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# Effect of Surfactants on Drug Absorption. I. Changes in Drug Stability in Both Bio and Synthetic Surfactants Solutions estimated by Thiol-Disulfide Exchange Reaction *in Vitro*

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Effects of surfactants on the thiol-disulfide exchange reaction consisting of thiamine disulfide compounds and thiols were studied as a model system representing changes in drug's stability which would be caused by drug-surfactant interaction. Addition of sodium laurylsulfate, sodium glycocholate or polysorbate 80 to the reaction mixture caused, in their own way, a decrease or an increase in the rate of the reaction. The effective factors affecting on the surfactant effects were found to be the lipophilic character and structure of the disulfide compounds and also the kind of thiols, *e.g.* glutathione, cysteine, cysteamine or thiophenol.

There have been many reports made concerning drug-surfactant interactions in reference to drug stability or drug absorption. Riegelman,[2] Mitchell, *et al.*[3] and several other workers[4–8] have investigated the hydrolysis of ester linkage of procaine or aspirin in micellar solution of nonionic surfactant. Yamada, *et al.*[9] proposed a pseudo two phase model[10] for polysorbate solution consisting of micellar and aqueous phases in order to account for drug absorption. Bates, *et al.*[11] reported the solubilizing action of bile salt micelles for some water-insoluble drugs such as griseofluvine with a suggested contribution of bile salts to drug absorption. Gedberg, *et al.*[12] and many others[13–18] dealt with a release of drugs from micellar phase in connection with a role of biosurfactant in the absorption of water-insoluble drugs. Several workers[19–28] further studied the effects of bile salts and suggested their direct action

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on the intestinal mucosa, which might alter membrane permeability so that drug absorption might be enhanced. These findings help to explain the drug absorption from solution containing either bile salts or a synthetic surfactant (s). However, there are cases in which both bile salts and a synthetic surfactant (s) are contained in solution. Further study on such cases seems necessary.

Presently, a drug-surfactant interaction *in vitro* was pursued centered on variations of drug stability. The thiol-disulfide exchange reaction[29-31] between thiamine disulfide compounds and thiols was taken up as a model reaction, which did not seem to have been applied in the study being concerned with drug-surfactant interaction, as depicted in equation (1), (2), and (3). It is generally understood that this nonenzymatic reaction, which results in the cleavage of disulfide bond, is one of the important steps in biotransformation of thiamine disulfide compounds as well as enzymatic one[30] which catalyzes the same reaction. It may also be considered that this reaction plays a role in the metabolism and absorption of the disulfide drugs in the intestine.

Sodium laurylsulfate (SLS), polysorbate 80 and sodium glycocholate (SGC) were used as surfactants.

$$B_1SSB_1 + RSH \longrightarrow B_1SSR + B_1 \quad (1)$$

$$B_1SSR + RSH \longrightarrow RSSR + B_1 \quad (2)$$

$$B_1SSB_1 + 2RSH \longrightarrow RSSR + 2B_1 \quad (3)$$

## Result and Discussion

### 1. Determination of the Rate Constant of the Reaction

Assuming that thiamine disulfide compound reacts with thiol under the condition that the latter compound is in large excess, the over all reaction may proceed in first order with respect to the disulfide compound at the initial period, since the second step can be negligible. Then the disappearance rate of the disulfide compound should be followed by equation (4).

$$\log C_0/C = kt \quad (4)$$

where $C_0$ is the concentration of benzoylthiamine disulfide (BTDS) at time $t=0$ and $C$ is the concentration of BTDS at some latter time, $t$. $k$ is the apparent first-order rate constant for the initial period. Thus, the rate constant, $k$, can be calculated by the plot of $\log C_0/C$ versus time $t$.

