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Thiamine Derivatives of Disulfide Type. II.[1] The Formation of Thiamine from the Disulfide Derivatives in Rat Intestine *in Vitro*[2]

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The formation of thiamine from disulfide derivatives, *i.e.*, thiamine tetrahydrofurfuryl disulfide, thiamine disulfide, thiamine benzyl disulfide, and thiamine propyl disulfide, was examined by rat intestine using a modified Wiseman–Smyth's apparatus. From the results obtained the following conclusions were drawn.

1) The rate of thiamine formation followed first order kinetics, and the derivatives with the larger rate constant had the larger partition coefficient for an organic solvent. It would mean that the interaction between lipophilic gut wall and the disulfide derivatives was the one of the important factor for the conversion.

2) The temperature effect on the conversion was examined between 27° and 47°. The contribution of chemical reaction and diffusion process was concluded from the apparent activation energy obtained.

3) Since the reduction was observed at both sides of intestine, the reaction mechanism would not relate directly to the absorption site of intestine but the general biological phenomenon which might be observed in other animal tissues.

In the proceeding paper,[1] the conversion of thiamine propyl disulfide (TPD) to free thiamine was studied kinetically using rat intestine attached to a modified Wiseman–Smyth's apparatus and the rate and the mechanism of the reaction was discussed. The conversion has been concluded that the chemical reactions as shown in Eq. (1) and (2) coupled with a enzymatic reaction represented by Eq. (3) which will be reported by the following paper in detail.

$$G(\text{-SH}) + B_1\text{SSPr} \longrightarrow B_1\text{SH} + G(\text{-SSPr}) \quad (1)$$

$$G(\text{-SSPr}) + G(\text{-SH}) \longrightarrow G(\text{-SS-})G + \text{PrSH} \quad (2)$$

$$G(\text{-SS-})G \longrightarrow 2G(\text{-SH}) \quad (3)$$

where G(–SH) is thiol group in gut tissue, B_1SSPr TPD, B_1SH thiamine, G(–SSPr) propyl disulfide derivative in gut wall, PrSH propylthiol, and G(–SS–)G thiol in gut wall in oxidized form, respectively.

The procedure used in the previous study was applied to other disulfide derivatives of thiamine, *i.e.*, thiamine tetrahydrofurfuryl disulfide (TTFD), thiamine disulfide (TDS), and thiamine benzyl disulfide (TBD), for the study of the conversion during the absorption of the drugs administered orally. The mechanism proposed was confirmed on the conversion of these compounds used in the present study.

1) Part I: H. Nogami, J. Hasegawa, and K. Noda, *Chem. Pharm. Bull.* (Tokyo), **17**, 219 (1969).
2) Presented before the Kanto Branch Meeting of Pharmaceutical Society of Japan, Tokyo, January, 1966.
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Experimental

Chemicals——TPD, TTFD, TBD, TDS, Thiamine hydrochloride, and 5,5′-dithiobis(2-nitrobenzoic acid) were as same as the previous report.[1]

Method——Experimental method and the circulation apparatus used were as same as reported previously.[1]

Animals——Normal adult male Donryu rats weighing 220—330 g were used. Intestine sample was prepared by the same procedure as reported in the proceeding paper.[1]

Analysis——Disulfide type thiamine derivatives were reduced to free thiamine with the treatment of cysteine. Free thiamine was oxidized to thiochrome and the optical density at 368 mμ was determined by Hitachi-Perkin-Elmer 139 spectrophotometer. Thiol group was determined spectrophotometrically by the method of Ellman.[4] Mercuric ion was determined by dithizone method as previously reported.[1]

Determination of Partition Coefficients——Ten ml of thiamine derivative solution in 0.1M phosphate buffer of pH 7.4 was shaken with 10 ml of organic solvent at 20°. After equilibrium was maintained, the concentration of thiamine derivative in aqueous layer was determined with the optical density at 275 mμ. Partition coefficient was calculated by Eq. (4).

$$\text{P. coef.} = \frac{\text{init. conc. of aq. soln.}}{\text{equilibrium conc. of aq. soln.}} - 1 \qquad (4)$$

Results and Discussion

As mentioned in the proceeding paper, the thiol compound(s) was secreted from intestinal tissue and thiamine was formed by an exchange reaction between the disulfide derivatives and the thiol compound(s). The effect may not be neglected especially in the case of TBD since it is impossible to use enough amount of the compound for disregard of the side reaction when the solubility of the compound is limitted. The amount of the thiol compound secreted during perfusion, therefore, was determined.

