Note

Conversion Ratio of Tryptophan to Niacin in Rats Fed a Vitamin B₁-Free Diet

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Summary The effect of a vitamin B₁-free diet on the conversion ratio of tryptophan to niacin in rats was investigated using the current methods for determination of the intermediate metabolites. Rats were fed with diets with or without B₁ for 33 days. The body weight gain and food intake in the B₁-free group were almost the same as the control group for 10 days, but, they steeply dropped after that time and the conversion ratio of tryptophan to niacin began to increase. The ratio reached 7-fold that of the control group on the last day of the experiment. This finding seriously differed from the previous reports, which described that B₁ was needed in the first part of tryptophan conversion to niacin and that the conversion ratio of tryptophan to niacin decreased in B₁-deficient rats. Furthermore, the activity of tryptophan oxygenase, in which it was reported that B₁ is required for the tryptophan catalytic reaction, did not decrease but increased even when the B₁-free diet was fed. These results suggest that there is a very small possibility of the direct involvement of B₁ in the conversion of tryptophan to niacin.

Key Words vitamin B₁, tryptophan metabolism, nicotinamide, conversion ratio of tryptophan to niacin

A coenzyme, NAD⁺, is derived not only from vitamin pyridine 3-carboxylic acid derivatives but also from an essential amino acid, Trp. These conversion pathways play a critical role when the body abruptly needs a lot of NAD⁺. It is well known that the vitamin B group is involved in the conversion pathway of Trp to NAD⁺: The reaction of kynurenine to 3-hydroxykynurenine (1) involves vitamin B₂ as a cofactor, and the reaction of 3-hydroxykynurenine to 3-hydroxyanthranilic acid (2) involves vitamin B₆. Furthermore, Junqueira and Schweigert (3) reported that the urinary excretion of niacin and its metabolite, MNA, in B₁-deficient rats

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Abbreviations: B₁, vitamin B₁; MNA, N¹-methylnicotinamide; Nam, nicotinamide; NiA, nicotinic acid; 2-Py, N¹-methyl-2-pyridone-5-carboxamide; 4-Py, N¹-methyl-4-pyridone-3-carboxamide; Trp, L-tryptophan.

decreased. Porter et al (4) also reported that the urinary excretion of kynurenine, kynurenic acid, and xanthurenic acid in B₁-deficient rats differed from that of control rats. Dalglish (5) reported that there was no excretion of N-formylkynurenine even when Trp was administered to B₁-deficient rats, and suggested that the reaction of Trp to N-formylkynurenine might decrease. Based on the results, a metabolic map indicating that B₁ is involved in the reaction of Trp to N-formylkynurenine, is shown on the cover of the book entitled "Nicotinic Acid" (6). However, the role of B₁ in the Trp conversion to niacin has not yet been clearly shown. Therefore, we fed rats a B₁-free diet to investigate the effect on the conversion ratio of Trp to niacin using the current methods for determination of the metabolites.

Vitamin-free milk casein, sucrose, L-methionine, Nam, Trp, and thiamine hydrochloride were purchased from Wako Pure Chemical Industries (Osaka, Japan). MNA chloride was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). 2-Py and 4-Py were synthesized by the methods of Pullman and Colowick (7) and Shibata et al (8), respectively. The mineral and vitamin mixtures were obtained from Oriental Yeast Kogyo (Tokyo, Japan). All other chemicals were of the highest purity available from commercial sources.

The animal room was maintained at around 22°C and about 60% humidity, and a 12-h light/12-h dark cycle was maintained. Body weight and food intake were measured daily around 09:00 a.m., and food and water were renewed daily. The care and treatment of the experimental animals conformed to the rules for ethical treatment of laboratory animals.

Male rats of the Wistar strain (4 weeks old) were obtained from Clea Japan (Tokyo, Japan) and immediately placed in individual metabolic cages (CT-10; Clea Japan). To accustom the rats to these conditions, they were initially fed ad libitum for 7 days with a 20% casein diet (+NiA & +B₁ diet) as shown in Table 1. They were then divided into four groups at 09:00 a.m. on day 0 (the first day of the experiment was defined as day 0), and fed diets with or without NiA and/or B₁ (+NiA & +B₁ diet, +NiA & -B₁ diet, -NiA & +B₁ diet, and -NiA & -B₁ diet; Table 1) ad libitum until 09:00 a.m. on day 34, namely for 33 days. Urine samples (09:00 a.m.-09:00 a.m.; 24-h urine) were periodically collected in amber bottles containing 1 mL of 1 M HCl and stored at -25°C until use. For example, urine samples on day 0 were collected from 09:00 a.m. on day 0 to 09:00 a.m. on day 1. The conversion ratio of Trp to niacin was calculated by the following equation: {(the sum of the urinary excretion of Nam, MNA, 2-Py and 4-Py (mol/daily urine)) × 100}/Trp intake during urine collection (mol/day). The contents of Nam, 2-Py, and 4-Py in the urine were simultaneously measured by the HPLC method of Shibata et al (8), while the content of MNA in the urine was measured by the HPLC method of Shibata (9). The Trp intake was calculated from the food intake. The content of B₁ in the urine on the last day of the experiment was measured by the method of Itokawa (10).