Based on this assumption, thiamine disulfide compound, BTDS, in the concentration of $7.43\times10^{-6}$M was reacted with glutathione (GSH) of $7.14\times10^{-4}$M (about 100 times as much as BTDS in molar concentration) in buffer or buffered SLS solution and the $\log C_0/C$ was plotted against time $t$. As shown in Fig. 1, the plots showed a good linearity at least within 5 minutes and it is apparent that the reaction proceeds in first order reaction respect to BTDS in this experimental condition. Such kinetical treatment was then applied to almost all of the experiments in this study.

### 2. Effects of SLS on the Reaction of Thiamine Disulfide Compounds with Thiol Compounds

Two symmetric thiamine disulfide compounds, BTDS and thiamine disulfide (TDS), were reacted with GSH in various concentration of SLS solution and the rate constants were calculated. Fig. 2 represents the effect of SLS concentration on the rate of the reaction between BTDS and GSH, together with the effect on the solubility of BTDS at 37° and pH 6.4. It was found that SLS decreased the rate constant of the reaction quantitatively until its

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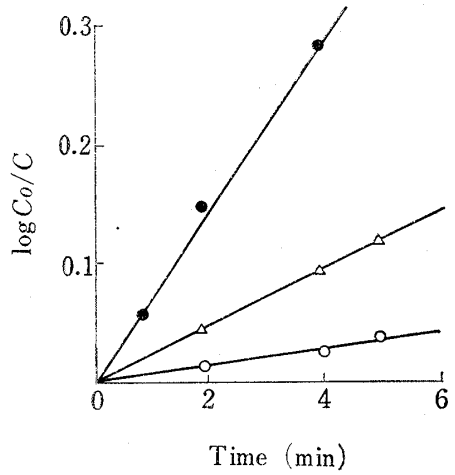


Fig. 1. First Order Plot of the Reaction of BTDS with GSH in Buffer and Buffered SLS Solution at 37° and pH 6.4

reaction; BTDS: $7.43\times10^{-6}$M, GSH: $7.14\times10^{-4}$M, ●: control, △: 0.02% SLS, ○: 0.05% SLS

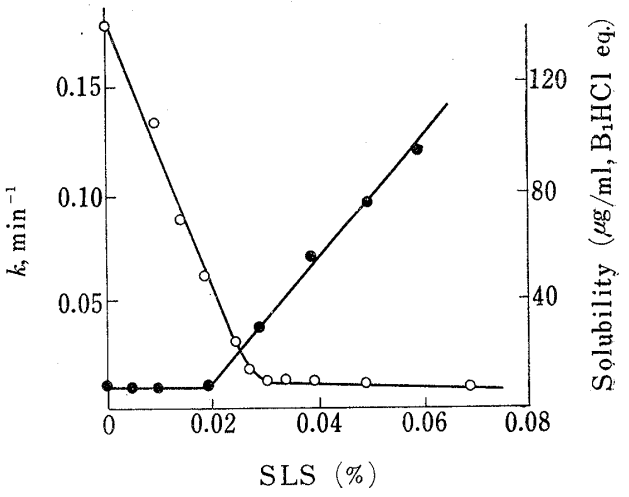


Fig. 2. Effect of SLS on the Rate of the Reaction of BTDS with GSH and on the Relation to the Solubility of BTDS in the Presence of SLS at 37° and pH 6.4

reaction; BTDS: $7.43\times10^{-6}$M, GSH: $7.14\times10^{-4}$M ○: $k$, ●: solubility of BTDS

concentration came up to around the critical micellar concentration (c.m.c), 0.02%, which was estimated from the solubilization curve. Above the c.m.c, there appeared to be little change in the rate constants with the increase of SLS concentration. It is apparent that SLS inhibits the reaction and its effect is the concentration dependent only below the c.m.c. These suppressing effects may be caused by possible interactions between BTDS and laurylsulfate anion ($LS^-$) such as complex formation which results in suppression in the rate of the reaction. This kind of complex formation is supported by the fact that salt formation between thiamine, the parent compound of BTDS, and $LS^-$ has already been evidenced by Utsumi, *et al.*[32] in its crystalized form. The BTDS-LS complex may form micelles, *i.e.*, they may aggregate themselves in an intact form above the c.m.c retaining their suppressing effect. Thus, it appears that micellar formation itself is not a sufficient factor to suppress the reaction.

