Table 1. Composition of the Diets

	Control	+0.04%	+0.08%	+0.10%	+0.20%	+0.50%	+0.80%	+1.00%
Casein	20	20	20	20	20	20	20	20
L-Methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
α-Cornstarch	46.9	46.86	46.82	46.8	46.7	46.5	46.3	46.2
Sucrose	23.4	23.4	23.4	23.4	23.4	23.3	23.2	23.1
Corn oil	5	5	5	5	5	5	5	5
Mineral mixture[a]	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mixture[a]	1	1	1	1	1	1	1	1
D-Biotin	0	0.04	0.08	0.10	0.20	0.50	0.80	1.00

Each value is expressed as g/100 g of diet.
[a]The compositions of the mineral and vitamin mixtures are described in Ref. 16.

Industries (Osaka, Japan). Corn oil was purchased from Ajinomoto (Tokyo, Japan). Gelatinized corn starch, the mineral mixture (AIN-93M)[16] and vitamin mixture (AIN-93-VX containing 25% choline bitartrate)[16] were obtained from Oriental Yeast (Tokyo, Japan). Thiamin hydrochloride ($C_{12}H_{17}ClN_4OS$-HCl, 337.27), riboflavin ($C_{17}H_{20}N_4O_6$, 376.37), cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$, 1355.40), nicotinamide (Nam; $C_6H_6N_2O$, 122.13), calcium pantothenate ($C_{18}H_{32}N_2O_{10}$-Ca, 476.54), folic acid (pteroylmonoglutamic acid; $C_{19}H_{19}N_7O_6$, 441.40), D(+)-biotin ($C_{10}H_{16}N_2O_3S$, 244.31), and L(+)-ascorbic acid ($C_6H_8O_6$, 176.13) were purchased from Wako Pure Chemical Industries. N^1-methylnicotinamide (MNA) chloride ($C_7H_9N_2O$-HCl, 159.61) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N^1-methyl-2-pyridone-5-carboxamide (2-Py; $C_7H_8N_2O_2$, 152.15) and N^1-methyl-4-pyridone-3-carboxamide (4-Py; $C_7H_8N_2O_2$, 152.15) were synthesized by the methods of Pullman and Colowick[17] and Shibata *et al.*,[18] respectively. All other chemicals used were of the highest purity available from commercial sources.

Animals. The care and treatment of the experimental animals conformed to the University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals.

Male Wistar rats (3 weeks old) were obtained from CLEA Japan (Tokyo, Japan) and placed in individual metabolic cages (CT-10; CLEA). They were divided into eight groups (such group consisting of four rats) and fed *ad libitum* for 28 days: one group with a 20% casein diet (used as a control group containing 0.00002% biotin), and the others with the same diet plus 0.04, 0.08, 0.10, 0.20, 0.50, 0.80 or 1.0% biotin (Table 1).

The room temperature was maintained at around 22 °C and 60% humidity, and a 12-h light (06:00–18:00)/12-h dark (18:00–06:00) cycle was maintained. The body weight and food intake were measured every 2 days at around at 10:00. Urine samples (24 h; 10:00–10:00) were collected in amber bottles containing 1 ml of 1 M HCl on the last day of the experiment, and were stored at −20 °C until needed.

The rats were killed by decapitation at around 10:00 on the last day (day 28), after the urine sample had been collected. Serum was collected to measure biotin, and was stored at −20 °C until needed. The liver, spleen, kidney, heart, lung, brain, testis and thigh muscle of each animal were removed, and a portion (about 0.5 g) was immediately treated as described next to measure biotin.

Analyses. Vitamin B_1: One milliliter of 1 M HCl was added to 9 ml of the 24-h urine sample. The urinary concentration of thiamin was determined by the HPLC post-labeled fluorescence method.[19]

Vitamin B_2: One milliliter of 1 M HCl was added to 9 ml of the 24-h urine sample. The urinary concentration of riboflavin was determined by the HPLC method.[20]

Vitamin B_6: One milliliter of 1 M HCl was added to 9 ml of the 24-h urine sample. The urinary concentration of the vitamin B_6 catabolite, 4-pyridoxic acid (4-PIC), was determined by the HPLC method.[21]

Vitamin B_{12}: Part of the 24-h urine samples was stored at −20 °C. The urinary vitamin B_{12} concentration was assayed by a microbiological method with *Lactobacillus delbrueckii* subsp. *lactis* ATCC 7830.[22]

Niacin: One milliliter of 1 M HCl was added to 9 ml of the 24-h urine sample. The quantity of Nam, 2-Py and 4-Py in the urine was measured simultaneously by the HPLC method of Shibata *et al.*[18] The content of MNA was measured by the method of Shibata.[23] The sum of Nam, MNA, 2-Py and 4-Py was used to represent the niacin catabolites.

Pantothenic acid: Part of the 24-h urine samples was stored at −20 °C. The content of pantothenic acid in the urine was directly measured by using *Lactobacillus plantarum* ATCC 8014.[24]

Folates: One milliliter of 1 M ascorbic acid was added to 9 ml of the 24-h urine sample. The urinary concentration of folates was determined by the microbioassay method with *Lactobacillus casei* ATCC 7469.[25]

Biotin: Part of the 24-h urine samples was stored at −20 °C. The content of biotin in the urine was directly measured by using *Lactobacillus plantarum* ATCC 8014.[26] To measure the free serum biotin content, 0.05 ml of serum was added to 1 ml of distilled water, and the mixture was heated for 5 min in a water bath at 100 °C. After cooling to room temperature, the solution was centrifuged at 9000 *g* for 10 min at 4 °C, and the resulting supernatant was used to measure biotin. To

Table 2. Effects of Biotin Intake on the Body Weight Gain and Food Intake

	Control	+0.04%	+0.08%	+0.10%	+0.20%	+0.50%	+0.80%
Initial body weight (g)	37.8 ± 0.5	37.6 ± 0.5	37.8 ± 1.2	37.9 ± 0.6	37.7 ± 0.8	37.6 ± 1.3	37.5 ± 0.6
Final body weight (g)	219.7 ± 4.2^{a}	208.1 ± 1.9^{ab}	179.6 ± 7.7^{bc}	165.2 ± 5.2^{c}	131.7 ± 9.7^{d}	120.9 ± 8.8^{d}	101.0 ± 0.9^{d}
Body weight gain (g/28d)	181.9 ± 4.1^{a}	170.5 ± 1.5^{ab}	141.8 ± 7.3^{bc}	127.3 ± 4.6^{c}	94.1 ± 9.8^{d}	83.3 ± 9.0^{d}	63.8 ± 0.1^{d}
Food intake (g/28d)	380.1 ± 6.9^{a}	360.6 ± 4.4^{ab}	323.4 ± 10.0^{b}	315.4 ± 9.9^{b}	256.4 ± 15.1^{c}	236.9 ± 16.6^{c}	203.9 ± 4.7^{c}

Each value is expressed as the mean ± SEM.
A different superscript letter means significant difference at $p < 0.05$.

