CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 18, No. 10 October 1970

Regular Articles

[Chem. Pharm. Bull. 18(10)1937—1944(1970)]

UDC 615.356 : 577.164.11.076.9

# Pharmacokinetics of Thiamine Derivatives. II.[1] Elimination of Thiamine Derivatives from the Blood of Rat[2]

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(Received December 13, 1969)

Some thiamine derivatives were compared pharmacokinetically about their elimination from blood after intravenous injection in rat. TPD (thiamine propyl disulfide), CCT (cyclocarbothiamine) and BTMP (S-benzoylthiamine monophosphate) were used as the typical thiamine derivatives.

1) It was found that the rates of conversion of TPD and CCT to thiamine in blood were rapid, but that of BTMP was slow.

2) TPD formed thiamine mainly through erythrocytes, but CCT formed thiamine mainly through plasma.

3) Volume of distribution (Vd or $V_1$) of TPD, BTMP, CCT and thiamine were 37.6, 93.5, 187.5 and 256.1 ml, respectively. After intravenous injection of TPD, BTMP, CCT and thiamine rate constants of elimination of thiamine ($k_{el}$ or $k'_{el}$) were 0.145, 0.151, 1.61 and 1.98 $hr^{-1}$ respectively. Elimination constant of TPD was nearly equal to that of BTMP, and that of CCT to that of thiamine.

4) TPD and CCT behaved almost same in blood *in vitro*, except elimination of them from blood.

5) Since both TPD and BTMP were considerably taken up into erythrocytes, and also their elimination constants were equal to each other, the elimination constant must mean the constant of transfer rate from erythrocytes to plasma.

6) Pharmacokinetic model was proposed for each derivative.

It has been known that a proper chemical modification of thiamine brings about the increase of absorption and retention in body.[4] Although absorption and excretion of chemically modified thiamine derivatives were reported by many workers,[5] comparison between various thiamine derivatives each other was scarcely investigated, except comparison between thiamine and the derivatives. Especially, pharmacokinetic comparison has not yet been

1) This paper forms Part XVIII of "Studies on Absorption and Excretion of Drug," by H. Nogami. Part XVII: H. Nogami, M. Hanano, S. Awazu and T. Fuwa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1168 (1970).

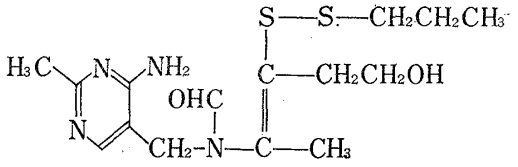
2) This work was presented at the 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969, being taken in part from the thesis of Tohru Fuwa for the degree of Doctor of Pharmaceutical Sciences, University of Tokyo, 1969.

3) Location: *Hongo, Tokyo.*

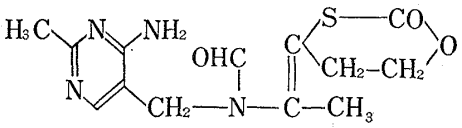
4) T. Matsukawa, S. Yurugi, H. Kawasaki, Y. Aramaki and J. Suzuoki, *Ann. Rep. Takeda Res. Lab.*, **12**, 1 (1953).

studied. Then, the present paper intends to describe and compare pharmacokinetically the processes of conversion of thiamine derivatives to thiamine in rat blood and elimination of thiamine derivatives from rat blood after intravenous injection of thiamine derivatives.

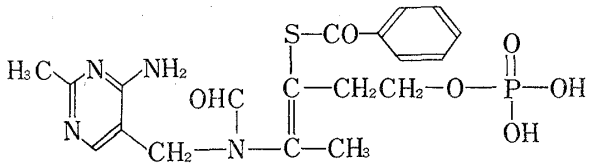
Thiamine derivatives used in this study were thiamine propyl disulfide (TPD), S-benzoylthiamine monophosphate (BTMP) and cyclocarbothiamine (CCT) (Chart 1).



thiamine propyl disulfide (TPD)



cyclocarbothiamine (CCT)



S-benzoylthiamine monophosphate (BTMP)

Chart 1. Chemical Structure of Thiamine Derivatives

## Experimental

**Experimental Procedure**——The male albino rats (Donryu) weighing 250—260 g were used. Administration of thiamine derivatives and sampling of blood were done by the previous method[6] under urethane anaesthesia (1 g/kg). Each dose was 1 mg. Only free thiamine in 0.1 ml of sampled whole blood was determined, since cocarboxylase formation was neglected.

