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Effect of Surfactants on Transmucosal Fluid Movement and Drug Absorption from Rat Small Intestine

Shikifumi Kitazawa, Masahiro Ishizu,[1a] and Keiko Kimura (née Kinouchi)[1b]

Department of Pharmacy, Kyoto University Hospital[1a] and Faculty of Pharmaceutical Sciences, Kyoto University[1b]

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Effects of surfactants on salicylamide absorption was investigated from the view point of the transmucosal fluid movement using the regular *in situ* recirculation perfusion technique with the rat small intestine. Surfactants used were polysorbate 80, Pluronic F 68 and Pluronic L 64, and they were dissolved in perfusates in appropriate concentrations. To obtain an accurate ratio of the fluid movement in the presence of surfactants, the cylinder method was developed originally in our laboratory, and validity of the method was demonstrated.

Based on the results obtained concerning the effect of surfactants on the drug absorption, the effect of the surfactant on the transmucosal fluid movement was apparently demonstrated. Since extent of the affection was unnegligible, it was suggested that the factor of the transmucosal fluid movement should be taken into considerations as well as the effect of the entrapment of the drug in micelle of the surfactant when the effect of the surfactant on drug absorption would be studied.

However, the mode of the affection was different from those brought about by osmotical change, and the surfactant inhibited only in the process of fluid absorption. Based on the difference in nature, the "water-molecule-holding" effect of the surfactant was considered and discussed.

Keywords——transmucosal fluid movement; surfactant's effect on drug absorption; *in situ* recirculation perfusion experiment; cylinder method; polysorbate 80; Pluronic F 68; Pluronic L 64; salicylamide absorption; water-molecule-holding effect of surfactant; intestinal drug absorption

Surfactants are one of the most important and most frequently used adjuvants in pharmaceutical preparations.[2] In fact, numbers of oral and parenteral solutions of oil-soluble vitamins, hormones and other drugs which are sparingly soluble in water have now been prepared using surfactants. In addition to such usage as solubilizer, the agents have been introduced in various dosage forms for diverse purposes, which are not necessary to repeat mention here.

These recent tendencies had stimulated studies related to the effect of these surfactants on the intestinal drug absorption and many publications of Kakemi,[3,4] and Gibaldi[5,6] had been accumulated in literature. After inquiring concerned publications, it was suggested as indicated in reviews of Gibaldi,[7] and Gibaldi and Feldman[8] that the absorption of drug might be subtly affected by the concentration of surfactant in the solution. When the concentration of the agent was less than the critical micelle concentration (c. m. c.) of the surfactant, an in-

1) Location: *a*) *Kawara-cho Shogoin, Sakyo-ku, Kyoto*; *b*) *Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto.*
2) K. Furuse, *Yukagaku*, **18**, 114 (1969).
3) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 861 (1965).
4) K. Kakemi, H. Sezaki, S. Muranishi, and A. Yano, *Chem. Pharm. Bull.* (Tokyo), **18**, 1563 (1970).
5) S. Feldman and M. Gibaldi, *J. Pharm. Sci.*, **58**, 425 (1969).
6) S. Feldman, M. Salvino, and M. Gibaldi, *J. Pharm. Sci.*, **59**, 705 (1970)
7) M. Gibaldi, *Federation Proceedings*, **29**, 1343 (1970).
8) M. Gibaldi and S. Feldman, *J. Pharm. Sci.*, **59**, 579 (1970).
9) V.T. Marchesi and G.E. Palade, *J. Cell Biol.*, **35**, 385 (1967).

crease in drug absorption might be observed and, on the other hand, in the case when the concentration was more than the c. m. c., drug absorption might be found decrease.

Mechanisms involving in the increasing in drug absorption were not elucidated clearly, however, Marchesi's findings[9] that surfactant, sodium lauryl sulfate, could stimulate both the Mg–ATPase and Na–K–ATPase in the membrane of erythrocyte ghost at low concentration and inactivations were observed at high concentration of the agent, suggested surfactant might activate enzymatic activities at low concentration, which would affect membrane transport of substances. On the other hand, the mechanism involved in the decreasing was demonstrated that amount of the drug to be absorbed might be decreased due to entrapment into micelle of the surfactant. The demonstration was seemed to be quite reasonable and, in fact, had been supported by many investigations.[10–13]

Authors of the present study had concentrated their attentions on the transmucosal fluid movement and the fluid movement had been revealed to affect on the drug absorption from the rat small intestine in unnegligible extent.[14,15] The present study was undertaken to elucidate the effect of surfactant on the transmucosal fluid movement.

Experimental

Drug and Surfactants——Along the line of purposes of the present study, it seemed to be preferable to choose a drug which has pK_a value nearer to the virtual pH[16] of the rat small intestine than sulfanilamide with which a series of studies concerning the transmucosal fluid movement had been conducted in this laboratory. Salicylamide was selected as the subjected substance, since pK_a of this substance is 8.2,[17] and it was considered that comparison in the absorption in both of the forms of unionized and ionized might be possible with perfusates having pH of not so far from the virtual pH of the rat small intestine. The assay method employed in determining the drug in the perfusates was followed to the method used in the report of Yamada[13] with some modifications, and it was found that the method was not interfered by existence of surfactants used in the present study.