Table 3. Effects of Biotin Intake on the Tissue Weights of Rats

	Control	+0.04%	+0.08%	+0.10%	+0.20%	+0.50%	+0.80%
Brain	1.05 ± 0.05	1.05 ± 0.01	1.03 ± 0.00	1.02 ± 0.01	0.96 ± 0.03	1.06 ± 0.02	1.00 ± 0.05
Liver	10.23 ± 0.58^{a}	10.64 ± 0.15^{a}	8.53 ± 0.52^{ab}	7.54 ± 1.03^{bc}	6.94 ± 0.53^{bc}	5.95 ± 0.55^{bc}	5.34 ± 0.29^{c}
Heart	0.85 ± 0.01^{a}	0.76 ± 0.02^{ab}	0.66 ± 0.02^{bc}	0.62 ± 0.04^{cd}	0.54 ± 0.04^{de}	0.49 ± 0.01^{e}	0.42 ± 0.02^{e}
Kidney	1.84 ± 0.03^{a}	1.72 ± 0.02^{ab}	1.53 ± 0.04^{b}	1.46 ± 0.10^{bc}	1.19 ± 0.08^{cd}	1.22 ± 0.06^{cd}	1.04 ± 0.03^{d}
Lung	1.61 ± 0.12^{a}	1.30 ± 0.07^{ab}	1.14 ± 0.05^{bc}	1.13 ± 0.17^{bc}	0.78 ± 0.09^{c}	0.73 ± 0.06^{c}	0.67 ± 0.00^{c}
Spleen	0.68 ± 0.01^{a}	0.69 ± 0.01^{a}	0.59 ± 0.01^{a}	0.53 ± 0.07^{ab}	0.41 ± 0.05^{bc}	0.29 ± 0.02^{c}	0.26 ± 0.02^{c}
Testis	2.32 ± 0.04^{a}	2.29 ± 0.03^{a}	2.24 ± 0.01^{a}	2.15 ± 0.16^{a}	1.91 ± 0.17^{a}	1.41 ± 0.10^{b}	0.81 ± 0.10^{c}

Each value is expressed in g as the mean ± SEM.
A different superscript letter means significant difference at $p < 0.05$.

measure the total biotin content in the tissues, a portion (about 0.5 g) of each tissue (liver, spleen, kidney, heart, lung, brain, testis and skeletal muscle) was homogenized with two volumes of 2.25 M H_2SO_4 and then hydrolyzed by autoclaving for 1 h at 121 °C and 2 atm. After cooling, the hydrolysate was centrifuged at 9000 *g* for 10 min at 4 °C, and the resulting supernatant was used to measure biotin. To measure the free biotin content in the liver, a portion of the liver was homogenized with two volumes of a 0.05 M potassium phosphate buffer (pH 7.0), the homogenate was centrifuged at 9000 *g* for 10 min at 4 °C, and the resulting supernatant was used to measure biotin.

Statistical analysis. Each value is expressed as the mean ± SEM. The statistical significance was determined by ANOVA, this being followed by Tukey's multiple-comparison test. $P < 0.05$ was considered to be statistically significant. Graph Pad Prism4.0 (Graph Pad Software, San Diego, CA, USA) was used for all the analyses.

Results

Effect of excessive biotin administration on the food intake and body weight gain in young rats

The 0.00002% biotin diet was set as the control because the AIN-93 diet recommended by AIN contains 0.00002% biotin.[16] The food intake and body weight gain were not significantly different between the 0.04% biotin-added and control groups, whereas the food intake and body weight gain in the group with the >0.08% biotin-added diets were significantly lower than those in the control group (Table 2). Diarrhea was observed in the young rats fed with the >0.50% biotin-added diets. One rat in four died with the 0.80% biotin-added diet, and two in four rats died with the 1.0% biotin-added diet. Therefore, the data for the 1.00% biotin-added diet group are not shown in Table 2.

Effect of excess biotin administration on the tissue weight of young rats

Table 3 shows the tissue weight of the rats fed on the biotin diets. The brain weight was not significantly different among the seven groups. The weights of other tissues, including the liver, heart, kidney, lung, spleen and testis, showed increasingly lower values in a dose-dependent manner. The tissue weights in the 0.04% biotin-added group were the same as those in the control group, and all tissue weights except the brain in the 0.50% and 0.80%-added groups were lower than those in the control group. The heart and kidney weights in the groups with the >0.08% biotin-added diets were lower than those in the control group, the liver and lung weights in the groups with the >0.10% biotin-added diets were lower, and the testis weights in the group with the >0.20% biotin-added diets were lower those in the control group.

Effect of excess biotin administration on the biotin concentration in the urine, tissues and blood

The effect of excess biotin on the concentration of biotin in the urine is shown in Fig. 1A. The urinary excretion of biotin increased with increasing to dietary intake of biotin. The urinary biotin excretion rate to biotin intake was 54.1 ± 5.8 in the control group, and 82.7 ± 2.5, 68.9 ± 5.7, 63.3 ± 2.5, 60.0 ± 2.6, 31.4 ± 6.8 and 29.8 ± 6.8% in the 0.04, 0.08, 0.10, 0.20, 0.50 and 0.80% biotin-added groups, respectively. The serum free biotin content also increased with increasing intake of biotin (Fig. 1B).

The liver total, bound and free biotin contents are shown in Fig. 2. These biotin contents in the liver increased in a dose-dependent manner, although the

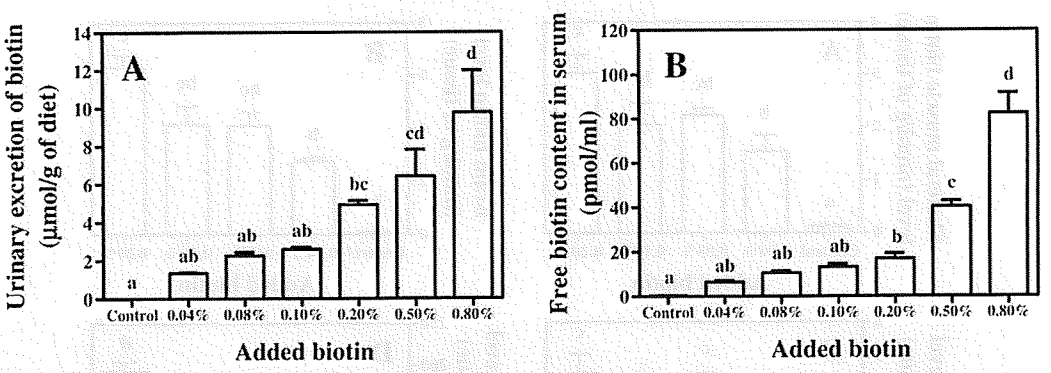

Fig. 1. Effects of Excessive Administration of Biotin on the Urinary Excretion of Biotin (A) and Free Biotin Content in the Serum (B).
The 24-hr urine samples were collected on the last day of the experiment, and then the serum was collected. Each bar is the mean ± SEM for 3 or 4 rats. A different superscript letter means significant difference at $p < 0.05$.

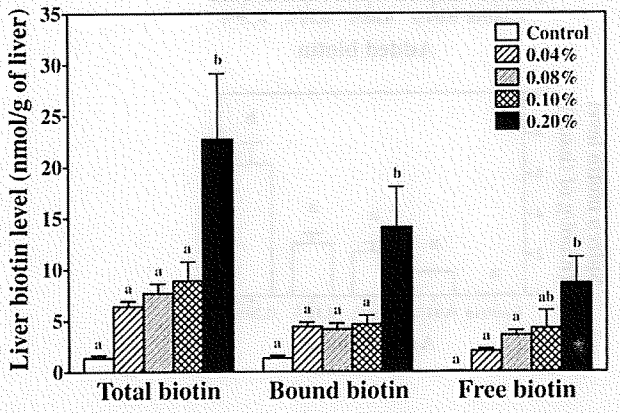

Fig. 2. Effects of Excessive Administration of Biotin on the Total, Bound and Free Biotin Contents in the Liver.
The serum and liver were collected on the last day of the experiment. Each bar is the mean ± SEM for 3 or 4 rats. A different superscript letter means significant difference at $p < 0.05$.

liver bound biotin contents were at the same level in the 0.04, 0.08 and 0.10% biotin-added groups. Ninety seven percent of the liver total biotin existed as bound biotin in the control group, and 30–50% of total biotin was of the bound type in the 0.04, 0.08, 0.10 and 0.20% biotin added-groups.

