ylsiloxane and everted intestine only when materials not interacting with the membranes were under examination. If membrane interaction occurs, it is difficult to draw conclusions about possible interactions between the drug and the substance added to the drug solution.

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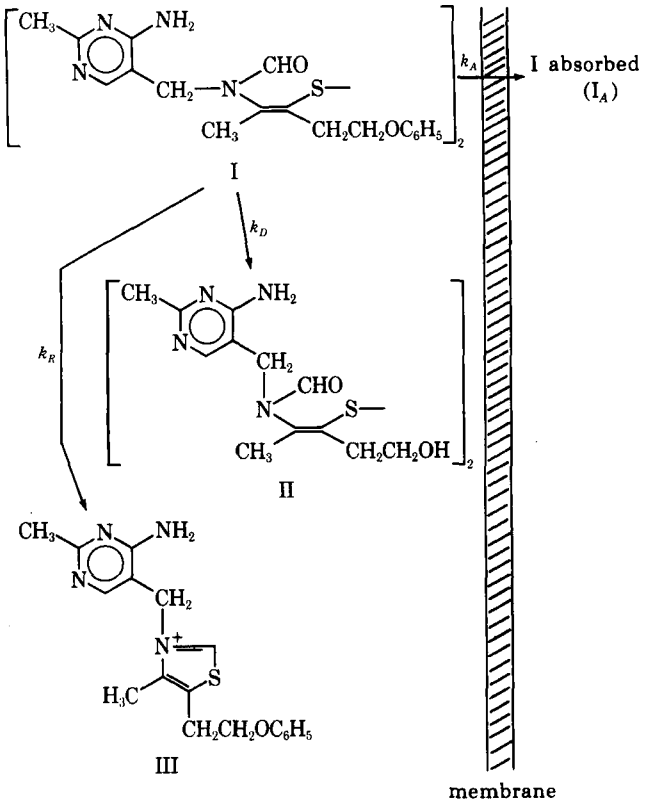
Surfactant Effects on Drug Absorption III: Effects of Sodium Glycocholate and Its Mixtures with Synthetic Surfactants on Absorption of Thiamine Disulfide Compounds in Rat

I. UTSUMI[x], K. KOHNO, and Y. TAKEUCHI

Abstract □ The effects of surfactants, such as sodium glycocholate and its related compounds or mixtures, on the absorption and metabolism of the model compounds, *O*-benzoylthiamine disulfide and thiamine disulfide were evaluated by both *in situ* and *in vivo* experiments using rats. The *in situ* results indicated that, in the presence of a biosurfactant, the increasing or decreasing effect of a synthetic surfactant on drug absorption and metabolism could be canceled by a possible formation of new mixed micelles consisting of the drug and both surfactants. Results were confirmed by oral experiments using the drug-micellar solution in rats.

Keyphrases □ Surfactants (sodium glycocholate, sodium lauryl sulfate, and polysorbate 80)—effects on absorption and metabolism of thiamine disulfide compounds, rats □ Drug absorption (thiamine disulfide compounds)—effects of biosurfactants and synthetic surfactants, rats □ Absorption (thiamine disulfide compounds)—effects of biosurfactants and synthetic surfactants, rats □ Thiamine disulfide compounds—effects of biosurfactants and synthetic surfactants on absorption and metabolism, rats □ Sodium glycocholate—effects on absorption and metabolism of thiamine disulfide compounds, rats

When a drug is entrapped into micelles, the thermodynamic activity (1) of the drug is decreased and the absorption is thereby suppressed while the stability of the drug is increased. However, previous studies (2) indicated that the reaction rates of the thiol–disulfide exchange reaction between thiamine disulfide compounds and thiols were influenced (acceleration or inhibition) by sodium lauryl sulfate, polysorbate 80, and sodium glycocholate, depending on the concentrations of surfactants, the lipophilic character of the disulfide, and the thiols used. These findings suggest that the thermodynamic activity of drugs is not always decreased by the solubilization or interaction with surfactants. Another study (3), which included kinetic treatment of the drug–surfactant interaction, indicated that *O*-benzoylthiamine disulfide (I) interacted with the lauryl sulfate anion to form a 1:2 molar complex and that this complex is