Nonionic surfactants of polysorbate 80, obtained from Tokyo Kasei Co., and Pluronic F 68 and L 64, obtained from Asahi Denka Industries Co., Ltd., of the most purified grade were used with appropriate concentrations.

Test Solutions——Two kinds of the test solutions were employed in the present study with purpose of investigating the absorption of the drug in both of ionized and unionized forms of the substance. One was sodium chloride solution and the other was carbonate–bicarbonate buffer solution having pH of 9.5 (1.70 g of sodium carbonate and 2.86 g of sodium bicarbonate were dissolved in 1000 ml of distilled water to obtain an isotonic solution).

In the first stage of the present study, the absorption of salicylamide was investigated without the surfactants. In these cases, the test solutions contained three different levels in concentration of respective components to obtain hypertonic, isotonic and hypotonic test solutions and the straight regression lines of absorption of each unionized and ionized form of the drug was obtained. Based on these lines, the absorption of salicylamide was determined following the protocol which was presented in the previous report.[15]

In the second stage of the study, the effect of the surfactant on the drug absorption was investigated in relation to the transmucosal fluid movement. Unless otherwise stated, the concentration of sodium chloride or buffer components was kept in low level to obtain hypotonic solution. The concentration of surfactant was set at an appropriate range depending upon the inhibitory characteristics of these surfactants, however, the concentrations were much higher than the c. m. c. of these surfactants.[18]

The osmolarity of these test solutions were determined before they were employed in the perfusion experiment. The determination was conducted using Advanced DigiMatic Osmometer of model 3D of Advanc-

10) J.A. Nissim, *Nature*, **187**, 308 (1960); *idem*, *Biochem. Pharmacol.*, **8**, 21 (1961).
11) C.B. Taylor, *J. Physiol.*, **165**, 199 (1963).
12) R.H. Engel and S.J. Riggi, *Proc. Soc. Exp. Biol. Med.*, **130**, 879 (1969).
13) H. Yamada and R. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **13**, 1279 (1965).
14) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).
15) S. Kitazawa, M. Ishizu, and E. Arakawa, *Chem. Pharm. Bull.* (Tokyo), **24**, 3169 (1976).
16) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 491 (1964).
17) T.R. Bates, D.A. Lambert, and W.H. Jones, *J. Pharm. Sci.*, **58**, 1468 (1969).
18) I.R. Schmolka and J.A. Raymond, *J. Am. Oil Chem. Soc.*, **42**, 1088 (1965).

ed Instruments Inc., Needham Heights, Mass. U. S. A. The osmolarity of these test solutions was almost the same as that of the test solution which did not contain surfactant. This evidence suggested that the surfactants employed in the present study did not affect on the osmolarity of the perfusate and all the perfusion experiments were conducted with osmotically equivalent perfusates in every cases.

Determining the Transmucosal Fluid Movement——Since phenol red which had been employed as an unabsorbable indicator of the transmucosal fluid movement had not been demonstrated not to be absorbed from the rat small intestine in the presence of surfactant and the same apprehension might be remained in the case of using other indicators,[19] authors were obliged to develop some other device which could determine the fluid movement without using these unabsorbable indicators.

As had been described in the previous paper,[14] the perfusion apparatus used in the experiment contained a conical flask of 50 ml volume as a reservoir of the perfusate. The flask was replaced by a graduated cylinder of 50 ml volume having a diameter of not more than 2 cm. The volume changes during the course of the perfusion were observed and were plotted in a graph having the volume of the perfusate in the cylinder on the vertical axis and the time on the horizontal axis. Since these plots formed a straight line, the line was extrapolated to the time zero and an intercept on the vertical axis was obtained.

The volume at the intercept would be regarded as the resultant volume subtracting volumes of perfusates in tubings of the perfusion apparatus and in lumen of the small intestine from the initial volume of the perfusate which was 40 ml, and the increase in volume of the perfusate due to fluid secretion from tissues in the animal body and the decrease due to absorption of the perfusate would be reflected on the volume change in the graduated cylinder. The ratio of the volume at 1 hr after the perfusion over the volume at the intercept might be regarded as the ratio of the transmucosal fluid movement occurred during the course of the perfusion experiment.

To demonstrate a validity of determining the fluid movement ratio with the device which was originally developed in our laboratory, ratios of the transmucosal fluid movement were investigated in perfusion experiment with the cylinder method and the regular phenol red method simultaneously. The results were presented and discussed in the section of "Results and Discussion."

Animal Procedures and Perfusion Experiment——Animal procedures employed in the present work were just the same as those in the previous report.[14] However, some modifications were adopted in the perfusion experiment due to the cylinder method. In case of the perfusion study using a nonabsorbable indicator, the initial sample of the perfusate should be taken up at a delayed period after the beginning of the perfusion, since it was considered to take some period of time to mix well the perfusate with the washings left in the small intestine and to obtain uniformity in concentration of the indicator in the perfusate. However, things were changed in the case of using the cylinder method. There were no necessities to set up a lag time in sampling and even to take up the initial sample at the beginning of the perfusion study. An original concentration of drug in the test solution might be regarded as the initial sample in the case of the indicator method, and the volume at the intercept might be used as substitute for the concentration of the indicator in the initial sample of the indicator method.