Eighty ml of Tyrode solution was perfused in serosal and mucosal sides and the time plot of thiol content was conducted. The result is given in Fig. 1, where the secretion was prosperous for initial twenty minutes and an equilibrium state was observed after that. Since the rapid reaction was expected between disulfide and thiol group, it would be reson-

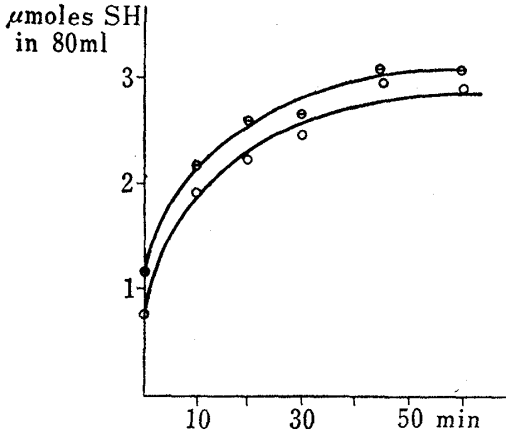

Fig. 1. Secretion of Thiol Compound(s) into Circulated Solution

⊖: mucosal solution ○: serosal solution

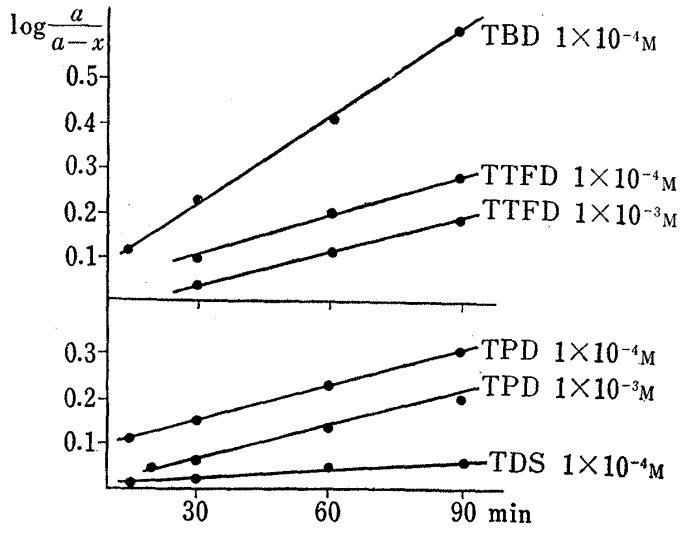

Fig. 2. First Order Plot for Reduction of Disulfide Type Thiamine Derivatives at Mucosal Side

4) G.L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).

able to assume that the formation of thiamine after twenty minutes is caused mainly by the coupled system of chemical and enzymatic reaction in intestinal tissue.

The formation of thiamine from disulfide derivative in mucosal solution was studied as follows. Eighty ml of thiamine derivative in Tyrode solution and 80 ml of Tyrode solution were circulated at mucosal and serosal sides, respectively, and the amount of thiamine in mucosal solution was determined after initial twenty minutes. When the reactions follow the relation shown by the Eq. (1), (2), and (3), the formation of thiamine should be represented by first order kinetics. The amount of drug transfered to serosal side was neglected since it was very few in per cent at the experimental condition. The result is given in Fig.2 in which linear plot was obtained as expected. The rate constant was calculated from the slope of straight line and shown Table I.