Fig. 3 illustrates the effects of SLS on the rate constant of the reaction between TDS and GSH and on the solubility of TDS at 37° and pH 6.4. This indicated that SLS exerted no influence on the rate constant of the reaction over the concentration range below the c.m.c, but a very slight influence in decreasing the rate of the reaction at the higher concentration. This situation appears to be quite different from that observed in the reaction of BTDS with GSH.

Structural analogues of BTDS, such as succinoylthiamine disulfide (SCTDS), propionylthiamine disulfide (PTDS) and butyrylthiamine disulfide (BuTDS), each of which has its own lipoid solubility, were reacted with GSH in 0.03% SLS solution. The degree of the suppressing action of SLS on each rate of the reaction was estimated by the ratio of $k$ in the SLS solution to that in the control solution, $k_{SLS}/k_{control}$. Fig. 4 represents the plots of $k_{SLS}/k_{control}$ *vs* logarithmic values of oil-water partition coeffcients of the analogues.[33] It was revealed that the suppressing action of SLS was increased with the increase of the lipoid solubility of each compound in the order: TDS<PTDS<BuTDS<BTDS. Contribution of such a lipophilic factor to the decreasing effect of SLS on the reaction rates clearly suggests the existence of hydrophobic interaction between the disulfide compounds and $LS^-$, although this inhibition was specific for the compounds that have the partition coefficients larger than unity.

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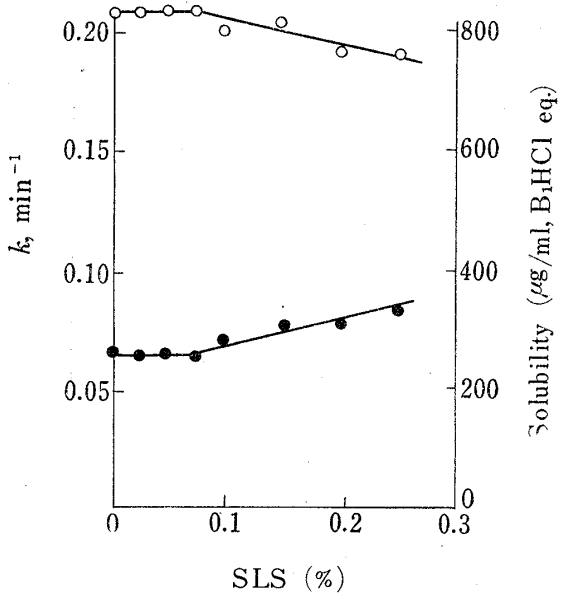


Fig. 3. Effect of SLS on the Rate of the Reaction of TDS with GSH and on the Relation to the Solubility of TDS in the Presence of SLS at 37° and pH 6.4

reaction; TDS: $7.43\times10^{-6}$M, GSH: $7.14\times10^{-4}$M
○: $k$, ●: solubility of TDS

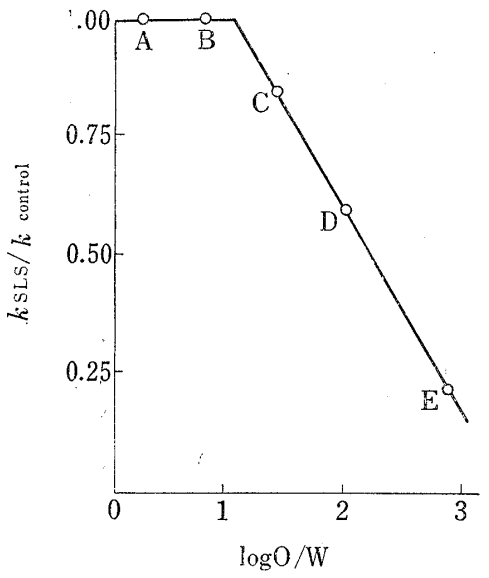


Fig. 4. Relationship between the Ratio of $k_{SLS}$ to $k_{control}$ and Oil-Water Partion Coefficient (O/W) at 37° and pH 6.4

reaction; the disulfide compounds: $7.43\times10^{-6}$M, GSH: $7.14\times10^{-4}$M, SLS: 0.03%
A: TDS, B: SCTDS, C: PTDS, D: BuTDS, E: BTDS

