THIAMINE METABOLISM IN MAGNESIUM-DEFICIENT RATS

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Experiments for investigating thiamine metabolism in magnesium-deficient rats were carried out, and the following results were obtained.
1. Thiamine concentration in the sciatic nerve, liver, heart, and kidneys of magnesium-deficient rats was lower than that in the magnesium-sufficient rats. A decrease in thiamine was not detected in the central nervous system of magnesium-deficient rats.
2. In subcellular fractions of the liver of magnesium-deficient rats, thiamine content was most markedly decreased in the mitochondrial fraction.
3. When thiamine-¹⁴C was administered to magnesium-deficient rats, radioactivity in 24-hour urine decreased, and the radioactivity in the blood, liver, kidneys, heart, and sciatic nerve increased as compared to magnesium-sufficient rats.
4. With either oral or parenteral administration of thiamine, thiamine content in the heart, liver, and kidneys decreased at the same level in magnesium-deficient rats.

A plausible mechanism is suggested by these results.

A series of studies in this laboratory clarified that thiamine concentration in the liver and kidneys of magnesium-deficient rats was lower than that of the magnesium-sufficient rats (1). This finding suggests that the metabolism of thiamine in magnesium-deficient rats differs from that in magnesium-sufficient rats. The present study is intended to clarify the differences of thiamine metabolism between magnesium-sufficient and -deficient animals.

MATERIALS AND METHODS

Animals and diets. Male rats of Wistar strain, weighing 80–110 grams, were housed individually in stainless steel cages with a raised wire bottom. Table 1 shows the composition of the diets fed ad libitum during the experiments. Growth

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Table 1. Composition of diet.

| Diet No. | Thiamine Magnesium | A (g/100 g diet) | B (g/100 g diet) | C (g/100 g diet) | D (g/100 g diet) |
|----------|--------------------|------------------|------------------|------------------|------------------|
|          |                    | sufficient       | deficient        | sufficient       | deficient        |
| Casein (Vitamin free) | 15.0              | 15.0             | 15.0             | 15.0             |
| Sucrose  | 68.3               | 68.9             | 68.3             | 68.9             |
| Olive oil| 10.0               | 10.0             | 10.0             | 10.0             |
| Salt mixture (Mg free) | 3.4              | 3.4              | 3.4              | 3.4              |
| Cellulose| 2.0                | 2.0              | 2.0              | 2.0              |
| Vitamin mixture (Thiamine free) | 0.5              | 0.5              | 0.5              | 0.5              |
| Choline chloride | 0.2              | 0.2              | 0.2              | 0.2              |
| MgCl₂·6H₂O | 0.6              | 0.6              | 0.6              | 0.6              |
| Thiamine HCl | 0.5              | 0.5              | 0.5              | 0.5              |

* a Purchased from Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A.
* b The Mg-free salt mixture contained: (milligrams in 100g diet) NaCl, 173; Na₂HPO₄·H₂O, 343; K₂HPO₄, 945; CaCO₃, 530; Fe(C₆H₅O₇)·5H₂O, 115; CaHPO₄·2H₂O, 1,285; MnSO₄, 4; KI, 0.3; CuSO₄·5H₂O, 0.3; ZnCO₃, 0.3; K₂A₁₂(SO₄)₄·24H₂O, 0.3; CoC₁₂·6H₂O, 0.3.
* c When included in the diet at a level of 0.5%, the thiamine-free vitamin mixture had the following contents in micrograms in 100g diet: riboflavin, 750; nicotinic acid, 5,000; pyridoxine, 500; cyanocobalamin, 5; pantothenic acid, 2,500; folic acid, 250; biotin, 40; ascorbic acid, 18,750; α-tocopherol, 500; menadione, 500; inositol, 9,000; retinyl palmitate, 6,250 I.U.; ergocalciferol, 500 I.U.

rates were similar to those outlined in previous papers (1–4).

Analytical methods. Thiamine was assayed by the thiochrome method of FUJIWARA and MATSUI (5). Radioactivity was determined by adding 0.2 ml of urine, blood, or tissue homogenate to 20 ml of counting solution which consisted of toluene—Triton X 100—PPO—dimethyl POPOP (6) using a liquid scintillation counter (Nuclear Chicago, Mark II). Sample quenching was corrected by the channel ratio technique. The counting efficiency of this method was approximately 80%.