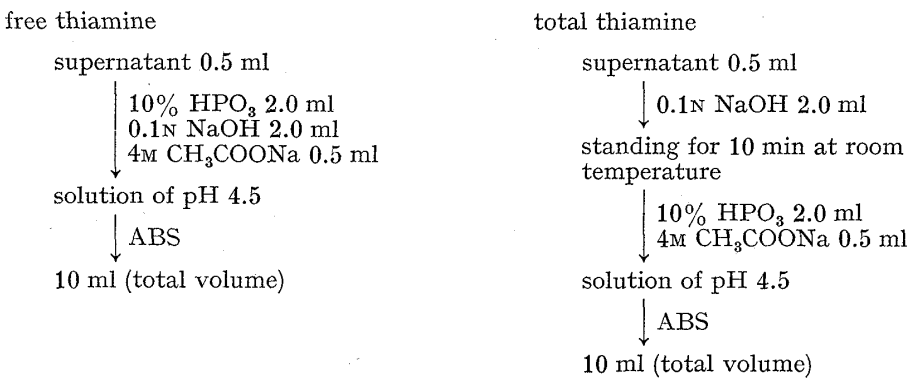



Chart 2. Separatory Determination Procedure for Free Thiamine and Cyclocarbothiamine

ABS: acetate buffer solution (pH 4.5)

5) C. Kawasaki and I. Tomita, *Vitamins* (Kyoto), **22**, 420 (1961); I. Utsumi, K. Harada, H. Kobayashi, K. Kohno, K. Yasuda, Y. Kondo and H. Hirao, *ibid.*, **25**, 71 (1962); C. Kawasaki, I. Tomita and T. Noriuji, *ibid.*, **25**, 112 (1962); T. Mineshita, M. Morita and T. Iwata, *ibid.*, **25**, 483 (1962); K. Takenouchi, K. Aso, S. Shimizu and T. Kobayashi, *ibid.*, **26**, 261 (1962); Y. Itokawa, *ibid.*, **28**, 564 (1963); K. Yoshikawa, *ibid.*, **31**, 101 (1965); K. Kohno, I. Saito and I. Utsumi, *ibid.*, **33**, 340 (1966); H. Bamba, K. Aso, K. Takenouchi and T. Shiozaki, *ibid.*, **35**, 195 (1967); K. Kohno, K. Noda and I. Utsumi, *ibid.*, **36**, 330 (1967); Y. Shiobara and M. Murakami, *ibid.*, **37**, 306 (1968); K. Aso, T. Shiozaki and K. Takenouchi, *ibid.*, **37**, 380 (1968); H. Shindo, K. Okamoto, J. Tohtsu and I. Takahashi, *ibid.*, **38**, 21 (1968).

6) H. Nogami, M. Hanano, S. Awazu and T. Iga, *Chem. Pharm. Bull.* (Tokyo), **18**, 228 (1970).

Other *in vitro* experiments were carried out by same method as the preceding paper.[7]

**Materials**——TPD, BTMP and CCT were supplied by Takeda Chemical Industries, Ltd., Sankyo Co., Ltd. and Yamanouchi Pharmaceutical Co., Ltd. respectively.

**Determination of Thiamine and CCT**——Free thiamine was determined by the previous method.[7] CCT was determined with the difference between free thiamine and total thiamine obtained after the complete hydrolysis in an alkaline solution. The reagents and procedures for the determination of free thiamine and total thiamine in a supernatant of erythrocytes suspension were shown in Chart 2.

Other procedures were as same as those on TPD.[7]

## Result and Discussion

**1) Formation of Thiamine from Thiamine Derivatives in Blood**——Since the fission of S–S and S–CO bonding of the thiamine derivatives are due to the reduction with thiol compounds or the hydrolysis in body,[8] the formation of thiamine from the derivatives was studied in blood *in vitro* as the first step of the comparison (Fig. 1).

It was found that in incubation at 37±0.1° TPD and CCT were converted rapidly to thiamine, but BTMP gradually. Since it was reported that the fission of thioester in BTMP was depressed with the adjacent phosphate group in BTMP,[9] it was assumed that BTMP was converted to thiamine as shown in Chart 3. Either of the two steps was considered to be the rate-determining step in the conversion process of BTMP, because thiamine appeared according to the first order kinetics as shown in Fig. 2.