Table I. Apparent Rate Constants of Thiamine Formation

Thiamine derivatives	k_{app} hr^{-1}	
	Mucosal side	Serosal side
TDS	0.05	0.05
TTFD	0.32	0.20
TPD	0.34	0.21
TBD	0.86	0.39

The same experiment mentioned was conducted in serosal solution. The result is given in Fig. 3. The rate constant from first order plot in the figure is given in Table I. The consistency of rate constant was observed within the concentration range of 10^{-3} to 10^{-4} molar for TPD and TTFD which might be one of the evidence of the relations shown by Eq. (1), (2), and (3).

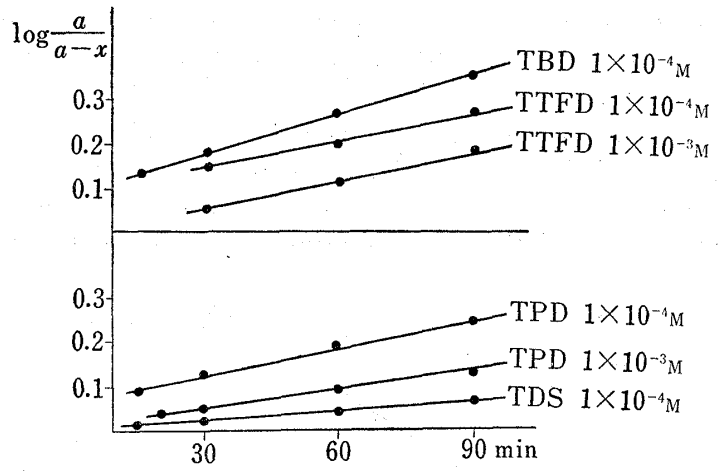

Fig. 3. First Order Plot for Reduction of Disulfide Type Thiamine Derivatives at Serosal Side

The distribution coefficient between some organic solvents and phosphate buffer solution is tabulated in Table II. The relation between rate constant and the coefficient is given in Fig. 4 and 5. As seen in the figures, the larger rate constant was found on TBD which showed higher distribution in organic solvents and the smaller rate was observed on TDS which showed hydrophilic character in distribution coefficient on the contrary. Many instances have been reported since the studies on the drug pene-

Table II. Partition Coefficient[a] of Thiamine Derivatives

Thiamine derivatives	Organic solvent			
	Benzene	Cyclohexane	Chloroform	Ethyl acetate
TDS	—	0.005	0.032	0.093
TTFD	0.003	0.173	14.8	1.17
TPD	0.014	0.847	35.9	7.53
TBD	0.03	4.23	26.2	47.8

a) 20°, organic solvent/0.1M phosphate buffer pH 7.4

tration by Overton[5] that the larger distribution to intestinal wall, the more rapid uptake of drugs.[6-8]

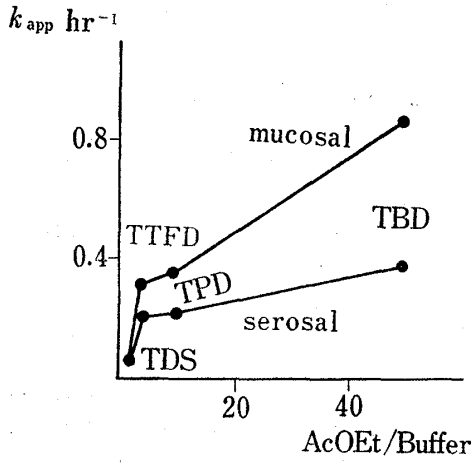

Fig. 4. Relationship between Apparent Rate Constant and Partition Coefficient

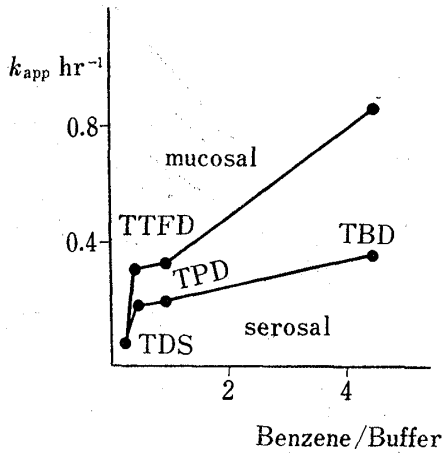

Fig. 5. Relationship between Apparent Rate Constant and Partition Coefficient

It may be concluded from the results mentioned that the interaction between disulfide derivatives and lipophilic mucosal membrane is the important factor for the conversion of thiamine derivatives.

