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**129. Kiichiro Kakemi, Takaichi Arita, and Shozo Muranishi: Absorption and Excretion of Drugs. XXVII.\*<sup>1</sup> Effect of Nonionic Surface-Active Agents on Rectal Absorption of Sulfonamides.**

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Surface-active agents have been used so widely as emulsifiers, solubilizers and formulation adjuncts in pharmaceuticals. Particularly nonionic surface-active agents are used as a base in high concentration or as an ingredient of base when drugs are applied to the rectum as a suppository.

Nissim<sup>1)</sup> and Taylor<sup>2)</sup> reported that the surface-active agents reduced the rat intestinal absorption of glucose and methionin, and Riegelman<sup>3)</sup> also described in his reports that the rectal absorption of triiodophenole was retarded by surface-active agents. However the cause of reduction was not presented in their papers. On the other hand, enhancement of drug absorption has been observed when surface-active agents were added to a medication.<sup>4)</sup> Much of the complexity with the surface-active agents might be due to different properties and concentrations of surface-active agents, or different method for experiments.

The present report describes the effect the various nonionic surface-active agents on the rectal absorption of sulfonamides and shows that the effect of nonionic surface-active agents is due to the entrapment of the drugs in the micelles that is in accord with experimental data.

### Experimental

**Absorption Experiments**—Procedure of the absorption experiments has been described previously,<sup>5)</sup> the recirculation fluid was collected completely by washing with water after recirculation for one hour, and sulfonamides were determined. The drug solutions which contained 0.5 mmole/L. of sulfonamides and various concentrations of nonionic surface-active agents were prepared with isotonic buffered solution as described in the previous report.<sup>5)</sup> And 0.5 mmole/L. of Yellow AB incorporated in the solutions.

**Surface Tensions**—Surface tension measurements were made at 20° with a Du Nouy interfacial tensiometer.

**Equilibrium Dialysis**—Visking cellulose tubings, 24/32 inches in diameter, were previously boiled in water three times for half an hour, followed by thorough rinsing each time. The dialysis bags prepared from these tubings were filled with 20 ml. of solution (sulfonamide and nonionic surface-active agent in buffered solution), and fastened with a rubber band around the bottle at the upper end. Approximately three days was allowed at 37° for equilibration against equal volumes of the buffered solution, which was accelerated by mechanical shaking. If necessary, the sulfonamides in both the dialyzed solutions and the dialyzates were then diluted, and determined by the regular procedure, and nonionic surface-active agents in the dialyzed solution were determined. The sulfonamides in the dialyzed solution represent the sum of both free and entrapped concentrations, and those in the dialyzates represent free drug concentrations.

**Solubilities**—The solubilities of sulfonamides at various concentrations of nonionic surface-active agent were studied as described in the previous report.<sup>6)</sup>

**Experiments with Sacs of the Rectum *in situ***—These experiments were employed for rectal absorption from the drug suspensions. An operation procedure for animal was described elsewhere. The proximal

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1) J. A. Nissim: Nature, 187, 308 (1960).

2) C. B. Taylor: J. Physiol. (London), 165, 199 (1963).

3) S. Riegelman, W. J. Crowell: J. Am. Pharm. Assoc., Sci. Ed., 47, 127 (1958).

4) P. M. Lish, J. H. Weikel: Toxic. Appl. Pharmacol., 1, 501 (1959).

5) K. Kakemi, T. Arita, S. Muranishi: This Bulletin, 13, 861 (1965).

6) *Idem*: *Ibid.*, 13, 969 (1965).

end of the male rat rectum was closed by a ligature, and 0.3 ml. of the drug suspension was filled, and then the distal end was ligatured closely. After one hour, a heart blood was taken for total (free and acetylated) sulfonamide determination. The drug suspensions were prepared with sulfisoxazole of 100 mesh particle size and nonionic surface-active agent to allow for equilibrium at 37°.

**Analytical Methods**—Sulfonamides in solution and total sulfonamides in blood were determined by the regular procedure as previously described<sup>7)</sup>. Nonionic surface-active agents were determined according to the method of Stevenson<sup>8)</sup>. Yellow AB was measured at 460 m $\mu$  after extraction with chloroform.

