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Studies on Absorption and Excretion of Drug. XIII.[1] The Sorption of Surface Active Agents into the Intestinal Tissue of Rat and Their Effect on the Drug Absorption *in Vitro*[2]

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1) The sorption of ionic surfactants (sodium lauryl sulfate, cetyltrimethylammonium bromide) into the intestinal tissue of the rat and the effect of sodium lauryl sulfate on the drug absorption were investigated above and below critical micelle concentration from the standpoint of kinetics *in vitro*.

2) The sorption of the surfactant into the intestinal tissue was concluded to follow the first order kinetics below critical micelle concentration, and the zero order kinetics above it.

3) The observed zero order rate constants were slightly larger than the calculated values in sodium lauryl sulfate and particularly cetyltrimethylammonium bromide.

4) The micelle formation of sodium lauryl sulfate decreased the absorption of nitrobenzene which distributed to the micelle. But the absorption of *o*-methoxybenzaldehyde which did not distribute to the micelle was not influenced by the micelle formation of surfactant.

The effect of various surface active substances on the drug absorption from the gastrointestinal tract or rectum has been investigated by many workers.[4–8] In these reports, surfactants affect increasingly or decreasingly the absorption of drugs depending on the concentration and the type of surfactant, the species of drug and also the combination of drug and surfactant. These studies attributed the decreasing effect to the micelle formation of surfactant and the solubilization of drug in it. Very few investigation concerning the sorption of surfactant itself into the intestinal tissue, however, are found in spite of the fundamental importance to elucidate the mechanism of surfactant effect.

The present investigation deals with the kinetic behavior of the ionic surfactants in the removed rat intestine. The role of the micelle formation in the intestinal sorption of surfactant is shown from the kinetic measurements of its disappearance from the intestinal lumen perfusing the solution above and below the critical micelle concentration. The effect of surfactant on the drug absorption is also examined in order to confirm the low sorption tendency of the micelle into the intestinal wall. The intestinal absorption of solubilizates from the micelle solution is compared between nitrobenzene and *o*-methoxybenzaldehyde, because they are different each other in the distribution coefficient between the micelle and water. In the present study, the effect of surfactant on the intestinal absorption is estimated in relation to the affinity of substance to the micelle.

The critical micelle concentration of ionic surfactants as sodium lauryl sulfate and cetyltrimethylammonium bromide is so variably influenced by the salt incorporated in the

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2) Presented before the Kanto Branch Meeting of Pharmaceutical Society of Japan, Tokyo, July 1965.
3) Location: *Hongo, Tokyo.*
4) G. Levy and R.H. Reuning, *J. Pharm. Sci.*, **53**, 1471 (1964).
5) K. Kakemi, T. Arita, H. Sezaki and I. Sugimoto, *Yakugaku Zasshi*, **84**, 1210 (1964).
6) K. Kakemi, T. Arita and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 976 (1965).
7) H. Yamada and R. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **13**, 1279 (1965).
8) H. Matsumoto, *Yakugaku Zasshi*, **86**, 590 (1966).

solution that it was determined by measuring the solubilized amount of sudan III in perfusion surfactant solution. Since their values of sodium lauryl sulfate and cetyltrimethylammonium bromide at 37° were 1.0 mM and 0.4 mM respectively, 0.1 and 0.5 mM as below critical micelle concentration, and 5.0 and 10.0 mM as above were examined for the sodium lauryl sulfate solution, and 0.1 and 0.2 mM as below, and 5.0 and 10.0 mM as above for cetyltrimethylammonium bromide solution.

The residual rates of sodium lauryl sulfate in inner solution (mucosal side) for various time in the perfusion experiments are shown in Fig. 1. In the case of 0.1 and 0.5 mM solution which is below the critical micelle concentration, the disappearance of the surfactant in perfusion solution originated in the sorption into the intestinal tissue follows an exponential curve, because the logarithm of residual rate *vs.* time plots are found to be straight lines as shown in Fig. 2. Moreover, although two straight lines are appeared not to be corresponding more or less to each other by the experimental error, the gradients of the straight lines are considered to be almost independent of the initial concentration of the surfactant. Thus, ths diappearance of sodium lauryl sulfate below the critical micelle concentration is allowed to be the first order process in view of kinetics.

