AFFINITY OF THIAMINE PROPYL DISULFIDE-S³⁵ TO ORGANS¹

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(Received May, 7, 1964)

The authors investigated in the foregoing paper (1) the distribution of S³⁵ in the blood after administration of TPD-S³⁵(inner) and TPD-S³⁵(outer) and found a difference in distribution between the two. In the present study, the authors investigated the distribution and retention of S³⁵ in various organs in the body and the replacement of thiamine.

EXPERIMENTAL

Methods

TPD-S³⁵(inner), TPD-S³⁵(outer) and thiamine-S³⁵ were used as described in the previous paper (1). The methods of determination were also the same, except for the fractional determination of thiamine, thiamine monophosphate and thiamine pyrophosphate, for which Fujiwara's method (2) was used.

The amount of S³⁵ is tentatively expressed as thiamine ($\mu\text{g}/100\text{ ml}$), calculated according to the following formula, as described in the previous paper (3).

$$\text{S}^{35} \text{ concentration in the organ } (\mu\text{g}/100 \text{ g}) = \text{amount of S}^{35} \text{ per g of the organ} \\ (\text{cpm}) \times \frac{\text{dose given } (\mu\text{g})}{\text{amount of S}^{35} \text{ given (cpm)}} \times 100$$

RESULTS

1. Experiment with TPD-S³⁵(outer)

Distribution of S³⁵ in Organs after Subcutaneous Injection—Five mg of TPD-S³⁵(outer) was given subcutaneously to Wistar strain white rats weighing about 300 g. The animals were sacrificed after 2 hours by decapitation, and the S³⁵ in the liver, spleen, intestine (10 cm of the upper part of the small intestine with the contents), kidney, lung, heart muscle (the heart was opened and the blood was thoroughly washed out with physiological saline), testicle, bone (right

¹ Allthiamine, a Newly Discovered Derivative of Thiamine. VII.

Following abbreviations are used: TPD, thiamine propyl disulfide; thiamine, thiamine hydrochloride; TMP, thiamine monophosphate; TDP, thiamine diphosphate.

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femur), muscle (right femoral muscle) skin (of the back) and brain was determined. In the control, 5 mg of TPD-S³⁵(inner) was given subcutaneously and

FIG. 1 *S*³⁵ in Various Organs Two Hours after Subcutaneous Injection of 5 mg Each of TPD-S³⁵(outer and inner) in a Rat

^a Including the content ■, TPD-S³⁵(outer); □, TPD-S³⁵(inner).

FIG. 2 *S*³⁵ in Various Organs Two Hours after Oral Administration of 5 mg Each of TPD-S³⁵(outer) and TPD-S³⁵(inner) in a Rat

^a Including the content ■, TPD-S³⁵(outer); □, TPD-S³⁵(inner).

S^{35} was determined in the same tissues. The results of comparison of thiamine in $\mu\text{g}/100\text{ ml}$ calculated tentatively from the S^{35} of TPD- S^{35} (outer) and (inner) after administration of TPD- S^{35} are shown in Fig. 1.

After injection of TPD- S^{35} (outer), S^{35} was found in the largest quantity in the kidney, followed in order by the blood, lung and liver. Distribution of the S^{35} from TPD- S^{35} (inner), on the other hand, was most marked in the liver, followed by the spleen, small intestine, kidney and blood.

Distribution of S^{35} in the Organs after Oral Administration—The animals were sacrificed two hours after oral administration of TPD- S^{35} (outer) and TPD- S^{35} (inner) as above and the quantities of S^{35} were compared. As shown in Fig. 2 the increase in S^{35} in various organs was generally slight. The S^{35} from TPD- S^{35} (inner) was found in the small intestine and liver in large quantities, while the increase in S^{35} from TPD- S^{35} (outer) was not marked.

2. S^{35} Concentration in the Organs after TPD- S^{35} Administration

One mg of TPD- S^{35} (outer) was injected in a group of 5 Wistar strain rats weighing about 300 g each. The animals were sacrificed 6, 24, 48, 72 and 96 hours after injection and the S^{35} in the liver, heart muscle, wall of the small intestine and typhlocolon (whole digestive tract excised, split lengthwise and the contents

FIG. 3 Change of S^{35} in Liver with Time after a Single Subcutaneous Injection of One mg Each of TPD- S^{35} (inner), TPD- S^{35} (outer) and Thiamine- S^{35} in a Rat —, TPD- S^{35} (inner); — —, thiamine- S^{35} ; - - -, TPD- S^{35} (outer).