## Results

### The Reduction of the Sulfonamide Absorption Rate by Nonionic Surface-Active Agent

The relationship between the concentration of Polysorbate 80 and the absorption rate of three unionized sulfonamides was shown in Fig. 1, where Polysorbate 80 was added to give the concentrations of 1, 3, 5, 10 and 20 g.%. Each spot in the figure represents the mean of four animals. Fig. 1 shows that the absorption rates of all

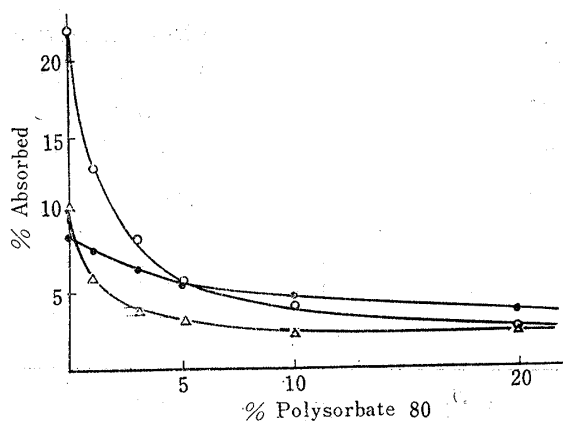


Fig. 1. Effect of Polysorbate 80 on Rectal Absorption of Various Unionized Sulfonamides

○—○ Sulfisoxazole pH 3.3  
 △—△ Sulfaethylthiadiazole pH 3.8  
 ●—● Sulfapyridine pH 5.5  
 Each spot is expressed as the mean of four animals.

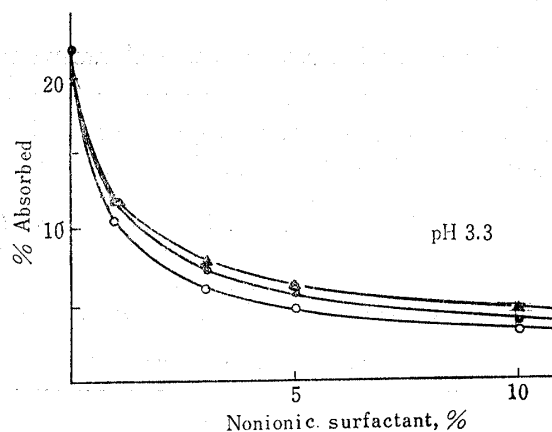


Fig. 2. Effect of Polyoxyethylene Sorbitan Alkyl Esters on Rectal Absorption of Unionized Sulfisoxazole

▲—▲ Polyoxyethylene sorbitan mono-laurate  
 ●—● Polyoxyethylene sorbitan mono-palmitate  
 ○—○ Polyoxyethylene sorbitan mono-stearate

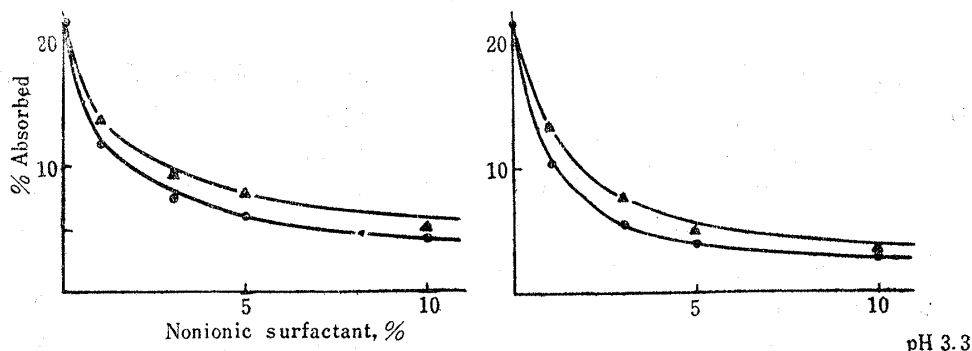


Fig. 3. Effect of Polyoxyethylene Alkyl Esters and Ethers on Rectal Absorption of Unionized Sulfisoxazole

▲—▲ Polyoxyethylene stearate (45)      ▲—▲ Polyoxyethylene lauryl ether (10)  
 ●—● Polyoxyethylene stearate (30)      ●—● Polyoxyethylene lauryl ether (15)

7) K. Kakemi, T. Arita, S. Muranishi: Arch. Pract. Pharm., 23, 39 (1963).

8) D.G. Stevenson: Analyst, 79, 504 (1954).

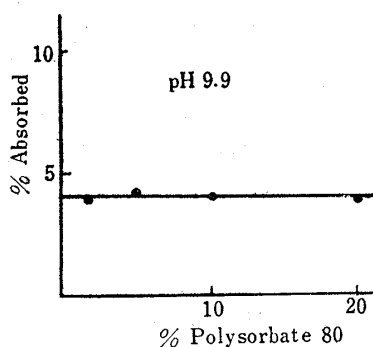


Fig. 4. Effect of Polysorbate 80 on the Rectal Absorption of Ionized Sulfisoxazole

in Fig. 4, indicating that the absorption of the ionized drug is not affected by the surface-active agent.