In the case of 5.0 and 10.0 mM solution which is above the critical micelle concentration, the residual rate *vs.* time plots are considered to be the straight lines as seen in Fig. 1. The gradient of the line varies for the initial concentration of sodium lauryl sulfate. Since the residual rates are identical between 5.0 mM in one hour and 10.0 mM in two hours, the sorption of the surfactant into the intestinal tissue can be concluded to follow the zero order kinetics.

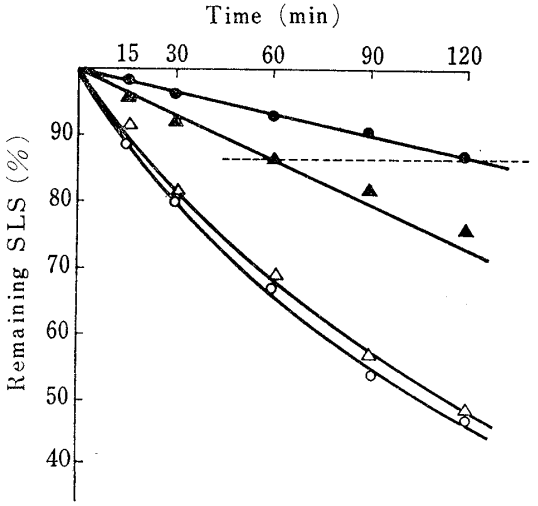

Fig. 1. The Percentage of Remaining Sodium Lauryl Sulfate in Perfusion Solution

—●— 10 mM SLS —○— 0.5 mM SLS
—▲— 5 mM SLS —△— 0.1 mM SLS
SLS: sodium lauryl sulfate

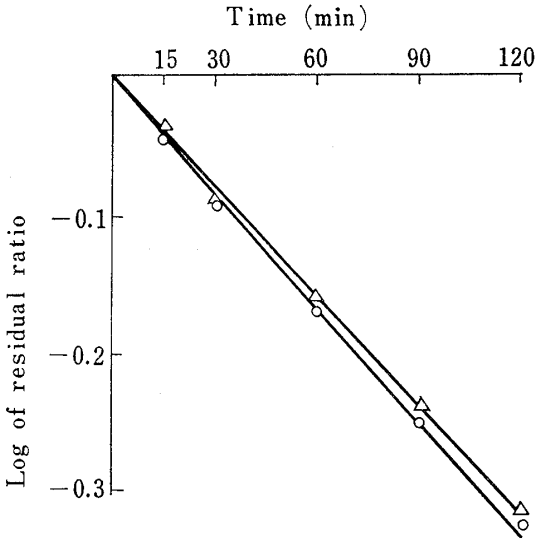

Fig. 2. Residual Ratio of Sodium Lauryl Sulfate plotted in the Logarithmic Scale

No surfactant transported through the intestinal tract was found in the outer solution which was soaking the serosal side of the intestine even after two hours continuous perfusion of surfactants in mucosal side. Furthermore, the evidence of the uptake of surfactant in the intestinal tissue was confirmed by the washing recovery experiment, that is, the amount of the disappeared surfactant calculated from the decrease of the concentration in inner solution was equal to that from the recovery of inner solution which was washed out four times with isotonic sodium chloride solution after perfusion experiment. The evidence of chemical and enzymatic stability of the surfactant solution was confirmed from the incubation experiment of the surfactant solution which was made by the isotonic phosphate buffer solution