The concentrations of biotin in the skeletal muscle, brain, heart, kidney, lung, spleen and testis also increased with increasing intake of biotin (Fig. 3). The levels of biotin in the skeletal muscle, brain, heart, kidney, lung and testis in the 0.04, 0.08, 0.10 and 0.20% biotin-added groups were significantly higher than those in the control groups. The spleen biotin content in the 0.04% biotin-added group was not significantly higher than that in the controls, however, the biotin contents in the 0.08, 0.10 and 0.20% biotin adding groups were significantly higher than that in the control group.

Effect of excess biotin administration on the urinary excretion of other water-soluble vitamins

A mega-dose of biotin did not greatly affect the urinary excretion of other water-soluble vitamins (Fig. 4). Only the urinary excretion of folates was significantly increased by feeding a diet containing up to 0.10% biotin. The urinary excretion of ascorbic acid tended to increase with increasing intake of biotin, but a small number of rats in each group failed to show any significant difference.

Discussion

We have previously reported that a 0.3% nicotinamide diet and 1.0% calcium pantothenate diet did not show any effect on the growth of young rats.[27,28] In the present study, an extremely high dose of biotin representing more than a 0.80% in the diet caused death, and more than 0.08% biotin-added diet retarded the growth of young rats. These results suggest that an excess biotin intake might cause some adverse effects on humans, and that setting UL for biotin would be important to prevent such dietary biotin-induced adverse effects. Although no adverse effects of biotin on humans have been reported, two studies have reported that subcutaneously administered biotin (50 and 100 mg/kg) to pregnant rats inhibited fetal and placental growth and resorption of fetuses and placentae.[12,13] The effects of excess biotin intake on the reproductive organs of male rats were not investigated in the present study, although the testis weights in the young rats fed with the diets containing more than 0.50% biotin were lower than those in the other groups. Whether an oral intake of high biotin by pregnant rats would affect the sex hormones, reproductive organs and fetal growth remains to be elucidated.

For increasing accumulation of biotin in the tissues was observed as the amount of biotin administered was increased. This phenomenon might have been due to too great an amount of biotin than was possible to metabolize and excrete. It is suggested that this accumulation was associated with the retardation of growth. The bound biotin content in the liver increased in the present study in a dose-dependent manner, and biotin quantification after SDS–PAGE separation showed that 40% of the accumulated biotin in the rat

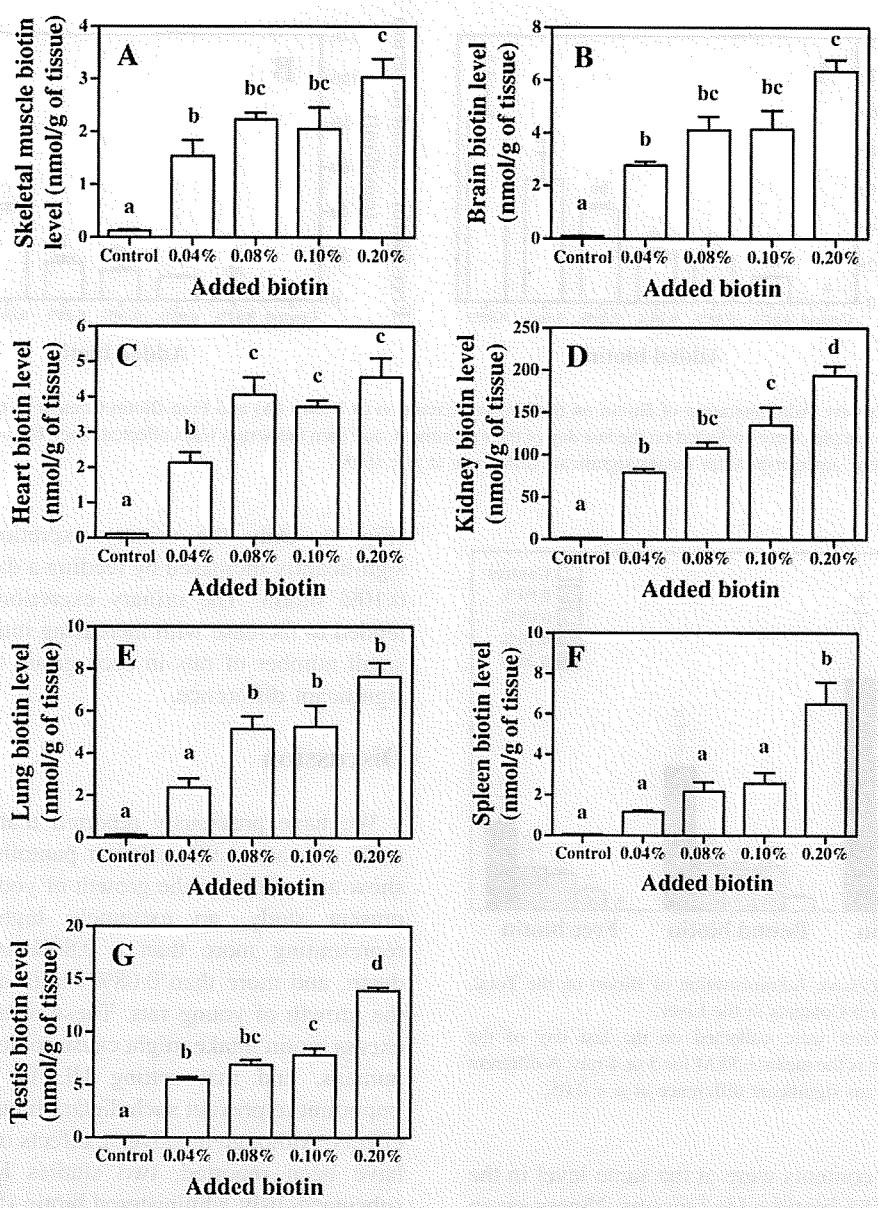

Fig. 3. Effects of Excessive Administration of Biotin on the Biotin Contents in the Muscle (A), Brain (B), Heart (C), Kidney (D), Lung (E), Spleen (F), and Testis (G).

The tissues were collected the last day of the experiment. Each bar is the mean ± SEM for 3 or 4 rats. A different superscript letter means significant difference at $p < 0.05$.

liver that have been overdosed with biotin was bound to protein (data not shown). Hymes *et al.* have proposed a reaction mechanism by which the enzyme, biotinidase (EC 3.5.1.12), mediates covalent binding of biotin to histones.[29] Biotinylation of histones might play a role in gene silencing,[30] cell proliferation,[31,32] and DNA repair or apoptosis.[30] Treatment of cell lines with a pharmacological concentration of biotin (10 pmol/ml) for several weeks had only a moderate impact on biotinylation of histones, whereas the biotinylation of carboxylases was strongly correlated with the biotin concentration in the culture media.[33–35] A pharmacological dose of dietary biotin (100 mg/kg) has decreased the abundance of biotinylated carboxylase in rat liver.[36] It is unclear whether an excess biotin intake wuold affect the biotinylation of histones, and how these changes to histones and some carboxylases are related to the detrimental effect of an excess biotin intake.