#### Absorption of Sulfonamide at a Concentration below the Critical Micelle Concentration

The absorption rate of sulfonamides was investigated at a very low concentration of nonionic surface-active agent below the critical micelle concentration, with the same manner in the previous section. The results obtained from surface tension measurement

TABLE I. The Absorption Rates of Sulfonamides at Concentration below the Critical Micelle Concentration

Concentration of Polysorbate 80 (%)	0	0.005	0.01
Surface tension (dyne/cm.)	50.1	44.3	43.6
Sulfisoxazole	22.1	21.8	22.5
Sulfaethylthiadiazole	10.5	10.9	10.8
Sulfapyridine	8.8	9.0	8.4

showed that the critical micelle concentration of Polysorbate 80 was 0.01%. The absorption rates of unionized sulfonamide, at the concentrations of 0, 0.005, and 0.01% of Polysorbate 80, were compared as shown in Table I, indicating that the absorption rates of sulfonamides were not changed by these concentrations of the agent, and suggesting that nonionic surface-active agent does not affect the drug absorption at a concentration below the critical micelle concentration.

#### Distribution of Sulfonamides between the Aqueous Phase and the Micellar Phase

Smith<sup>9)</sup> reported that the distribution of organic solute to the soap micelles was explained by postulating adsorption of the solute on the micelles. It is convenient to consider the drug as being distributed between two phases, the water and the micelles. Therefore, the portions of drug in the water and in the micelles are considered to be in equilibrium according to the adsorption isotherm of Freundlich (1),

$$M = K_m \cdot F^{1/n} \cdot S \quad (1)$$

where M is the concentration of drug in micelles ( $\mu\text{g./ml.}$ ), F is the concentration of free drug in the water ( $\mu\text{g./ml.}$ ), S is the concentration of nonionic surface-active agent (g.%) and  $K_m$ , n are the constants.

By taking the logarithm of Eq. (1), Eq. (2) is obtained.

$$\log M/S = 1/n \log F + \log K_m \quad (2)$$

9) E.L. Smith : J. Phys. Chem., 36, 1672 (1932).

According to this equation, a plot of  $\log M/S$  against  $\log F$  is a straight line, and the constants may be evaluated from the slope  $1/n$  and the Y-intercept  $\log K_m$ .

To determine the quantitative relationship between drug in micelles and free drug in the water, equilibrium dialysis study and solubility study were applied. The equilibrium dialysis followed the method, which was employed by Yang<sup>10)</sup> and Patel<sup>11)</sup>. The solutions containing 0.25 or 0.5 mmole/L. of sulfonamide and 0.1 to 20% of nonionic surface-active agent were used in the dialysis bags. The results from dialysis equilibria of four unionized sulfonamide using Polysorbate 80 solutions are shown in Fig. 5. In each sulfonamide a good linearity exists among the spots, and the slopes of the

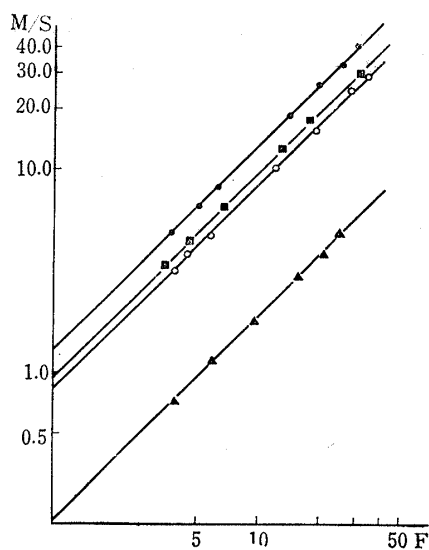


Fig. 5. Equilibrium Dialysis Curves of Various Unionized Sulfonamides with Polysorbate 80

- Sulfaethylthiadiazole pH 3.8
- Sulfamonomethoxine pH 3.8
- Sulfisoxazole pH 3.3
- ▲—▲ Sulfapyridine pH 5.5

