The fact that the tissue affinity of thiamine propyl disulfide (TPD) is superior to that of thiamine hydrochloride has been shown by many studies in this country as well as in foreign countries. Especially, the distribution of thiamine in the blood after administration of TPD is worthy of note, i.e., the thiamine concentration in the blood, particularly in blood cells, is markedly higher after administration of TPD than after administration of thiamine hydrochloride. However, the mechanism of penetration of TPD into blood cells in such high concentrations remained to be elucidated. In order to clarify this, the authors gave TPD-S\textsuperscript{35} (outer), TPD-S\textsuperscript{35}(inner) and thiamine-S\textsuperscript{35} to animals under different conditions, investigated the distribution of both S\textsuperscript{35} and thiamine and obtained several interesting findings.

**EXPERIMENTAL**

**Methods**

The method of S\textsuperscript{35} determination was described in the foregoing paper (1). Thiamine was determined by the thiochrome method of Fujiwara and Matsui (2). The concentration of S\textsuperscript{35} was expressed as thiamine (\(\mu g/100\text{ ml}\)) and was calculated from the cpm of S\textsuperscript{35} according to the following formula:

\[
S^{35}\text{ concentration (}\mu g/100\text{ ml}) = \frac{(\text{amount of } S^{35} \text{ in 1 ml}) \times \text{dose given (}\mu g\text{)}}{\text{amount of } S^{35}\text{ given}} \times 100
\]

When the amount of S\textsuperscript{35} is expressed in this way after administration of TPD-S\textsuperscript{35}(inner), it can be regarded as being roughly equivalent to the amount of thiamine.

In the case of TPD-S\textsuperscript{35}(outer), however, it is not related to the actual amount of thiamine when the amount of S\textsuperscript{35} is expressed as thiamine, \(\mu g/100\text{ ml}\), as TPD is assumed to be split into thiamine and propyl-S\textsuperscript{35} in the body. But it seems convenient to tentatively express the S\textsuperscript{35} of TPD-S\textsuperscript{35}(outer) as thiamine in the comparative study of the distribution of propyl-S\textsuperscript{35} and thiamine.

1. Allithiamine, a Newly Discovered Derivative of Thiamine. VI.
2. 藤原元典, 笠川栄成, 細川喜明, 池田 博.
RESULTS

1. Distribution of $S^{35}$ in the Blood after Administration of TPD-$S^{35}$(outer)

(a) Blood Concentration of $S^{35}$ after Administration of TPD-$S^{35}$(outer)

Subcutaneous Injection——Five mg of TPD-$S^{35}$(outer) was given subcutaneously in rabbits weighing about 3 kg and the blood was collected from the ear vein after 0.5, 3, 5 and 24 hours. Five ml of the blood (to which 0.02 ml of a heparin solution had been added to prevent coagulation) was centrifuged at 3,000 rpm for 30 minutes to separate the blood cells from the plasma. The cells were washed with 5 ml of physiological saline solution, and the whole was centrifuged at 3,000 rpm for 30 minutes. The supernatant was added to the plasma. This procedure was repeated twice. The $S^{35}$ in the separated cells and in the plasma was determined and the thiamine in 5 ml of the blood, collected at the same time, was determined by the thiochrome method.

As shown in Table I, thiamine was found in the blood cells in an extremely large quantity. It was 400 µg/100 ml after 30 minutes and considerably high values were noted after 3 and 5 hours, while thiamine concentration in the plasma was low. On the contrary, the amount of $S^{35}$ in the plasma was extremely high, 700 µg/100 ml after 30 minutes, while it was several times less in the cells.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Concentration of $S^{35}$ and Thiamine in Blood Cells and Plasma after Subcutaneous Injection of 5 mg TPD-$S^{35}$(outer) in a Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time after injection</td>
<td>Radioactivity$^a$</td>
</tr>
<tr>
<td>hr</td>
<td>µg/100 ml</td>
</tr>
<tr>
<td>0.5</td>
<td>141</td>
</tr>
<tr>
<td>3</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
</tr>
<tr>
<td>24</td>
<td>14</td>
</tr>
</tbody>
</table>

$^a$ Calculated tentatively as thiamine from $S^{35}$

$^b$ Determined by the thiochrome method

Oral Administration——Blood was collected after 0.5, 3, 5 and 24 hours after oral administration of 5 mg (in 5 ml) of TPD-$S^{35}$(outer) by a stomach tube in rabbits weighing about 3 kg.