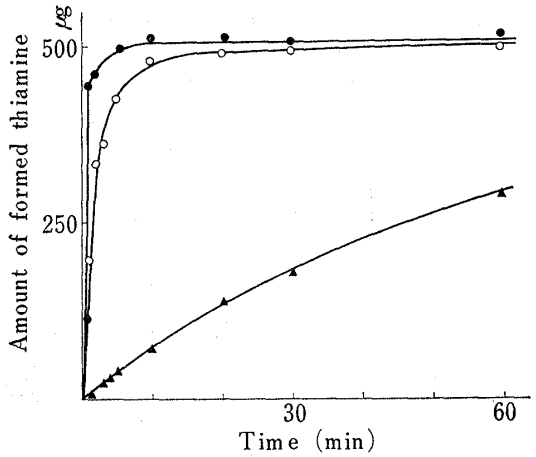



Fig. 1. Formation of Thiamine from Thiamine Derivatives in Rat Blood at 37±0.1°

initial amounts: amounts of thiamine derivatives equivalent to 500 μg thiamine hydrochloride
initial volume: 10 ml
—●—: thiamine propyl disulfide (TPD)
—○—: cyclocarbothiamine (CCT)
—▲—: S-benzoylthiamine monophosphate (BTMP)

BTMP ⟶ SBT ⟶ thiamine

Chart 3. Conversion Process of S-Benzoylthiamine Monophosphate to Thiamine in Blood

BTMP: S-benzoylthiamine monophosphate
SBT: S-benzoylthiamine

**2) Effect of Blood Components on Conversion of Thiamine Derivatives to Thiamine**——The effect of erythrocytes and plasma on the conversion was studied separetely. The results were given in Table I and II.

The remarkable difference was observed between the conversions of TPD and CCT. In 100 fold dilution experiment, erythrocytes had the effect on conversion of TPD, on the other hand, plasma had the effect on conversion of CCT. As for CCT, this result agreed with that of Murakami, *et al.*[10]

**3) Elimination of Thiamine Derivatives from Blood after Intravenous Injection**——Fig. 3 shows the time course of concentration of thiamine in blood after intravenous injection of thiamine or thiamine derivatives in rat.

7) H. Nogami, M. Hanano, S. Awazu and T. Fuwa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1168 (1970).
8) C. Kawasaki, M. Kondo and S. Katsuno, *Vitamins* (Kyoto), **37**, 431 (1968); H, Shindo, K. Okamoto, J. Tohtsu and I. Takahashi, *Vitamins* (Kyoto), **38**, 21 (1968); H. Nogami, J. Hasegawa and K. Noda, *Chem. Pharm. Bull.* (Tokyo), **17**, 219 (1969).
9) M. Yamazaki, *Vitamins* (Kyoto), **38**, 12 (1968).
10) M. Murakami, Y. Shiobara, N. Sato, H. Homma, R. Hattori and K. Yogi, *Vitamins* (Kyoto), **33**, 413 (1966).

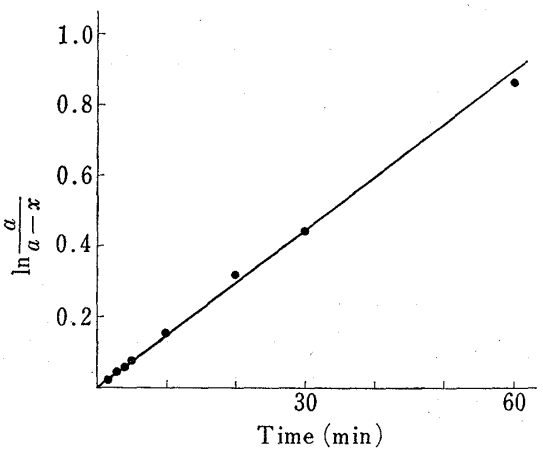



Fig. 2. First Order Plot of Formation of Thiamine from S-Benzoylthiamine Monophosphate in Rat Blood

initial amount: amount of S-benzoylthiamine monophosphate equivalent to 500 $\mu$g thiamine hydrochloride
initial volume: 10 ml
$a$: initial amount of S-benzoylthiamine monophosphate
$x$: amount of formed thiamine

