It has been demonstrated that a large amount of thiamine is excreted in the urine without degradation when the vitamin is subcutaneously injected. On the other hand, a relatively small amount of thiothiamine appears in the urine, when it is given by injection, suggesting the in vivo decomposition of thiothiamine. However, in the case of oral administration, thiamine is largely excreted in the feces, whereas thiothiamine mainly in the urine. It is therefore presumed that the thiocompound is readily absorbed from the intestinal tract (2). Since thiothiamine has no vitamin activity, it is of interest to investigate the metabolism of the thiocompound as compared to that of thiamine. In the present paper some findings will be described on the metabolite of thiothiamine in rats.

EXPERIMENTAL

1. Material

Ten male rats weighing from 150 to 200 g were fed a thiamine-deficient diet and 30 mg of thiothiamine in 3 ml water acidified with hydrochloric acid was intraperitoneally injected every day. The urine was collected in a bottle containing dilute HCl and toluene.

2. Paper Chromatography of Urine

Fresh urine, obtained as described above, was subjected to an ascending paper chromatography using acetic acid-n-butanol-water (1:4:5) or water-saturated n-butanol as a solvent system and the spots were visualized with Dragendorff reagent.

Fig. 1 shows the results of the urine following thiothiamine administration after developing with acetic acid-butanol-water. The spot, $R_F$ around 0.9, seems to be due to a derivative of thiazole, while that at $R_F$ 0.67—0.69 corresponds to thiothiamine. Further, the spot around $R_F$ 0.3 was always seen. Besides, the spots, $R_F$ 0.3—0.4, 0.1—0.2 and 0—0.1, possibly due to pyrimidine derivatives, were eventually observed.

In the control urine of the animals fed a thiamine-free diet receiving no thiothiamine, no compounds positive with Dragendorff reagent were detected by paper chromatography.
A. Developed that water-saturated butanol
Urine, thiothiamine intraperitoneally injected.
Urine, thiothiamine orally given.
Thiothiamine
Th-SH
HO-Th-S

B. Developed with acetic acid-n-butanol-water (1:4:5)
Urine, thiothiamine intraperitoneally injected.
Urine, thiothiamine orally given.
Thiothiamine
Th-SH
HO-Th-S

Thiothiamine (Rabbit urine subcutaneous injection)

Fig. 1 Paper Chromatography of the Urine after Administering Thiothiamine Intraperitoneally or Orally to a Rat
The spots were located with Dragendorff reagent.

The urine of the rat after receiving thiothiamine orally was tested also for Dragendorff, but it was negative. In the case of the rabbits receiving a subcutaneous injection of thiothiamine the spot positive with Dragendorff reagent was detected at around $R_f$ 0.8, close to the spot for thiothiamine, largely in agreement with the case of rats. After a subcutaneous injection of 500 mg thiothiamine to rabbit weighing 1.5 kg, 181.5 mg (average of 4 cases) of the thiocompound appeared in 24-hour urine, and after an intraperitoneal injection of 50 mg thiothiamine to a rat, the thiocompound in the 24-hour urine was about 18 mg, a roughly the same amount per kg of the body weight.