Instead of GSH, several thiol compounds (thiophenol, cysteine, and cysteamine) were used for an interest of their reactivities with BTDS in SLS solution. Since the reaction of thiophenol could not be explained by the first order kinetics, probably due to the fact that thiophenol has a large lipophilic nature which might be favoured to its own interaction with SLS, the data were expressed in terms of the % reduction of BTDS in given time as listed in Table I. It can be seen from these data that SLS markedly decreased the rate of the reaction between BTDS and cysteine. This decreasing effect of SLS was quite similar with that observed in the case of GSH, but was quite unlike the case of cysteamine whose reaction was increased by SLS. For this reason, it is presumed that the loss of the reactivities of GSH and cysteine for BTDS may be due to an electrostatic repulsion of carboxyl group by $LS^-$ surrounding complex, and that the increase of the reactivity of cysteamine may be due to a good affinity of amino group to $LS^-$ or to the complex.

Table I. Effect of SLS on the Reaction Rates between BTDS and the Several Thiols at 37° and pH 6.4

| Thiols | Reaction time (min) | % of thiamine formed SLS (%) | | | |
|---|---|---|---|---|---|
| | | 0 | 0.015 | 0.03 | 0.05 |
| GSH | 5 | 27.0 | 13.0 | 8.5 | 6.5 |
| Cysteine | 5 | 35.5 | 17.0 | 12.5 | 11.8 |
| Cysteamine | 5 | 57.5 | 84.0 | 88.5 | 87.0 |
| Thiophenol | 2 | 77.0 | 70.0 | — | 56.0 |

reaction; BTDS: $7.43\times10^{-6}$M; Thiols: $7.14\times10^{-4}$M

Fig. 5 represents the effect of SLS on the rate constant of the reaction between BTDS and cysteamine and on the relation to the solubility of BTDS relative to the concentration of SLS at 37° and pH 6.4. SLS did not decrease but accelerated the reaction over the con-

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centration range used. The rate of the reaction attained the maximum value at 0.03% SLS solution around the c.m.c, where the increase in the rate constant of the reaction was up to 2.6 fold of that in the absence of SLS.

A concept that regards a complexing agent as a kind of catalytic agent has appeared in past works. Bender, *et al.*[34,35] reported that an acceleration of hydrolytic reaction of phenylacetate ester was caused by a formation of its inclusion compound with cyclodextrin and that the compound was considered as a model for hydrolytic enzyme-substrate complex. In this respect, the acceleration of BTDS-cysteamine exchange reaction by SLS might be considered as a potential model for a reducing enzyme-substrate complex.

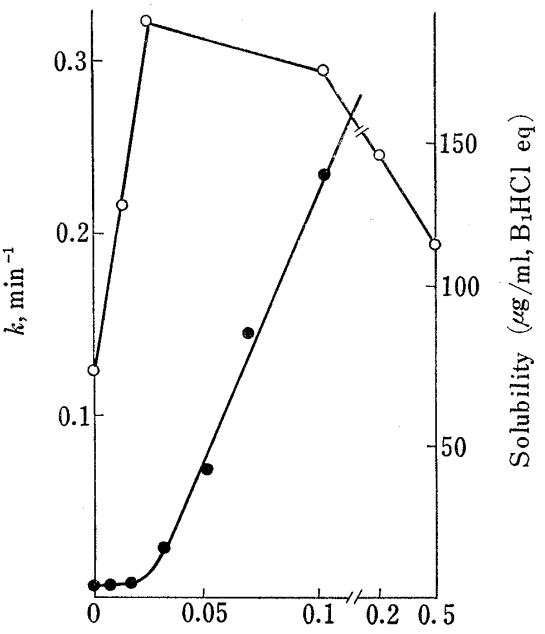


Fig. 5. Effect of SLS on the Rate of the Reaction of BTDS with Cysteamine and on the Relation to the Solubliity of BTDS in the Presence of SLS at 37° and pH 6.4

reaction; BTDS: $7.43\times10^{-6}$M, cysteamine: $7.14\times10^{-4}$M
○: $k$, ●: solubility of BTDS