Scheme I

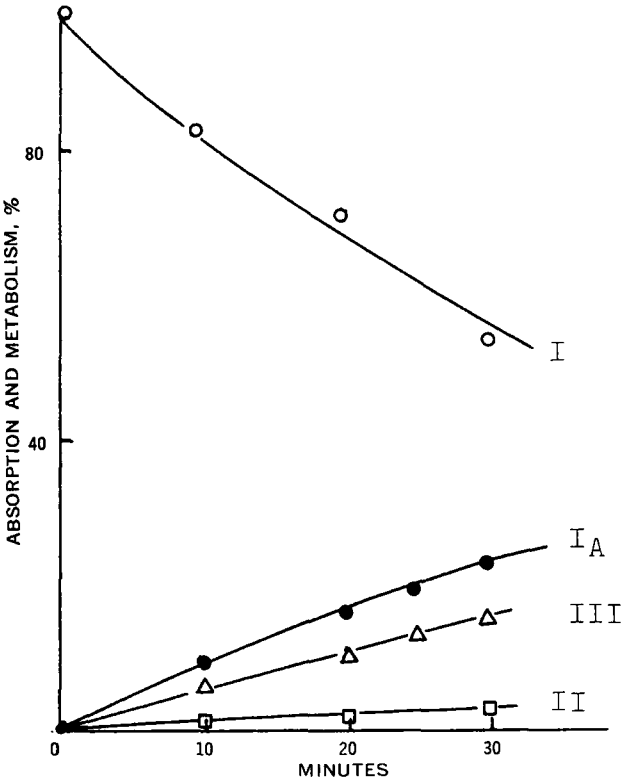


Figure 1—*Typical examples of the time course of absorption and metabolism of* O-*benzoylthiamine disulfide* (*I*).

broken up by the addition of sodium glycocholate, a biosurfactant, to produce new mixed micelles composed of the thiamine derivative and the surfactants.

On the basis of these observations, it was presumed that a biosurfactant could have a complicated effect, different from the effect of single species of the synthetic surfactant, on drug absorption and metabolism in the intestinal tract when synthetic surfactant-containing drug preparations were administered. To clarify this presumption, the effects of biosurfactants and synthetic surfactants on drug absorption were examined *in situ* and *in vivo* in rats. The results of the *in vitro* (2), *in situ*, and *in vivo* experiments also were compared to obtain a correlation of surfactant effects among the experiments.

EXPERIMENTAL

Materials—The drugs, *O*-benzoylthiamine disulfide (I) and thiamine disulfide (II), were the same preparations as in previous studies (2, 3). Sodium lauryl sulfate[1] was recrystallized from 85% acetone solution for use. Polysorbate 80[1] was used without purification. Sodium glycocholate, a chromatographically pure sample, was synthesized by the method introduced by Norman (4).

Measurements of Absorption and Metabolism—Apparent first-order rate constants of absorption and metabolism (hydrolysis and reduction) of the materials were determined by an *in situ* circulation method. The perfusate (pH 6.4) consisted of 10 μg/ml of I or II (equivalent to thiamine hydrochloride) and the isotonic phosphate buffer solution, with or without surfactant (2). The volume of the circulated solution (perfusate) was 50 ml. The small intestine of an anesthetized rat was ligated at the lower portion of the duodenum to prevent the bile fluid from entering the perfusate and at the upper portion of the ileocaecum by cannulation with a glass tube connected to a silicone tube. Both tubes were introduced into the reservoir flask containing the perfusate, thereby making a closed system through which the perfusate was circulated. The solution was kept at 37°; before circulation the intestinal tract was washed to expel the intestinal contents with 70 ml of the buffer solution free from thiamine compound and surfactant. The circulation through the intestine was done by a miniflow pump[2] at a speed of 5 ml/min. Constant agitation of the perfusate was accomplished by directing air into the reservoir flask. One milliliter of each sample was taken at a certain interval and immediately put into 4 ml of 25% KCl solution containing 0.1 *N* HCl to inhibit the enzymatic hydrolysis and reduction of I. The concentrations of I and its metabolites in each sample were assayed by means of the thiochrome and acylthiochrome methods (5). Phenol red was used as an indicator for the correction of the water absorption, since the total recovery of the phenol red added into the circulation fluid was not influenced by the surfactant after circulation for 30 min.

