STUDIES ON HYDROXYETHYLTHIAMINE AND RELATED COMPOUNDS

I. VITAMIN B₁ ACTIVITY OF HYDROXYALKYLTHIAMINE

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On the basis of experiments with model compounds, Breslow proposed a mechanism of action of thiamine in which α -hydroxyethyl-2-thiamine pyrophosphate (HET-PP) was suggested to be a key intermediate of pyruvate metabolism (1). Later, Krampitz *et al.* succeeded in synthesizing the DL-hydroxyethylthiamine (DL-HET), the biological activity of which was found to be approximately 80% of that of thiamine when tested with thiamine-requiring microorganisms, *Lactobacillus fermenti* or *Lactobacillus viridescens* (2). They also demonstrated that DL-HET and thiamine were equally effective in reactivating yeast carboxylase. Thereby it was pointed out that the synthetic HET they used was a racemic form and very probably only one antipode was active in these biological systems. In view of the important role of HET-PP played in the metabolic process of pyruvate, it seemed of great interest to determine the optical form of HET which is really biologically active.

In the course of our investigation on chemical and biological properties of hydroxyethylthiamine derivatives, optical resolution of synthetic DL-HET was undertaken, which resulted in successful isolation of both isomers in pure state.

Hence, as a first step to the elucidation of biological properties of optically active HET, these isomers, together with their two higher alkyl homologues, DL-hydroxypropylthiamine (DL-HPT) and DL-hydroxyisobutylthiamine (DL-HIBT), were examined for their vitamin B₁ activity, using thiamine-deficient rats and rice-birds as test animals.

EXPERIMENTAL

1. Materials

Compounds used were as follows.

DL-HET, mp 218° (dec); D-HET, mp 218° (dec), $[\alpha]_D^{22} = +12.3^\circ$ (in 0.1 N HCl); L-HET, mp 218° (dec), $[\alpha]_D^{22} = -13.5^\circ$ (in 0.1N HCl); DL-HPT, mp 207° (dec); DL-HIBT, mp 217° (dec).

DL-HET, DL-HPT and DL-HIBT were synthesized in our laboratory from thiamine and corresponding aldehyde according to the method of Miller *et al.* (3).

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Optical resolution of DL-HET was carried out by Mr. Matsumoto with dibenzoyl-D-tartaric acid, the details of which will be published elsewhere.

2. Growth-promoting Effect on Thiamine-deficient Rats

Male Wistar strain rats, 4 weeks old, maintained on a thiamine-deficient diet were placed individually in wide-meshed, wire-bottomed cages to prevent coprophagy.

Thiamine-deficient diet (Table 1) was prepared according to the description of

TABLE 1
Composition of Thiamine-free Diet for Rats

Component	Amount
	<i>g</i>
Corn starch	272
Casein	72 ^a
Soybean oil	24
Cod liver oil	9.6
Mineral mixture	16 ^b
Vitamin mixture ^c	

^a Casein was treated overnight with 2% NaHSO₃ solution to destroy thiamine, repeatedly washed with water and dried.

^b The composition of the mineral mixture was the following: NaCl 20.2, MgSO₄·7H₂O 63.5, K₂HPO₄ 111.5, Ca-lactate 28.7, Na₂HPO₄·12H₂O 52.6, CaHPO₄·2H₂O 132.3, Fe-citrate 13.8.

^c The vitamin mixture contained the following components, in mg: riboflavine 4.0, pyridoxine 5.0, nicotinic acid 20.0, Ca-pantothenate 20.0, choline chloride 90.0, inositol 118.0, DL-methionine 24.0, cyanocobalamin 0.0024.

Yamashita (4), with the modification that vitamin B group substances other than thiamine were coated on sucrose granule and mixed with the diet so that each animal can receive sufficient amount of them. Food and water were given *ad libitum* and body weights were measured daily at 9:00 a.m.

At the time their gain of body weight leveled, the animals were divided, with respect to weight, into groups of 8 rats each and the compounds were administered daily by mouth, usually in a dose equivalent to 10 µg of thiamine in 0.2 ml of water. The control animals received 0.2 ml water instead. The observation was made over a period of one month.

3. Preventive Effect on the Thiamine-deficiency of Rice-birds

Male rice-birds (*Uroloncha striata* var. *domestica*), ranging in body weight

TABLE 2
Composition of Thiamine-free Diet for Rice-birds

Component	Amount
	<i>g</i>
Powdered rice ^a	3.7
Casein ^a	0.15
Sand	0.1
Calcium lactate	0.05
Ascorbic acid	0.001
Cod liver oil	one drop per week

^a Immersed in 2% NaHSO₃ solution overnight, washed thoroughly with water and dried.

from 12 to 13 g were fed on a thiamine-deficient diet, the composition of which is shown in Table 2.

Each bird, five in one group, received test compounds daily in a dose equivalent to 3 μ g of thiamine dissolved in 0.05 ml water. Body weights were measured every three days and observation was made over a period of 20 days.

RESULTS

1. Effect on Growth Curves of Thiamine-deficient Rats

The rats kept on a thiamine-free diet maintained normal rate of growth for about 10 days, followed by gradual decrease of body weight.