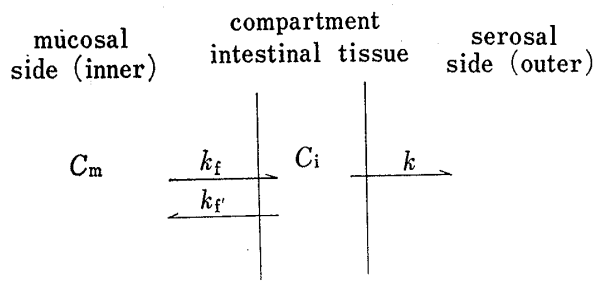

Chart 1. Model of Drug Absorption in Removed Intestine

perfused beforehand in the intestine. Therefore, the disappeared amount from inner solution during perfusion was allowed to be due to the uptake into intestinal tissue and not to the absorption through the intestine.

The experimental system with removed intestine can be schematized by three compartments in which the surfactant is assumed to distribute homogeneously for the sake of simplification as shown in Chart 1.

The concentrations of surfactant in the intestinal tissue were not determined in this experiment and therefore C_i in Chart 1 is a dummy at the convenience of theoretical consideration. The velocity of the uptake of the surfactant from the inner solution into the intestinal tissue is expressed as $k_f C_m$ from the diffusion equation where $k_{f'}$ and C_m are the rate constant of this process and the concentration of surfactant in the inner solution, respectively, and in the same manner kC_i expresses the velocity of the release from the intestinal tissue to the outer solution. As mentioned above, the release to the outer solution was not observed and this would mean the very small value of the rate constant, k. The value of $k_{f'}$ will be also small as shown in the result of washing recovery. When $k_{f'}$ and k is negligible compared with the uptake rate constant, k_f, the decreasing concentration of the surfactant in the inner solution which is below the critical micelle concentration will be expressed by the first order rate process as follow,

$$2.303 \log(C_m/C_0) = -k_f t \qquad (1)$$

where C_0 is the initial concentration of the surfactant in the inner solution.

The equilibrium constant of the surfactant concentration between the inner solution and the intestinal tissue is expressed by the ratio of k_f to $k_{f'}$. Since $k_{f'}$ is very minor, the removed intestine should have large capacity of the uptake of the surfactant. This would be important and interesting point concerning the interaction of surfactant to the gut, though the large capacity may be particular to the removed intestine.

Above the critical micelle concentration, the free surfactant ion and the micelle are in equilibrium. In this case, the free ion but micelle can only be sorbed into the intestine as shown in dialysis experiment. If it is real in this removed intestine, the decrease of the concentration of the inner solution will follow a zero order kinetics, that is a constant rate sorption, since the concentration of the free surfactant ion above the critical micelle concentration will be constant and equal to the critical value. That is

$$-(dC_m/dt) = k_f C^* \qquad (2)$$

where C^* is the critical micelle concentration.

The Integration of Eq. 2 gives,

$$C_m/C_0 = 1 - k_f C^* t/C_0 = 1 - Kt/C_0 \qquad (3)$$

where C_0 and K are the initial concentration and the rate constant (zero order), respectively.

The next equation gives the relationship between k_f determined by the experiment below the critical micelle concentration and K above that.

$$k_f C^* = K \qquad (4)$$

Table I shows the comparison between the observed rate constants and the calculated ones. The observed zero order rate constants from Eq. 3 are slightly larger than the calculated

value from Eq. 4 in sodium lauryl sulfate and particularly cetyltrimethylammonium bromide. In the case of sodium lauryl sulfate, the difference between the observed value and the calculated one is considered to be not so much large. Therefore, the weak interaction of the micelle of ionic surfactants, particularly cetyltrimethylammonium bromide, and the intestinal tissue might be concluded.