<table>
<thead>
<tr>
<th>Table II</th>
<th>Concentration of $S^{35}$ and Thiamine in Blood Cells and Plasma after Oral Administration of 5 mg TPD-$S^{35}$(outer) in a Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time after injection</td>
<td>Radioactivity$^a$</td>
</tr>
<tr>
<td>hr</td>
<td>µg/100 ml</td>
</tr>
<tr>
<td>0.5</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>24</td>
<td>7</td>
</tr>
</tbody>
</table>

$^a$ Calculated tentatively as thiamine from $S^{35}$

$^b$ Determined by the thiochrome method
rabbits. The blood cells were separated from the plasma as described above and S\textsuperscript{35} and thiamine were determined.

As listed in Table II, the concentration of S\textsuperscript{35} and thiamine in the cells and plasma were lower than those after subcutaneous injection, though the distribution of thiamine and S\textsuperscript{35} in the cells and plasma showed a similar trend.

**In vitro Experiment** — Change in the S\textsuperscript{35} in the early stage after administration of TPD was investigated in an attempt to ascertain the mode of penetration of TPD into the blood cells. To 9 tubes containing 5 ml of physiological saline solution were added 10 µg of TPD-S\textsuperscript{35}(outer) (1 ml = 9,200 cpm), and one ml of normal blood, the mixture was incubated at 38° for 2, 4, 6, 10, 15, 30, 60, 90 and 120 minutes, respectively, followed by centrifugation at 3,000 rpm for 30 minutes to separate the cells from the plasma and the radioactivity of S\textsuperscript{35} was determined. As shown in Table III and Fig. 1, the concentration of S\textsuperscript{35} in the cells was very low 2 minutes after addition of TPD-S\textsuperscript{35} (outer), but rose gradually, reaching a peak in 15—30 minutes and it decreased gradually thereafter.

**Table III**

<table>
<thead>
<tr>
<th>Time of incubation (min)</th>
<th>cpm of blood cells (a)</th>
<th>cpm of plasma (b)</th>
<th>Total cpm (b)</th>
<th>a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>748</td>
<td>8,236</td>
<td>8,984</td>
<td>8.3</td>
</tr>
<tr>
<td>4</td>
<td>1,540</td>
<td>7,160</td>
<td>8,700</td>
<td>17.7</td>
</tr>
<tr>
<td>6</td>
<td>1,981</td>
<td>6,181</td>
<td>8,162</td>
<td>24.3</td>
</tr>
<tr>
<td>10</td>
<td>2,157</td>
<td>6,661</td>
<td>8,818</td>
<td>24.2</td>
</tr>
<tr>
<td>15</td>
<td>2,490</td>
<td>6,300</td>
<td>8,790</td>
<td>28.6</td>
</tr>
<tr>
<td>30</td>
<td>2,490</td>
<td>5,884</td>
<td>8,374</td>
<td>29.0</td>
</tr>
<tr>
<td>60</td>
<td>1,371</td>
<td>6,300</td>
<td>7,671</td>
<td>17.9</td>
</tr>
<tr>
<td>90</td>
<td>1,056</td>
<td>7,356</td>
<td>8,412</td>
<td>12.6</td>
</tr>
<tr>
<td>120</td>
<td>1,360</td>
<td>7,426</td>
<td>8,786</td>
<td>15.4</td>
</tr>
</tbody>
</table>

**Fig. 1**  
**Penetration of TPD-S\textsuperscript{35}(outer) into Blood Cells**

S\textsuperscript{35} in blood cells and supernatant after incubation of TPD-S\textsuperscript{35}(outer) in a suspension of blood cells

\(\times\), total;  
○, supernatant;  
●, blood cells.

The concentration of S\textsuperscript{35} in the supernatant was temporarily decreased at the early stage, but increased gradually. The total amount, however, remained practically constant.
(b) Behavior of $\text{S}^{35}$ in Plasma after Administration of TPD-$\text{S}^{35}$ (outer)

The finding that the majority of $\text{S}^{35}$ was found in the plasma after administration of TPD-$\text{S}^{35}$ (outer) was worthy of note and the substance in the plasma with which $\text{S}^{35}$ had combined was further studied by paper ionophoresis.