3. Isolation of Thiothiamine Metabolites

The 24-hour urine, 1.2 l, eliminated by ten rats during 20 days receiving 6 g of thiothiamine in all contained 1.3 g of the thiocompound. From this material, several metabolites were separated by the procedure shown in Fig. 2. The urine alkalinized with sodium hydroxide and saturated with NaCl was extracted with ether. The ether phase was dehydrated with anhydrous sodium sulfate and kept in a refrigerator to yield white powder. It was recrystallized twice from 60% per cent ethanol, giving the crystal melting at 238°. It showed no depression of the melting-point after admixture with an authentic thiothiamine, and gives a Dragendorff-positive spot at $R_f$ 0.68. It has two maxima in the ultraviolet absorption spectrum at 240 and 320 mμ in agreement with those of thiothiamine. The residue, Fraction I of Fig. 2, was a dark red liquid, water-insoluble, and gives a positive Dragendorff reaction. Its $R_f$ value was 0.25—0.35. The ultraviolet absorption spectrum in $N$ HCl shows an absorption maximum at 262—265 mμ, suggesting a pyrimidine derivative. The aqueous phase after extraction with ether was shaken with isobutanol. The extract was dried with
anhydrous sodium sulfate and evaporated to dryness below 50°. The residue was dissolved in absolute ethanol, and the solution was filtered. The ethanol was evaporated at 40° and water was added to the residue, whereby two fractions were separated, water-soluble Fraction II and water-insoluble Fraction III. The latter fraction is positive with the Dragendorff reagent, giving $R_f$ value higher than thiothiamine, suggesting a thiazole derivative. It was therefore subjected to cellulose column chromatography, using a water-saturated butanol as a solvent and each 10-ml fraction was collected. The Fractions Nos. I–IV, strongly positive for Dragendorff reaction, were combined and concentrated in vacuo to yield a resin-like residue. In paper chromatogram of the residue, two spots, positive with the Dragendorff reagent, were detected at $R_f$ values of 0.88 and 0.95. By ether extraction of the residue, the fraction $R_f$ 0.88, moved largely to the ether phase (Fraction IV), whereas the fraction $R_f$ 0.95 was largely extracted with ethanol (Fraction V). However, the separation was not complete. The Fractions IV and V were separately subjected to paper chromatography using the filter paper, Toyo Roshi No. 50, 40×40 cm, and the resulting two bands, $R_f$ 0.88 and 0.95 respectively, were separately cut out and each fraction was extracted and concentrated. After rechromatography of each fraction, two spots, $R_f$ 0.88 and 0.95, appeared in each of them, suggesting the two compounds to be derived from the same substance. Ikehata (5) observed that 2-mercaptop-4-methyl-5-β-hydroxyethylthiazole (Th-SH) gave two spots on the paper chromatogram, and that at $R_f$ 0.88 gave with the Dragendorff reagent
stronger color reaction than the other. The authors examined three Th-SH preparations. The sample No. 1 gave two spots in the paper chromatogram, agreeing with the results of Ikehata, whereas the samples No. 2 and No. 3 gave only one spot at RF 0.88. The melting-points of the samples were all 157—159°. The RF value of the sample No. 1 and those of Fractions IV and V agreed well as shown in Fig. 3. The ultraviolet absorption spectra of the portions, RF 0.88 and 0.95, isolated by

\[
\begin{array}{|c|}
\hline
\text{Th-SH} \\
\text{Th-SH}+R_F 0.88 \\
\text{Th-SH}+R_F 0.95 \\
\hline
\end{array}
\]

\[
\begin{array}{|c|}
\hline
\text{HO-Th-S} \\
\text{HO-Th-S}+R_F 0.88 \\
\text{HO-Th-S}+R_F 0.95 \\
\hline
\end{array}
\]

**FIG. 3** Comparison of the RF Value of Th-SH (Preparation 1), with those of RF 0.88 and 0.95 Substances by the Paper Chromatography Using Acetic Acid-n-Butanol-Water (1:4:5). The spots were located with Dragendorff reagent.

**Fig. 4** The UV-Spectra of Th-SH and RF 0.95 and 0.88 Substances in 1 N HCl

**Fig. 5** Comparison of UV-Spectra of Th-SH, and RF 0.95 and 0.88 Substances (Ethanol solution)

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1 Kindly given by Mr. K. Miyatake, Dai-Ichi Pharm. Co. Ltd.
2 Kindly given by Mr. K. Miyatake, Dai-Ichi Pharm. Co. Ltd. in 1960.
3 Kindly supplied by Mr. S. Yoshida, Sankyo Co. Ltd. in 1960.
repeating paper chromatography twice and those of the authentic samples of Th-SH and 4-methyl-4-hydroxy-5-β-hydroxyethylthiazolidone (2) (OH-Th-S) were compared as given in Figs. 4 and 5. The spectra of the portions, $R_f$ 0.88 and 0.95, showed equally a maximum at 320 m$\mu$ in N HCl, ethanol or methanol, agreeing with Th-SH, but the absorbances at below 290 m$\mu$ were strongly disturbed by impurities. The ultraviolet spectrum in methanol was almost the same with that in ethanol. The tests for sulfhydryl group, e.g., the reaction with iodine, sodium azide and nitroprusside, were positive in both Fractions IV and V. The former two reactions were stronger in Fraction IV, but the nitroprusside reaction was slightly positive in Fraction V.