The beginning of the perfusion study was set at the time when the lumen of the small intestine was filled for the first time with the perfusate.

As have been mentioned so far, it was suggested to be necessary that the flow of the perfusate in the small intestine should be kept smooth during all courses of the perfusion experiment. Care should be concentrated in the animal procedures to obtain the smooth flow in the circulating system adopted with the cylinder method.

Determining of the Drug Entrapped in Micelle——To estimate an amount of the drug which was entrapped in micelle of surfactant, an apparent distribution constant, K_m, was obtained with an equilibrium dialysis study using the test solutions which were about to be employed in the perfusion experiment. Visking cellulose tubings of 8/32 inches in diameter, obtained from Visking Co., Chicago, Illinois, U. S. A., was soaked in boiling water for a half an hour and washed with distilled water for three times. Dialysis bags prepared with the tubings were filled with 5 ml of the test solution. The dialysis was proceeded in a test tube containing the equal volume of the test solution which did not contain the drug and the surfactant.

After shaking the test tube in an incubator of Taiyo Co. at 37° for one hour, the dialyzed drug which was in the solution outside of the bag was measured and the concentration of the free drug in the dialyzed medium was obtained. The concentration of the drug in micelle was also obtained after appropriate calculations. Using the results, the apparent distribution constant was calculated following an equation, $K_m = M/(F \times S)$, developed by Kakemi and his co-workers,[20] where M was the concentration of the drug in micelle (mcg/ml), F was the concentration of the free drug in the dialyzed medium and S was the concentration of the surfactant in the test solution (g %).

19) D.L. Miller and H.P. Schedl, *Gastroenterol.*, **62**, 48 (1972).
20) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 976 (1965).

Results and Discussion

Validity of the Cylinder Method

As the cylinder method for determining the ratio of the transmucosal fluid movement was originally developed and was arbitrarily terminated in our laboratory, confirmations on the validity of the method should be demonstrated at this stage of the study. A series of regular perfusion experiments using perfusates containing different concentrations of sodium chloride and fixed concentration of phenol red were conducted, and the ratios of the fluid movement were determined following both of the cylinder method and the indicator method. The results were illustrated in a graph which had the ratio obtained by the indicator method on the vertical axis and by the cylinder method on the horizontal axis, and Fig. 1 was obtained.

As was evident from Fig. 1, scattered plots had a straight regression line ($y=0.998x+0.015$, $n=18$, $r=0.989$) with a slope of almost 1. This evidence demonstrated that the cylinder method had an enough validity in being applied as a method of determination of the fluid movement with almost the same extent of accuracy as that of the indicator method which had been employed in a series of this study in our laboratory, and that the cylinder method was applicable in any case of the bidirectional transmucosal fluid movement since a straight regression line was able to be illustrated over a wide range of the ratio from 0.6 to more than 1.2 which included fluid absorption and fluid secretion in the small intestine.

The advantage of applying the cylinder method in the perfusion experiment would be decrease in times of sampling taken up from the perfusate for the purpose of determining the ratio of the fluid movement. The initial samples for determining both of the fluid movement and the drug absorption which were indispensable in the indicator method were not needed in the cylinder method. The protocol suggested that the indispensable sampling in the cylinder method would be limited to only one sampling which should be taken up in the final stage of the perfusion for the determination of the concentration of drug in the final perfusate. These

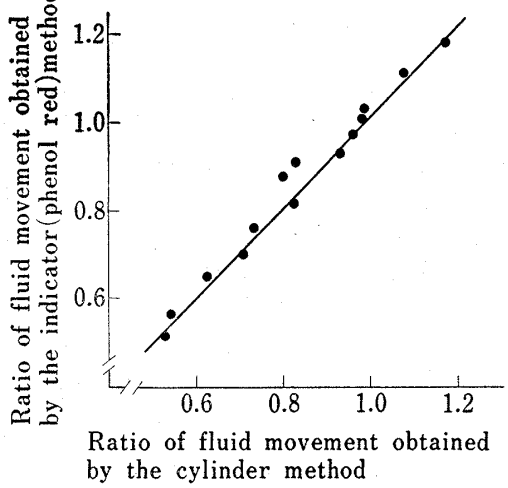

Fig. 1. Relationship of Ratios of the Transmucosal Fluid Movement obtained between by the Indicator Method and by the Cylinder Method

The regression line in the figure was obtained by the least squares method.