As a preliminary experiment designed to decide the dosage, three graded levels of thiamine (5, 10 and 20 μ g) were administered orally daily, starting on the 16th day. As is shown in Fig. 1, the administration of thiamine brought about immediate recovery of growth rate. Although 5 μ g of thiamine seemed insufficient to ensure maximal growth, the body weight curve of those receiving 20 μ g was in no way different from that of normal animals, while administration of 10 μ g resulted in weight gain slightly less than that of 20 μ g. Out of these observations, the dose was fixed at 10 μ g in the subsequent experiments.

FIG. 1 Effect of Different Levels of Thiamine on the Growth Rate of Rats Maintained on a Thiamine-deficient Diet

Each curve represents the average of eight animals. The arrow indicates the start of administration.

Daily dose: ○, 20 μ g; ×, 10 μ g; △, 5 μ g; ●, control.

FIG. 2 Growth-promoting Effect of DL-HET on Thiamine-deficient Rats

Daily dose: 10 μ g thiamine equivalents.

○, thiamine; △, DL-HET; ●, control.

FIG. 3 *Growth-promoting Effect of D-HET and L-HET on Thiamine-deficient Rats*

Daily dose: 10 μ g thiamine equivalents.

○, thiamine; △, D-HET; ×, L-HET; ●, control.

FIG. 4 *Growth-promoting Effect of DL-HPT and DL-HIBT on Thiamine-deficient Rats*

Daily dose: 10 μ g thiamine equivalents.

○, thiamine; △, DL-HPT; ×, DL-HIBT; ●, control.

Fig. 2 and 3 show the results obtained with DL-HET and its optically active isomers. The effect of DL-HET was indistinguishable from that of thiamine. Unexpectedly, however, no difference was observed between D- and L-HET, both isomers showing the effects comparable to that of thiamine.

FIG. 5 *Preventive Effect of HET on the Thiamine Deficiency of Rice-birds*

Each curve is the average of five birds.

Daily dose: 3 μ g thiamine equivalents. ○, thiamine; ●, DL-HET; △, D-HET; ×, L-HET.

With regards to alkyl homologues, DL-HPT accorded growth equal to that of thiamine, whereas the effect of DL-HIBT was very close to that of thiamine (Fig. 4).

2. Effect on Thiamine-deficient Rice-birds (Figs. 5 and 6)

Rice-birds are known to develop thiamine deficiency easily and its daily requirement has been established as $3\text{ }\mu\text{g}$ fairly accurately. Under our experimental conditions, the birds in the control group showed a marked decrease of body weight and all of them died on the 10th and 11th days, revealing typical symptoms of thiamine deficiency. Those which had received thiamine, DL-HET, D-HET, L-HET and DL-HPT remained healthy through the whole experimental period of 20 days, while DL-HIBT was less effective and three out of five died within the period. Thus, also in this case, both D- and L-HET could replace thiamine equally well, no difference between the two being detected.

FIG. 6 Preventive Effect of DL-HPT and DL-HIBT on the Thiamine Deficiency of Rice-birds

Each curve is the average of five birds and cross indicates death.

Daily dose: $3\text{ }\mu\text{g}$ thiamine equivalents. ○, thiamine; △, DL-HPT;

—●—, DL-HIBT; ---●---, control.

DISCUSSION

The importance of HET-PP in the pyruvate metabolism has been well established by Krampitz *et al.* (2) and Holzer *et al.* (5) and most likely either one of its optical isomers, D- or L-HET-PP, must be actually functioning in the enzymic process.

In this connection, Holzer *et al.* reported that, from the reaction mixture containing synthetic DL- C^{14} -HET-PP and pyruvic decarboxylase, acetaldehyde was isolated in the yield of 43–47%, while from C^{14} -pyruvate, the yield amounted to 84% (6). This led them to postulate that only one antipode entered into the reaction.

Surprisingly, in our experiments, both isomers of DL-HET were entirely in-

distinguishable from each other in their growth-promoting effect on the thiamine-deficient rats and on average days of survival of the rice-birds. This seems to imply that in living body there operates certain mechanism, enzymic or non-enzymic, by which inactive antipode is converted into its biologically active counterpart.

It is also worthy of note that DL-HPT and DL-HIBT were found to have more or less activity of vitamin B₁. Of these two, DL-HIBT had been reported by Krampitz *et al.* to be 2.5 times as active as thiamine when assayed with *L. fermenti* or *L. viridescens* (2).

The cause of thiamine activity of these compounds is of great interest, which will be the subject of further investigation.

SUMMARY

1. Optical isomers of hydroxyethylthiamine (DL-, D- and L-HET), together with its two alkyl homologues, DL-hydroxypropylthiamine (DL-HPT) and DL-hydroxyisobutylthiamine (DL-HIBT), were examined for their vitamin B₁ activity, using thiamine-deficient rats and rice-birds as test animals.

2. When orally administered, in a daily dose equivalent to 10 μ g of thiamine, to rats which had been maintained on a thiamine-deficient diet, all these compounds showed perfect thiamine activity, bringing about immediate increase of growth rate. Their effects were indistinguishable from that of thiamine and, unexpectedly, no difference was observed between the activity of D- and L-isomers of HET.

3. To rice-birds fed a thiamine-deficient diet, each compound was orally administered in a daily dose equivalent to 3 μ g of thiamine. While the control birds died on the 10th and 11th days with noticeable decrease in body weight, those receiving test compounds other than DL-HIBT, as well as thiamine, remained healthy through the experimental period of 20 days. The effect of DL-HIBT, however, was less marked and three out of five died within the period.

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