The present experiment using young rats clearly indicated that an excessive oral intake of biotin retarded the body weight gain and food intake. Judging from the results of the body weight gain and food intake, the no observed adverse effect level (NOAEL) in young rats was 0.04% in the diet, and the lowest observed adverse effect level (LOAEL) was 0.08% in the diet. Young rats in the 0.04% biotin group consumed about 6.83 g/day of their diet during days 0 to 28, the mean body weight during that period being about 177.8 g. Therefore, the

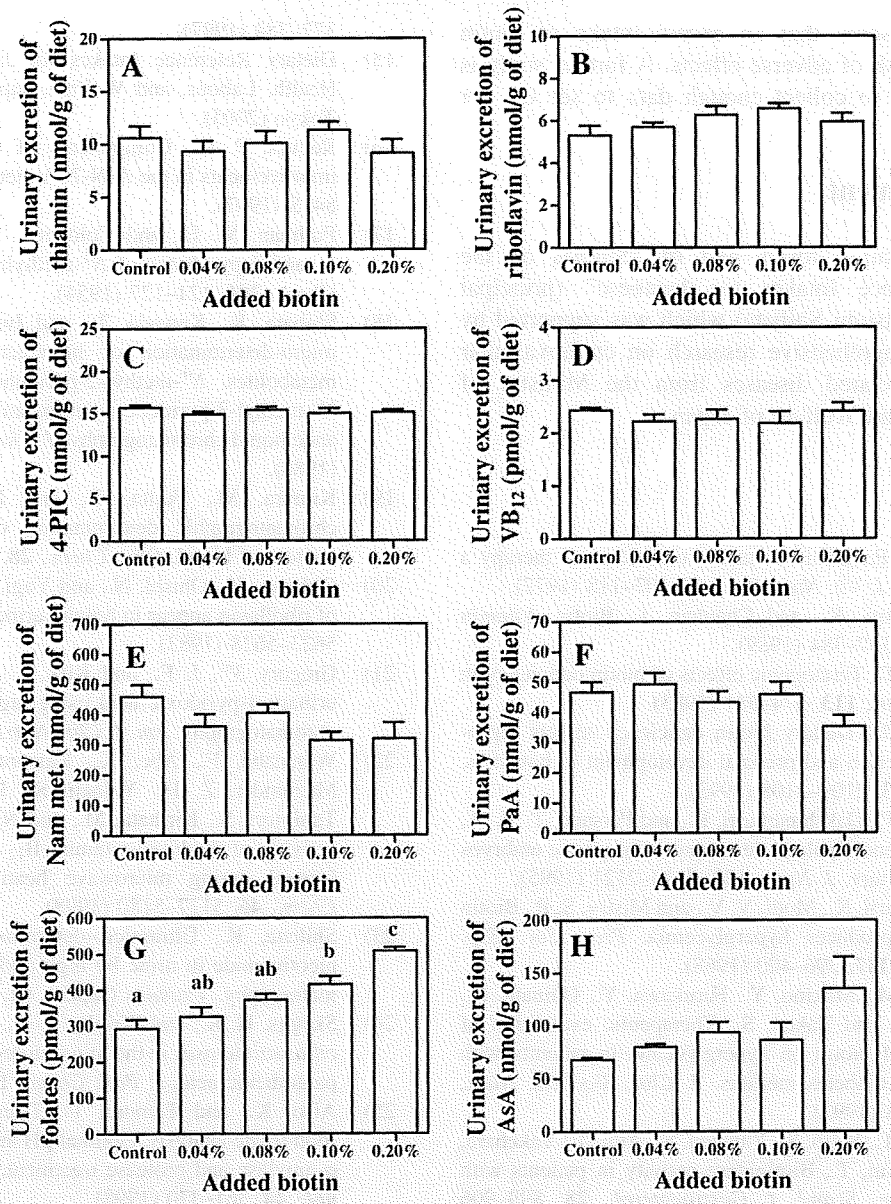

Fig. 4. Effects of Excessive Administration of Biotin on the Urinary Excretion of Thiamin(A), Riboflavin (B), 4-Piridoxic Acid (4-PIC), a Metabolite of Vitamin B_6 (C), Vitamin B_{12} (D), Sum of the Nicotinamide Metabolites, MNA, 2-Py and 4-Py (E), Pantothenic Acid (PaA) (F), Folates (G), and Ascorbic Acid (AsA) (H).

The 24-hr urine samples were collected the last day of the experiment. Each bar is the mean ± SEM for 3 or 4 rats. A different superscript letter means significant difference at $p < 0.05$.

biotin intake was calculated as 38.4 mg/kg body weight/day. Young rats in the 0.08% biotin group consumed about 11.76 g/day during days 0 to 28, the mean body weight during that period was being about 149.7 g. Therefore, the biotin intake was calculated as 79.2 mg/kg body weight/day. Although the present study clearly showed that the 79.2 mg/kg body weight/day oral intake of biotin caused adverse effects, the present study investigated the acute, but not chronic, effects of excess biotin intake on the body weight gain, food intake, tissue weight, tissue biotin content and urinary excretion of water-soluble vitamins, and not the histopathology nor production toxicity. Furthermore, the results of the present study were obtained from a limited number of animals, four rats in each group. A further study is needed to set more accurate NOAEL and LOAEL.

A single oral administration of 20 mg of biotin or 4.5 mg intravenously to healthy adults caused no adverse effect.[37] An oral intake of 1.2 mg/day of biotin by healthy adults for 14 days also did not cause any adverse effect.[38] Since the data on adverse effects from a high biotin intake are not sufficient for a quantitative risk assessment, UL for biotin has not been derived in USA and Japan.[15,39] The data from human studies plausibly show the low risk of several mg of biotin intake, but our

results clearly show that an excess intake of biotin increased the risk of adverse effects. A further study is therfore needed to collect enough data to set UL for biotin.

Acknowledgment

This investigation forms part of "Studies on the Dietary Reference Intakes for Japanese" (principal investigator, Katsumi Shibata) which was supported by a grant for comprehensive research on cardiovascular and life-style related diseases from the Ministry of Health, Labor and Welfare of Japan.

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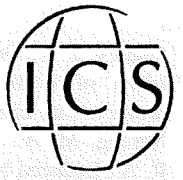

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Characterization of tryptophan–niacin metabolism in rats fed with an excessive tryptophan diet

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Abstract. We investigated tryptophan–niacin metabolism in rats when fed with an excessive tryptophan diet. Male rat of the Wistar strain (3 weeks old) were divided into the four groups of five rats each, and one of the group was fed with a 20% casein diet added with 0, 0.5, 1, 2, and 5% tryptophan for 30 days. The last day urine samples (24-h urine) were collected for analyses for the metabolites of tryptophan such as kynurenic acid (KA), xanthurenic acid (XA), 3-hydroxyanthranilic acid (3-HA), quinolinic acid (QA), nicotinamide (Nam), N^1-methylnicotinamide (MNA), N^1-methyl-4-pyridone-3-carboxamide(4-Py), and N^1-methyl-2-pyridone-5-carboxamide (2-Py). The urinary excretion of KA, XA, and 3-HA were increased according to the intake of tryptophan. However, the excretion of QA was almost the same in the groups between the groups of 2 and 5% tryptophan diets. The sum metabolites of Nam+MNA+2-Py+4-Py were almost the same in the 1–5% tryptophan diets. The value of (2-Py+4-Py)/MNA decreased sharply in the 0.5 and 1% Trp diets. Therefore the adverse effects of dietary Trp were observed from the diet containing 0.5% Trp, which was calculated as 0.45 g/kg of rat body weight. © 2007 Published by Elsevier B.V.