Measurement of Urinary Excretion of Thiamine—A rat was fixed on a board and anesthetized at the femoral area by a local anesthetic. A needle for subcutaneous injection, connected to a silicone tube (2φ), was introduced into the femoral vein and the animal was continuously infused with 0.9% NaCl solution (37°) at the speed of 0.067 ml/min using the miniflow pump. About 3–10 ml of urine was easily collected. The animals received 2 ml of a micellar solution of sodium lauryl sulfate, polysorbate 80, or sodium glycocholate containing I (100 μg/ml) by stomach tube. Excreted urine was then collected for 7 hr after administration, and the amount of thiamine excreted was determined.

Kinetic Model for Absorption and Metabolism—In the intestinal tract, I is absorbed in an intact form but is partially metabolized by two processes (Scheme I) (6). One process is a reduction, being considered nonenzymatic, to form *O*-benzoylthiamine (III); the other is an enzymatic hydrolysis of ester linkage (deacylation) to form the less absorbable II.

If it is assumed that the absorption and metabolism of I are

[1] Nikko Chemicals Co. Ltd.

[2] Sharp Co. Ltd.

Table I—Effects of Sodium Lauryl Sulfate on Rate Constants for Absorption and Metabolism of *O*-Benzoylthiamine Disulfide[a] (I)

System	Number of Rats	Rate Constants, hr^{-1}		
		k_A	k_R	k_D
Control[b]	3	0.40 ± 0.03	0.37 ± 0.05	0.68 ± 0.03
0.015% sodium lauryl sulfate	3	0.34 ± 0.08	0.79 ± 0.09	0.12 ± 0.12
0.1% sodium lauryl sulfate	3	0.18 ± 0.03	0.76 ± 0.14	0.10 ± 0.08

[a] I in perfusate: 10 μg/ml (50 ml), pH 6.4, 37°. [b] Without surfactant.

described as a first-order process and the absorption of the metabolites is negligible because of their low concentration in the circulation fluid, initial rate constants for the absorption and metabolism can be calculated. The disappearance rate of I from the *in situ* circulation fluid can be expressed by Eq. 1:

$$d[I]/dt = -(k_A + k_R + k_D)[I] \quad \text{(Eq. 1a)}$$

$$= -k[I] \quad \text{(Eq. 1b)}$$

where k_A, k_R, and k_D are the apparent first-order rate constants for absorption, reduction, and deacylation, respectively. Brackets show molar concentrations. The rates of absorption and metabolism are defined in Eqs. 2–4, from which Eqs. 5–7 are derived:

$$d[I_A]/dt = k_A[I] \quad \text{(Eq. 2)}$$

$$d[II]/dt = k_D[I] \quad \text{(Eq. 3)}$$

$$d[III]/dt = k_R[I] \quad \text{(Eq. 4)}$$

$$[I_A]/[II] = k_A/k_D \quad \text{(Eq. 5)}$$

$$[I_A]/[III] = k_A/k_R \quad \text{(Eq. 6)}$$

$$[III]/[II] = k_R/k_D \quad \text{(Eq. 7)}$$

where I_A refers to I absorbed in an intact form. According to Eqs. 5–7, the ratios of I_A and metabolites at time t can be replaced by the ratios of the rate constants. By combining these equations with Eq. 1, the rate constants were calculated. These were further corrected by fitting the analog computer-generated curves to the experimental data points at time intervals. The analog computer program for the kinetic model is shown in Scheme II.

Figure 1 shows a typical example of the data, in which it is observed that the theoretical line correlates well with the experimental data calculated.