In vitro Experiment——To 2 ml of the venous blood of a normal healthy subject (to which 0.02 ml of heparin solution had been added to prevent coagulation) was added 500 $\mu$g (39,112 cpm) of TPD-$\text{S}^{35}$ (outer), and incubated at 37˚C for 2 hours at pH 7.0. The blood cells were then separated from the plasma by centrifugation. Paper ionophoresis was carried out with the plasma using veronal buffer solution of pH 9.0, the paper was stained with a protein-staining agent (to one g of bromphenol blue, and a saturating amount of mercuric chloride, ethanol was added up to 100 ml). Plasma protein fractions were determined with the filter paper electric photometer and the radioactivity of the same filter paper was determined with Actigraph. As summarized in Fig. 2, $\text{S}^{35}$ was found in the serum albumin fraction, no radioactivity being detectable in any other fractions.

In vivo Experiment——Ten mg (12,400,000 cpm) of TPD-$\text{S}^{35}$ (outer) was given subcutaneously in rabbits, the blood was collected after 30 minutes, the plasma was separated and paper ionophoresis was carried out. As shown in Fig. 3, $\text{S}^{35}$ was found in the albumin fraction only as in the case of human blood.

![Fig. 2 In vitro Reaction of TPD-$\text{S}^{35}$ (outer) with Human Plasma](image)

![Fig. 3 In vivo Reaction of TPD-$\text{S}^{35}$ (outer) with Rabbit Plasma](image)

2. Distribution of $\text{S}^{35}$ in Blood after Administration of TPD-$\text{S}^{35}$ (inner) and Thiamine-$\text{S}^{35}$

(a) Change of $\text{S}^{35}$ in Blood after Administration of TPD-$\text{S}^{35}$ (inner) or Thiamine-$\text{S}^{35}$
**Subcutaneous Injection** — Five mg each of TPD-S\(^{35}\) (inner) or thiamine-S\(^{35}\) was given subcutaneously, the blood cells were separated from the plasma and the S\(^{35}\) was determined. As shown in Tables IV and V, an extremely high level of S\(^{35}\) was found in the cells, decreasing gradually with time, while the S\(^{35}\) in the plasma was very low in the case of TPD-S\(^{35}\) (inner) injection. Following thiamine-S\(^{35}\) injection, however, S\(^{35}\) in the cells was very low, the majority being found in the plasma.

### Table IV

**Amount of S\(^{35}\) and S\(^{35}\) Concentration in Blood Cells and Plasma after Subcutaneous Injection of 5 mg TPD-S\(^{35}\) (inner) in a Rabbit**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>S(^{35}) in blood cells</th>
<th>S(^{35}) in plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/ml</td>
<td>µg/100 ml</td>
</tr>
<tr>
<td>0.5</td>
<td>3,692</td>
<td>462</td>
</tr>
<tr>
<td>3</td>
<td>2,281</td>
<td>285</td>
</tr>
<tr>
<td>5</td>
<td>1,300</td>
<td>163</td>
</tr>
<tr>
<td>24</td>
<td>155</td>
<td>19</td>
</tr>
<tr>
<td>48</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td>96</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>192</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1 µg TPD-S\(^{35}\) (inner) = 800 cpm

*a* Calculated from S\(^{35}\)

### Table V

**Amount of S\(^{35}\) and Concentration of S\(^{35}\) in Blood Cells and Plasma after Subcutaneous Injection of 5 mg Thiamine-S\(^{35}\) in a Rabbit**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>S(^{35}) in blood cells</th>
<th>S(^{35}) in plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/ml</td>
<td>µg/100 ml</td>
</tr>
<tr>
<td>0.5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>96</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>192</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1 µg thiamine-S\(^{35}\) = 660 cpm

*a* Calculated from S\(^{35}\)

**Oral Administration** — Blood was collected 0.5, 3 and 5 hours, respectively, after oral administration of 5 mg each of TPD-S\(^{35}\) (inner) or thiamine-S\(^{35}\) to rabbits and the distribution of S\(^{35}\) in the cells and plasma was determined. As listed in Table VI, following the administration of TPD-S\(^{35}\), a far higher concentration of S\(^{35}\) was obtained in the cells and plasma than in the case of thiamine-S\(^{35}\) and the decrease in concentration was slow, whereas in the case of thiamine-S\(^{35}\), S\(^{35}\) was found in the plasma at a very low concentration, no radioactivity being found in the cells.
TABLE VI
Concentration of S\textsuperscript{35} in Blood Cells and Plasma after Oral Administration of 5 mg Each of TPD-S\textsuperscript{35}(inner) or Thiamine-S\textsuperscript{35} in a Rabbit

