the case of hydrogenation of 2-methyl-1,2,3,4-tetrahydro-5-isoquinolinol[1],*1) and these observations relatively agree with Cocker's recent investigation[12] that hydrogenation of 2,3-xylenol over Raney nickel in methanol at 150° and 90 atm. gave four isomeric alcohols of 2,3-dimethylcyclohexanol and that the *cis-cis* form (XXId) was obtained in yield greater than the *trans-trans* form (XXIb) (Chart 5), although *trans*-decahydroisoquinoline was mainly produced by similar hydrogenation of isoquinoline.[13]

The author wishes to express his cordial gratitude to Prof. Kimoto for his kind guidance and unfailling encouragement throughout the course of this research, and for his revision of this manuscript. He is also indebted to the members of Analytical Center, University of Kyoto for elemental microanalyses and Dr. Y. Sawa, Dr. K. Tori, Shionogi Research Laboratory, Shionogi & Co., Ltd. for the measurements of NMR spectra and kind advices concerning assignments of these spectra. Thanks are also due to Mr. H. Yoshimits uand Mrs. H. Yoshimitsu, K. Nishijima and S. Kabuto for their excellent technical assistances.

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[Chem. Pharm. Bull. 15(2) 172～178 (1967)]

UDC 615.77-092

24. Kiichiro Kakemi, Hitoshi Sezaki, Shozo Muranishi, and Hidefumi Matsui : Absorption and Excretion of Drugs. XXIX.*1 Effect of Surface-active Agents on Rectal Absorption of Sulfisoxazole from Oily Base.

(*Faculty of Pharmaceutical Sciences, Kyoto University**2)

Effect of various types of surface-active agents on the rectal absorption of sulfisoxazole from cocoa butter was investigated by the blood level determination with rabbits. Blood levels were in general increased with increase of surface-active agent, and decreased by addition of the large amount. Surface-active agent accelerated the release of the drug from the base to dissolution medium. On the other hand, surface-active agent reduced the absorption rate of the drug from aqueous solution. These results suggest that the increase of the drug absorption at the low concentrations of surface-active agents is due to release acceleration, but the reduction effect on drug absorption in their higher concentrations is due to predominance of absorption reduction over release acceleration. Cetyltrimethyl ammonium bromide especially stimulated the drug absorption in the very low concentrations.

(Received May 10, 1966)

Surface-active agents are often used as emulsifiers and solubilizers in oily base suppository.[1] Their addition to oily base is presumed to affect the drug absorption to some extent from the rectum. Schroff[2] and Oesch[3] actually confirmed that salicylic acid, sodium salicylate and sodium iodide were rapidly absorbed by emulsifying the

*1 Part XXVIII. Yakugaku Zasshi, **86**, 278 (1966).
*2 Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto (掛見喜一郎, 瀬崎 仁, 村西昌三, 松井秀文).
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aqueous solution of the drug in cocoa butter with lecithin or cholesterol. On the other hand, in the previous work[4] of this series, the authors reported that the rectal absorption of sulfonamides from aqueous solution was reduced by the addition of surface-active agents such as Polysorbate 80 due to the entrapment of the drug in micelles. Furthermore, the previous study[5] concerning the rectal absorption from oily solution demonstrated the operation of the two ways of the passage of the drug, namely *via* secreting fluid to the rectal membrane and direct passage of the drug from oil.

Secreting fluid in the rectal lumen plays a significant role in the drug absorption from oily base, and the release of drug from oily base to secreting fluid could be a rate limiting step. Addition of surface-active agent to oily base perhaps affects the release of drug from the base to secreting fluid in the rectum. Besides, the direct action of surface-active agents on the gastro-intestinal mucosa varies with their kinds as reported by Nissim.[6] Thus the absorption mechanism in the absorption site is conceivable to be fairly complicated. No studies, however, have been conducted on the effect of surface-active agent on the rectal absorption from oily base.

The present paper describes the effect of various types of surface-active agents on the rectal absorption of sulfisoxazole from cocoa butter in rabbits. Polyoxyethylene sorbitan monostearate (nonionic surface-active agent), sodium lauryl sulfate (anionic surface-active agent), cetyltrimethyl ammonium bromide and lecithin (cationic surface-active agent) were used. It was found that the addition of small amount of surface-active agents in general accelerated the drug absorption, while the addition of their large amount reduced it.