FIG. 4 Change of S^{35} in the Heart Muscle with Time after a Single Subcutaneous Injection of One mg Each of TPD- S^{35} (outer) TPD- S^{35} (inner), and Thiamine- S^{35} in a Rat —, TPD- S^{35} (inner); — —, thiamine- S^{35} ; - - -, TPD- S^{35} (outer)

washed out with physiological saline), brain and blood (2 ml) was determined. Similar experiments were carried out with TPD-S³⁵(inner) and thiamine-S³⁵. The S³⁵ concentration in each organ after administration of TPD-S³⁵(outer), TPD-S³⁵(inner) and thiamine-S³⁵ with the lapse of time was examined.

As summarized in Figs. 3—8, in the liver and heart muscle, the S³⁵ of TPD-S³⁵(inner) was found in larger quantities and retained for a longer period of time than the S³⁵ of TPD-S³⁵(outer), while in the blood and intestinal tract, the S³⁵ of TPD-S³⁵(outer) was found in larger quantities than that of TPD-S³⁵(inner). This was especially marked in the blood. In the brain, however, there was no marked difference between the two. Comparison of TPD-S³⁵(inner) and thiamine-S³⁵ showed that the penetration of S³⁵ into the tissues and the retention were much higher in the former than the latter in all the cases.

3. Replacement of Thiamine in the Organs after Continuous Subcutaneous Injection of TPD-S³⁵ or Thiamine-S³⁵

The affinity of TPD-S³⁵(inner), TPD-S³⁵(outer) and thiamine-S³⁵ to the tissues after a single injection in animals was clarified by Experiment 2, in which the accumulation in the tissues after continuous administration was investigated.

FIG. 5 Change of S³⁵ Concentration^a in the Small Intestine with Time after a Single Subcutaneous Injection of One mg Each of TPD-S³⁵(inner), TPD-S³⁵(outer) and Thiamine-S³⁵ in a Rat

---, TPD-S³⁵(outer); —·—, thiamine-S³⁵; —, TPD-S³⁵(inner)

^a Calculated as thiamine from S³⁵

FIG. 6 Change of S³⁵ Concentration^a in the Wall of Typhlocolon with Time after a Single Subcutaneous Injection of One mg Each of TPD-S³⁵(inner), TPD-S³⁵(outer) and Thiamine-S³⁵ in a Rat

---, TPD-S³⁵(outer); —·—, thiamine-S³⁵; —, TPD-S³⁵(inner)

^a Calculated as thiamine from S³⁵

FIG. 7 Change of S^{35} Concentration^a in the Blood with Time after a Single Subcutaneous Injection of One mg Each of TPD- S^{35} (inner), TPD- S^{35} (outer) and Thiamine- S^{35} in a Rat

---, TPD- S^{35} (outer); - · -, thiamine- S^{35} ; —, TPD- S^{35} (inner)

^a Calculated as thiamine from S^{35}

FIG. 8 Change of S^{35} Concentration^a in the Brain with Time after a Single Subcutaneous Injection of One mg Each of TPD- S^{35} (inner), TPD- S^{35} (outer) and Thiamine- S^{35} in a Rat

---, TPD- S^{35} (outer); - · -, thiamine- S^{35} ; —, TPD- S^{35} (inner)

^a Calculated as thiamine from S^{35}

Continuous Subcutaneous Injection of TPD- S^{35} (outer)—One mg of TPD- S^{35} (outer) was injected subcutaneously every day consecutively in 4 rats, which were sacrificed on the 1st, 3rd, 5th and 10th day, respectively, and the S^{35} in various organs was determined with the results shown in Table I.

TABLE I
 S^{35} Concentration^a in various Organs 3 Hours after Subcutaneous Injection of One mg TPD- S^{35} (outer) a Day Consecutively in a White Rat

Organ	Duration (days)			
	1	3	5	10
	μg per 100 g			
Liver	210	240	269	266
Kidney	377	231	235	388
Heart muscle	87	65	104	96
Brain	78	200	134	123
Testicle	35	156	95	133
Spinal cord	64	120	127	100

^a Indicated tentatively as thiamine concentration assuming that one g-atom of S^{35} corresponds to one mole of thiamine.