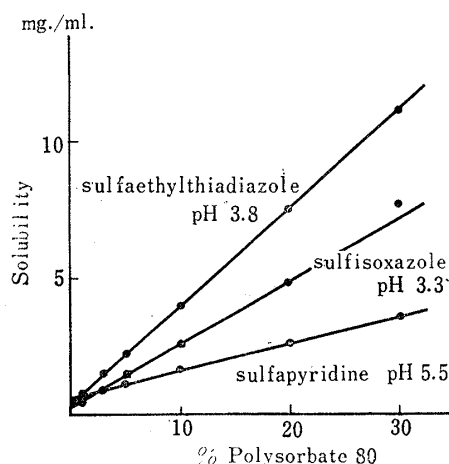


Fig. 6. The Solubility of Sulfonamides in Buffered Solutions containing Various Concentrations of Polysorbate 80

lines ( $1/n$ ) are almost identical and very nearly 1.0 as can be seen in Fig. 5. Thus neglecting the  $n$  in Eq. (1), gives,

$$M = K_m \cdot F \cdot S \quad (3)$$

where  $K_m$  is the apparent distribution constant.

The  $K_m$ 's obtained from Y-intercepts were 1.30 on sulfaethylthiadiazole, 0.95 on sulfisoxazole and 0.19 on sulfapyridine.

On the other hand, according to solubility study, the good linearity exists between the concentration of Polysorbate 80 and solubility of sulfonamides as shown in Fig. 6. In this figure, the concentration of free sulfonamide is a constant value corresponding to their water solubility, and the concentration of sulfonamide in micelles can be calculated as the difference between the total sulfonamide in solution and the free sulfonamide. Fig. 7 shows a plot of  $M/F$  against  $S$ , derived from solubility data, and in this case the slope of the line represents  $K_m$ . The apparent distribution constants,  $K_m$ 's obtained from two studies are summarized in Table II, and a comparison of values

10) J. T. Yang, J. F. Foster : J. Phys. Chem., 57, 628 (1953).

11) N. K. Patel, H. B. Kostenbauder : J. Am. Pharm. Assoc., Sci. Ed., 47, 289 (1958).

TABLE II. Values of Apparent Distribution Constant on Polysorbate 80

	Km from dialysis	Km from solubility
Sulfisoxazole	0.95	0.87
Sulfaethylthiadiazole	1.30	1.22
Sulfapyridine	0.19	0.20

of dialysis and solubility indicates fairly good agreement, showing that  $K_m$ 's are supported by two studies. The distribution data of unionized sulfisoxazole on polyoxyethylene sorbitan alkyl esters by equilibrium dialysis are shown in Fig. 8, and the similar data on polyoxyethylene alkyl esters and ethers are shown in Fig. 9. In these data, a good linearity exists among the spots, and the slopes of the lines are almost

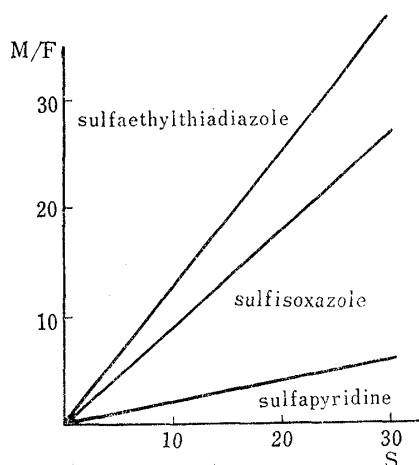


Fig. 7. Plot of M/F against S, Derived from Solubility Data

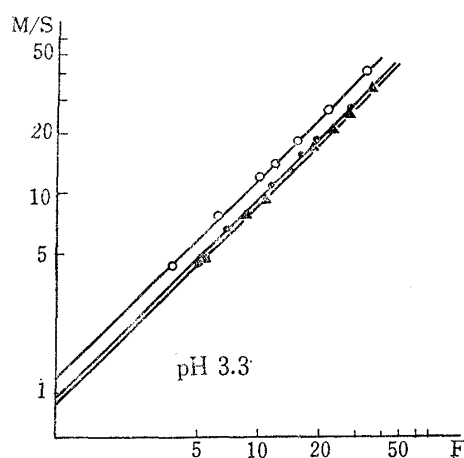


Fig. 8. Equilibrium Dialysis Curves of Unionized Sulfisoxazole with Various Polyoxyethylene Sorbitan Alkyl Esters

- Polyoxyethylene sorbitan mono-stearate
- Polyoxyethylene sorbitan mono-palmitate
- ▲—▲ Polyoxyethylene sorbitan mono-laurate