Experimental

Absorption Experiments from Suppositories with Rabbits——Three male rabbits weighing 2.5 to 3.0 kg were used for one experiment. Seventy two hours prior to the administration of a cocoa butter suppository, food, but not water, was removed from the cages. The animal was positioned in the holder for the allotted time period, a suppository of 300 mg. was administered to the rectum, and the distal end of the animal rectum was closed by ligature. After 0.5, 1, 2, 4, 6, 8 hours the samples of blood were taken for total (free and acetylated) sulfonamide determination.[7]

Preparations of Suppositories——The suppositories were prepared by incorporation of 10% of sulfisoxazole as follows. The melted cocoa butter was mixed with sulfisoxazole and a surface-active agent of appropriate amount, and poured into a mold.

Release Experiments *in vitro*——Three hundreds mg. of each suppository was placed in a cylindrical filter paper (used for Soxhlet extraction) of Toyo No. 84. The filter paper was used to reduce the difficulty of withdrawal of the samples. The filter paper with suppository was placed in a 100 ml. beaker, containing 50 ml. of normal saline solution, equipped with a constant temperature bath set at $37 \pm 0.5°$. The stirrer was regulated at the same speed at about 200 r.p.m. Samples were taken at varied time intervals for sulfisoxazole determination. The assay of sulfonamide has been described.[8] The amounts determined for the second, third, fourth and fifth samples withdrawn were corrected for the amount of sulfisoxazole lost from the dissolution medium by previous withdrawals. This correction is summarized by the following expression :

$$A_n = C_n \times V_n + C_{n-1} + C_{n-2} + C_{n-3} + \cdots\cdots\cdots$$

where A_n is the amount released (mg.) for the $_n$-th withdrawal, C_n and C_{n-1} are the concentrations (mg./ml.) actually determined respectively for the $_n$-th and before the $_n$-th sample, and V_n is the volume of dissolution medium (ml.) remained at the $_n$-th withdrawal.

Absorption Experiments from Aqueous Solution in Rats——Procedure of the absorption experiments *in situ* have been described previously.[4] The drug solutions which contained 0.5 m mole/L. of sulfonamides and various concentrations of ionic surface-active agents were prepared with isotonic buffered solution.

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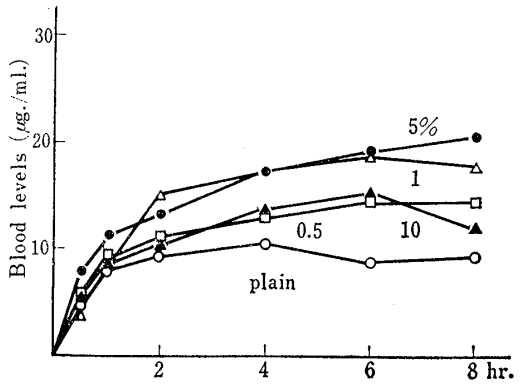

Fig. 1. The Effect of Polyoxyethylene Sorbitan Monostearate on the Blood Levels of Sulfisoxazole after Rectal Administration of a Cocoa Butter Suppository in Rabbits

The numbers in the figure indicate the concentration of surface-active agent in cocoa butter.

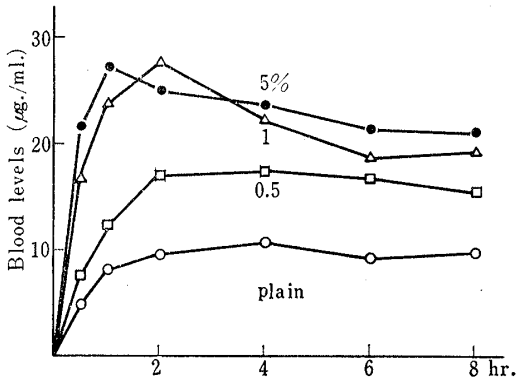

Fig. 2. The Effect of Sodium Lauryl Sulfate on the Blood Levels of Sulfisoxazole after Rectal Administration of Cocoa Butter Suppository in Rabbits