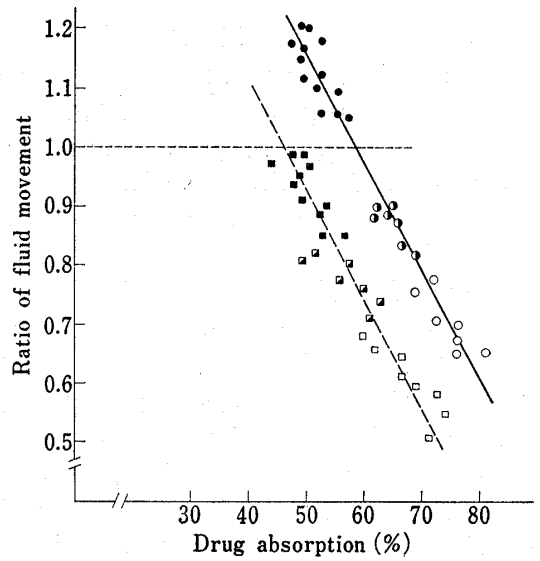

Fig. 2. Relationship between the Ratio of the Transmucosal Fluid Movement and the Absorption of Salicylamide in both Forms of Unionized and Ionized

The relationship of the unionized form was represented as a solid line and that of the ionized form was represented as a broken line.
key;
●: hypertonic sodium chloride solution
◑: isotonic sodium chloride solution
○: hypotonic sodium chloride solution
■: isotonic carbonate-bicarbonate buffer solution of pH 9.5
◪: 3/4 of the isotonic of the buffer solution
□: 2/3 of the isotonic of the buffer solution

advantages would indicate that the perfusion experiment might be conducted with the whole volume of the perfusate which was 40 ml at the initial stage of the experiment without taking a certain volume of the perfusate as the sample in the course of the perfusion experiment.

These lines of advantages suggested that the developed method might be applicable in all of the perfusion studies in replace of the indicator method, however, a technical point should be remarked in employing the new method. The point was concerned in the processes of the animal procedures. As evident from descriptions mentioned so far, the cylinder method was depended on a constant flow of the perfusate not only in the small intestine of the subjected animal but also all the way including the pathway in the perfusion apparatus. Any particulate which would disturb the flow should be left from the tract before the perfusion experiment. Moreover, cares should be concentrated in curvatures of the small intestine in the abdomen of the animal. Without these causions, meniscus of the perfusate in the cylinder might be twisted up and down and a straight regression line in the graph indicating the time course change of the perfusate in the cylinder would be difficult to obtain.

These lines of evidences demonstrated that the cylinder method had enough validity as one of the method determining ratio of the fluid movement in *in situ* recirculation perfusion experiment under well treated conditions.

Absorption of Salicylamide

Along with a purpose of investigating the absorption of the drug in an intact condition, that is without surfactants, the perfusion experiments using the two kinds of test solutions were conducted at the first stage of the present investigation.

To obtain hypertonic, isotonic and hypotonic perfusates, 1.2%, 0.9% and 0.6% of sodium chloride solutions were applied. However, in the case of the buffer solution of carbonate-bicarbonate, three levels in concentration having all hypotonic tonicities were prepared, since sodium carbonate exerted toxic effect at the isotonic concentration as had been demonstrated in the previous report.[15] The results of these experiments were illustrated in Fig. 2 in the same manner as described in the previous report.[14]

As was evident in Fig. 2, two straight regression lines of the unionized form of the drug, $y=-0.019\,x+2.10$ ($n=24$, $r=-0.932$), and the ionized form of the drug, $y=-0.019\,x+1.91$ ($n=25$, $r=-0.910$), were obtained and, moreover, they were exactly parallel to each other in a wide range of the ratio of the fluid movement.

The regression lines had intercepts on a line indicating 1.0 in the ratio of the fluid movement. Following the protocol proposed in the previous report,[15] the absorption of salicylamide from the perfusate containing sodium chloride, in which the drug was expected to be in unionized form, was 58% and from the perfusate of pH 9.5 buffer solution in which the drug was expected to be in ionized form was 46.5%. The difference in the absorption might be explained as the pH-partition hypothesis of Brodie, Schanker and their coworkers.[21]

Although the absorption of the drug in hypertonic perfusate of pH 9.5 buffer solution was not conducted due to anxiety of appearance of toxic effect to the animal, the intact absorption of salicylamide was thus investigated and it was found that the absorption of the drug was also influenced by the transmucosal fluid movement like other drugs which had been investigated in a series of studies in our laboratory, and that the absorption was also affected by pH of the perfusate, suggesting that the absorption of salicylamide might obey the pH-partition hypothesis.

Degree of Entrapment of Salicylamide in Micelle of Surfactants

Before starting the investigations on the effect of surfactant on the drug absorption, it would be desirable to know approximate degree of entrapment of the drug both in forms of

21) P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exper. Therap.*, **119**, 361 (1957); L.S. Schanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *ibid.*, **120**, 528 (1957).

Table I. The Distribution Constants (K_m) of Unionized and Ionized of Salicylamide in Micelle of the Surfactants Used in the Present Study

Surfactant	K_m	
	Unionized	Ionized
Polysorbate 80	0.36	0.08
Pluronic F68	0.12	0.076
Pluronic L64	0.085	0.001

The result is an average of more than 10 experiments.

unionized and ionized in these surfactants used in the present study. Following an equilibrium dialysis method determining distribution constant of a drug in micelle which was developed by Kakemi and his co-workers,[20] apparent distribution constant, K_m, were calculated and listed in Table I.

As was evident in Table I, the unionized drug behaved to be entrapped much more than the ionized drug in every case of the surfactant. The difference in K_m between unionized and ionized form of the drug was almost a figure, however, the ratio of K_m values of unionized over ionized revealed that there might not be an uniformity in the difference in entrapment between the unionized and the ionized form of the drug.