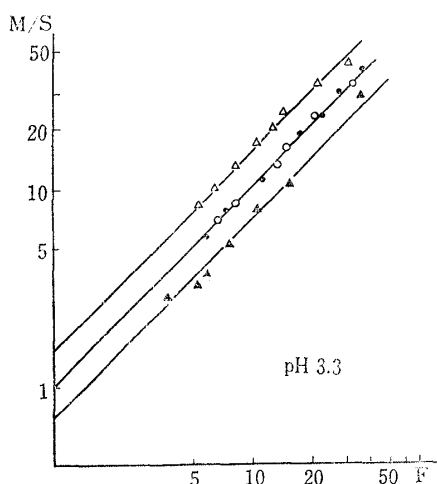


Fig. 9. Equilibrium Dialysis Curves of Unionized Sulfisoxazole with Polyoxyethylene Alkyl Ethers and Ethers

- △—△ Polyoxyethylene lauryl ether (10)
- Polyoxyethylene lauryl ether (15)
- Polyoxyethylene stearate (30)
- ▲—▲ Polyoxyethylene stearate (45)

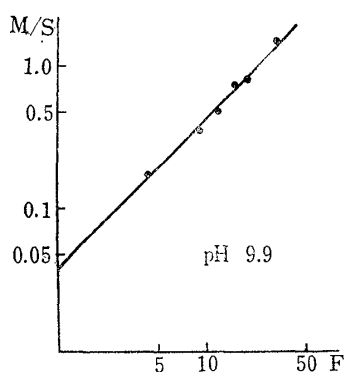


Fig. 10. Equilibrium Dialysis Curves of Ionized Sulfisoxazole with Polysorbate 80

TABLE III. Values of Apparent Distribution Constant on Unionized Sulfisoxazole

Nonionic surfactant	Km
Polyoxyethylene sorbitan mono-laurate	0.90
Polyoxyethylene sorbitan mono-palmitate	0.95
Polyoxyethylene sorbitan mono-stearate	1.20
Polyoxyethylene stearate (30)	1.00
Polyoxyethylene stearate (45)	0.70
Polyoxyethylene lauryl ether (10)	1.50
Polyoxyethylene lauryl ether (15)	1.00

identical and vary nearly 1.0. The apparent distribution constants, Km's on various nonionic surface-active agents with unionized sulfisoxazole obtained from equilibrium dialysis are summarized in Table III. The distribution data of the anion of sulfisoxazole on Polysorbate 80 by equilibrium dialysis are shown in Fig, 10, indicating that Km is very small (0.04).

The above results show that the amount of sulfonamide entrapped in micelles is increased with increase of the concentration of nonionic surface-active agent, the magnitudes of entrapment of unionized sulfonamide in micelles of Polysorbate 80 are arranged in decreasing order as sulfaethylthiadiazole, sulfisoxazole and sulfapyridine, and ionized sulfonamide is scarcely distributed to micelles. A comparison of Km's and absorption curves in Figs. 1, 2, 3 and 4 indicate a close relationship between absorption reduction and entrapment in micelles; the greater the Km, the greater the reduction effect on absorption rate.

#### Absorption of Drug Entrapped in Micellar Phase

The results obtained above, suggested that the drug entrapped in micelles was poorly absorbed or not absorbed. And then to determine whether the drugs in micelles are absorbed or not, 30% solution of Polysorbate 80 containing sulfonamide and 20% solution of Polysorbate 80 containing Yellow AB, azo dye, were employed. In these solutions the drug exists almost entrapped in micelles as shown in Table IV, where Yellow AB was completely entrapped in micelles by equilibrium dialysis. The results of absorption experiments indicate as shown in Table IV that the drug in micelles of Polysorbate 80 is absorbed about 1.5%, and suggest that the drug in micelles is absorbed

TABLE IV. Absorption Rates of Substances in Micelles using Polysorbate 80

	Polysorbate 80 (%)	F/M	% Absorbed (mean of 5 rats)
Yellow AB	20	0	1.6
Sulfaethylthiadiazole	30	0.026	1.4
Sulfisoxazole	30	0.036	1.6

TABLE V. Absorption Rates of Yellow AB in Micelles using 20% Solution of Various Surfactants

Nonionic surfactant	% Absorbed
Polyoxyethylene sorbitan mono-laurate	2.1
Polyoxyethylene sorbitan mono-palmitate	1.6
Polyoxyethylene sorbitan mono-stearate	1.5
Polyoxyethylene stearate (30)	2.0
Polyoxyethylene lauryl ether (10)	2.0
Polyoxyethylene lauryl ether (15)	1.6

with very difficulty, but the absorption rates are almost constant independent on the kinds of drugs.