The rats were sacrificed by decapitation after the last day of the experiment (day 34 at 09:00 a.m.). The liver of each animal was removed, and a portion

Table 1. Compositions of the diets.

	20% Casein diet (+NiA & +B ₁) (%)	2% Casein diet (+NiA & -B ₁) (%)	20% Casein diet (-NiA & +B ₁) (%)	20% Casein diet (-NiA & -B ₁) (%)
Vitamin-free casein	20	20	20	20
L-Methionine	0.2	0.2	0.2	0.2
Gelatinized cornstarch	45.9	45.9	45.9	45.9
Sucrose	22.9	22.9	22.9	22.9
Corn oil	5	5	5	5
Mineral mixture ¹	5	5	5	5
Vitamin mixture ¹	1	0	0	0
Vitamin mixture ¹ (vitamin B ₁ -free)	0	1	0	0
Vitamin mixture ¹ (NiA-free)	0	0	1	0
Vitamin mixture ¹ (vitamin B ₁ - and NiA-free)	0	0	0	1

¹ These compositions are those of Oriental Yeast Kogyo (Tokyo).

(approximately 1 g) was immediately homogenized with a Teflon-glass homogenizer in five volumes of cold 50 mM KH₂PO₄-K₂HPO₄ buffer (pH 7.0). These homogenates were used as the enzyme source for measuring Trp oxygenase (11).

The significance of difference between values was evaluated by analysis of variance and Duncan's multiple range test (12).

The effects of B₁ deficiency in the presence and absence of NiA on the body weight and daily food intake are shown in Fig. 1A and B. After the rats were fed B₁-free diets for around 10 days, the body weight and food intake began to decrease. That is, the rats fell into a B₁-deficient state. If B₁ is needed for the conversion of Trp to niacin, the supplementation of NiA to the B₁-free diet would cause a partial recovery of body weight gain in young rats. But, its addition did not contribute to body weight gain as shown in Fig. 1A. This means that there is a very small possibility that B₁ is involved in the conversion of Trp to niacin.

The periodical changes in urinary excretion of the sum, which is Nam + MNA + 2-Py + 4-Py, and the conversion ratio of Trp to niacin are shown in Fig. 2. The sum in the groups fed the B₁-free diet became higher after 10 days than that in the groups fed the B₁-containing diet. Therefore, the conversion ratio in the B₁-free group also increased after 10 days and reached 7-fold of the control group on the last day of the experiment, which was not consistent with a previous report (3). The conversion ratio could not be calculated in the NiA-containing groups because Nam and its metabolites originated not only from Trp but also from NiA in the diet.

Fig. 1. Effect of feeding the B₁-free diet with or without NiA on the gain in body weight (A) and food intake (B). ○, +NiA & +B₁; ●, +NiA & -B₁; □, -NiA & +B₁; ■, -NiA & -B₁. Each point is the mean for five rats.

Fig. 2. Effect of feeding the B₁-free diet on urinary excretion of the sum (A) and the conversion ratio of Trp to niacin (B). ○, +NiA & +B₁; ●, +NiA & -B₁; □, -NiA & +B₁; ■, -NiA & -B₁. Each point is the mean $\pm$ SE for five rats. The sum is Nam + MNA + 2-Py + 4-Py.

The activity of Trp oxygenase did not decrease but increased as the result of feeding a B₁-free diet ($1.11 \pm 0.05 \mu\text{mol/h/g}$ of liver in the group fed the -NiA & +B₁ diet vs. $2.71 \pm 0.23 \mu\text{mol/h/g}$ of liver in the group fed the -NiA & -B₁ diet). The difference between the two groups was statistically significant, although Dalglish (5) reported that B₁ was needed for this enzyme reaction.

From these results, it was concluded that B₁ is not directly involved in the conversion of Trp to niacin, and, therefore, the metabolic map (6) indicating that B₁ is involved in the reaction of Trp to N-formylkynurenine is not correct. However, it might be possible that the flux of Trp and its intermediates to niacin in the liver increased during B₁ deficiency because the conversion ratio of Trp to niacin significantly increased in B₁-deficient rats (Fig. 2B).

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