Differences in K_m were also observed between these nonionic surfactants. Polysorbate 80 exhibited the largest values and Pluronic L 64 exhibited the smallest value of K_m when the drug was subjected in the unionized form. The same inclination was also observed in the case of the ionized form of salicylamide. These values were expected to influence the drug absorption in more or less extent.

Salicylamide Absorption in the Presence of Polysorbate 80

As indicated in the case of the buffer solution, concentrations of the buffer components were obliged to keep less than a half of the isotonicity. As the result of this, absorption of fluid would be proceeded during the course of the perfusion experiment. To make it possible to compare the effect of the surfactants on the ionized form of the drug with that of the unionized form, all the experiments were conducted with hypotonic perfusates.

Being along with the purpose of the experiment, concentrations of sodium chloride in the perfusate were fixed 0.9%, 0.6%, and 0.45%. Polysorbate 80 was added to respective perfusate in concentrations of 2.5%, 5%, 10%, and 20%. The *in situ* recirculation perfusion experiments were conducted using these perfusates, and the results were illustrated in the same manner as described in the previous report.[14]

Figure 3 shows the results obtained with the perfusates containing 0.9% of sodium chloride. Plots were not on the original regression line of salicylamide which was illustrated as a solid line in Fig. 2, and all the plots were appeared in a region where the absorption was decreased, that is, on the left hand side of the original regression line. The distance between plots and the regression line increased with increasing in concentration of the surfactant in the perfusate. These lines of evidences strongly supported findings of Yamada,[13] and others concerning the effect of surfactant on the drug absorption, which cited that drug absorption decreased in the presence of surfactant and that the absorption decreased more and more with increasing in concentration of the surfactant.

However, detailed observations of these plots in Fig. 3 revealed that these plots would have a regression line and, as the results of calculations, an equation $y=-0.0029\,x+1.02$ ($n=27$, $r=-0.893$) was obtained. As was evident from Fig. 3 and also from the slope of the equation, -0.0029, ratios of the transmucosal fluid movement was apparently increased with increasing in concentration of the surfactant in the perfusate.

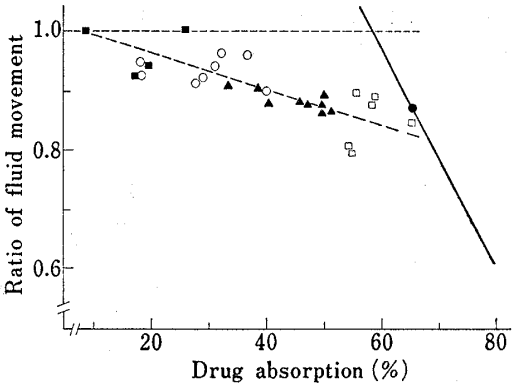

Fig. 3. Effect of Polysorbate 80 on the Salicylamide Absorption from the Perfusate Containing 0.9% of Sodium Chloride

The solid line in the figure represents the solid line of the original regression lines obtained in Fig. 2.
key: The per cent of the surfactant contained in the perfusate was indicated by the following symbols.
●: 0%, □: 2.5%, ▲: 5%, ○: 10%, ■: 20%

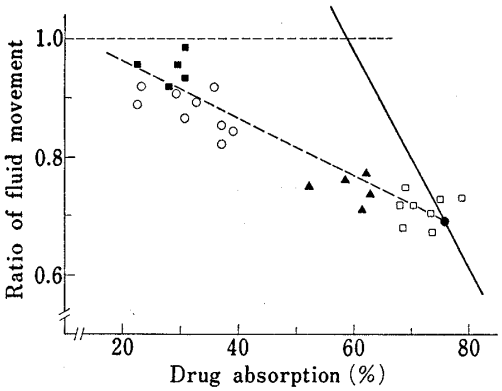

Fig. 4. Effect of Polysorbate 80 on the Salicylamide Absorption from the Perfusate Containing 0.6% of Sodium Chloride

The explanations and the key of the figure are the same as that of Fig. 3.

Although osmolarities of the perfusates were almost the same indifferent to the concentration of surfactant in the perfusate, it was not understandable that the ratio was increased, in other words, fluid absorption was apparently suppressed and the extent of the suppression was increased with increasing in concentration of the surfactant. This evidence suggested that surfactant might affect the transmucosal fluid movement which had been demonstrated to influence on the absorption of substance in the small intestine.

Figure 4 and Fig. 5 show the effect of the surfactant on the drug absorption when the concentration of sodium chloride was decreased to 0.6% and 0.45%, respectively. As expected, ratios of the transmucosal fluid movement decreased with decreasing in concentration of the sodium chloride in the perfusate, and the plots presenting the results obtained with the perfusates without the surfactant, which was presented as large solid circle, moved gradually along the regression line illustrated as solid line to the direction indicating that the transmucosal fluid movement was increased. Essentially similar patterns to that obtained in Fig. 3 were presented concerning the effect of the surfactant on the drug absorption, and the regression lines of the surfactant in the perfusate containing 0.6% sodium chloride was $y=-0.0048\,x+1.06$ ($n=23$, $r=-0.921$) and $y=-0.0057\,x+1.02$ ($n=21$, $r=-0.879$) in the case of 0.45% of sodium chloride. Considering based on differences in the slope of the regression line, the effect of the surfactant on the suppression of the transmucosal fluid movement was more exaggerated when the fluid moved more in extent.