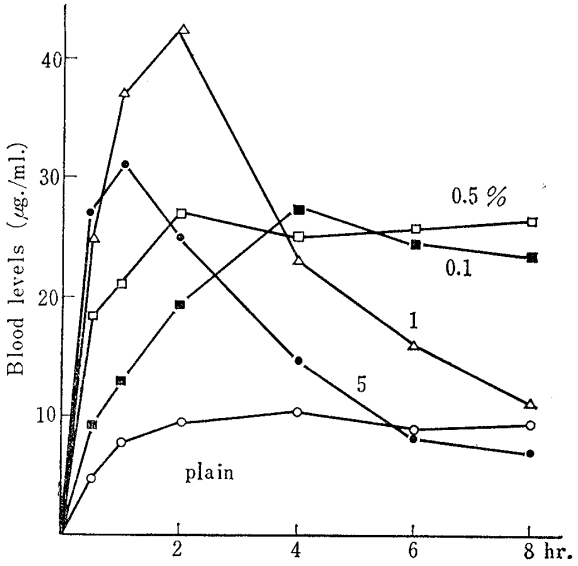

Fig. 3. The Effect of Cetyltrimethyl Ammonium Bromide on the Blood Levels of Sulfisoxazole after Rectal Administration of Cocoa Butter Suppository in Rabbits

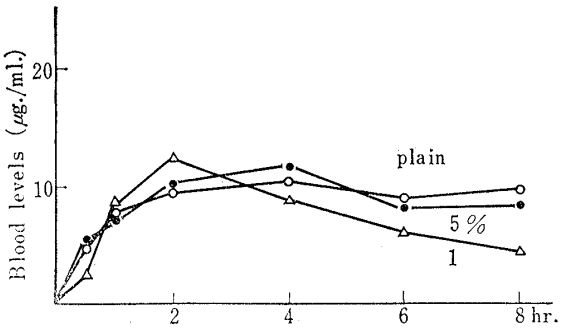

Fig. 4. The Effect of Lecithin on the Blood Levels of Sulfisoxazole after Rectal Administration of Cocoa Butter Suppository in Rabbits

Results

Comparison of Blood Levels in the Rabbit Following Rectal Administration of Cocoa Butter Suppository

Three hundred mg. of the suppository with surface-active agents to give the concentration of 0, 0.5, 1, 5 or 10%, was administered to rabbit, and after the appropriate time period, a sample of blood was taken for comparison of total sulfisoxazole levels.

The blood level *versus* time curves obtained with various types of surface-active agents are shown in Figs. 1~4. Each spot in the figures represents the average of the three animals. Fig. 1 shows the data obtained with polyoxyethylene sorbitan monostearate. The blood levels of sulfisoxazole gradually increased with increasing the

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concentration of the agent. But, when the agent increased to 5%, the difference in blood level curves was hardly observed. On the contrary, at 10% level, the decrease in blood levels was observed.

Fig. 2 shows the data obtained with sodium lauryl sulfate. Between 0.5 and 1% of the surfactant, the blood levels gradually increased with increasing the concentration, but no marked difference between the blood levels at 1% and 5% was observed. This tendency is similar to polyoxyethylene sorbitan monostearate.