Furthermore, Yellow AB was employed again for the absorption experiments of the drug in micelles of the various other nonionic surface-active agents. The absorption rates of Yellow AB were 1.5 to 2.1% in various nonionic surface-active agents as shown in Table V. These results show that the absorption rate of the drug in micelles is almost independent upon the kinds of the surface-active agents.

### The Enhancement of Sulfonamide Absorption by Solubilization

The solubilization of sulfisoxazole by Polysorbate 80 can be explained by Fig. 11.  $B_1$  is a concentration of sulfisoxazole, just solubilized in water at the concentration  $S_2$  of Polysorbate 80. When the surface-active agent is incorporated at the concentration  $S_1$ , a suspension is considered as the three-phase system consisting of the free sulfisoxazole in the solution (F), the drug entrapped in micelles (M), and the solid of the drug dispersed in the solution (H). M increases with increasing Polysorbate 80 up to  $S_2$ , and F is constant. Above  $S_2$ , the drug is completely solubilized and therefore M/F increases with increasing Polysorbate 80; the free drug concentration decreases. Among three components, the free sulfisoxazole is readily absorbed, and the solid of sulfisoxazole is not absorbed. If the drug in micelles is absorbed a little, it would be expected that the absorption rate increases in the concentration of surface-active agent below  $S_2$ , and decreases above  $S_2$ .

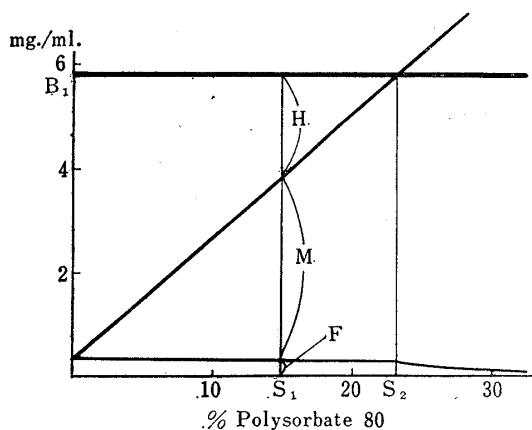


Fig. 11. Diagram Representing Sulfisoxazole in Solution of Polysorbate 80

H : solid of sulfisoxazole  
M : sulfisoxazole in micelles  
F : free sulfisoxazole in solution

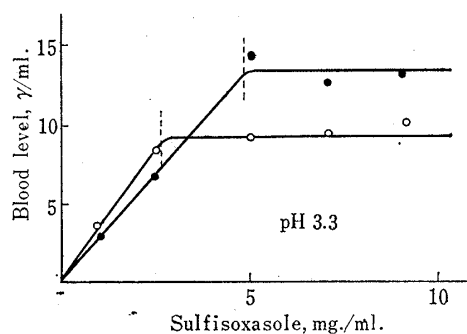


Fig. 12. Effect of Amount of Sulfisoxazole with a Constant Concentration of Polysorbate 80 on Blood Levels

○—○ Polysorbate 80 10%  
●—● Polysorbate 80 20%

To compare the amounts absorbed from these dispersed solution or solubilized solutions, the blood samples of rats were taken at one hour after administration of dispersed solution into sac of the rectum for total sulfonamide determination. Fig. 12 shows the relation between the blood levels and the administration doses of sulfisoxazole. At 10% of Polysorbate 80, the blood levels increased with increasing the concentration of sulfisoxazole up to its solubility (2.6 mg./ml. at 10% Polysorbate 80), but the blood levels were almost constant in the region above its solubility. Further, at 20% of Polysorbate 80, the blood levels were almost constant in the region above its solubility (5.0 mg./ml. at 20% Polysorbate 80). These indicate that the amount of absorbed drug depends upon its solubilized amount and the concentration of nonionic surface-active agent, as the solubility of sulfonamide is usually constant at 37°. And therefore the results indicate that the solid of drug dispersed is not related to absorption for one hour in this experimental condition.

The relationship between the blood level and the concentration of Polysorbate 80 was observed as shown in Fig. 13, when sulfisoxazole of constant concentration, above its solubility in water, was administered. When sulfisoxazole administered was 5 mg./ml., the blood levels present a linear increase with increasing Polysorbate 80 up to 20%, as expected. 5 mg. of sulfisoxazole is just solubilized in 1 ml. of water at the concentration of 20% of Polysorbate 80, as can be seen in Fig. 12. In the region above 20% of Polysorbate 80, the reduction effect for absorption was observed as described in the previous section. At 2.5 mg./ml. of sulfisoxazole solution, the blood levels were increased up to about 10% of Polysorbate 80 and reduced in the region above the concentration. At 1.0 mg./ml. of sulfisoxazole solution, a similar result was obtained.