S^{35} was found in the largest quantity in the kidney, followed by the liver. No trend for accumulation of S^{35} in the organs was noted.

Continuous Subcutaneous Injection of TPD- S^{35} (inner)—A similar experiment was carried out with 1 mg of TPD- S^{35} (inner) and the S^{35} was determined

together with the total thiamine by the thiochrome method. As can be seen from the Tables II and III, the thiamine in the organs was found to have been

TABLE II
S³⁵ a, Total Thiamine and Replacement Ratio^b in Various Organs 3 Hours after Subcutaneous Injection of One mg TPD-S³⁵(inner) a Day Consecutively in a White Rat

Case I

Organ		Duration (days)			
		1	3	5	10
		<i>μg per 100 g</i>			
Liver	S ³⁵	507	684	634	704
	Total thiamine	1129	1195	1484	981
	Replacement ratio (%)	44.9	57.2	42.7	71.8
Heart muscle	S ³⁵	84	240	500	950
	Total thiamine	387	479	507	930
	Replacement ratio (%)	21.8	50.1	98.6	100.0
Brain	S ³⁵	38	172	236	236
	Total thiamine	271	353	299	240
	Replacement ratio (%)	14.0	48.7	78.9	98.3

^a Calculated as thiamine S³⁵

^b The ratio of S³⁵ to the total thiamine

TABLE III
S³⁵ a, Total Thiamine^b, Free Thiamine^b and Replacement Ratio^c in Various Organs 3 Hours after Subcutaneous Injection of One mg TPD-S³⁵(inner) a Day Consecutively in a White Rat

Case II

Organ		Duration (days)			
		1	3	5	10
		<i>μg per 100 g</i>			
Liver	S ³⁵	388	457	706	1250
	Total thiamine	1412	1905	1348	1593
	Free thiamine	313	145	210	259
	Replacement ratio (%)	27.5	24.0	52.4	78.5
Heart muscle	S ³⁵	50	339	493	667
	Total thiamine	713	790	678	749
	Free thiamine	30	147	143	90
	Replacement ratio (%)	7.0	42.9	72.2	89.1
Brain	S ³⁵	27	172	274	354
	Total thiamine	318	494	238	384
	Free thiamine	37	28	41	63
	Replacement ratio (%)	8.5	34.8	81.0	92.2
Femoral muscle	S ³⁵	21	42	43	76
	Total thiamine	130	126	84	141
	Free thiamine	0	51	26	60
	Replacement ratio (%)	16.2	33.3	51.2	53.9

^a Calculated as thiamine from S³⁵

^b Determined by the thiochrome method

^c The ratio of S³⁵ to the total thiamine

replaced by the newly ingested TPD-S³⁵(inner) by the continuous subcutaneous injection of TPD-S³⁵(inner). It was interesting that in the heart and brain, the ratio of replacement became very high on the 5th to 10th day, though it was low on the first day. Thus, it was found that thiamine was completely replaced by TPD-S³⁵(inner) in 10 days. In the liver and femoral muscle, to which the transfer of TPD-S³⁵(inner) was high from the beginning, such a high ratio of replacement as in the heart and brain was not noted.

Continuous Subcutaneous Injection of One mg Thiamine-S³⁵—The results of continuous administration of thiamine-S³⁵ under the same conditions are listed in Table IV. It was found that the ratio of replacement was much lower with thiamine-S³⁵ than with TPD-S³⁵(inner).

TABLE IV
S³⁵ a, Total Thiamine^b, Free Thiamine^b and Replacement Ratio^c in Various Organs 3 Hours after Subcutaneous Injection of One mg Thiamine-S³⁵ a Day Consecutively (in White Rat)

Organ		Duration (days)			
		1	3	5	10
		<i>μg per 100 g</i>			
Liver	S ³⁵	347	331	347	443
	Total thiamine	1411	1362	938	1472
	Free thiamine	175	154	92	102
	Replacement ratio (%)	24.6	24.3	37.0	30.1
Heart muscle	S ³⁵	13	117	108	65
	Total thiamine	387	517	317	496
	Free thiamine	57	7	21	22
	Replacement ratio (%)	3.4	22.6	34.1	13.1
Brain	S ³⁵	17	74	99	76
	Total thiamine	289	397	286	308
	Free thiamine	47	11	10	9
	Replacement ratio (%)	5.9	18.6	34.6	24.7
Femoral muscle	S ³⁵	0	9	34	8
	Total thiamine	88	103	88	64
	Free thiamine	17	5	14	6
	Replacement ratio (%)	0	8.7	38.6	12.5