Keywords: Quinolinic acid; N^1-methylnicotinamide; N^1-methyl-2-pyridone-5-carboxamide; Tryptophan; Niacin; N^1-methyl-4-pyridone-3-carboxamide

1. Introduction

Niacin, serotonin and melatonin are very important bioactive compounds, which derive from an essential amino acid, tryptophan. Niacin is concerned with various metabolisms as a vitamin. Serotonin is involved in relieving pain, hypnosis, and tranquilizes as a

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doi:10.1016/j.ics.2007.07.019

neurotransmitter. Melatonin is a pineal hormone which works the rhythm of sleep. Based on these facts, tryptophan is widely found on the market as supplement. The adverse effects of tryptophan are not well known. We started to research the metabolism change of tryptophan by an excessive intake of tryptophan.

2. Materials and methods

2.1. Chemicals

Vitamin-free milk casein, sucrose, L-methionine, Gelatinized cornstarch, L-tryptophan (Trp), nicotinamide (Nam), and quinolinic acid (QA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Kynurenic acid (KA), xanthurenic acid (XA), 3-hydroxyanthranilic acid (3-HA), and N^1-methylnicotinamide (MNA) chloride were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N^1-Methyl-2-pyridone-5-carboxamide (2-Py) and N^1-methyl-4-pyridone-3-carboxamide (4-Py) were synthesized by the methods of Pullman and Colowick [1] and Shibata et al. [2], respectively. The mineral (AIN-93-G-MX) and niacin-free vitamin (AIN-93-VX) mixtures were obtained from Oriental Yeast Kogyo (Tokyo, Japan). All other chemicals used were the highest purity available from commercial sources.

2.2. Animal and diet

The care and treatment of the experimental animals conformed to the University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals. Twenty male rats of the Wistar strain (3 weeks old obtained from Clea, Japan) were divided into four groups of five each, and placed in an individual metabolic cage (CT-10 for rats; Clea Japan). One of the groups was fed with a 20% casein diet as a control group and others were fed with test diets which added the 20% casein diet to 0.5, 1, 2, and 5% Trp (Table 1), and allowed free access to food and water. The animal room was maintained at the temperature of around 20 °C with 60% humidity and a 12 h light/12 h dark cycle (light onset at 6:00 a.m.). Body weight and food intake were measured daily at around 9:00 a.m., and food and water were

Table 1

	Ctrl diet (%)	Test diet (Ctrl diet+Trp) (%)			
		+0.5% Trp	+1.0% Trp	+2.0% Trp	+5.0% Trp
Casein	20	20	20	20	20
L-Methionin	0.2	0.2	0.2	0.2	0.2
Gelatinized cornstarch	45.9	45.4	44.9	43.9	40.9
Sucrose	24.4	24.4	24.4	24.4	24.4
Corn oil	5	5	5	5	5
Mineral mixture (AIN-93-G-MX)	3.5	3.5	3.5	3.5	3.5
Vitamin mixture (AIN-93-VX niacin free)	1	1	1	1	1
Trp	0	0.5	1	2	5

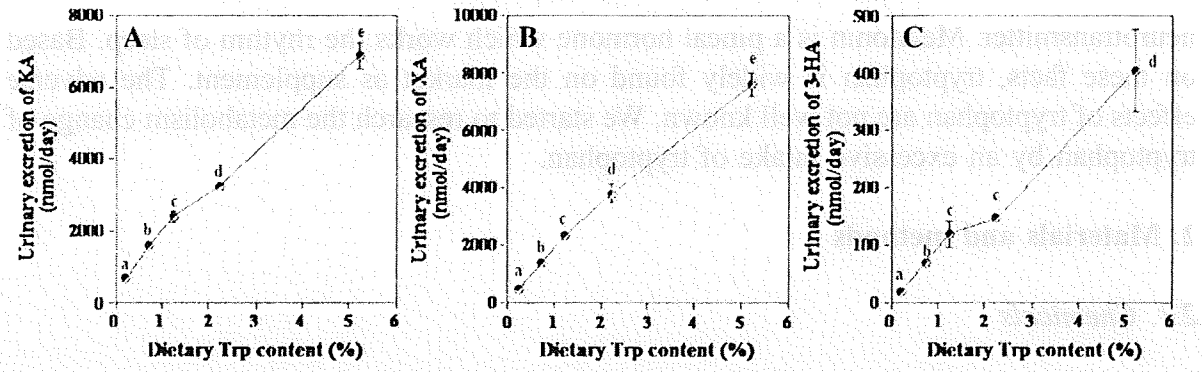

Fig. 1. Effect of Trp intake on the urinary excretion of KA (A), XA (B), and 3-HA (C) in rats. A different letter on the circle means a significant difference at $p<0.05$, as determined by Tukey–Kramer multiple comparisons test.

renewed daily. The experimental period was for 30 days. Urine samples (10:00 a.m.–10:00 a.m.; 24-h urine) were collected on the last day. Urine samples were stored at −20 °C until needed.

2.3. Analyses

The urinary contents of Nam, 2-Py, and 4-Py were simultaneously measured by the HPLC method of Shibata et al. [2], and those for MNA by the method of Shibata [3]. Urinary concentration of 3-HA was measured by the HPLC method of Shibata and Onodera [4], while the urinary concentration of KA was measured by the method of Shibata [5]. The urinary concentration of XA was measured by the method of Shibata and Onodera [6], and QA was measured by the method of Mawatari et al. [7].

3. Results and discussion

Trp has widely appeared on the market and consequently, there is a risk of taking excessive amounts of Trp. Hence, it is important to know what is happening when taking excessive Trp. As the first experiment of the series of studies on the adverse effects of Trp,

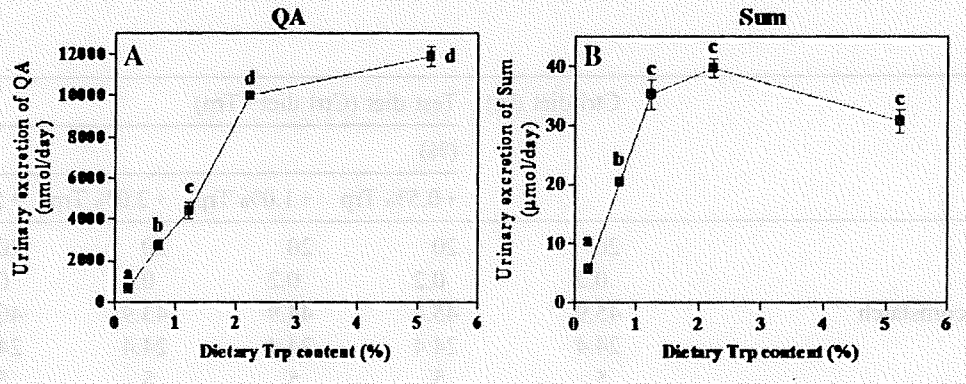

Fig. 2. Effect of Trp intake on the urinary excretory of QA in rat. (A) Sum=Nam+MNA+2-Py+4-Py. (B) A different letter on the circle means a significant difference at $p<0.05$, as determined by Tukey–Kramer multiple comparisons test.

we investigated the Trp-niacin metabolism in rats when fed an excessive Trp diet. The body weight gain and food intake of the rats fed with the 5% Trp-added diet was the lowest of all the groups. The data showed a clear adverse effect in rats fed with the 5% Trp-added diet. The urinary excretion of such Trp catabolites as KA, XA, and 3-HA were increased according to the intake of Trp (Fig. 1).