The effect of temperature on the reaction was examined. Mercuric chloride dissolved in physiological saline was circulated for two hours in both sides of intestine for the examination of reactivity of thiol group in intestinal wall which would be assumed as an index of temperature change of gut wall. The decrease of mercuric ion during the circulation is given in Table III from which it may be concluded that the reactivity did not change within the temperature range experimented.

Table III. Decrease of Mercuric Ion after Circulation of Gut Lumen for 2 Hours

Temperature (°C)	Decrease of mercuric ion (μmoles)
27	25.6
37	24.4
47	17.2

The time plot of thiamine formation as first order reaction is shown in Fig. 6 and 7 which was determined from 27° to 47° in the mucosal 10^{-3} molar TPD or TTFD solution when Tyrode solution was prefused at serosal side. An Arrhenius plot is given in Fig. 8 and 9 where the rate constant was calculated from the slope of the straight line in the proceeding figures. Apparent activation energy was 4.3 and 22.4 kcal/mole for TPD and 5.0 and 26.9 kcal/mole for TTFD, respectively, when it was calculated from the slope of two lines seen in the figures.

If we can assume no effect of temperature on the intestinal tissue of rat in experimental conditions, it may be concluded that the values of apparent activation energy obtained at

5) E. Overton, *Viertejahrsshr. Naturforsh. Ges. Zurich*, **40**, 159 (1895).
6) R. Höber and J. Höber, *J. Cell. Comp. Physiol.*, **10**, 401 (1937).
7) C.A.M. Hogben, L.S. Schanker, D.J. Tocco, and B.B. Brodie, *J. Pharmacol. Exper. Therap.*, **125**, 275 (1959).
8) L.S. Schanker, *J. Pharmacol. Exper. Therap.*, **126**, 283 (1959).