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>TPD-S\textsuperscript{35} concentration</th>
<th>Thiamine-S\textsuperscript{35} concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood cells (µg/100 ml)</td>
<td>Plasma (µg/100 ml)</td>
</tr>
<tr>
<td>0.5</td>
<td>41.7</td>
<td>35.0</td>
</tr>
<tr>
<td>3</td>
<td>45.8</td>
<td>29.0</td>
</tr>
<tr>
<td>5</td>
<td>62.0</td>
<td>15.0</td>
</tr>
</tbody>
</table>

(b) Behavior of S\textsuperscript{35} in Blood Cells and Plasma after Administration of TPD-S\textsuperscript{35}(inner) and Thiamine-S\textsuperscript{35}

In order to investigate with what substance S\textsuperscript{35} combines in the blood after

---

**Fig. 4** Reaction of Human Blood with TPD-S\textsuperscript{35}(inner) or Thiamine-S\textsuperscript{35}

1. reaction of TPD-S\textsuperscript{35}(inner) and blood cells; II, reaction of thiamine-S\textsuperscript{35} and plasma. A, Actigram; B, paper chromatogram
Condition of paper chromatography: developing solvent, butanol-acetic acid-water [4:1:5]
Condition of Actigraph: slit, 1/8 in.; scan, speed, 6 in./hr; time const., 20 sec.; count rate range, 300 cpm
Detecting reagent: E, mixture of bromphenol blue and mercuric chloride; F, potassium ferricyanide-NaOH reagent

---

**Fig. 5** In vitro Reaction of TPD-S\textsuperscript{35}(inner) with Human Blood Cells
Condition of paper isophoresis: solvent, acetate buffer solution, pH 4.5; 400 V; 5 hr.
Condition of Actigraph: slit, 1/8 in.; scan, speed, 6 in./hr; time const., 20 sec.; count rate range, 300 cpm
administration of TPD-S\textsuperscript{35}(inner) or thiamine-S\textsuperscript{35}, 500 \(\mu g\) each of TPD-S\textsuperscript{35}(inner) or thiamin-S\textsuperscript{35} (1 \(\mu g\) TPD-S\textsuperscript{35} = 800 cpm, 1 \(\mu g\) thiamine-S\textsuperscript{35} = 600 cpm) was added to 2 ml of the blood taken from a normal healthy subject as in the case of TPD-S\textsuperscript{35}(outer) and incubated at 37\(^\circ\) for 2 hours (pH 7.0) in a water bath. The cells were separated from the plasma and paper chromatography using a developing solvent of butanol-acetic acid-water (4:1:5) or paper ionophoresis using acetate buffer solution, pH 4.5, at 400 volts for 5 hours was carried out and the radioactivity was determined with Actigraph with the results given in Figs. 4 and 5.

In both the cases of TPD-S\textsuperscript{35} (inner) and thiamine-S\textsuperscript{35} administrations, the radioactivity was found only in the part corresponding to thiamine, no radioactivity being detectable in hemoglobin in the cells or in plasma proteins.

**DISCUSSION**

It is worthy of note that the thiamine part of TPD-S\textsuperscript{35}(outer) penetrates in large amounts into the blood cells, while the propyl-S\textsuperscript{35} group remains in the plasma. As already shown by Nanjo (3), the TPD-reducing effect of plasma is weak, while that of the cells is far stronger. Therefore, splitting of TPD at \(\text{-S-S-}\) linkage in the blood is assumed to be due to the activity of the cells.

From the finding in the previous experiment that the concentration of \(\text{S}\text{\textsuperscript{35}}\) in the cells rapidly increased and then decreased, while that in the plasma was increased gradually after administration of TPD-S\textsuperscript{35}(outer), it can be assumed that TPD, having penetrated into the cells, is split into the thiamine part and the propylmercapto group only after contact with the cells, though it is unknown whether the lining or the surface of the cells is involved.