The Fraction IV was purified by repeated paper chromatography and to the portion $R_f$ 0.88 dissolved in ethanol was added a saturated solution of picric acid in ethanol, melting at 117°, which showed no depression after admixture with the picrate of Th-SH. The elementary analysis showed N 13.73%, calculated from $C_{12}H_{12}N_4O_8S$, 13.86%. The $R_f$ value of the picrate was about 0.8, detectable as a yellow spot, and was the positive with the Dragendorff reagent. Besides, a yellow spot was observed at $R_f$ 0.6, corresponding to picric acid, and a Dragendorff-positive spot at $R_f$ 0.88, corresponding to Th-SH, suggesting the separation of picric acid in the course of paper chromatography. The same finding was also observed in the paper chromatography of Th-SH picrate, sample No. 2.

**DISCUSSION**

As already described in the preceding paper (2), urinary excretion of thiothiamine was roughly 30 per cent of the dose administered orally or subcutaneously in 24 hours and the amount excreted in the urine was always much less than after administering thiamine, suggesting more degradation of the thiocompound in the body. In order to examine the nature of the metabolite, 30 mp of the thiothiamine was injected intraperitoneally and the urine was subjected to paper chromatography to detect the metabolite. Thus the spot corresponding to Th-SH was always detectable. The compound was isolated and definitely identified as Th-SH. The most plausible metabolic pathway is as shown in Fig. 6, the hydrolysis into 2-methyl-4-amino-5-hydroxymethylpyrimidine (OMP) and Th-SH. In the case of thiamine, the urinary metabolite, pyramine, is shown to be OMP (6). In the case of thiothiamine, the hydrolysis of the same kind is easily presumed. Assuming that thiothiamine is less toxic than thiamine, and the injected thiocompound is more easily degraded than thiamine, a large amount of toxic OMP is expected to be produced and the low toxicity of thiothiamine is hardly understandable.

![Fig. 6 Degradation of Thiothiamine](image)
Further, assuming that the degradation of thiothiamine is a simple hydrolysis, the production of OMP equivalent to Th-SH is expected to be produced but it was not detectable.

Ikehata (5) reported from the results of paper chromatography and ultraviolet absorption spectra that thiothiamine was hydrolyzed by thiaminase II into OMP and Th-SH. Since Th-SH, but not OMP, could be detected by the authors in animal experiments, the degradation of thiothiamine in animals may not be so simple as in bacteria and the pyrimidine derivative once produced is considered to be metabolized further.

**SUMMARY**

Thiothiamine was intraperitoneally injected in rats and the urinary metabolites were investigated by paper chromatography using acetic acid-n-butanol-water and three spots at \( R_f 0.88-0.95, 0.7 \) and 0.3 were detected. The spot at \( R_f 0.7 \) corresponded to thiothiamine. The alkaninized urine was extracted with ether, then with iso-butanol. Thiothiamine was obtained from the ether phase. The isobutanol layer was evaporated and the residue was subjected to cellulose column chromatography to yield oily substances of \( R_f 0.88 \) and 0.95. Further purification by paper chromatography led to the isolation of a compound positive for sulfhydryl group with a maximum at 320 m\( \mu \) in ultraviolet absorption spectrum. It was identified as 2-mercapto-4-methyl-5-\( \beta \)-hydroxyethylthiazole by elementary analysis, mixed melting point and paper chromatography. Further confirmation was made with its picrate.

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