RESULTS AND DISCUSSION

Effect of Sodium Lauryl Sulfate *(In Situ)*—Table I represents the effect of sodium lauryl sulfate, in concentrations below and above the critical micelle concentration (CMC), on the absorption and metabolism of I in the small intestine of rats. Sodium lauryl sulfate decreased the absorption rate constant with the increase of concentration in the perfusate. This retardation is considered due to the same mechanism that results in a suppression of the reaction rate between I and glutathione or cysteine in the solution of sodium lauryl sulfate *in vitro*, namely, the formation of a 1:2 complex of I with the lauryl sulfate anion in the circulation fluid (3). This consideration is also supported by the reported observation that the absorption of the entrapped drug into surfactant micelles is negligible (7, 8).

Sodium lauryl sulfate also decreased the rate constant for the enzymatic deacylation of I in concentrations below and above the CMC. This effect can be explained by the same idea of complexation as applied to retardation of the absorption rate. Another possible explanation of the suppression of hydrolysis is a denaturative inactivation of the intestinal esterase by sodium lauryl sulfate. In an experiment with an intestinal homogenate, however, practically no inactivation of the esterase activity was observed for a commonly used substrate, methyl butyrate, in the presence of sodium lauryl sulfate below the CMC under similar condition[3].

In contrast to the inhibition of absorption and hydrolysis, the reduction rates were found to increase about two times as much as the control value in the presence of sodium lauryl sulfate with its concentrations below and above the CMC (Table I). Such a reducing-accelerating action of sodium lauryl sulfate was also observed in the *in vitro* experiment, in which sodium lauryl sulfate accelerated the reaction between I and cysteamine (2).

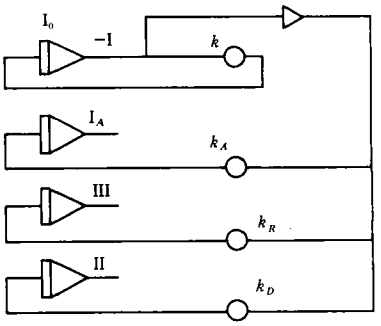
A reducing substance of low molecular weight was detected in the fluid that was circulated without I in the small intestine of rats using a gel filtration technique[3]. Thus, it may be feasible that the reductive cleavage of I in the intestinal tract *in situ* is carried out by a cysteamine-like substance.

Table II data show the effects of sodium lauryl sulfate on the absorption and metabolism of II. The intestinal behavior of II is expected to be affected to a lesser degree than I in the presence of sodium lauryl sulfate since it has a looser interaction with the lauryl sulfate anion (2). In fact, sodium lauryl sulfate produced little decrease on the rate constants for the absorption and reduction of II, consistent with the previous *in vitro* results (2).

From the observations, it is clear that sodium lauryl sulfate has similar characteristics in its effect on the absorption and the metabolism of I and II as observed in the *in vitro* experiment in the reactions of thiamine disulfide compounds with thiols (2, 3).

Effect of Sodium Glycocholate *(In Situ)*—Table III represents the rate constants for the absorption and metabolism of I in the circulation fluid with or without sodium glycocholate. Sodium glycocholate did not influence the absorption rate at the concentration of 0.015% (below the CMC) but raised it by approximately 50% at a higher concentration than 0.1%, where it formed micelles including some portion of I.

Based on these findings, it is suggested that sodium glycocho-



Scheme II—Analog computer program for kinetic model of absorption and metabolism of O*-benzoylthiamine disulfide (I)*

Table II—Effect of Sodium Lauryl Sulfate on Rate Constants for Absorption and Metabolism of Thiamine Disulfide[a] (II)

System	Number of Rats	Rate Constants, hr^{-1}	
		k_A	k_R
Control[b]	3	0.27 ± 0.01	0.25 ± 0.05
0.015% sodium lauryl sulfate	3	0.30 ± 0.07	0.26 ± 0.03
0.1% sodium lauryl sulfate	3	0.22 ± 0.04	0.25 ± 0.08

[a] II in perfusate: 10 μg/ml (50 ml), pH 6.4, 37°. [b] Without surfactant.

[3] Unpublished data.