The results with cetyltrimethyl ammonium bromide are shown in Fig. 3. The

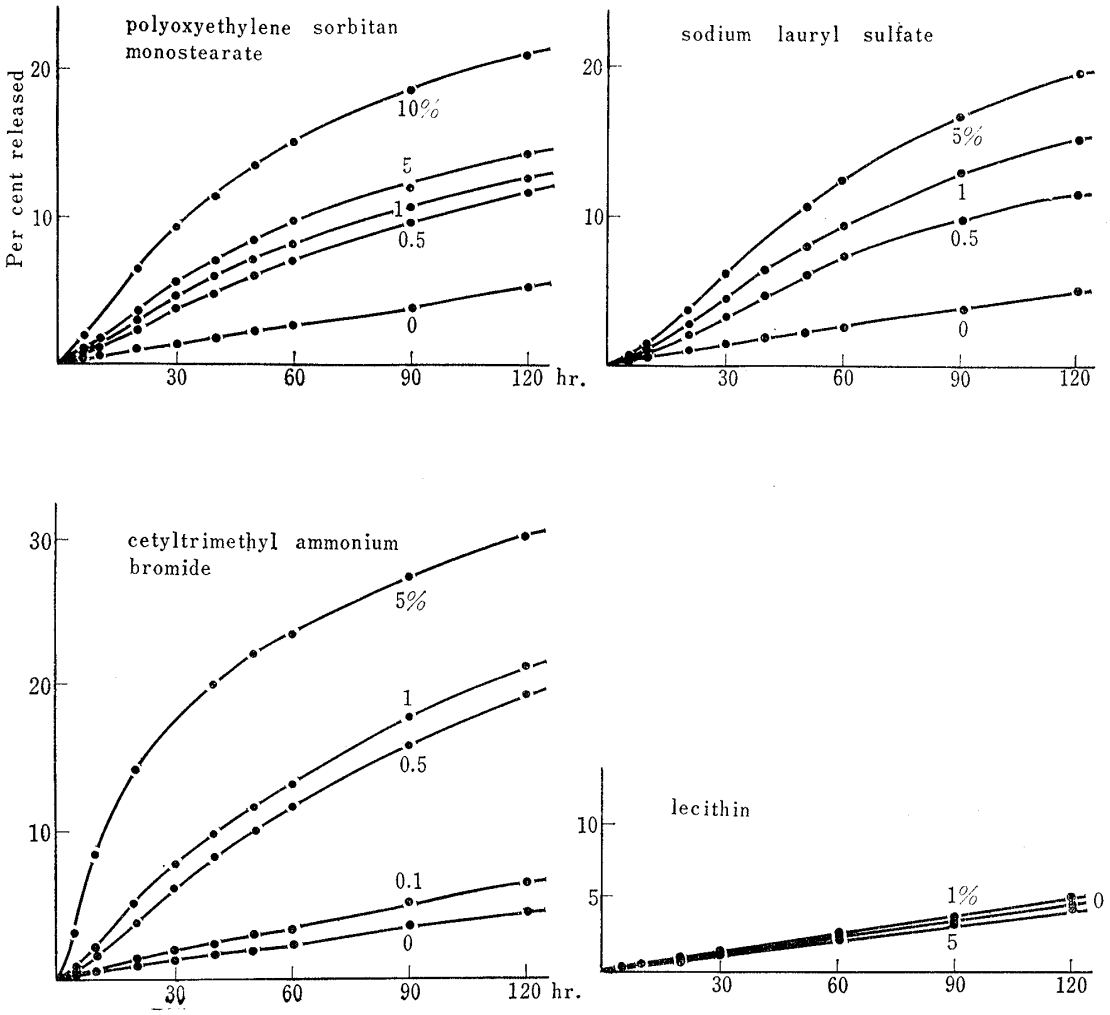

Fig. 5. Effect of Surface-active Agents on the Release of Sulfisoxazole from Cocoa Butter

The numbers in the figures indicate the concentration of surface-active agents in cocoa butter.

blood levels increased with increase of cetyltrimethyl ammonium bromide up to 1%, and the degree of the blood level increase is more remarkable than sodium lauryl sulfate. But the blood levels were reduced by the addition of 5% of cetyltrimethyl ammonium bromide. Although the maximum blood levels at 1% and 5% were considerably high, their elimination rates were observed to be rapid.

From the results obtained, it is concluded that although the effect of surface-active agents on the absorption rate of sulfisoxazole from cocoa butter suppository varies to some extent with their nature, the small amount of surface-active agents tends to accelerate the absorption in general whereas large amount reduces it.

Effect of Surface-active Agent on the Release of Sulfisoxazole from Cocoa Butter Suppository *in vitro*

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The process of absorption of sulfisoxazole proceeds mainly from oil to secreting fluid, and then to the rectal membrane.[5] Therefore, the increase of the release of sulfisoxazole could be presumed to result in increase of their absorption. There is, as yet, no satisfactory explanation for the relationship between release and absorption of drug.

To approach to the condition of drug release from cocoa butter to secreting fluid in rabbit, the amounts released to saline solution were periodically determined at 38°, where cylindrical filter paper of Toyo No. 84 was applied in order to avoid mixing of oil with saline.

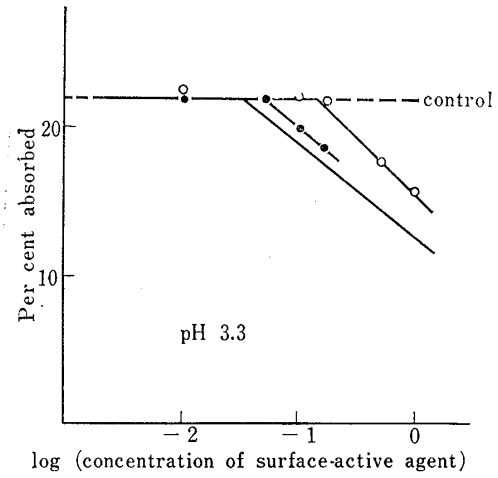

Fig. 6. Effect of Anionic Surface-active Agent on Rectal Absorption of Sulfisoxazole in Rats