Table I. Observed and Calculated Zero Order Rate Constants (K_{obs} and K_{calc}), and First Order Rate Constants (k_f) of Sodium Lauryl Sulfate and Cetyltrimethylammonium Bromide

	SLS	CTABr
K_{obs} (mole 1^{-1} hr^{-1})	7.2×10^{-4}	9.2×10^{-4}
K_{calc} ($=k_fC^*$)	4.0×10^{-4}	2.0×10^{-4}
k_f (hr^{-1})	4.0×10^{-1}	5.0×10^{-1}

SLS : sodium lauryl sulfate CTABr : cetyltrimethylammonium bromide

The absorption of solubilized substances from the intestine was further investigated in order to estimate the role of the micelle of ionic surfactant in the intestinal sorption. If the micelle of sodium lauryl sulfate can not transfer to the intestinal tissue as mentioned above, the absorption rate of solubilized substance is influenced by the distribution coefficient of substance between water and the micelle. The absorption rate will decrease with increasing the solubilized amount in the micelle. This phenomena of nonionic surfactants have already been reported on dialysis experiments of benzocaine by Matsumoto, *et al.*[9] and on the intestinal absorption of salicylamide by Yamada, *et al.*[7] In the present study, the absorption of nitrobenzene and *o*-methoxybenzaldehyde, which are quite different from each other in the distribution to the micelle, were investigated.

The concentration decrease of nitrobenzene in the inner solutions of various sodium lauryl sulfate levels are shown in Fig. 3. These inner solutions were prepared as follows. Nitrobenzene was dissolved at the concentration of two millimolar in isotonic phosphate buffer solutions with and without 0.5 mM, 5.0 mM and 10.0 mM of sodium lauryl sulfate. Then these solutions were allowed to stand overnight before the perfusion experiment so that the solubilization was sufficiently accomplished. The experimental procedure of the perfusion was the same as the surfactant itself. From the experimental results, it is obvious that the surfactant does not affect the absorption of nitrobenzene below the critical micelle concentration, but does above the concentration. The decreasing effect becomes strong with increasing the concentration of the surfactant above the critical micelle concentration. In Table II, the distribution coefficient of nitrobenzene was listed which was determined by the procedure

Table II. Distribution Coefficient of Nitrobenzene and *o*-Methoxybenzaldehyde between Aqueous and Micellar Phases of Sodium Lauryl Sulfate Solution

	C aq. (mM)	*C* mic. (mM)	*C* mic./*C* aq.
Nitrobenzene	0.8	1.2	1.50
o-Methoxybenzaldehyde	*ca.* 2.0	0	0

C aq. : concentration of nitrobenzene or *o*-methoxybenzaldehyde in aqueous phase per unit volume of the solution
C mic. : concentration of nitrobenzene or *o*-methoxybenzaldehyde in micellar phase per unit volume of the solution

9) H. Matsumoto, H. Matsumura and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **14**, 391 (1966).