Table III—Effect of Concentration of Sodium Glycocholate on Rate Constants for Absorption and Metabolism of *O*-Benzoylthiamine Disulfide[a] (I)

System	Number of Rats	Rate Constants, hr^{-1}		
		k_A	k_R	k_D
Control[b]	3	0.40 ± 0.03	0.37 ± 0.05	0.68 ± 0.03
0.015% sodium glycocholate	3	0.38 ± 0.05	0.36 ± 0.07	0.20 ± 0.06
0.1% sodium glycocholate	3	0.38 ± 0.02	0.32 ± 0.01	0.22 ± 0.04
0.17% sodium glycocholate	3	0.62 ± 0.04	0.36 ± 0.03	0.18 ± 0.02
0.25% sodium glycocholate	3	0.57 ± 0.06	0.40 ± 0.01	0.08 ± 0.07
0.34% sodium glycocholate	3	0.55 ± 0.02	0.32 ± 0.01	0.15 ± 0.03
0.5% sodium glycocholate	3	0.62 ± 0.07	0.23 ± 0.07	0.12 ± 0.02

[a] I in perfusate: 10 μg/ml (50 ml), pH 6.4, 37°. [b] Without surfactant.

Table IV—Effect of Polysorbate 80 on the Rate Constants for the Absorption and Metabolism of *O*-Benzoylthiamine Disulfide[a] (I)

System	Number of Rats	Rate Constants, hr^{-1}		
		k_A	k_R	k_D
Control[b]	3	0.40 ± 0.03	0.37 ± 0.05	0.68 ± 0.03
0.015% polysorbate 80	3	0.37 ± 0.05	0.32 ± 0.04	0.67 ± 0.05
0.5% polysorbate 80	3	0.17 ± 0.01	0.15 ± 0.05	0.26 ± 0.03

[a] I in perfusate: 10 μg/ml (50 ml), pH 6.4, 37°. [b] Without surfactant.

late, a main component of bile salts, does not decrease the absorption of I in concentrations above the CMC but, instead, increases the absorption. This enhancing effect above the CMC is quite the reverse of that observed with sodium lauryl sulfate. As reviewed by Gibaldi (9), it is known that bile acid salts alter membrane permeability toward certain drugs; therefore, the absorption-enhancing effect of sodium glycocholate is attributable to a membrane-altering action of sodium glycocholate.

Unabsorbed I underwent a lesser deacylation in the presence of sodium glycocholate than in the solution free from surfactant. Increased stability was also observed in the reduction process. The stabilizing action of sodium glycocholate possibly enhances the absorption of I because of a persistently high concentration of I at the absorption site.

It may be concluded that sodium glycocholate is an effective surfactant for increasing the absorption of I, enhancing the absorption rate at the same time as it suppresses hydrolysis.

Effect of Polysorbate 80 *(In Situ)*—The influence of polysorbate 80 on the absorption and metabolism of I is shown in Table IV.

Polysorbate 80 decreased the absorption rate constant at the 0.5% concentration (above the CMC) where the micelles are formed but did not decrease the rate constant at the 0.015% concentration (below the CMC). These results suggest that polysorbate 80 inhibits absorption by means of the uptake of I into micelles. Polysorbate 80 also decreased the rates of hydrolysis and reduction, but only at the 0.5% concentration. The inhibition is considered to result from the same factor suggested for the absorption-inhibiting effect. The immobilization was reported previously with other drugs (1, 7, 8, 10–12).

Effect of Binary Surfactant Mixtures *(In Situ)*—Because of the interesting effects of the mixture of sodium lauryl sulfate and sodium glycocholate on the reaction of I with glutathione noted in the previous *in vitro* experiment (3), the effect of the binary mixtures of sodium glycocholate with either sodium lauryl sulfate or polysorbate 80 on the intestinal behavior of I *in situ* was investigated.