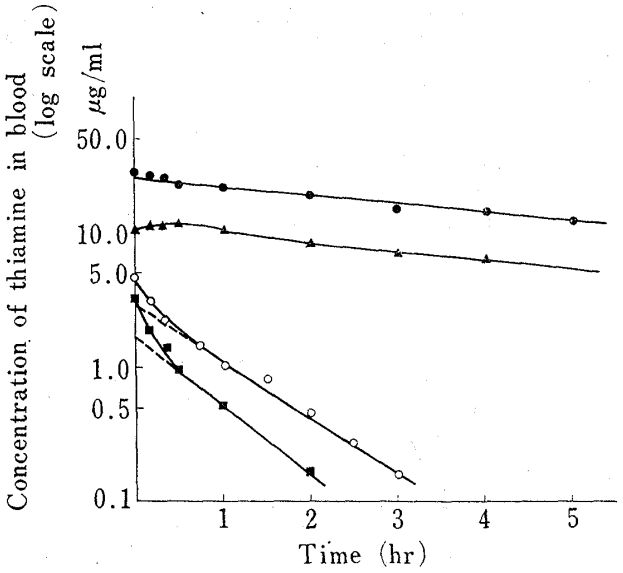



Fig. 3. Concentration of Thiamine in Blood after Intravenous Injection of Thiamine Derivatives Equivalent to 1 mg Thiamine Hydrochloride in Rat

—●—: thiamine propyl disulfide (TPD)
—▲—: S-benzoylthiamine monophosphate (BTMP)
—○—: cyclocarbothiamine (CCT)
—■—: thiamine

Table I. Formation of Thiamine from Thiamine Derivatives in Erythrocytes Suspension Variously Diluted with Krebs Ringer Phosphate Buffer Solution (pH 7.3)

| Thiamine derivatives | Dilution ratio of erythrocytes | | |
|---|---|---|---|
| | 1 | 1/10 | 1/100 |
| TPD | 50.0[a] | 50.0 | 48.0 |
| CCT | 40.7 | 8.5 | 1.5 |
| BTMP[b] | 0 | 0 | 0 |

initial amounts: amounts of thiamine derivatives equivalent to 50 $\mu$g thiamine hydrochloride
initial volume: 5 ml
incubation time: 30 min
*a*) All figures show amounts of formed thiamine equivalent to amount of thiamine hydrochloride ($\mu$g).
*b*) data estimated from the report by H. Shindo, *et al.*[11]
TPD: thiamine propyl disulfide
CCT: cyclocarbothiamine
BTMP: S-benzoylthiamine monophosphate

Table II. Formation of Thiamine from Thiamine Derivatives in Plasma Solution Diluted 100 Fold with Krebs Ringer Phosphate Buffer Solution (pH7.3)

| Thiamine derivatives | Time (min) | | |
|---|---|---|---|
| | 20 | 30 | 60 |
| TPD | 3.5[a] | 3.9 | 3.2 |
| CCT | 3.2 | 4.2 | 10.5 |
| BTMP | 0 | 0 | 0 |

initial amounts: amounts of thiamine derivatives equivalent to 50 $\mu$g thiamine hydrochloride
initial volume: 5 ml
incubation time: 30 min
*a*) All figures show amounts of formed thiamine equivalent to amount of thiamine hydrochloride ($\mu$g).

The time course of CCT was very similar to that of thiamine. But the courses of TPD and BTMP were remarkably different to above. CCT and thiamine itself were eliminated from blood according to two compartments model, since their time courses were composed of two exponential phases (see later section). On the other hand, TPD seemed to be eliminated according to one compartment model, since the time course was composed of one exponential phase. BTMP had a peak in the time course in Fig. 3 and it could be attributed to the gradual conversion to thiamine in blood as shown in Fig. 1.

The pharmacokinetic constants were obtained and given in Table III.