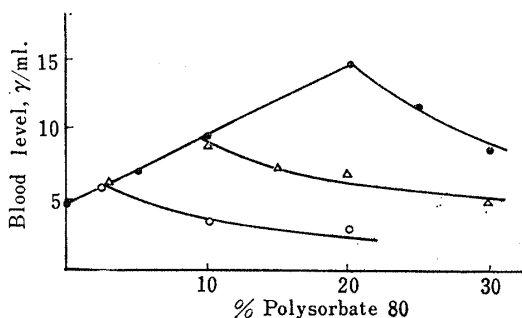


Fig. 13. Effect of Polysorbate 80 on Blood Levels of Sulfisoxazole

●—● 5 mg./ml.    △—△ 2.5 mg./ml.  
○—○ 1 mg./ml.  
Each spot is expressed as the mean of three animals.

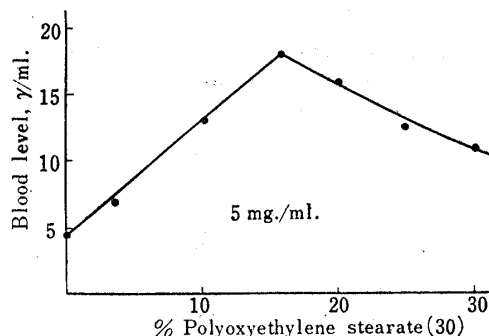


Fig. 14. Effect of Polyoxyethylene Stearate (30) on Blood Levels of Sulfisoxazole

Fig. 14 shows the effect of polyoxyethylene stearate (30) on the blood level of sulfisoxazole. A similar tendency was obtained to the effect of Polysorbate 80.

### Discussion

From the results obtained above, it is suggested that the free sulfonamide in the solution is readily absorbed, but the drug in micelles is poorly absorbed since the micelles are too large to pass through the rectal membrane.

Thus, the mechanism of drug absorption in the presence of nonionic surface-active agent will be represented as pictured diagrammatically in Fig. 15: the free drug (F) can be in general absorbed more readily than the drug in micelles (M), and there is an equilibrium relationship between the free drug and the drug in micelles, depending on the concentration of nonionic surface-active agent.

It is possible to derive a formula, which will express mathematically the absorption rate of drug,\*<sup>3</sup>

\*<sup>3</sup> Let  $f$  and  $m$  be the free drug and the drug in micelles fractions respectively. Therefore,

$$f + m = 1 \tag{i}$$

From Eq. (3),

$$m = K_m \cdot f \cdot S \tag{ii}$$

thus 
$$f = \frac{1}{1 + K_m \cdot S} \tag{iii}$$

The total absorption rate is given by

$$A_T = A_f \cdot f + A_m \cdot m \tag{iv}$$

Substituting Eq. (ii) and Eq. (iii), gives formula (4)

$$A_T = \frac{A_f}{1 + K_m \cdot S} + \frac{A_m \cdot K_m \cdot S}{1 + K_m \cdot S} \tag{4}$$



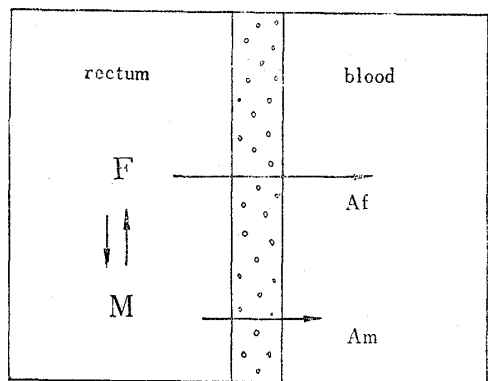


Fig. 15. Model Illustrating the Mechanism of Drug Absorption in the Presence of Nonionic Surface-active Agent

$$A_T = \frac{A_f \cdot S}{1 + K_m \cdot S} + \frac{A_m \cdot K_m \cdot S}{1 + K_m \cdot S} \quad (4)$$

where  $A_T$  is the total theoretical absorption rate of drug,  $A_f$  is the observed absorption rate of free drug,  $A_m$  is the observed absorption rate of drug in micelles and  $S$  is the concentration of nonionic surface-active agent. Thus if  $A_f$ ,  $A_m$  and  $K_m$  are known,  $A_T$  at a certain value of  $S$  can be calculated.