Table V presents the values for the rate constants for the absorption and metabolism of I in solution with and without binary mixtures. The rate constant for absorption was 0.38 ± 0.03 hr^{-1} in the presence of 0.1% sodium lauryl sulfate and 0.5% sodium glycocholate, so there was no decrease as compared with the control value of 0.40 ± 0.03 hr^{-1}. Since sodium lauryl sulfate strongly decreases the absorption rate of I (Table I), it is apparent that sodium glycocholate reduces the absorption-inhibiting effect of sodium lauryl sulfate. This antagonistic effect was also observed in the reduction process, in which sodium glycocholate reduced the rate-increasing effect of sodium lauryl sulfate. On the other hand, sodium glycocholate did not influence the absorption-inhibiting action of polysorbate 80. The difference in the effect of sodium glycocholate on the absorption between the two binary systems suggests that the nonionic surfactant, polysorbate 80, differs from the ionic one, sodium lauryl sulfate, in its way of forming mixed micelles.

The detailed study on the absorption rate of I, which was determined to be a function of concentration of sodium glycocholate in a medium with 0.1% sodium lauryl sulfate, indicated that sodium glycocholate at a concentration above 0.25%, at which the molar ratio of sodium glycocholate to sodium lauryl sulfate was 1.5, was able to inhibit the action of sodium lauryl sulfate and thereby increase the decreased rate of absorption up to approximately the control level (Table VI). In an identical experiment (Table VI), sodium glycocholate reduced the increased reduction rate at the minimum concentration of 0.17% which was approximately the same as that observed for the absorption rate.

A similar antagonistic phenomenon was observed in the *in vitro* experiment (3) concerning the reaction of I with glutathione, in which sodium glycocholate reduced the rate-increasing effect of I. As predicted previously (3), the role of sodium glycocholate in binary surfactant mixtures is explained in terms of a possible formation of mixed micelles composed of I, sodium lauryl sulfate, and sodium glycocholate. Similarly, the absorption and reduction of I in the present investigation can be controlled by at least mixed micelles possibly increasing the mobility of I into the membrane surface.

***In Vivo* Studies**—The evaluation of the absorption of I by the rat was made by measuring the amount of urinary excretion of thiamine, which was ascribed to administered I. Urinary excretion data are shown in Table VII. When a control solution containing 200 μg of I was administered, 18.1 ± 1.8% of the dose was recovered from the urine during 7 hr after administration. When I was given in the micellar solution of 0.1% sodium lauryl sulfate, 30.4 ± 7.7% was recovered. It was evident that the absorption increased about two times as much as that in the control solution. Thus, sodium lauryl sulfate apparently had an enhancing action on the absorption of I *in vivo*, whereas it decreased the rate of absorption in the circulation experiment *in situ*. The reversed situation suggests that the effect of sodium lauryl sulfate *in vivo* is ex-

Table V—Effect of the Binary Surfactant Mixtures on the Rate Constants for the Absorption and Metabolism of *O*-Benzoylthiamine Disulfide[a] (I)

System	Number of Rats	Rate Constants, hr^{-1}		
		k_A	k_R	k_D
Control[b]	3	0.40 ± 0.03	0.37 ± 0.05	0.68 ± 0.03
0.1% sodium lauryl sulfate–0.5% sodium glycocholate	3	0.38 ± 0.07	0.30 ± 0.07	0.04 ± 0.08
0.5% polysorbate 80–0.5% sodium glycocholate	3	0.20 ± 0.02	0.13 ± 0.04	0.06 ± 0.01

[a] I in perfusate: 10 μg/ml (50 ml), pH 6.4, 37°. [b] Without surfactant.

Table VI—Effect of Concentration of Sodium Glycocholate on the Absorption of *O*-Benzoylthiamine Disulfide (I) from Mixtures of Sodium Lauryl Sulfate and Sodium Glycocholate[a]

System	Number of Rats	Rate Constants, hr^{-1}	
		k_A	k_R
Control[b]	3	0.40 ± 0.03	0.37 ± 0.05
0.1% sodium lauryl sulfate	3	0.18 ± 0.03	0.76 ± 0.14
0.1% sodium lauryl sulfate–0.1% sodium glycocholate	3	0.22 ± 0.03	0.50 ± 0.07
0.1% sodium lauryl sulfate–0.17% sodium glycocholate	3	0.23 ± 0.01	0.39 ± 0.03
0.1% sodium lauryl sulfate–0.25% sodium glycocholate	3	0.37 ± 0.05	0.36 ± 0.06
0.1% sodium lauryl sulfate–0.34% sodium glycocholate	3	0.43 ± 0.07	0.39 ± 0.03
0.1% sodium lauryl sulfate–0.5% sodium glycocholate	3	0.38 ± 0.07	0.38 ± 0.07

[a] I in perfusate: 10 μg/ml, pH 6.4, 37°. [b] Without surfactant.