^a Calculated as thiamine from S³⁵

^b Determined by the thiochrome method

^c The ratio of S³⁵ to the total thiamine

4. Form of Thiamine in the Organ after Administration of TPD-S³⁵(inner)

Turnover of thiamine in the organ was rapid with administration of TPD-S³⁵(inner) as described above. Fractional determination of thiamine esters was carried out by the method of Fujiwara and Nakamura (1) in order to investigate the forms of the replaced thiamine esters in the tissues. As shown in Table V, the majority of thiamine in the organs was found to be in the form of thiamine pyrophosphate 3 hours after injection.

TABLE V

Total Thiamine, S³⁵ ^a and Replacement Ratio^b of Thiamine, TMP and TDP Fractions in Various Organs 3 Hours after Subcutaneous Injection of One mg TPD-S³⁵(inner) a Day Consecutively for 10 Days in a White Rat

Organ	Form of thiamine	Total thiamine	S ³⁵	Replacement ratio
		<i>μg per 100 g</i>		<i>%</i>
Liver	Thiamine	33.6	49.2	5.6
	TMP	79.5	12.5	1.4
	TDP	762.3	667.2	76.2
	Total	875.4	728.9	83.3
Heart muscle	Thiamine	33.0	6.6	0.9
	TMP	30.5	0.0	0.0
	TDP	688.2	655.9	88.4
	Total	741.7	662.5	89.3
Brain	Thiamine	0.0	0.0	0.0
	TMP	30.9	33.1	10.2
	TDP	292.4	275.4	85.2
	Total	323.3	308.5	95.4

^a Calculated as thiamine from S³⁵

^b The ratio of S³⁵ in thiamine, TMP and TDP fractions to the total thiamine in the organ.

DISCUSSION

The S³⁵ of TPD-S³⁵(inner) penetrated into the liver and heart at higher concentration and was retained for a longer time than the S³⁵ of TPD-S³⁵(outer). In the blood and intestinal tract, however, this relation was reversed. In the brain, the affinity of S³⁵ of TPD-S³⁵(outer) and TPD-S³⁵(inner) was almost the same. The difference in S³⁵ distribution in the organs between the two suggests a special affinity differing according to the organs similar to the combination of S³⁵ of TPD-S³⁵(outer) with plasma albumin only, as observed in the previous experiment.

It is interesting that the ratio of replacement of thiamine in the organs was high in the heart and brain when TPD-S³⁵(inner) was given every day consecutively. This may probably be due to the strong uptake of thiamine by the heart and brain and appears to be closely related to cardiac beriberi, a fulminating type of beriberi. The low thiamine replacement in the organs by thiamine-S³⁵ appears to show a weaker affinity of thiamine to the tissues.

The finding that the accumulation of S³⁵ in the organs was not prominent when TPD-S³⁵(outer) was given every day consecutively may explain the fact that the body odor is not increased when TPD is ingested every day.

SUMMARY

The S³⁵ in various organs was determined after administration of TPD-S³⁵(inner), TPD-S³⁵(outer) and thiamine-S³⁵ in the rats and the following results were obtained.

1. Distribution of the S^{35} of TPD- S^{35} (inner) and TPD- S^{35} (outer) differs according to the organ. The former is retained for a longer period of time in the liver and heart and the latter in the blood and intestinal tract.

2. It was found that the replacement ratio of S^{35} was higher in the heart and brain than in other organs, when TPD- S^{35} (inner) was given subcutaneously every day consecutively.

3. The accumulation of S^{35} in the organs was not increased when TPD- S^{35} (outer) was given subcutaneously every day consecutively, differing from that of TPD- S^{35} (inner) given in the same way.

REFERENCES

1. Fujiwara, M., Sasakawa, S., Itokawa, Y., and Ikeda, K., *J. Vitaminol.* **10**, 70 (1964).
2. Fujiwara, M., and Nakamura, K., *Vitamins* **28**, 340 (1963).
3. Fujiwara, M., Sasakawa, S., Itokawa, Y., and Ikeda, K., *J. Vitaminol.* **10**, 55 (1964).