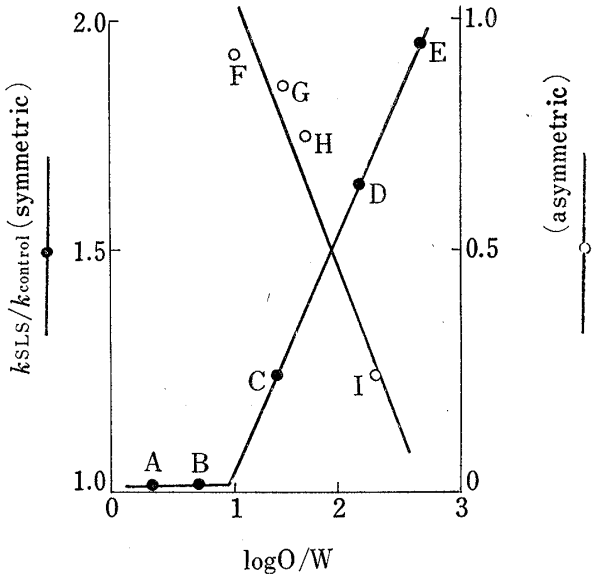


Fig. 6. Relationship between the Ratio of the Reaction Rate in 0.03%SLS to That in the Control and Oil-Water Partition Coefficient

reaction; the disulfide compounds: $7.43\times10^{-6}$M, cysteamine: $7.14\times10^{-4}$M, pH 5.8, 37°
symmetric; A: TDS, B: SCTDS, C: PTDS, D: BuTDS, E: BTDS
asymmetric; F: thiamine tetrahydrofurfuryldisulfide (TTFD), G: thiamine propyldisulfide (TPD), H: thiamine benzyldisulfide (TBzD), I: benzoylthiamine propyldisulfide (BzTPD)

There is another type of thiamine derivatives, which are commonly called "asymmetric thiamine disulfide compound," besides the symmetric TDS analogues. Fig. 6 illustrates the effects of SLS on the reductive cleavage of both types of thiamine derivatives by cysteamine at 37° and pH 5.8. It is apparent that SLS decreased the rate of the reaction in the asymmetric compound but increased it in the symmetric one in proportion to the magnitude of their partition coefficients. Therefore it may be concluded that the SLS effects are also changed by the structural differences between the symmetric and asymmetric thiamine disulfide compounds, although the reason for this is not clear at present.

### 3. Effects of SGC on the Reaction of Thiamine Disulfide Compounds with Thiol Compounds

An anionic biosurfactant, SGC, is one of the main components of the bile salts and is seen as exerting an influence upon the drug behaviour. Fig. 7 represents the effect of SGC on the

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rate constant of the reaction between BTDS and GSH or cysteamine together with a solubilization curve of BTDS by SGC. In both cases, the reaction rates decreased linearly up to the concentration of about 0.02% SGC and then became nearly constant in the vicinity of the c.m.c, but increased with further increase of SGC concentration. The c.m.c for SGC was 0.1%, as determined from the solubilization curve of BTDS at 37° and pH 6.4, and this value was in close agreement with the concentration where the slope of the plot of the rate of the reaction became upward. Thus the suppressing action of SGC at the low concentration was thought to be enfeebled at the high concentration above the c.m.c, but this kind of phenomenon was not found in the case of SLS as previously shown in Fig. 2. It is also worthy to note that the effect of SGC is not dependent on the thiol compound used, whereas the effect of SLS is reversed in the reaction with GSH and in that with cysteamine as shown in Table I.