Matsukawa and Yurugi (4) showed that the propylmercapto group of TPD forms \(S\text{-Propylmercapto-L-cysteine}\) by combining with cysteine in the reduction experiment of TPD with cysteine. The following \textit{in vitro} experiment was carried out under the assumption that the \(\text{-SH}\) group takes an important part in the reduction of TPD. After addition of 2.5 mg of TPD to blood in 5 ml of physiological saline solution, \(p\)-chloromercuribenzoate was added to block the effect of the \(\text{SH}\) group and the uptake of the thiamine part of TPD by blood cells and the free thiamine in the supernatant were compared with the case
without \( p \)-chloromercuribenzoate. It was found that not only the uptake of the thiamine part of TPD by the cells was undoubtedly reduced by adding \( p \)-chloromercuribenzoate, but also the reduction of TPD itself was markedly inhibited as can be seen from the results shown in Table VII. The effect of the SH group of the blood cells is therefore responsible for penetration of the thiamine part of TPD into the cells (5).

**Table VII**

*Effect of \( p \)-Chloromercuribenzoate on Penetration of Thiamine Part of TPD into Blood Cells*

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Blood cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine found in</td>
<td>( \mu g )</td>
<td>( \mu g )</td>
</tr>
<tr>
<td>( 1.0 ) ml blood</td>
<td>110</td>
<td>52</td>
</tr>
<tr>
<td>With PCBM</td>
<td>229</td>
<td>410</td>
</tr>
<tr>
<td>Without PCBM</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>( 0.5 ) ml blood</td>
<td>112</td>
<td>184</td>
</tr>
</tbody>
</table>

PCBM 2.5 mg was added and the whole was incubated at 37\(^\circ\)C for 10 min.

It was reported that \( S^{35} \) was taken up not only by albumin, but also by globulin by Sachs (6), using \( Na_2S^{35}O_4 \) and Bronsky (7) using both cysteine-\( S^{35} \) and methionine-\( S^{35} \). It is an interesting finding that \( S^{35} \) was taken up only by albumin in the case of TPD-\( S^{35} \) (outer).

Fujiwara (8) reported the adsorption of allicin, an odor principle of garlics, by blood protein. Albumin was assumed to take an important part in this case. Fujiwara et al. demonstrated that allicin combined with the protein of the blood cells forming alillithiamine by the reaction with thiamine as shown in the following formulae.

\[
\text{Protein} + \text{allicin} \rightarrow \text{allicin-protein complex}
\]

\[
\text{Allicin-protein complex} + \text{thiamine} \xrightarrow{pH 8.0, 37^\circ} \text{allithiamine}
\]

However, further study seems to be necessary whether this relationship can also be applied to the combination of \( S^{35} \) with albumin or not.

The finding that most of the \( S^{35} \) is found in the blood cells after subcutaneous injection of TPD-\( S^{35} \) (inner) is coincident with the results obtained by determining thiamine by the thiochrome method reported by many other investigators.

After oral administration of TPD-\( S^{35} \) (inner), \( S^{35} \) penetrates into the blood cells while no penetration of the isotope was found after administering thiamine-\( S^{35} \). The difference between the two shows that a considerable amount of TPD passes through the intestinal wall without splitting into thiamine and propylmercaptan in the intestinal tract or intestinal wall. Nose et al. (9) reported recently that TPD was confirmed after infusing TPD into the intestinal tract, collecting the blood of the vein from the intestine, deproteinizing immediately at low temperature, and determination by bioautography, a finding supporting our results given above.
SUMMARY

In order to investigate in detail the increase in blood thiamine level after administration of thiamine propyl disulfide, TPD-S\(^{35}\) (outer), and TPD-S\(^{35}\) (inner), and of thiamine-S\(^{35}\) in rabbits, the distribution of S\(^{35}\) in the blood was examined and the following interesting findings were obtained.

1. The thiamine part of TPD was shown to be found in large quantities in blood cells, while the majority of S\(^{35}\) was found in plasma after oral or parenteral administration of TPD-S\(^{35}\) (outer).

2. S\(^{35}\) was found in the albumin fraction rather than in the globulin fraction of plasma after administration of TPD-S\(^{35}\).

3. After parenteral administration of TPD-S\(^{35}\) (inner), S\(^{35}\) was found mainly in blood cells, and extremely poorly in plasma.

4. The SH group in blood cells is shown to be involved in the mechanism of the penetration of the thiamine part of TPD into blood cells.

5. S\(^{35}\) was increased in blood cells after oral administration of TPD-S\(^{35}\) (inner), though it was less than after subcutaneous injection, whereas the increase in S\(^{35}\) was scarcely noted in blood cells after administration of thiamine-S\(^{35}\). The finding suggests that TPD passes through the intestinal tract without alternation.

REFERENCES