Thus, it might be that the enzyme activities concerning Trp to 3-HA are enough to be able to metabolize the diet containing up to 5% Trp. However, the excretion of QA was almost the same in the groups between 2 and 5% Trp diets (Fig. 2A). Thus, it might be that the metabolism of 3-HA to QA was saturated in the 5% Trp diet. It means the enzyme of α-amino-β-carboxymuconate-ε-semialdehyde decaraboxylase activity increased. The sum metabolites of Nam+MNA+2-Py+4-Py were almost the same in the 1 and 5% Trp diets, which showed the metabolism of QA to nicotinic acid mononucleotide was saturated in the 2 and 5% Trp diet. It means quinolinate phosphoribosyl transferase QPRT activity saturated in 1% Trp diet. (Fig. 2B) The value of (2-Py+4-Py)/MNA decreased sharply in the 0.5 and 1% Trp diets.

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総　説

生理活性ミネラルとB群ビタミンの生体利用率との関係*

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Vitamins (Japan), **82** (2), 115-125 (2008)

Relationship between Bioactive Minerals and Bioavailability of B-group vitamins

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In Japan, two basic texts are used for nutritional guidance, Standard Tables of Food Composition in Japan and Dietary Reference Intakes for Japanese, 2005. The "Food Composition Tables" describe the nutrient values of foods, and "Dietary Reference Intakes" reports the values that humans can use. Synthetic vitamins have been often used in experiments to determine vitamin requirements, and the bioavailability of synthetic vitamins is considered to be 100%. However, the bioavailability of a vitamin from foods may not be 100% because most vitamins existing in nature are bound to proteins, sugars, or other compounds. Although it is necessary to determine the bioavailability of vitamins in food consumed, no method has been established. We introduce a practical method to determine the bioavailability of vitamins in food. In particular, we are also interested in the effects the dietary intake of minerals and micronutrients exerts on the bioavailability of vitamins. We believe that the results of these studies will contribute to the maintenance of public health in Japanese.

Key words: bioavailability, vitamin, food, mineral, urine

(Received June 20, 2007)

はじめに

本論文は，2006年2月10日に開催されたビタミンB研究委員会主催のシンポジウム「B群ビタミンと生理活性ミネラル」での講演で，著者らが担当した「生理活性ミネラルとB群ビタミンの生体利用率との関係」を文章化したものである．講演した内容は，すでに確立されたものばかりでなく，著者らのみが提唱している内容も含まれている．

食品中に含まれるビタミン含量が明らかにされたのと同時に，摂取した食品由来のビタミンが，どの程度我々の体が消化・吸収し，かつ利用しているのかに関して，多くの栄養学者・医学者が興味をもっていたが，十分な情報が得られないまま，今日に至っている．

「食品成分表」の値は，化学的な方法により，食品中のビタミンをできる限りより多く抽出できる方法を駆使し，かつビタミン型(遊離型のビタミン)にまで分解したのち，測定した値である．いわゆる資源としての数値が記載されている．一方，「食事摂取基準」の数値は，ヒトが利用できる値が記載されている．たとえば，あるヒトが

*本論文はビタミンB研究委員会シンポジウム「B群ビタミンと生理活性ミネラル」(平成18.2.10大阪)の講演内容をまとめたものである．

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1日の食事から2mgのチアミンを摂取しても，このヒトが消化・吸収し，かつ利用できたチアミンの量は1mgにすぎない可能性がある．

文献検索ソフトを利用して，「bioavailability」と「vitamin」をキーワードにして調べると，葉酸とビタミン B_6 に関する論文が多く検索され，次にナイアシンが検索される．これらのほとんどの論文は，著者ら（おそらく多くの人々）が知りたい「習慣的に食べている1日食事由来のビタミンがどの程度我々の体で消化・吸収され，かつ利用されているかを知りたい」に関する情報を定量的に与えてくれない．いずれも，定性的な現象を報告しているにすぎない．動物性食品に含まれるB群ビタミンの利用性は高いが，植物性食品は低い，というものである．たとえば，トウモロコシの「ぬか」に含まれるナイアシン，チアミン，パントテン酸の利用性が「ぬか」の製粉状態により異なるとか[1]，食物繊維の存在はビタミン B_6 の吸収を阻害するとか[2]，ヨーグルトの摂取はチアミン，リボフラビン，ビタミン B_6 栄養状態を低下させる傾向があるとか[3]，牛乳の摂取が食品中の葉酸の消化・吸収率を高めるとか[4]，植物食品中にはビタミンと糖類が結合したもの[5][6]，あるいはタンパク質と結合したもの[7]が報告されており，これらの結合型ビタミンは消化されにくいため，吸収が悪いことが報告されている[8][9]．さらに，まぐろ，パン，ピーナッツバター中のビタミン B_6 の栄養有効性を相対的に求めた結果，まぐろ中のビタミン B_6 が他の食品よりも高かったという報告もある[10]．

1．生体利用率とは

食事から摂取したビタミンをヒトがどの程度消化・吸収し，かつ利用されているかを表す言葉は統一されておらず，成書をみると，生理活性，生物有効性，生物学的有効性，生体利用度，栄養有効性，栄養効率，生体利用率などの名称が使用されている．そこで，「日本人の栄養所要量－食事摂取基準－策定検討会」では，食事から摂取したビタミンをヒトがどの程度消化・吸収し，かつ利用されているかを表す言葉として，「生体利用率」を使用することを決めた[11]．今までにも「生体利用率」という言葉は使用されているが，この「生体利用率」に関する定義は，研究者間でまちまちである．血液中の値を指標とした数値をいう研究者もいる．尿中の値を指標とした数値をいう研究者もいる．実験動物を使用する場合は，臓器中の値あるいは体重の増加量を指標とすることができるが，ヒトを被験者とする場合は，血液と尿に限られる．したがって，ヒトを被験者として，栄養素の利用性を調べる実験において，著者らは，血液中の値を指標とする場合は「消化・吸収率」とし，尿中の値を使用する時は「生体利用率」とすべきであると，提案している．