These characteristic actions of SGC are considered to be specific for this biosurfactant.

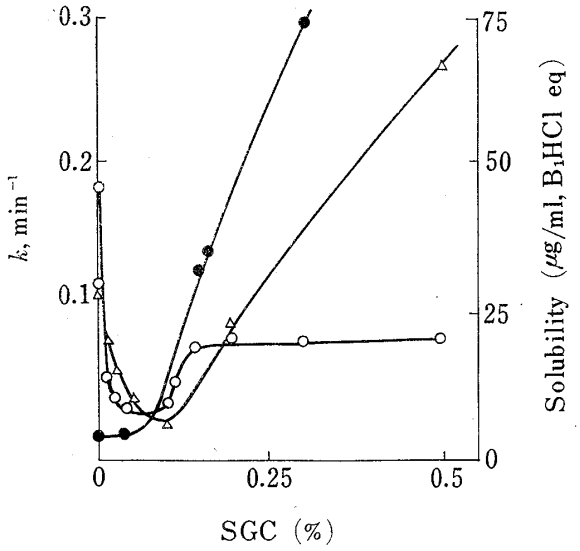


Fig. 7. Effect of SGC on the Rate of the Reaction between BTDS and Thiols at 37° and pH 6.4

reaction; BTDS: $7.43\times10^{-6}$M, thiols: $7.14\times10^{-4}$M
△: cysteamine, ○: GSH
●: solubility of BTDS

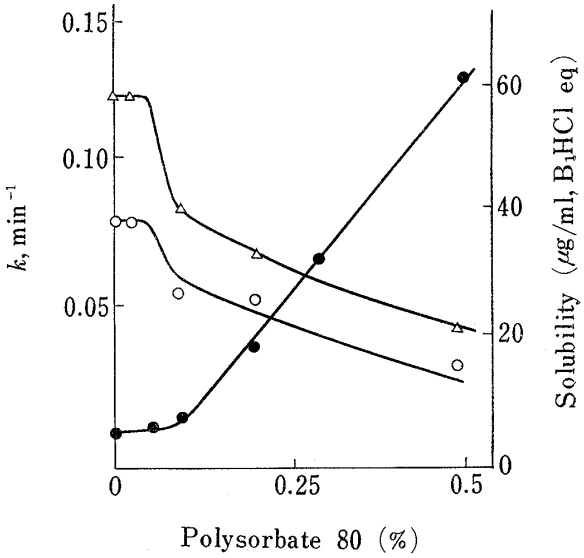


Fig. 8. Effect of Polysorbate 80 on the Reaction Rates between BTDS and Thiols and on the Relation to the Solubility of BTDS at 37° and pH 5.8

reaction; BTDS: $7.43\times10^{-6}$M, thiols: $7.14\times10^{-4}$M
△: cysteamine, ○: GSH
●: solubility of BTDS

## 4. Effects of Polysorbate 80 on the Reactions of BTDS with Thiol Compounds

Polysorbate 80, a well known nonionic surfactant, is likely to have an influence on the thiol-disulfide exchange reaction. The effects of polysorbate 80 on the rate of the reaction and on the relation to the solubility of BTDS at 37° and pH 5.8 are indicated in Fig. 8. According to the results, polysorbate 80 did not decrease the rates of the reaction until the concentration came up to around 0.1% which was in close agreement with c.m.c, obtained from the solubilization data, but decreased them above the c.m.c in both the reactions of BTDS with GSH and with cysteamine. Then, it appears that these suppressing actions of polysorbate 80 above the c.m.c may be due to the incorporation of BTDS into the micelles.