Statistical procedure. Significant differences were considered statistically, based on the method of one factor ANOVA (7).

Thiamine-¹⁴C. Thiamine-¹⁴C was synthesized and donated by the Takeda Pharmaceutical Co., Japan.

RESULTS

1. Thiamine concentration

Four groups of five rats each were fed four different synthetic diets (Table 1). Group 1, a thiamine-sufficient magnesium-sufficient diet (diet A); group 2, a thiamine-sufficient magnesium-deficient diet (diet B); group 3, a
thiamine-deficient magnesium-sufficient diet (diet C); and, group 4, a thiamine-
deficient magnesium-deficient diet (diet D). After four weeks on these dietary regimens the rats were sacrificed. The brain, spinal cord, sciatic nerve, heart, liver, and kidneys were removed, and thiamine concentrations were determined. As seen in Table 2 and similar to findings in a previous paper (1), thiamine concentration in the heart, liver, kidneys, and sciatic nerve in the thiamine-adequate magnesium-deficient rats (group 2) was lower than in the magnesium-sufficient rats (group 1). On the other hand, no significant difference was observed between magnesium-deficient and -sufficient rats in the central nervous system, i.e., brain and spinal cord. Although thiamine levels in all tissues decreased markedly in the thiamine-deficient groups 3 and 4 as compared to thiamine-sufficient groups, the thiamine concentrations in the liver, heart, kidneys, and sciatic nerve in the thiamine-deficient magnesium-deficient rats (group 4) were lower than in the thiamine-deficient, magnesium-sufficient rats (group 3) as shown in Table 2.

2. Thiamine-\(^{14}\)C loading to magnesium-deficient rats

To further investigate thiamine metabolism in magnesium-deficient rats, the above dietary regimens were carried out for four weeks to other rat groups (groups 5–9, diets A–D). After four weeks, 1 mg of thiamine-\(^{14}\)C (1.4 mCi/mmole) was injected intraperitoneally after which a 24-hr urine specimen was collected. The rats were then sacrificed, and the radioactivity in various tissues was determined.

As represented in Table 3, radioactivity in the 24-hr urine specimen was highest in thiamine-sufficient magnesium-sufficient rats (group 5) followed by thiamine-sufficient magnesium-deficient rats (group 6), thiamine-deficient magnesium-sufficient rats (group 7), and thiamine-deficient magnesium-deficient rats (group 8). In contrast, thiamine-\(^{14}\)C in the blood, like a mirror image, was lowest in thiamine-sufficient magnesium-sufficient rats and highest in thiamine-deficient magnesium-deficient rats. A similar tendency was seen in thiamine-\(^{14}\)C levels in the heart, liver, kidneys, and sciatic nerve, although a significant difference was not observed. In the brain and spinal cord, however, there was no significant difference in thiamine-\(^{14}\)C levels between magnesium-sufficient and magnesium-deficient rats, as shown in Table 3.

3. Subcellular distribution of thiamine in the liver of magnesium-deficient rats

To clarify the difference in thiamine distribution in liver subfractions of magnesium-deficient rats and magnesium-sufficient rats, two groups of 4 rats each were put on a thiamine-sufficient magnesium-sufficient diet (group 9: diet A) and a thiamine-sufficient magnesium-deficient diet (group 10: diet B) for 30 days. After sacrifice, the liver was removed and homogenized in a 0.25 M sucrose solution containing Tris buffer (0.5 mM) and EDTA (40 \(\mu\)M) at a pH of 7.4. Nuclei, mitochondria, microsome, and supernatant fractions were separated centrifugally
Table 2. Thiamine levels in various tissues. Values represent Mean ± SEM of 5 rats. Data were considered statistically based on the method of one factor ANOVA. The same alphabetical superscripts (a, b, c) following mean and SEM denote no significant difference between groups in each tissue; different alphabetical superscripts denote significant difference (p < 0.05) between groups. (e.g., entries with superscript "a" showed no significant differences on comparison with groups marked "a"; those with superscript "b" showed significant differences on comparison with groups not marked "b").