—○— sodium lauryl sulfate
—●— sodium dioctyl sulfosuccinate
——— Polysorbate 80

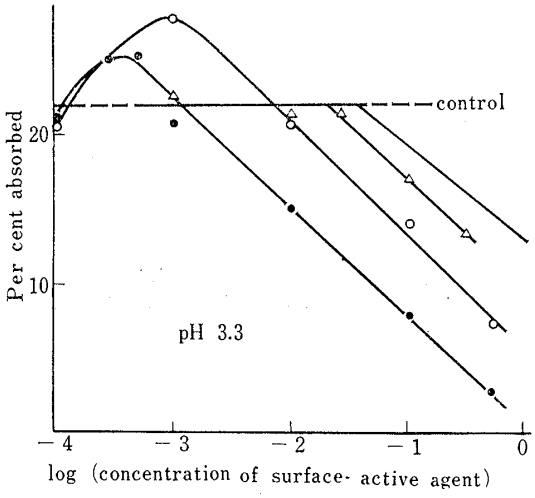

Fig. 7. Effect of Cationic Surface-active Agent on Rectal Absorption of Sulfisoxazole in Rats

—●— cetyltrimethyl ammonium bromide
—○— benzethonium chloride
—△— lecithin
——— Polysorbate 80

The results obtained are summarized in Fig. 5. Curves in the figure represent the percentage of sulfisoxazole released with time. The addition of the surface-active agents such as polyoxyethylene sorbitan monostearate, sodium lauryl sulfate and cetyltrimethyl ammonium bromide resulted in marked increase : the released amounts increased gradually with increasing these agents. The percentage of the drug release within 2 hours at 5% of the agent was 14.0 on polyoxyethylene sorbitan monostearate, 19.7 on sodium lauryl sulfate and 30.0 on cetyltrimethyl ammonium bromide respectively. The addition of cetyltrimethyl ammonium bromide gave the highest percentage of drug release. Lecithin did not affect the drug release.

Effect of Ionic Surface-Active Agents on Rectal Absorption of Sulfisoxazole from Aqueous Solution in Rat

Not only a drug, but a surface-active agent must be released from the suppository into secreting fluid. Nonionic surface-active agents released in secreting fluid reduce the drug absorption due to drug entrapped in micelles.[4] No studies, however, reported on the effect of ionic surface-active agent on drug absorption from aqueous solution. The *in situ* perfusion experiments were followed using rat. The results obtained were shown in Figs. 6 and 7, representing the percentage of absorption with logarithm of concentration of the surface-active agent. Sodium lauryl sulfate was found to reduce the absorption rate of sulfisoxazole in the concentration more than about 0.2%, but hardly affect it less than 0.2%. Sodium dioctyl sulfosuccinate also reduced the rate

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of the drug absorption in the concentrations from 0.05% to 0.2%, but did not affect it less than 0.05%. The critical micell concentrations (CMC) of these anionic surface-active agents are 0.2% on sodium lauryl sulfate and 0.03% on sodium dioctyl sulfosuccinate. These indicate that the reduction of drug absorption by the anionic surface-active agent occurs in the concentration above CMC. The data with Polysorbate 80 obtained in the previous report[4] are shown together in Figs. 6 and 7, indicating that the absorption rate-concentration curves of the anionic surface-active agents are very similar in tendency to this curve.

On the other hand, cetyltrimethyl ammonium bromide and benzethonium chloride were found to cause a stimulation of the absorption of sulfisoxazole in the region of very low concentrations as can be seen in Fig. 7. This stimulation effect on drug absorption is not observed with nonionic and anionic surface-active agents. At higher concentrations, cetyltrimethyl ammonium bromide and benzethonium chloride, however, reduced sulfisoxazole absorption.

Also lecithin was observed to reduce sulfisoxazole absorption similar to nonionic surface-active agent.

Discussion

From the fact that the reduction of sulfisoxazole absorption by anionic surface-active agents, such as sodium lauryl sulfate and sodium dioctyl sulfosuccinate, occurred in the concentration above CMC, it is suggested that the depression effect of anionic surface-active agents depends upon entrapment of the drug in micelles similarly to nonionic surface-active agents. The stimulation effect of cationic surface-active agents, such as cetyltrimethyl ammonium bromide and benzethonium chloride, indicates the same result reported by Nissim[9] and Taylor,[10] in which cetyltrimethyl ammonium bromide caused an enhancement of glucose transport from the intestinal tract at low concentrations. The mechanism of these stimulation phenomena, however, has not been elucidated.