As mentioned above, many studies demonstrated that surfactant decreased drug absorption when the concentration of the surfactant was more than the c. m. c., and concerning the mechanism involved in these decreasing effects, they pointed that the drug was entrapped in micelle of the surfactant. In the present study, the decreasing effect of surfactant on the drug absorption was apparently demonstrated, however, it was suggested in Fig. 3 to Fig. 5 that the mechanism might not be solely the entrapment, since all the regression lines obtained in the presence of the surfactant had certain slopes. This evidence strongly suggested that the transmucosal fluid movement did affect on the absorption in addition to the entrapment, since the regression line in the presence of the surfactant might be parallel to the horizontal axis and might not have slope if the entrapment were only the mechanism which decreased the drug absorption.

These lines of evidences and considerations encouraged authors to speculate further in advance in concerning degree of influence of these two factors of the fluid movement and the entrapment on the drug absorption. When the slope of the regression line was gentle and approached to the horizontal axis, the effect of the entrapment might be dominant. On the other hand, when the slope of the regression line of the surfactant was steep and approached to the original regression line of the drug, the effect of the fluid movement might be dominant.

These speculations were supported in the case when the drug absorption was investigated with using perfusate in which the drug was in the form of ionized form and was demonstrated that the fraction of the drug in micelle was less than that of unionized form as presented in Table I. The results were plotted in Fig. 6.

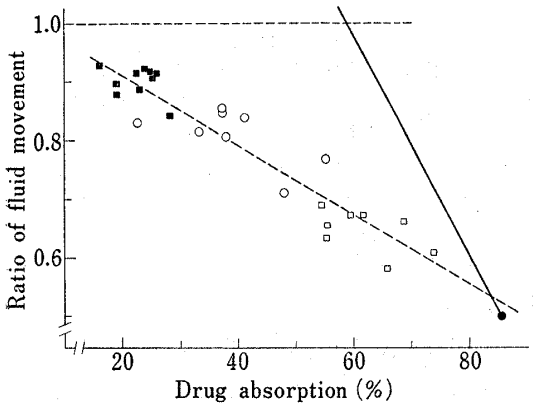

Fig. 5. Effect of Polysorbate 80 on the Salicylamide Absorption from the Perfusate Containing 0.45% of Sodium Chloride

The explanations and the key of the figure are the same as that of Fig. 3.

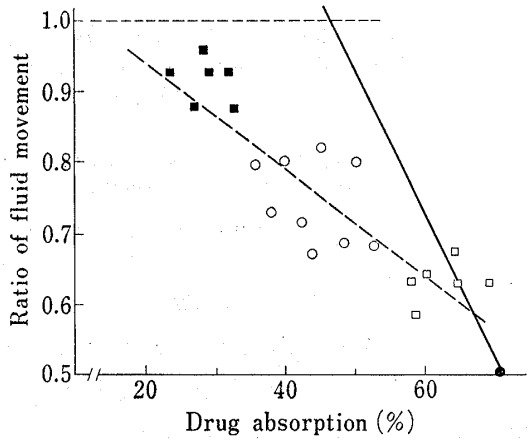

Fig. 6. Effect of Polysorbate 80 on the Salicylamide Absorption from the Perfusate of pH 9.5 Buffer Solution having a Half of the Isotonicity

The solid line in the figure represents the broken line of the original regression lines obtained in Fig. 2. The key of the figure was the same as that presented in Fig. 3.

As was evident in Fig. 6, absorption of the drug was decreased with increasing in concentration of the surfactant in the perfusate, and also the absorption was apparently affected by the fluid movement. Supposing from the slope of the regression line, $y=-0.0075x+1.09$ ($n=26$, $r=-0.898$), the affection of the fluid movement might be greater than any of those investigated so far. The steepness in slope of the regression line might be brought about by the reason that the fraction of the drug in micelle was smaller in comparison with the unionized form as presented in Table I.

Salicylamide Absorption in the Presence of Pluronic F 68

Another run of the *in situ* recirculation perfusion experiment was conducted using Pluronic F 68 in place of polysorbate 80. As had been examined, the fraction of the drug including both of ionized and unionized form in micelle was much less than those of polysorbate 80. The slope of the regression line was expected to be steep as that of the ionized form of the drug in the perfusate containing polysorbate 80.

As illustrated in Fig. 7, the slope of the regression line, $y=-0.010x+1.28$ ($n=21$, $r=-0.913$), was enough steep as that illustrated in Fig. 6. These inclinations in steepness of slopes of regression line would become most significant when the ionized drug was in the perfusate containing Pluronic F 68, which was illustrated in Fig. 8. The slope of the regression line, $y=-0.013x+1.4$ ($n=26$, $r=-0.936$), was -0.013 and almost similar to that of the original regression line of the drug.