| Group | Diet | No. | Thiamine | Magnesium | Heart | Liver | Kidneys | Brain | Spinal cord | Sciatic nerve |
|-------|------|-----|----------|-----------|-------|-------|---------|-------|-------------|---------------|
| 1     | A    | sufficient | sufficient | 533 ± 31a | 1,067 ± 54a | 651 ± 98a | 301 ± 7a | 253 ± 7a | 167 ± 12a |
| 2     | B    | sufficient | deficient  | 261 ± 25b | 558 ± 68b | 253 ± 30b | 297 ± 13a | 257 ± 20a | 105 ± 10b |
| 3     | C    | deficient  | sufficient | 103 ± 12c | 184 ± 24c | 89 ± 12c | 96 ± 11b | 94 ± 4b | 68 ± 8c |
| 4     | D    | deficient  | deficient  | 51 ± 13d  | 80 ± 11d  | 38 ± 11d  | 103 ± 8b | 91 ± 8b | 36 ± 4d |

(µg/100 g wet weight)

Table 3. Radioactive thiamine in urine, blood, and tissues 24-hr after injection of thiamine-14C (1 mg). Values represent Mean ± SEM of 4 rats. Different letter superscripts (a, b, c) denote significant differences (p < 0.05) in each tissue.

| Gr. No. | Diet | V.B. | Mg | Urine 24-hr (µg) | Blood Liver (µg/dl) | Kidneys (µg) | Heart (µg) | Brain (µg) | Spinal cord (µg/100 g wet weight) | Sciatic nerve (µg/100 g wet weight) |
|---------|------|------|----|----------------|----------------------|-------------|------------|-----------|----------------------------------|----------------------------------|
| 5       | A    | +    | +  | 692 ± 31a      | 2.75 ± 0.61a         | 1.97 ± 0.36a | 1.14 ± 0.32a | 1.26 ± 0.12a | 0.55 ± 0.06a                       | 0.44 ± 0.03a                        |
| 6       | B    | +    | -  | 521 ± 48b      | 7.92 ± 0.75b         | 2.87 ± 0.24b | 1.86 ± 0.21ab | 1.45 ± 0.08b | 0.58 ± 0.07a                       | 0.39 ± 0.03a                        |
| 7       | C    | -    | +  | 362 ± 58b      | 8.34 ± 0.65b         | 3.75 ± 0.30b | 2.93 ± 0.35b | 1.63 ± 0.13ab | 1.03 ± 0.04b                       | 0.72 ± 0.05b                        |
| 8       | D    | -    | -  | 160 ± 32a      | 14.92 ± 1.35a        | 5.76 ± 0.64a | 3.48 ± 0.41ab | 2.00 ± 0.08b | 1.10 ± 0.10b                       | 0.77 ± 0.06b                        |

(µg/100 g wet weight)
by the method of Hogeboom (8). Thiamine concentration in various fractions of the liver was determined and is shown in Table 4. The thiamine level in the mitochondrial fraction of the liver was decreased most significantly in magnesium-deficient rats as compared to the magnesium-sufficient ones. In other fractions no marked difference was observed.

4. Comparison between oral and parenteral administration of thiamine to magnesium-deficient or -sufficient rats

In experiments 1-3 herein, it is obvious that a magnesium deficiency results in a thiamine deficiency. There are two probable factors concerning the aetiology of this phenomenon: (1) magnesium deficiency inhibits the intestinal absorption of thiamine; (2) magnesium plays a role in the binding of thiamine and tissue protein.

For elucidation, rats were separated into four groups of five each (groups 11-14), and various synthetic diets (diet A-D) were administered. Food consumption of groups 11 and 12 (orally thiamine-administered rats) was measured daily to determine the thiamine intake. To groups 13 and 14 (parenterally

| Group No. | Diet | Thiamine | Magnesium | Nuclear | Mitochondrial | Microsomal | Supernatant |
|-----------|------|----------|-----------|---------|---------------|------------|-------------|
| 9         | A    | sufficient | sufficient | 252±34  | 384±42        | 38±8       | 283±31      |
| 10        | B    | sufficient | deficient  | 194±25  | 186±34\(^a\)  | 34±12      | 234±29      |

Table 4. Distribution of thiamine in liver cell subfractions. Values represent Mean ± SEM of 4 rats.