In Figs. 16 and 17, a comparison is made between the theoretical absorption rates and the experimentally determined absorption rates over the various concentrations of nonionic surface-active agents. An excellent agreement

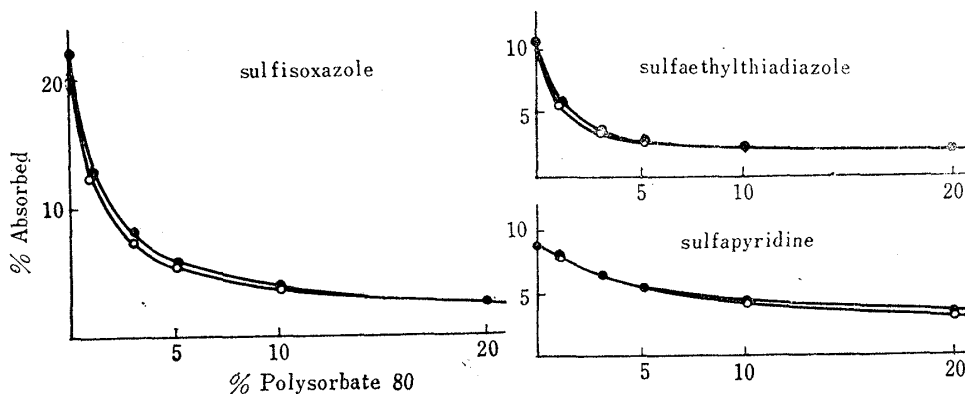


Fig. 16. Comparison between Observed Curves and Calculated Curves on Polysorbate 80

●—● Observed    ○—○ Calculated

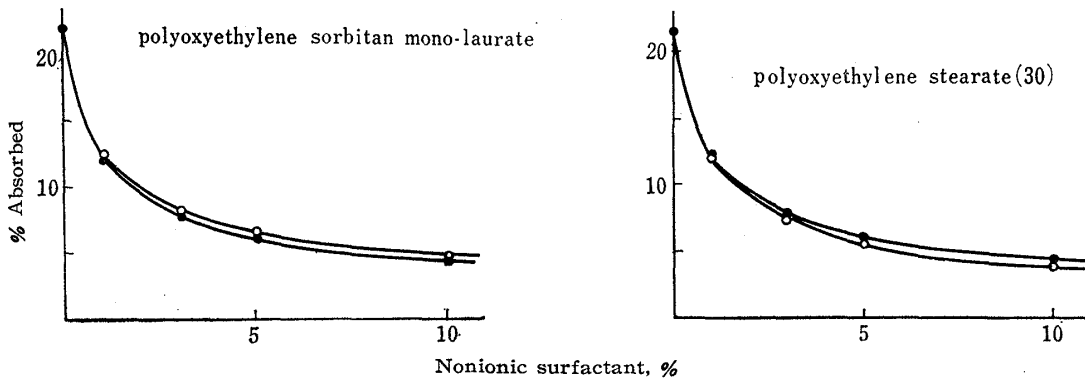


Fig. 17. Comparison between Observed Curves and Calculated Curves on Sulfisoxazole

●—● Observed    ○—○ Calculated

is obtained for all the sulfonamides and for all the nonionic surface-active agents. From the evidence obtained thus, one might conclude that the mechanism of the drug absorption in the presence of nonionic surface-active agent can be interpreted satisfactorily by separating the absorption of the free drug and of the drug in micelles.

As the other factors affecting on the drug absorption with surface-active agent, the following can be considered: a surface tension lowering, a specific interaction

between sulfonamide and nonionic surface-active agent, and a damage to rectal mucous membrane by nonionic surface-active agent. However, a surface tension does not particularly affect the drug absorption below critical micelle concentration in the present study. The evidence of specific interaction between sulfonamide and monomeric nonionic surface-active agent to form complexes was not obtained from solubility and ultraviolet absorption spectrum. Concerning damage to mucous membrane, Nissim<sup>12)</sup> has confirmed that nonionic surface-active agent like Polysorbate 80 exhibit no action to the gastrointestinal mucous membrane. Polysorbate 80 showed no histological injury to the rectal mucosa. Therefore it seems that these factors do not affect on the drug absorption.

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### Summary

The rectal absorption of sulfonamides were reduced by the addition of various nonionic surface-active agents. This reduction depends upon the entrapment of drug in micelles. The drug in micelles was also absorbed a little, and therefore the enhancement of drug absorption were obtained by solubilization of the drug remained as a solid in solution. The mechanism of drug absorption in the presence of nonionic surface-active agent can be explained by the concept that there are two ways of the passage of the free drug and of the drug in micelles.

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12) J. A. Nissim : Nature, 187, 305 (1960).