次に，著者らが提案している生体利用率の概念を詳細に述べたい．例をニコチンアミドとする．ニコチンアミドはそのままの形では機能を果たすことはできないので，細胞内では，多くは補酵素型として存在している（表1）．さらに，その機能を発揮するために，アポ酵素と結合し，ホロ酵素の一部として存在していなければならない．我々が食事として摂取する状態では，もちろん，加工程度，保存状態に依存するが，ニコチンアミドをはじめとしてB群ビタミンは，ホロ酵素の一部として存在している割合が高い．ニコチンアミド補酵素の場合は，組織を強酸性下で処理し，酵素タンパク質を変性・失活させることで，酵素タンパク質から遊離してくる．単なる熱処理だけでは遊離しにくい．ニコチンアミドを例として，ビタミンの体内運命（利用性）を図1～図4の4枚の図で説明する．図1は消化・吸収過程の概略図である．ホロ酵素の成分として摂取されたNADは，胃の酸性条件下で遊離する．遊離したNADは消化酵素成分であるNADピロホスファターゼ，ヌクレオシダーゼにより加水分解され，ニコチンアミドが遊離する．なお，NMN（ニコチンアミドモノヌクレオチド）→NR（ニコチンアミドリボシド）→ニコチンアミドの両反応を触媒する酵素は，小腸膜酵素であると思われる[12]．ニコチンアミドの血液中への転送は，エネルギー非依存的かつ非飽和型である[13]．血液から臓器・組織へのニコチンアミドの移行は単純拡散によって行われるが（図2），細胞はニコチンアミドを速やかに補酵素型に合成することで細胞内捕捉している[14]（図3）．補酵素型となったのちは，アポ酵素と結合し，ホロ酵素となり機能を果たす．そして，一定時間働けば，役目を終えて，タンパク質部分はプロテオソーム系で処理され，一方，NADは，図4に示したようにニコチンアミド，MNA（N^1-メチルニコチンアミド），2-Py（N^1-メチル-2-ピリドン-5-カルボキサミド），4-Py（N^1-メチル-4-ピリドン-3-カルボキサミド）へと代謝され，尿中に排泄される．図1～図4に示した過程をすべて含めた概念として「生体利用」と呼ぶことを提案したい．簡潔に述べれば，「消化・吸収」と「吸収後の体内利用」を合わせた概念である．ビタミンの「生体利用率」とは，ヒトが摂取した食事に含まれるビタミンがどの程度ヒトが利用できたのかという数値である．つまり，「（体内で利用されたビタミン量／摂取ビタミン量）」である．

表1．ラット肝臓中のナイアシン関連化合物含量

	概数（nmol/g）
$NAD^+ + NADH$	700
$NADP^+ + NADPH$	300
遊離ニコチンアミド	400
総ニコチンアミド	1400

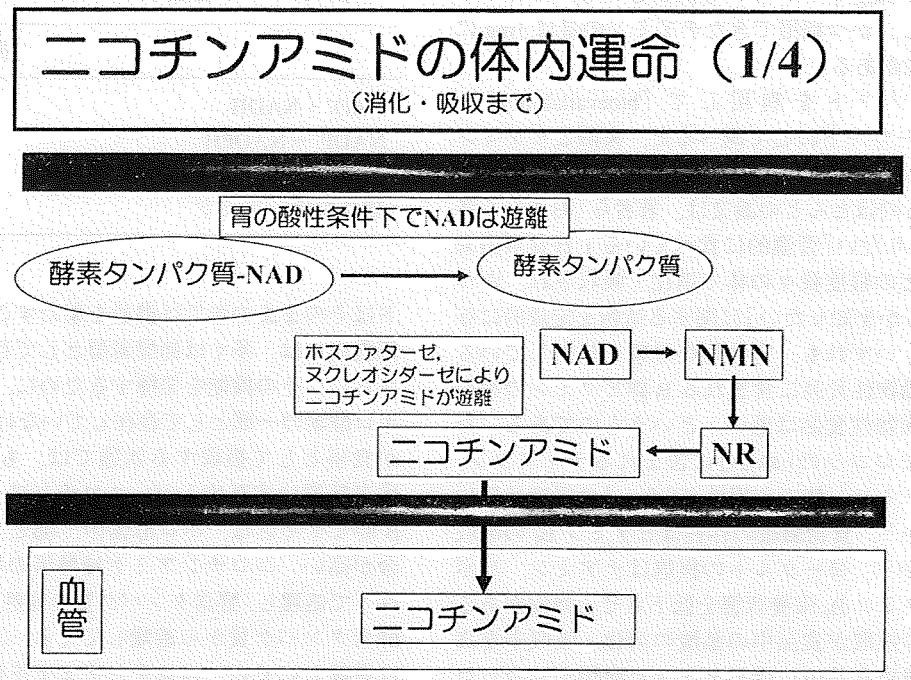

図1. ニコチンアミドの体内運命（1/4）（消化・吸収まで）

NAD = nicotinamide dinucleotide; NMN = nicotinamide mononucleotide; NR = nicotinamide riboside.

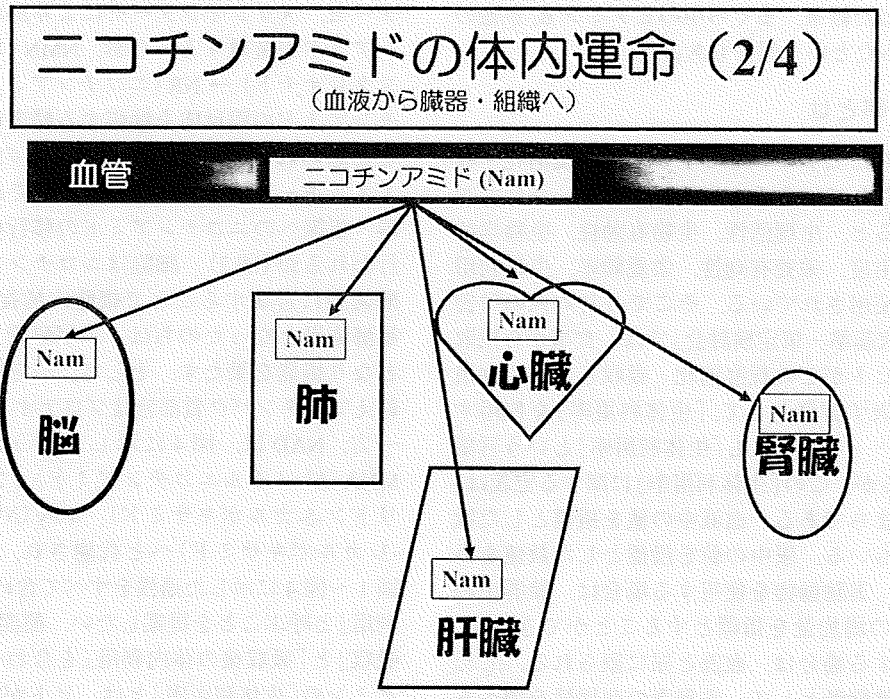

図2. ニコチンアミドの体内運命（2/4）（血液から組織へ）

Nam = nicotinamide;

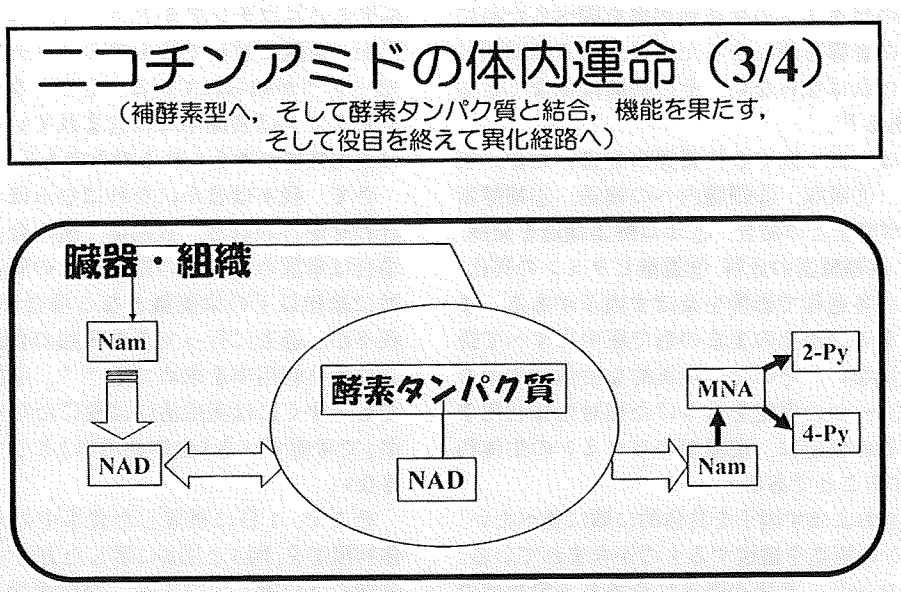

図 3．ニコチンアミドの体内運命（3/4）補酵素型へ，そして酵素と結合，機能を果たす，そして役目を終えて異化経路へ）
MNA = N^1-methylnicotinamide; 2-Py = N^1-methyl-2-pyridone-5-carboxamide; 4-Py = N^1-methyl-4-pyridone-3-carboxamide.