\(^a\) Significant difference (p<0.01) compared with group 9.

| Gr. No. | Diet | Thiamine injection | Brain | Heart | Liver | Kidneys |
|---------|------|---------------------|-------|-------|-------|---------|
| 11      | A    | +                   | 298±12\(^a\) | 506±55\(^a\) | 932±28\(^a\) | 525±35\(^a\) |
| 12      | B    | +                   | 295±12\(^a\) | 252±34\(^b\) | 511±31\(^b\) | 360±44\(^b\) |
| 13      | C    | +                   | 290±10\(^a\) | 492±50\(^a\) | 870±44\(^a\) | 604±62\(^a\) |
| 14      | D    | +                   | 303±13\(^a\) | 249±33\(^b\) | 488±41\(^b\) | 358±46\(^b\) |

Table 5. Comparison between oral and parenteral routes of thiamine to both magnesium-deficient and -sufficient rats. Values represent Mean±SEM of 5 rats. Different letter superscripts (a, b) denote significant differences (p<0.05) in each tissue.
thiamine-administered rats) the same amount of thiamine was given by intraperitoneal injection. These routines were carried out for 30 days. Twenty-four hours after the last treatment the rats were sacrificed, and the liver, kidneys, and brain were removed and thiamine concentration was determined.

As indicated in Table 5, with both oral and parenteral administration of thiamine, thiamine levels in the liver and kidneys of magnesium-deficient rats decreased significantly as compared to the magnesium-sufficient rats. No significant difference was seen among the groups as far as thiamine levels in the brain.

**DISCUSSION**

This study reconfirms evidence that thiamine and magnesium are metabolized interdependently (1-4, 9-15). The phenomenon that magnesium deficiency causes a thiamine deficiency is explained by two facts. (1) Total thiamine content in the heart, liver, kidneys, and sciatic nerve of magnesium-deficient rats was lower than in that of magnesium-sufficient rats. (2) Radioactivity in the 24-hr urine specimen after loading of thiamine-\(^{14}\)C was lower in the magnesium-deficient rats as compared to the magnesium-sufficient rats. In contrast, radioactivity in the blood and tissues was higher in the magnesium-deficient rats than in the magnesium-sufficient rats.

Regarding magnesium-deficient rats, there was no significant difference of thiamine levels in the tissues of orally thiamine-administered and parenterally administered rats. This fact coupled with our previous observation that the \textit{in vitro} addition of thiamine pyrophosphate to tissue homogenates of thiamine-deficient magnesium-deficient rats failed to restore transketolase activity (1) support the view that magnesium plays some role in binding thiamine with tissue proteins, and accordingly thiamine deficiency occurred in magnesium-deficient rats. The hypothesis that magnesium deficiency may inhibit intestinal absorption of thiamine should be discarded.

The result that uptake of thiamine-\(^{14}\)C in tissues is higher in magnesium-deficient rats than in magnesium-sufficient rats is rather contradictory to this hypothesis. The reason could be tentatively explained as follows: as magnesium levels in soft tissues, \textit{i.e.}, brain, liver, kidneys \textit{etc.}, are still within normal range after one month of a magnesium-deficient diet (3), the bulk of apoenzymes in magnesium-deficient rats remains intact and requires thiamine as coenzyme.

In contrast to soft tissues, magnesium levels in serum and bone decreased markedly in thiamine-sufficient magnesium-deficient rats, and this decrease of magnesium was rather low in thiamine-deficient magnesium-deficient rats (3). These findings suggest that thiamine accelerates the magnesium deficiency. They also support the above hypothesis that thiamine requires magnesium for the binding of thiamine and protein and, consequently, that magnesium deficiency in magnesium-deficient rats is accelerated by excess thiamine.
Among various subcellular fractions in the liver, thiamine content in the mitochondrial fraction was decreased most markedly in magnesium deficiency. It is feasible that magnesium deficiency primarily inhibits mitochondrial thiamine-related enzyme systems.

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