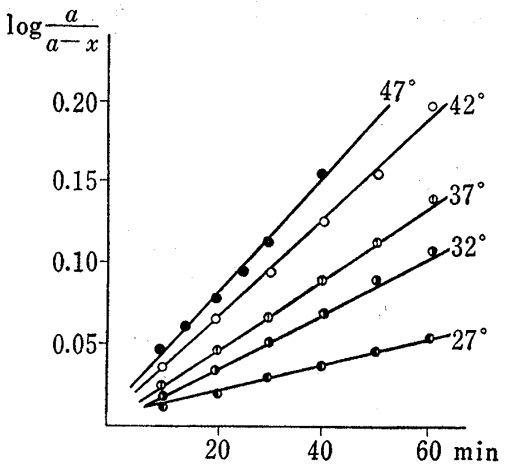

Fig. 6. First Order Plot for Thiamine Formation from TPD at Various Temperature

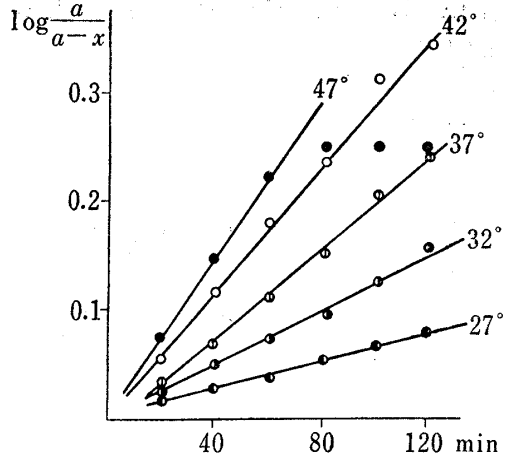

Fig. 7. First Order Plot for Thiamine Formation from TTFD at Various Temperature

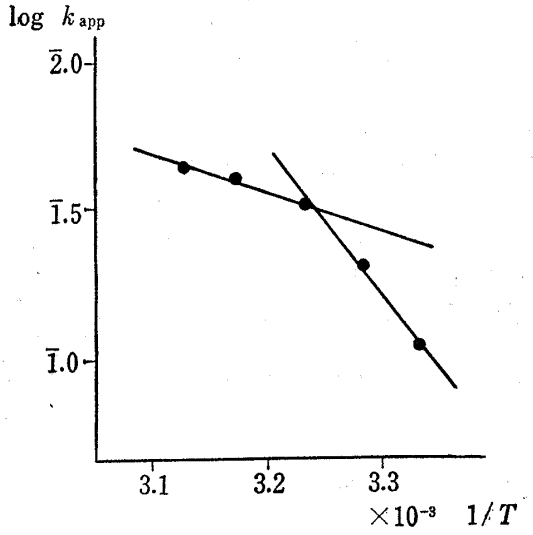

Fig. 8. Arrhenius Type Plot for Thiamine Formation from TPD

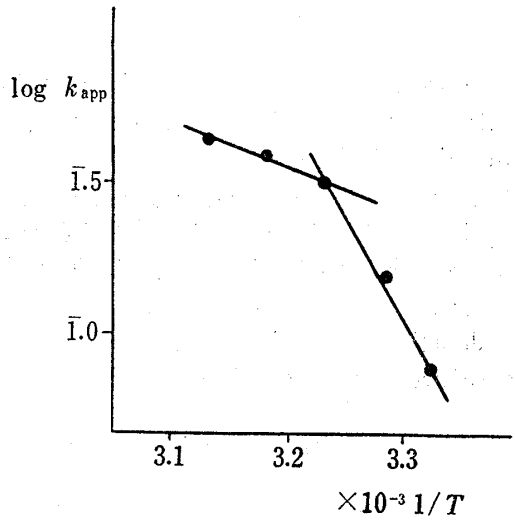

Fig. 9. Arrhenius Type Plot for Thiamine Formation from TTFD

lower and higher temperature region are the evidence of contribution of chemical reaction and diffusion process, respectively, since 22.4 and 26.9 or 4.3 and 5.0 kcal/mole are probable magnitude for a thiol-disulfide exchange reaction or the physicochemical process. The further examination would be required, however, for the propriety of the assumption mentioned above. From the experimental results, the conversion of thiamine derivatives of disulfide type to thiamine may be represented as follows. In the gut, the thiol-disulfide exchange reaction between disulfide type derivative and unknown thiol compound(s) secreted in mucosal solution may not be neglected, but the interaction between the derivative and lipophilic gut wall may be the important factor for conversion since the partition coefficient of the derivative was related to the rate of conversion. It was not discriminated from the experimental results that whether thiol-group in small molecule like glutathione or the one in protein molecule contributs to the exchange reaction in gut wall, but the both reactions probably may occur. It was assumed, however, that glutathione content in animal tissue was 20 to 100 mg per cent,[9,10] therefore, the main portion contributed to this exchange reac-

9) H.H. Tallan, S. Moore, and W.H. Stein, *J. Biol. Chem.*, 211, 927 (1954).
10) S.K. Bhattacharya, J.S. Robson, and C.P. Stewart, *Biochem. J.*, 62, 12 (1956).

tion might be glutathione. It was also reported[11] that the reactivity of thiol group in native albumin molecule was not evident as compared to the one in denaturated albumin. Since the conversion process mentioned was observed not only at mucosal side but also at serosal side of intestine, it meant the mechanism would not directly related to the absorption site of gastrointestinal tract but would be the general phenomenon which might be observed in other tissues of animal body.

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11) I. Utsumi, K. Harada, and K. Kohno, *Vitamins* (Kyoto), **26**, 128 (1962).