Table III. Volume of Distribution and Elimination Constants of Thiamine and Thiamine Derivatives

| | Vd (ml) | $V_1$ (ml) | $k_{el}$ or $k_{el}'$ ($hr^{-1}$) |
|---|---|---|---|
| TPD | 37.6 | | 0.145 |
| BTMP | 93.5 | | 0.151 |
| CCT | | 187.5 | 1.61 |
| Thiamine | | 256.1 | 1.98 |

Vd: volume of distribution calculated from one compartment model
$V_1$: volume of distribution calculated from two compartments model (central compartment)
$k_{el}$: rate constant of elimination of thiamine after intravenous injection of TPD or BTMP or CCT
$k'_{el}$: rate constant of elimination of thiamine after intravenous injection of thiamine

As for two compartments model, the constants were obtained by an iterative least square method using a digital computer.[12]

In BTMP, time course of thamine was assumed to obey a consecutive reaction kinetics (BTMP $\xrightarrow{k_a}$ $B_1$ $\xrightarrow{k_b}$) ($B_1$: thiamine, see later section). The usual equation (Eq. 1) was used to obtain pharmacokinetic constants.

$$C_T = \frac{k_a}{k_a - k_b} \cdot \frac{X_o}{V_d} \{\exp(-k_b t) - \exp(-k_a t) \quad (1)$$

where $C_T$, $X_o$ and $V_d$ are the concentration of thiamine in blood at time $t$, the initial amount of BTMP and the volume of distribution of BTMP respectively.

The elimination constant ($k_b$) was found to be 0.151 $hr^{-1}$ using the data after appearance of a peak in the time course. The volume of distribution was calculated from the concentration ($C_o$) extrapolated to time 0 in Fig. 3. Since the time when the maximum concentration appeared was 30 minutes, the ratio of $k_a/k_b$ was found to be about 50, putting $k_b$=0.151 $hr^{-1}$ into Eq. 2.

$$T_{max} = \frac{2.303 \log k_b/k_a}{k_b - k_a} \quad (2)$$

where $T_{max}$ is the time having the maximum concentration of thiamine in blood. As the ratio of $k_a/k_b$ was large enough to assume $k_a/(k_a - k_b)$ equal to 1, exp $(-k_a t)$ was negligible compared with exp $(-k_b t)$ and the volume of distribution was obtained by the equation of (DOSE/$C_o$) where DOSE is the administered dose ($\mu$g).

**4) Volume of Distribution and Elimination Constant ($k_{el}$ or $k'_{el}$)[13] (Table III)**——Since $V_1$ of thiamine was nearly equal to body volume[14] and about 13 times larger than blood

11) H. Shindo, K. Okamoto, J. Tohtsu and I. Takahashi, *Vitamins* (Kyoto), **38**, 21 (1968).
12) H. Nogami, M. Hanano, S. Awazu and H.H. Moon, *Chem. Pharm. Bull.* (Tokyo), **17**, 2097 (1969).
13) $k_{el}$: Rate constant of elimination of thiamine after intravenous injection of TPD, BTMP, and CCT, $k'_{el}$: Rate constant of elimination of thiamine after intravenous injection of thiamine.
14) Since the body weight of each rat was about 250 g, body volume was considered to be about 250 ml.

volume,[15] the compartment 1 (so called central compartment) was assumed to include other tissues in addition to blood. It was reported in the preceding paper[7] that TPD was converted rapidly to thiamine by erythrocytes and at that time about half amount of formed thiamine was distributed in erythrocytes and the other half in plasma. And, in the present study, thiamine in erythrocytes was found to transfer to plasma very slowly and then be rapidly eliminated from plasma, because $k_{el}$ of TPD was much smaller than that of thiamine. Vd[16] of TPD was about 15% of body volume[14] and about 2 times of blood volume.[15] Half amount of administered TPD disappeared so rapidly that Vd of TPD should be divided by 2 in order to obtain true volume of distribution of TPD. Thus, actual Vd, was very similar to the blood volume. It meant that TPD located almost in blood.

Pharmacokinetic constants of CCT were very similar to those of thiamine, therefore CCT behaves like thiamine in blood.

BTMP was found to have the very similar elimination constant to that of TPD, showing that the elimination process was the same as that of TPD which will be presented in the later section.

**5) Kinetics of Elimination of Each Thiamine Derivative from Blood**——In view of the results of thiamine formation from the derivatives *in vitro* and of time courses of intravenously injected derivatives together, the following schemes were considered as a possible behavior of the derivatives in blood.

**Thiamine Propyl Disulfide**

It was reported in the preceding paper[7] that TPD was converted to thiamine by thiol compounds in erythrocytes in a mode of second order reaction kinetics and taken up into erythrocytes instantaneously. In the case of the intravenous injection of TPD, the rate constant of formation of thiamine from TPD in blood was estimated roughly as about 97.7 $hr^{-1}$ from the data in Fig. 1 assuming the first order reaction.