## Conclusion

SLS was found to exert variety of influences on the rate of the reaction between the thiamine disulfide compounds and several thiol compounds. The lipophilic character of the disulfide compound is one of the factors that determine SLS effect and it suggests a contribution of a hydrophobic interaction to the possible formation of the complex between the

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disulfide compound and $LS^-$. The structural difference of the disulfide compound as well as the thiol compound is also a factor in changing the rate of the reaction which was accelerated or suppressed by SLS.

Similarly SGC was found to effect the rate of the reaction between BTDS and GSH or cysteamine but in a more complicated manner than that of SLS over the concentration below and above the c.m.c.

Polysorbate 80, in the concentration above its c.m.c, suppresses the reaction and its inhibitory effect is supposedly due to the well known drug-entrapping action of the micelles of a nonionic surfactant.

These findings in the reaction behaviour are believed to give a kind of prediction of the drug's stability, suggesting some implications about the role of the surfactant applied to pharmaceutical preparations.

Synergistic or antagonistic effect of the bio and synthetic surfactants on the exchange reaction will be described in a later paper, together with a detailed nature of the complex between BTDS and $LS^-$.

## Experimental

**Reagents**——Symmetric and asymmetric thiamine compound. BTDS, mp 146—147°, BuTDS, mp 130—136°, PTDS, mp 134—135°, SCTDS, mp 116—118°, TDS, mp 169—170°, thiamine propyldisulfide (TPD), mp 128—129°, thiamine benzyldisulfide (TBzD), mp 152° and benzoylthiamine propyldisulfide (BTPD), mp 80—90° were used. Thiol compounds: Commercial samples which were of a special grade were used.

SLS: Commercial sample was recrystallized from 80% acetone two times and then 90% acetone, mp 190°.

SGC: Chromatographically pure sample was synthesized by using the method introduced by Norman.[36]

Polyoxyethylene sorbitan monooleate (Polysorbate 80): The compound prepared by Nikko Chemicals Co., Ltd., was used without further purification.

**Drug Solution**——$1.04 \times 10^{-5}$M of the thiamine disulfide compounds were dissolved in 100 ml of 0.02N HCl solution. Each solution was diluted 10 fold with regard to the constituents by addition of isotonic buffer solution[37] of pH 5.8 and 6.4, which contains SLS, polysorbate 80 or SGC at a given concentration (w/v). Each thiol was dissolved in the same buffer solution in the concentration of $2.50 \times 10^{-3}$M.

**Kinetic Studies**——Five ml of buffer or buffered SLS solution containing $1.04 \times 10^{-5}$M of thiamine disulfide compound was added to the test tube which was placed in a thermostatically controlled water bath at 37°. Two ml of buffer or buffered SLS solution containing $2.50 \times 10^{-3}$M of thiol compound was then rapidly added to the test tube. One ml of Each sample was withdrawn at zero time and then at suitable time intervals. Each of them was immediately put into 9 ml of 0.1N HCl solution containing 25% KCl to stop the reaction and then assayed for free thiamine formed. The concentration of thiamine was determined by the conventional thiochrome method.

**Solubility**——Ten ml of buffer or buffered SLS solution containing 100 ml of BTDS or TDS in the sealed ampules were shaken for 3 days at 37° and pH 6.4. After equilibrium was attained, the solution contained in ampules was filtered through No. 5C Toyo filtered paper. Aliquot of filtrate was diluted with 0.1N HCl solution containing 25% KCl. One ml of each disulfide sample was placed in Nesler's tube and then 1 ml of 1M sodium acetate buffer solution, pH 4.6, and 1 ml of 2% $Na_2S_2O_3$ aqueous solution were added in turn. Each Nesler's tube was then kept for 20 min at 60° to create cleavage of the disulfide bond of thiamine disulfide compound. After cooling at room temperature 0.1N HCl solution containing 25% KCl was added to bring the total volume to 10 ml and then the concentration of thiamine in the filtered solution was determined.

**Partition Coefficient**——Data of the partition coefficients of the thiamine disulfide compounds reported by K. Harada, *et al.*[30] were used here, and unknowns for some compounds were determined in the same procedure.

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37) K. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964).

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