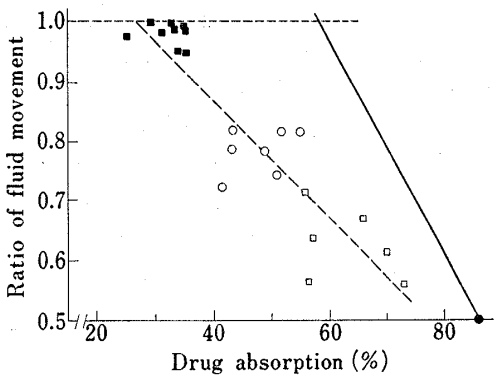

Fig. 7. Effect of Pluronic F 68 on the Salicylamide Absorption from the Perfusate Containing 0.45% of Sodium Chloride

The solid line in the figure represents the solid line of the original regression lines obtained in Fig. 2.
key: The per cent of the surfactant contained in the perfusate was indicated by the following symbols.
●: 0%, □: 2.5%, ○: 10%, ■: 20%

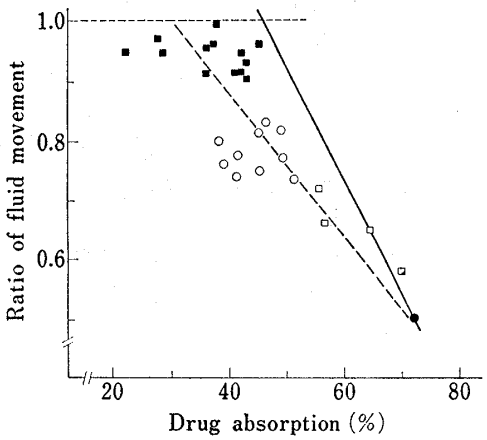

Fig. 8. Effect of Pluronic F 68 on the Salicylamide Absorption from the Perfusate of pH 9.5 Buffer Solution having a Half of the Isotonicity

The solid line in the figure represents the broken line of the original regression lines obtained in Fig. 2.
The key of the figure was the same as that presented in Fig. 7.

Salicylamide Absorption in the Presence of Pluronic L 64

Pluronic L 64 was introduced into our studies in place of Pluronic F 68. However, concentration of the surfactant was obliged to be decreased, since the absorption of the drug was decreased almost to zero percent when the surfactant was existed in the same concentration as those of polysorbate 80 and Pluronic F 68. A series of 0.1%, 0.25%, 0.5%, 1.0% and 2.5% in concentration of the surfactant was undertaken in runs of the perfusion experiment. The results obtained with the perfusates containing sodium chloride were illustrated in Fig. 9.

Contrary to our expectations, the regression line of these plots was not a straight line but had curvature having two asymptotic lines of the original regression line of the drug and a horizontal line indicating 1.0 in the ratio of the fluid movement.

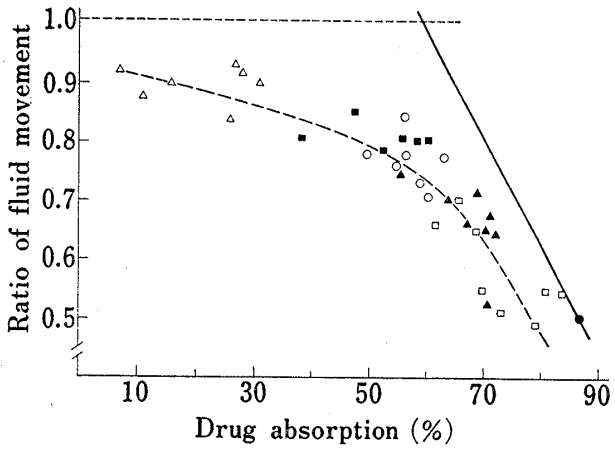

Fig. 9. Effect of Pluronic L 64 on the Salicylamide Absorption from the Perfusate Containing 0.45% of Sodium Chloride

The solid line in the figure represents the solid line of the original regression lines obtained in Fig. 2.
key: The per cent of the surfactant contained in the perfusate was indicated by the following symbols.
●: 0%, □: 0.1%, ▲: 0.25%, ○: 0.5%, ■: 1.0%, △: 2.5%

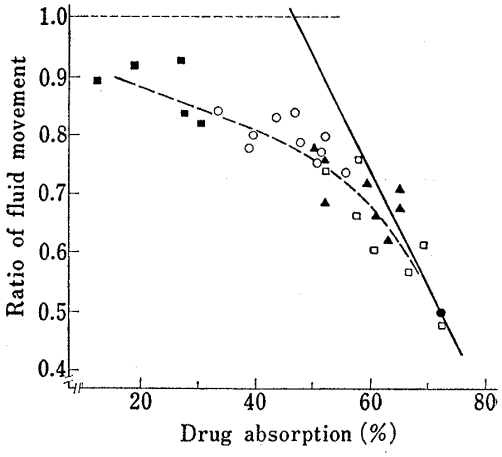

Fig. 10. Effect of Pluronic L 64 on the Salicylamide Absorption from the Perfusate of pH 9.5 Buffer Solution having a Half of the Isotonicity

The solid line in the figure represents the broken line of the original regression lines obtained in Fig. 2.
The key of the figure was the same as that presented in Fig. 9.