Table VII—Urinary Excretion of Thiamine after Administration of *O*-Benzoylthiamine Disulfide in Micellar Solutions[a]

Micellar Solution	Urinary Excretion of Thiamine, %
Control[b]	18.1 ± 1.8
0.1% sodium lauryl sulfate	30.4 ± 7.7
0.5% polysorbate 80	14.4 ± 3.5
0.5% sodium glycocholate	24.7 ± 4.2

[a] Dose: 200 μg/2 ml (pH 6.4). [b] Without surfactant.

plained by at least two mechanisms. These may both be inferred from the *in situ* results of the surfactant effect on the absorption of I. First, there may be a breakdown of the micellar complex between I and the lauryl sulfate anion, formation of which is the cause of the decrease in the absorbability of I, due to the occurrence of a biosurfactant such as bile salts in the rat intestine. A possible biosurfactant effect, *i.e.*, canceling the action of sodium lauryl sulfate, is demonstrated by the *in situ* experiment (Tables V and VI). Second, in the intestinal tract *in vivo*, the biosurfactant as well as the synthetic one may act to protect I from its enzymatic deacylation and may increase the absorption. Unlike the case of sodium lauryl sulfate, polysorbate 80 decreased the absorbability of I *in vivo*, and this is consistent with the result observed in the *in situ* experiment.

When I was given in the micellar solution of 0.5% sodium glycocholate, the urinary excretion of thiamine was 24.7 ± 4.2% and little increase in absorbability was observed. Since sodium glycocholate showed an increasing effect on the absorption of I *in situ*, the *in vivo* effect is acceptable.

SUMMARY AND CONCLUSION

The role of biosurfactants and synthetic surfactants in the absorption of thiamine derivatives was investigated by both *in situ* and *in vivo* experiments using rats.

In the *in situ* experiment, sodium lauryl sulfate at concentrations above and below the CMC decreased the rates of absorption and hydrolysis of I but accelerated the rate of the reduction. Polysorbate 80 decreased all of these rates. In contrast to these two synthetic surfactants, sodium glycocholate produced a considerable increase in the absorption rate of I. The addition of a 0.1% sodium lauryl sulfate–0.5% sodium glycocholate mixture did not influence the absorption rate constant of I, indicating that sodium glycocholate inhibited the absorption-inhibiting effect of sodium lauryl sulfate. This antagonistic effect was not observable in the mixed system of 0.5% polysorbate 80 and 0.5% sodium glycocholate.

In the *in vivo* studies, sodium lauryl sulfate in 0.1% micellar solution showed an increase in the absorbability of I about twice that of the solution without surfactant. Such an increase was the reverse of the *in situ* effect of sodium lauryl sulfate on the absorption of I. It follows that endogenous bile salts in the intestine participate in the formation of mixed micelles including I and sodium lauryl sulfate. This kind of mechanism is acceptable according to the *in situ* results of the absorption and metabolism of I in binary mixtures of sodium lauryl sulfate and sodium glycocholate and in consideration of the stabilizing action of their mixtures on the hydrolysis of I. Sodium glycocholate in 0.5% micellar solution showed the enhancing action on the absorption of I *in vivo*. Administration of I in micellar solution of 0.5% polysorbate 80 resulted in lowered absorption, and this finding agreed with the results of the *in situ* experiment.

The observations of the effects of sodium lauryl sulfate, sodium glycocholate, and polysorbate 80 on the absorption of I indicate that special biosurfactant effects should be considered when evaluating the individual effect of the surfactant administered with the drug.