Since the half amount of TPD was taken up into erythrocytes, 48.9 $hr^{-1}$ was given to both $k_1'$ and $k_2'$. As mentioned in the previous section, the behavior of thiamine in blood after intravenous injection of thiamine obeyed the two compartments model and the elimination rate was very rapid, because thiamine was not taken up into erythrocytes. Since half amount of thiamine was taken up into erythrocytes after intravenous injection of TPD, the elimination of thiamine was slow and was described as one compartment model. Namely, the slow elimination of thiamine was rate-determined by the slow transfer rate of thiamine from erythrocytes. And the transfer rate constant was found 0.145 $hr^{-1}$ from Fig. 3 as shown in Table III, since it was nearly equal to $k_{el}$ of TPD. A thinkable whole scheme of elimination of intravenously injected TPD was shown in Chart 4.

The phenomenon that TPD maintains a thiamine concentration in blood for a long time was explained by the slow transfer rate of converted thiamine from erythrocytes to plasma.

**Cyclocarbothiamine**

As mentioned above, CCT was converted rapidly to thiamine in blood like TPD as shown in Fig. 1. But the difference was that erythrocytes had little effect on the conversion of CCT. Though TPD was fully converted to thiamine in the 100 fold diluted erythrocytes suspension in 30 minutes, CCT was hardly influenced by erythrocytes in the same condition (Table I). Even when the amount of erythrocytes increased, the formation of thiamine from CCT was too slow to explain the result in Fig. 1.

On the other hand, as for plasma effect, it was found that CCT was converted much more rapidly thanTPD in plasma when CCT was incubated in the 100 fold diluted plasma solution, and about twenty percent of it was converted to thiamine in one hour (Table II).

15) The blood volume of rat weighing about 250 g was considered to be about 20 ml.
16) $DOSE/C_0$.

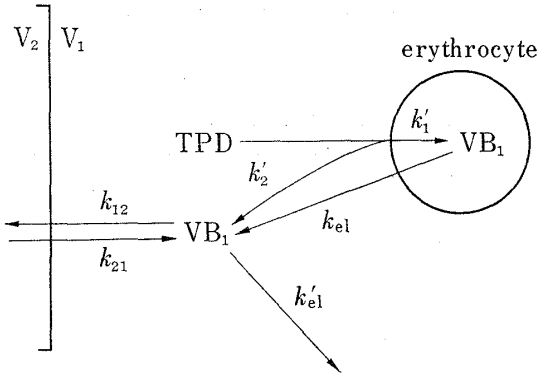



Chart 4. Scheme of Elimination of Thiamine Propyl Disulfide Injected Intravenously

TPD: thiamine propyl disulfide
$VB_1$: thiamine
$V_1$: central compartment
$V_2$: tissue compartment

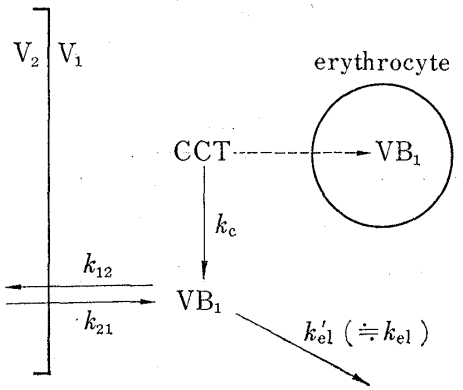



Chart 5. Scheme of Elimination of Cyclocarbothiamine Injected Intravenously

CCT: cyclocarbothiamine
$VB_1$: thiamine
$V_1$: central compartment
$V_2$: tissue compartment

Therefore, it was concluded that CCT was mainly converted to thiamine in plasma. The rate constant $k_c$ in Chart 5 of formation of thiamine from CCT in blood was obtained as 25.4 $hr^{-1}$ from Fig. 1 assuming the first order reaction. Other pharmacokinetic constants were calculated by previous method[11] using time course of thiamine elimination after intravenous injection of CCT. The fast conversion of CCT in plasma explained well that intravenously injected CCT behaved very similar to thiamine as shown in Fig. 3, since $k_c$ was large and then $k_{el}$ of CCT was nearly equal to $k_{el}'$ of thiamine. A thinkable whole scheme of elimination of intravenously injected CCT was shown in Chart 5.