Based on K_m value of the surfactant, amount of the drug in micelle was considered much scarce even in the unionized form. Reflecting the evidence, the initial portion of the regression curve was almost parallel to the original regression line. As the concentration of the surfactant was increased up to 0.5%, the curve began to vent to the left hand side and approached to the line indicating 1.0 in the ratio of the fluid movement.

Almost similar pattern in the regression curve was revealed in the case of the ionized form as illustrated in Fig. 10. However, the initial portion of the curve was almost overlapped to the original regression line. This evidence demonstrated that, although the surfactant contained the minimum amount of the drug in micelle and did not affect tonicities of the perfusate, the surfactant did decrease the drug absorption, and that mechanism of the decreasing might be due to the decreasing in the transmucosal fluid movement, which was brought about by the addition of the surfactant.

The curved line was obtained in the case of this surfactant which was quite different from other surfactants used so far in the present study. However, the same phenomena might be possible to occur when the concentration of surfactant was increased more than 20% in the perfusate, which was not undertaken in the present study, since viscosity of the perfusate was increased extraordinary and other factors relating the viscosity[22] should be taken into considerations. As the results of these curvatures, plots were never placed in the region of fluid secretion which was above a straight line indicating 1.0 in the ratio of the fluid movement. This evidence suggested that the surfactant apparently induced the inhibition of the fluid absorption but might not induce fluid secretion into lumen of the small intestine.

Surfactant and the Transmucosal Fluid Movement

Based on evidences obtained in the present study, it was demonstrated that, in hypotonic conditions where fluid absorption was well progressed, surfactant inhibited the fluid absorption and the extent of the inhibition increased with increasing in concentration of the surfactant in the perfusate. These phenomena seemed to be the same as those observed in the previous studies[14,15] with glucose and other electrolytes. In the cases of glucose and other electrolytes, osmolarity of the perfusate was increased with increasing in concentration of these solutes, however, osmolarity was not changed significantly in these cases of the surfactants. This evidence suggested that the inhibition in the transmucosal inflow induced by the presence of surfactant was not due to tonicity change in the perfusate. Reflecting the suggestion, surfactant could not induce the fluid secretion even in concentration as high as 20% in the cases of polysorbate 80 and Pluronic F 68.

These lines of evidences made authors' attentions turn from osmolarity of the perfusate to another possibility which would be brought about in the presence of surfactant. The first possibility was alternation of absorbability of the drug in the presence of the surfactant. Alternation in partition coefficient of salicyclic acid in the presence of polysorbate 80 was reported Hikal and his coworkers.[22] However, they showed the coefficient was increased with increasing in concentration of the surfactant, and they could not obtain a parallel relationship between the coefficients and the drug absorption. Surfactant might alternate partition coefficient of salicylamide, however, supposing the results obtained by Hikal, *et al.*, the direction of the alternation would not be favourable in explanation both of decrease in the drug absorption and the transmucosal fluid movement.

The second possibility was damages in the epithelial layer of the small intestine due to the surfactant. Nadai and his co-workers[23,24] demonstrated histological changes of the epithelial surface of the rat small intestine after the perfusion of fluid containing ionic and nonionic sur-

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factants. However, if the damages were so serious as they indicated, the tissues would secrete fluid as Dennis[25] had demonstrated. As the results of the fluid secretion from the damaged tissues, the ratio of the fluid movement should be above 1.0 when the perfusion was conducted with perfusates containing high concentration of the surfactant. Based on these speculations, the histological change might not behave dominant role at least in decreasing both of the drug absorption and the transmucosal fluid inflow.

The third possibility was entrapment of water molecules in micelle of the surfactant. That substances are entrapped in micelle of surfactant is well known evidence. This entrapment of substance might accompany entrapment of water molecules, since strict selection between substance and water molecule might not be undertaken in forming micelle of the surfactant. According to Florence,[26] Becher and Arai,[27] shape of micelle of such nonionic surfactant would be an oblate spheroid or "log-boom" and there might be room for entrapment of water molecule in the micelle. Moreover, surfactant has both hydrophobic and hydrophilic radicals in a large molecule, and it is easily understood that water molecules will be held, attached or entrapped not only in micelle but also around of the molecule of the surfactant, in other words, surfactant will be hydrated in aqueous solution. This effect was terminated for the sake of convenience as "water-molecule-holding" effect of the surfactant. Water molecules which would be moved freely and bidirectionally depending on the tonicity of the perfusate would be held by the surfactant. As the result of these holding, the amount of free water would be decreased with increasing in the amount of surfactant in the perfusate. As the result of such decreasing in the fluid absorption, the drug absorption would be decreased. Since surfactant was not absorbed through the epithelial layers of the small intestine, water molecule held by the surfactant would remain in the lumen of the intestine. Thus the "water-molecule-holding" effect of the surfactant was considered most appropriate in explanation not only the decrease in both of the drug absorption and the transmucosal fluid inflow but all the phenomena disclosed in the present study without any contradictions.

Some of the surfactant, dioctyl sodium sulfosuccinate and others, are used widely in clinical medicine as an effective fecal softener or laxative agent.[28–30] However, mechanisms of such surfactants are not elucidated clearly. The "water-molecule-holding" effect of the surfactant which was proposed originally in the present study might open one of the ways elucidating mechanisms involved in fecal softening property of the surfactants.

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