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Antibacterial Efficiency of Mercurials

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Abstract □ The minimum inhibitory concentrations (MIC) of five different mercurials against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were determined. Addition of sodium thioglycolate to the nutrient medium inactivated the mercurials, since higher MIC's were obtained. The extent of inactivation did not follow the stoichiometric relation with all tested mercurials and was different for each organism. The survivors-time relations of these compounds were measured using different concentrations of thioglycolate in the diluent. The sulfhydryl compound plays a role in the revival of damaged cells. The percentage of survivors was influenced by thioglycolate concentration; the highest survivors were obtained at an optimal concentration which was the same for all mercurials but changed with the test organism. *Ps. aeruginosa* was much more sensitive to the effect of the tested mercurials than was *Staph. aureus*. Mercuric chloride was the most efficient bactericide, followed by the phenylmercuric salts; thimerosal was the least. The possible mechanisms involved for such differences in efficiencies are discussed.

Keyphrases □ Mercurial compounds—antibacterial efficiency against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, effect of sodium thioglycolate in nutrient medium, role of sulfhydryl group □ Antibacterial efficiency—five mercurial compounds tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, effect of sodium thioglycolate in nutrient medium □ Thioglycolate (sodium)—effect on antibacterial efficiency of five mercurial compounds

The antibacterial action of mercurial compounds has been widely reported (1–7). Their mode of action was due to interference with essential metabolites and enzymes having sulfhydryl groups (8–11) or to reaction with the DNA (12) and RNA (13) of the cell. However, the antibacterial activity of these agents was found to be influenced by: *(a)* the chemical structure of the compound (14), *(b)* the type of test organism used [Gram-positive bacteria were reported to be more sensitive to mercurials than were Gram-negative ones (15)], and *(c)* the type of the quenching agent used to stop their effect, *e.g.*, sodium thioglycolate was a more efficient antagonizing agent than glutathione (11). Furthermore, previous work (16) with phenylmercuric nitrate showed that its antibacterial activity was greatly reduced by sodium thioglycolate when used in the diluent and at an optimum concentration. In this same study, thioglycolate was involved in the recovery of the damaged bacteria previously exposed to the effect of the mercurial.

Table I—Minimum Inhibitory Concentrations (MIC) of Different Mercurials against *Staph. aureus* and in the Presence of Sodium Thioglycolate

Mercurial Compound	Thioglycolate Concentration 0% MIC[a]	0.001% MIC	0.001% Hg to —SH[b]	0.003% MIC	0.003% Hg to —SH
Mercuric chloride	8.0	15	1:3	30	1:2.5
Thimerosal	1.0	4	1:12	7	1:14
Phenylmercuric acetate	0.5	4	1:8	7	1:13
Phenylmercuric borate	0.5	4	1:8	7	1:13
Phenylmercuric nitrate	0.5	4	1:8	7	1:13

[a] Micrograms per milliliter, average of six replicates. [b] Ratio of inactivated mercury molecules to thioglycolate molecules as given by: inactivated mercury molecules = (MIC of mercurial with thioglycolate − MIC without thioglycolate)/molecular weight of mercury compound.

In the present investigation, five commonly used mercurial compounds, one of which is inorganic, were tested for their antibacterial efficiencies on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, using sodium thioglycolate solution at different concentrations as the antagonizing agent. The purposes of this investigation were to study the effect of thioglycolate concentration on the antibacterial activity of mercurial compounds and to evaluate the antibacterial efficiency of these agents at the thioglycolate concentrations tested.

EXPERIMENTAL

Reagents—The following were used: mercuric chloride[1], phenylmercuric acetate[2], phenylmercuric nitrate[2], thimerosal[2], phenylmercuric borate[3], and thioglycollic acid[2] [neutralized aseptically with 1 *N* NaOH to prepare the stock sodium thioglycolate solution (16)].

Organisms—*Staphylococcus aureus* (NCTC 4163) and *Pseudomonas aeruginosa* (NCTC 7244) were used.

[1] Analar, British Drug Houses.
[2] Laboratory reagent grade, British Drug Houses.
[3] Laboratory reagent grade, Koch-Light Ltd.