsorbed on the mucosal membrane. Therefore, the surfactant solution in the large intestine was withdrawn with the aid of air from a hypodermic syringe. Animals were left intact for a certain period of time after the withdrwal of the surfactant and the drug solution which contains no surfactant was recirculated through the surfactant treated intestine in a usual manner and the absorption was studied. It is worthy to note that the absorption enhancing effect of the surfactant observed in sulfisoxazole and salicylic acid is time-dependent (Fig. 9). The concentration of the surfactant used for the pretreatment was 3.0%. As shown in Fig. 1, 2, and 3, the absorption inhibiting effect is operating at this concentration. Therefore, absorption promoting and inhibiting effects may be operative at the same time at this surfactant concentration and the observed net absorption modifying effect dependent on the relative magnitude of each. The enhancing effect tends to increase with the time after the surfactant treatment. This slow-acting characteristics of Polysorbate 80 is interesting from the standpoint of the nature of its membrane action.

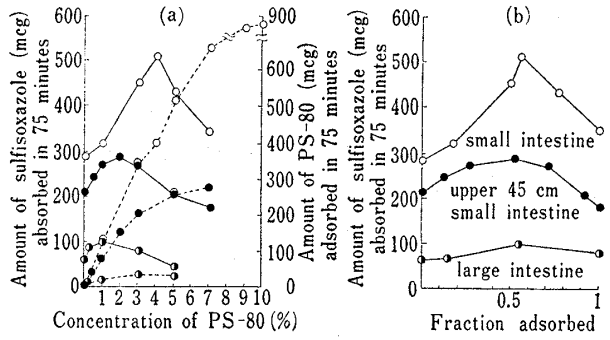

Fig. 10. Effect of Polysorbate 80 on the Absorption of Sulfisoxazole from the Different Segments of the Rat Intestine

(a) Plots showing the absorption of sulfisoxazole (—) and the amount adsorbed of Polysorbate 80 on the absorptive membrane (---) as a function of the initial concentration of the surfactant. Amount of the surfactant disappeared from the intestinal lumen during 75-minute recirculation was taken as the amount adsorbed.
(b) Plots showing 75-minute absorption of sulfisoxazole from the solution recirculating through the rat intestinal lumen as a function of the fraction saturated with the surfactant. The latter value was obtained by dividing the amount of adsorption at each surfactant concentration by the maximum value of adsorption at the corresponding intestinal segment.

Finally, absorption modifying effect of Polysorbate 80 on sulfisoxazole was examined with different regions of the intestine. As shown in Fig. 10-a, the absorption of sulfisoxazole was dependent on the concentration of Polysorbate 80 as was observed in the large intestine although the maximum points in the absorption fall on different surfactant concentrations depending on the region of the intestine. When plotted, however, on the amount of Polysorbate 80 per area basis, the maxima fall on the same point (Fig. 10-b), the point of half-saturation. This means that the disappearance of the drug is most influenced at the point where half the saturable amount of the surfactant was adsorbed onto the membrane.

It has been suggested that the mechanism of influence of surface-active absorption promoters, effective as they are under conditions of negligible lipid solubility of the drug, is to render the membrane a less effective barrier tothe penetration of such normally poorly absorbable drugs. Although the present findings may not directly explain the complex *in vivo* absorption promotion or inhibition by the surfactant, they should contribute significantly to the understanding of baseline behaviors.