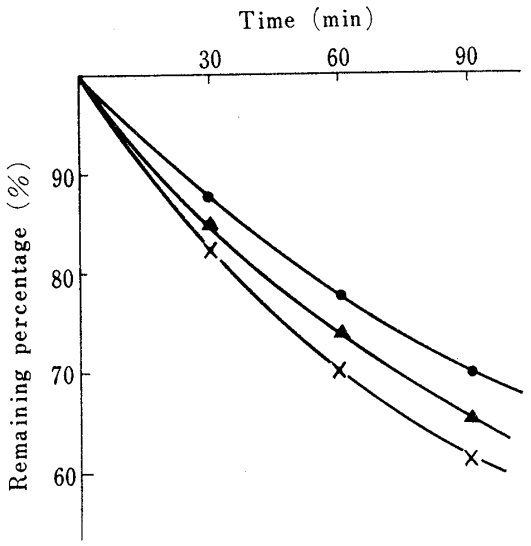

Fig. 3. Fffect of Sodium Lauryl Sulfate on Intestinal Absorption of Nitrobenzene

—●— 10 mM SLS
—▲— 5 mM SLS
—×— 0.5 mM SLS, no SLS

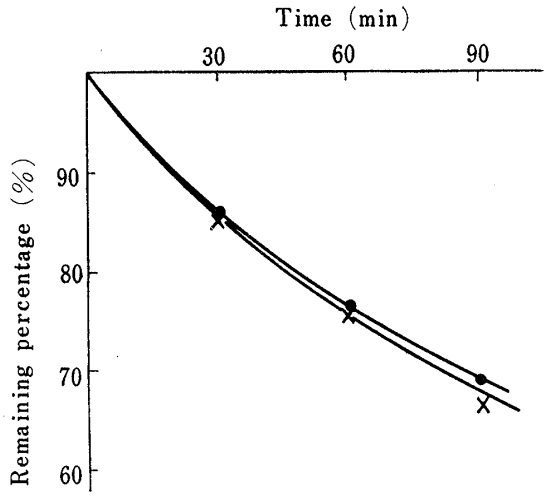

Fig. 4. Effect of Sodium Lauryl Sulfate on intestinal Absorption of *o*–Methoxybenzaldehyde

—●— 10 mM SLS
—×— no SLS

reported in the previous study with two millimolar of nitrobenzene in fifteen millimolar sodium lauryl sulfate solution at room temperature. The evidence of the distribution to micelle is obvious from this determination.

The experimental results of the intestinal absorption of *o*-methoxybenzaldehyde are shown in Fig. 4. The composition of perfusion solution and the experimental procedure were quite same as the case of nitrobenzene. The decreasing rate of the concentration of *o*-methoxybenzaldehyde in the inner solution during the perfusion experiment was not influenced by the surfactant, no matter how increasing the concentration of the surfactant unlike nitrobenzene.

As seen in Table II, it is obvious that *o*-methoxybenzaldehyde does not distribute to the micelle of sodium lauryl sulfate. The determination procedure was same as nitrobenzene.

These experimental results of the intestinal absorption using the solubilized substances indicate evidently that the micelle formation of sodium lauryl sulfate decrease the absorption of incorporated substance when it distribute to the micelle in the significant amount. A scheme of the mechanism of surfactant effect on the intestinal absorption is summarized in Chart 2. In the case of anionic surfactant, the free ion is only sorbed into the intestinal tissue. Above the critical micelle concentration, the free ion of the surfactant is in equilibrium with the micelle and is a constant concentration, the critical micelle concentration. The movement of surfactant from the micelle to the free ion is rapid enough and thus the rate of the intestinal sorption becomes constant, namely the zero order kinetics. The solubilized substance in the surfactant solution distributes between the micelle and water phases. The substance in water phase is absorbed through the intestinal membrane and thus the micelle formation decrease the absorption rate by lowering the activity of the substance in the surfactant solution.

micelle-forming surfactant $\rightleftarrows$ free surfactant $\longrightarrow$ surfactant in intestinal tissue
solubilized substance $\rightleftarrows$ free substance $\longrightarrow$ substance in intestinal tissue

Chart 2. Behavior of Ionic Surfactant and Solubilizate in Intestine

The intestinal sorption of cetyltrimethylammonium bromide was also investigated as same experimental procedure as sodium lauryl sulfate. The results were shown in Fig. 5 and 6. Since the tendency of the decrease of the concentration in the inner solution was

quite same as sodium lauryl sulfate, the sorption mechanism can be elucidated as same as the anionic surfactant below and above the critical micelle concentration. In comparison with the anionic surfactant, the calculated zero order rate constant of the cationic surfactant is greatly different from the observed one.

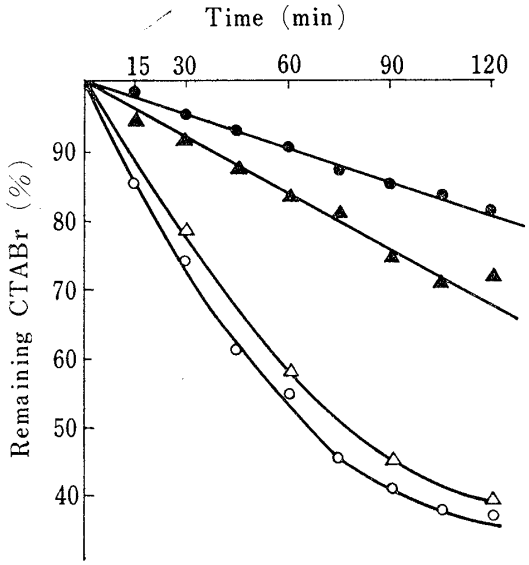

Fig. 5. The Percentage of Remaining Cetyltrimethylammonium Bromide in Perfusion Solution