The process of drug absorption from suppositories is more complex than absorption from aqueous solutions. Besides, addition of surface-active agent to suppository base further complicates the nature of rectal absorption. In order to be absorbed, sulfisoxazole must be released from cocoa butter, and this process is influenced to varying degrees by surface-active agent, and the drug amount released in general increases with increasing the surface-active agent incorporated as shown in Fig. 5.

In order to compare these release amounts with results obtained in the absorption experiments, an area under a blood level *versus* time curve from $t=0$ to $t=8$ on the abccissa was estimated graphically. The area is a relative measure of the amount of a given drug absorbed.[11~13] The area and the percentage of release at 2 hours in the presence of various surface-active agents are listed in Table I. When these areas were plotted against the percentage released, it was found that a direct relationship did not exist between these quantities as shown in Fig. 8. Although both the area and the release amount increased by the addition of low concentrations of polyoxyethylene sorbitan monostearate and sodium lauryl sulfate, the area did not increase proportionally to the release amount by their concentrations. Furthermore, the area increased markedly beyond expectation from the amount released at the addition of low concentration of cetyltrimethyl ammonium bromide. These results indicate that the

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Table I. Relationship between Release from Cocoa Butter and Rectal Absorption

Surface-active agent added	Conc. of surface-active agent	Per cent released at 2 hr.	Area under blood level-time curve from $t=0$ to 8 hr. (μg.×hr./ml.)
Polyoxyethylene sorbitan monostearate	0	4.7	71.2
	0.5	11.4	96.6
	1	12.3	117.5
	5	14.0	118.9
	10	20.6	95.9
Sodium lauryl sulfate	0.5	9.7	121.9
	1	13.5	167.1
	5	19.7	178.2
Cetyltrimethyl ammonium bromide	0.1	5.9	174.7
	0.5	19.1	201.5
	1	21.2	195.6
	5	30.0	130.3
Lecithin	1	4.8	64.7
	5	4.4	75.0

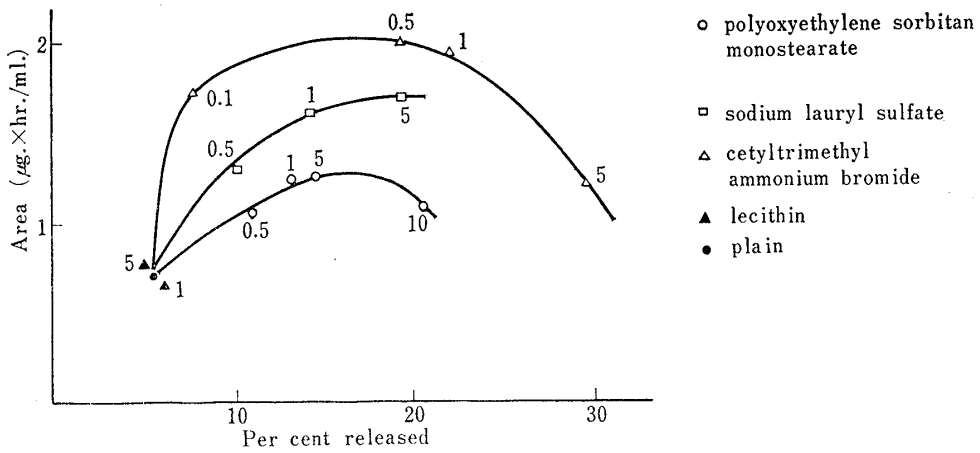

Fig. 8. Showing the Relationship between Area under Blood Level-Time Curve from $t=0$ to 8 hours and Per Cent released for 2 hours

The numbers in the figure indicate the concentration of surface-active agent in cocoa butter.

effect of surface-active agents on the absorption rate of sulfisoxazole does not depend only upon the release rate from cocoa butter.

When a suppository is administered to the rectum, it is considered that surface-active agents are also released to varying degree according to their type, concentration, and physical properties. If the nonionic surface-active agent in secreting fluid is concentrated above CMC, absorption rate of a drug will be reduced mainly due to entrapment in micelles. Accordingly it is postulated that, when a small amount of surface-active agents is added to the suppository, the enhancing effect on release rate exceeds the reduction effect on absorption rate owing to their lower concentration in secreting fluid. On the contrary, when a large amount of surface-active agent is added, the reduction effect on drug absorption occurs on the whole, due to predominance of absorption reduction over release acceleration. Stimulation effect of cetyltrimethyl ammonium bromide on the drug absorption was also demonstrated in the absorption experiment from cocoa butter.

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