## S-Benzoylthiamine Monophosphate

BTMP is so hydrophile due to phosphate group that dephosphorylation to SBT probably occurs before the uptake into erythrocytes, as Shindo, *et al.*[11] reported that BTMP could not be taken up into human erythrocytes without phosphatase and that SBT was taken up rapidly into erythrocytes. The same procedure being assumed, the scheme in Chart 6 was proposed to express the BTMP's behavior when it was intravenously injected.

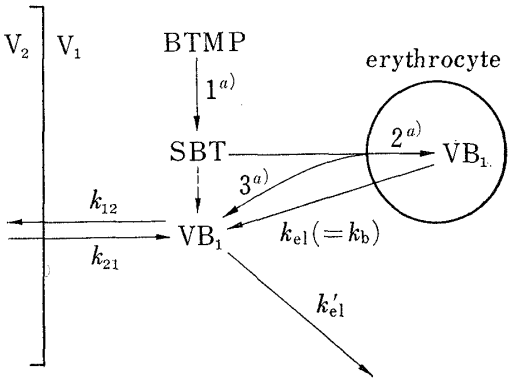



Chart 6. Scheme of Elimination of S-Benzoylthiamine Monophosphate Injected Intravenously

BTMP: S-benzoylthiamine monophosphate
SBT: S-benzoylthiamine
$VB_1$: thiamine
$V_1$: central compartment
$V_2$: tissue compartment
a) $k_a$ which is defined in page 8 of this paper expresses the rate constant of either formation step of SBT (step 1) or formation step of $VB_1$ (both step 2 and step 3).

The process of thiamine formation was found to follow the first order kinetics of which rate constant ($k_a$) was 0.860 $hr^{-1}$ *in vitro* (Fig. 2). However, the rate constant ($k_a$) was increased to have $50 \times 0.151$ $hr^{-1}$ *in vivo*, since $k_a/k_b$ was about 50. $k_a$ expresses the rate constant of the rate-determining step in Chart 3, but it is not found which step is rate-determining one. $k_b$ is $k_{el}$ itself of BTMP and its value is 0.151 $hr^{-1}$ (Table III). This value expresses the transfer rate constant of thiamine from erythrocytes like TPD and it fits the value (0.145 $hr^{-1}$) obtained in TPD section.

For the summation of the present paper, the pharmacokinetic constants for thiamine derivative were shown in Table IV.

Table IV. Pharmacokinetic Constant for Each Thiamine Derivative

| Thiamine derivatives | Pharmacokinetic constants | Thiamine derivatives | Pharmacokinetic constants |
|---|---|---|---|
| TPD | $k_1'=48.9\ hr^{-1}$ | CCT | $Vss^{a)}=320.8$ ml |
| | $k_2'=48.9\ hr^{-1}$ | | $k_c=25.4\ hr^{-1}$ |
| | $k_{el}=0.145\ hr^{-1}$ | Thiamine | $k_{12}=1.94 hr^{-1}$ |
| BTMP | $k_{el}=0.151\ hr^{-1}$ | | $k_{21}=3.27\ hr^{-1}$ |
| CCT | $k_{12}=2.30\ hr^{-1}$ | | $k_{el}'=1.98\ hr^{-1}$ |
| | $k_{21}=3.24\ hr^{-1}$ | | $V_1=256.1$ ml |
| | $k_{el}=1.61\ hr^{-1}$ | | $V_2=151.9$ ml |
| | $V_1=187.5$ ml | | $Vss^{a)}=408.0$ ml |
| | $V_2=133.4$ ml | | |

See Charts 4—6 in this paper about the meaning of each pharmacokinetic constant.
TPD: thiamine propyl disulfide BTMP: S-benzoylthiamine monophosphate
CCT: cyclocarbothiamine *a)* Vss: $V_1+V_2$

It was concluded, therefore, that the proper lipophilicity which assures the uptake of thiamine into erythrocytes and the liability to be converted to thiamine were necessary for the presence of the long half lives of the thiamine derivatives.

**Acknowledgement** This work was supported by the Grant-in Aid for Scientific Research provided by the Ministry of Education to which the authors are grateful. We are also grateful to Takeda Chem. Ind., Ltd., Sankyo Co., Ltd. and Yamanouchi Pharm. Co., Ltd. for their kind supply of the TPD, BTMP and CCT.