—●— 10 mM CTABr —○— 0.2 mM CTABr
—▲— 5 mM CTABr —△— 0.1 mM CTABr
CTABr: cetyltrimethylammonium bromide

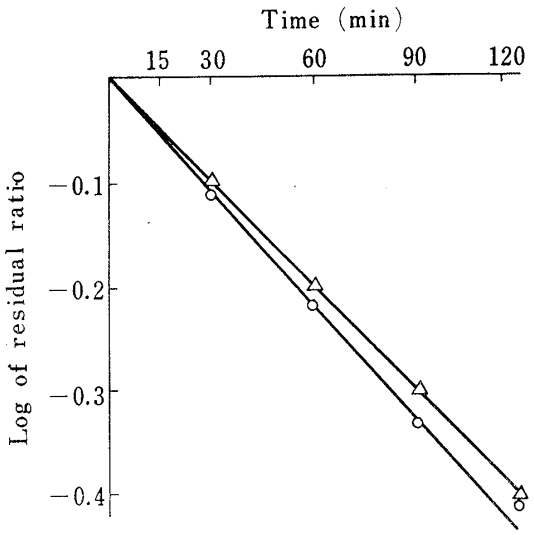

Fig. 6. Residual Ratio of Cetyltrimethylammonium Bromide plotted in the Logarithmic Scale

Experimental

Experimental Procedure——The small intestines were removed from fasted male albino rats (Donryu) weighing 200—300 g by the dissection under anaesthesia with pentobarbiturate. The intestine was set in the perfusion tube described in the previous paper.[10] Both inner and outer solutions were sampled at definite intervals and assayed. The outer solution was Tyrode's incubation solution. The inner solution was made with isotonic phosphate buffer solution (pH 6.4) containing surfactant, NaCl and the compounds to examine the absorbability.

Surfactants and the Test Compounds——Sodium lauryl sulfate was washed with ether and recrystalized from ethanol, mp 187°. Cetyltrimethylammonium bromide was washed with ether and recrystalized from aqueous solution of acetone, mp 237°. Nitrobenzene and *o*-methoxybenzaldehyde was guaranteed reagents.

Determination of the Critical Micelle Concentration (cmc)——Sudan III solubilization method was used for cmc determination of surfactant in the isotonic phosphate buffer solution at 37°.[11]

Assay Procedure——Sodium lauryl sulfate was assayed as described in the previous paper[11] from adequately diluted sample. Cetyltrimethylammonium bromide was assayed by Few's method[12] as follow. To 1 ml of diluted sample in 50 ml glass stoppered bottle add 10 ml of 0.1 mM orange II solution dissolved in 0.1 M NaCl aqueous solution and 15 ml of Chloroform. Then, shake it during 20 min and centrifuge during 5 min. Remove the aqueous layer and measure the optical absorbance at 485 mμ by spectrophotometer (Hitachi Perkin-Elmer 139 UV-VIS Spectrophotometer). Nitrobenzene wasassayed from directly diluted sample at 268 mμ. The blank was taken from the same experimental procedure using the surfactant solution without nitrobenzene. *o*-Methoxybenzaldehyde was assayed as same as nitrobenzene at 323 mμ.

Determination of Distribution Coefficient of Solubilizates between Aqueous and Micellar Phases——Distribution coefficients of nitrobenzene and *o*-methoxybenzaldehyde between aqueous and micellar phases were determined by the procedure reported in the previous study.[13]

Acknowledgement This work was supported by the Grant-in Aid for Scientific Research provided by the Ministry of Education to which the authors are grateful.

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