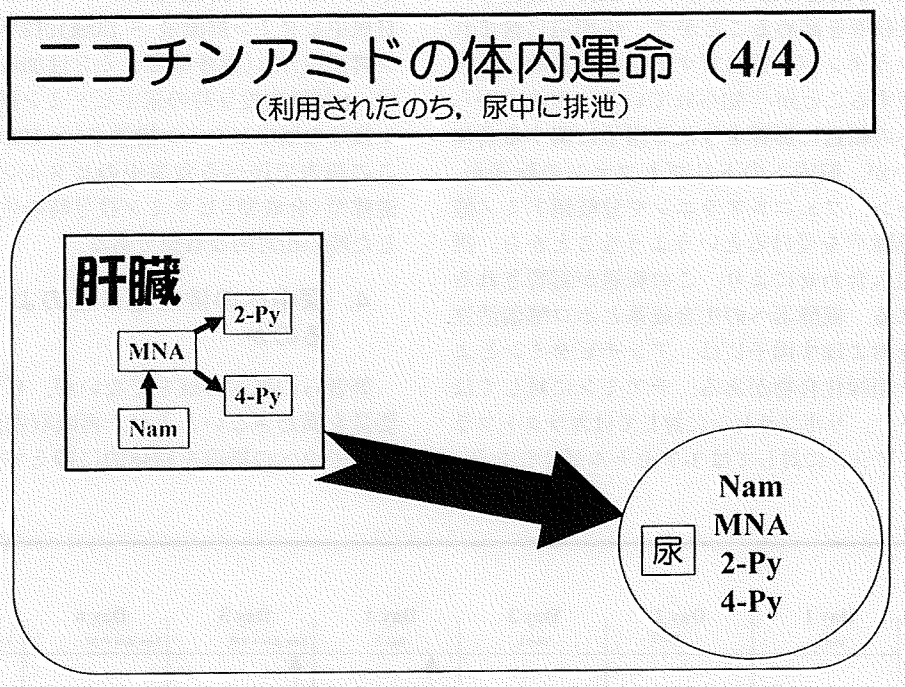

図 4．ニコチンアミドの体内運命（4/4）（利用されたのち，尿中に排泄）

2．摂取したビタミン量をどのようにして測定するのか

この問題は，技術的には簡単である．1 日に摂取する食事を倍量作り，半分を食べ，半分を分析用に供すればよい．

3．体内で利用されたビタミン量をどのようにして測定するのか

知りたい情報は，1 日に食べた食事由来のビタミンのうち，どの程度が図 1 ～図 4 に示した代謝運命をたどったのか，という数値を測定しなければならない．1 日に

食べた食事由来のビタミンの生体利用率を調べるためには，前歴の食事の影響を消し去るために，ある期間同じ食事を摂取しなければならない．その期間は少なくとも4日間は必要である[15]．

図1～図4では，真っ直ぐな代謝運命を書いたが，実際には，①消化，②吸収，③細胞内への輸送，④補酵素への合成，⑤アポ酵素との結合，⑥ホロ酵素機能を発揮，⑦ホロ酵素終末，⑧補酵素の分解，⑨遊離ビタミンの異化，⑩尿中への排泄の各過程で影響を及ぼす因子がある．また，摂取したビタミンがそのままの形で尿中にすべて排泄されるわけではない．そこで，現実的な生体利用率を求めるためには，一つの仮定をもうけた相対生体利用率を提案したい．その仮定は，遊離型のビタミンの生体利用率を100%とすることである．

各過程で影響をおよぼす因子を具体的に順に述べたい．食品にはビタミンの構造を破壊するものが含まれている．たとえば，thiaminase[16]，果物や野菜に含まれる耐熱性のポリフェノールがチアミンを破壊することが[17]，果物に含まれる有機酸が亜鉛依存性のコンジュガーゼ[18](ポリグルタミン酸型葉酸を吸収可能なモノグルタミン酸型葉酸に変換する酵素)を阻害することが[19]，牛乳が食品中の葉酸の消化・吸収率を高めることが[20]，生卵白に含まれるアビジンというタンパク質はビオチンと特異的に結合して吸収を阻害することが[21]知られている．血液中から臓器・組織中への転送に影響を与える因子に関する具体的な情報はないが，脳内へのトリプトファンの転送が，血液中のチロシン，フェニルアラニンや分岐鎖アミノ酸の量によって影響[22]を受けるというようなことから，摂取した食品の組み合わせにより，この転送が影響されるものと考えられる．補酵素への生合成系とホロ酵素活性の両方に影響をおよぼす因子には，アンチビタミンとよばれるビタミン類縁化合物がある．チアミンに対してはピリチアミンが[23]，リボフラビンに対してはガラクトフラビンが[24]，ナイアシンに対しては3-アセチルピリジン[25]，6-アミノニコチンアミド[26]，イソニコチン酸ヒドラジド[27]が，葉酸に対しては4-アミノプテリン[28]，メトトロキセート[29]が知られている．しかしながら，これらのアンチビタミンは食品中には含まれていないため，現実的には薬剤被害が考えられるのみである．

さて，我々は豊かになればなるほど種々の食品の組み合わせが行われる．食品は，組み合わせによって，ある場合は単独の栄養価の総和以上の効果がでる場合と，反対に総和以下の栄養価となる場合がある．したがって，我々が，過去に行ってきた単独の食品中の一つのビタミンの生体利用率を求めても[30][31]，学問的には価値があっても，すぐには実生活には役にたたないし，「食事摂取基準」で考慮すべき「生体利用率」として使用することもできない．

そこで，1日に摂取した食事中の水溶性ビタミンの生体利用率を，図5と図6に示した方法で求める実験方法を，著者らは提案している[32]．試験食投与時の尿中ビタミン排泄率＝(試験食投与時の1日尿中ビタミン排泄量)/(試験食中のビタミン含量)．遊離型ビタミン投与の尿中ビタミン排泄率＝{遊離型ビタミン付加によって増大した1日尿中ビタミン量(遊離型ビタミン付加試験食投与時の1日尿中ビタミン排泄量－試験食投与時の1日尿中ビタミン排泄量)}/(遊離型ビタミン付加量)．生体利用率(%)は，(試験食投与時の尿中ビタミン排泄率/遊離型ビタミン投与の尿中ビタミン排泄率)×100から求めた．つまり，この報告で述べる食事中のビタミンの生体利用率とは，遊離型(合成型)ビタミン投与時の生体利用率を100%とした時の相対的な数値である．

4. 尿中への排泄量はどのように調節されているのか

著者らの推測の域をでないが，ビタミンの摂取量が必要量を満たさないときは，再吸収系が能動的にはたらいて，尿中への排泄を積極的に抑えている．一方，体内飽

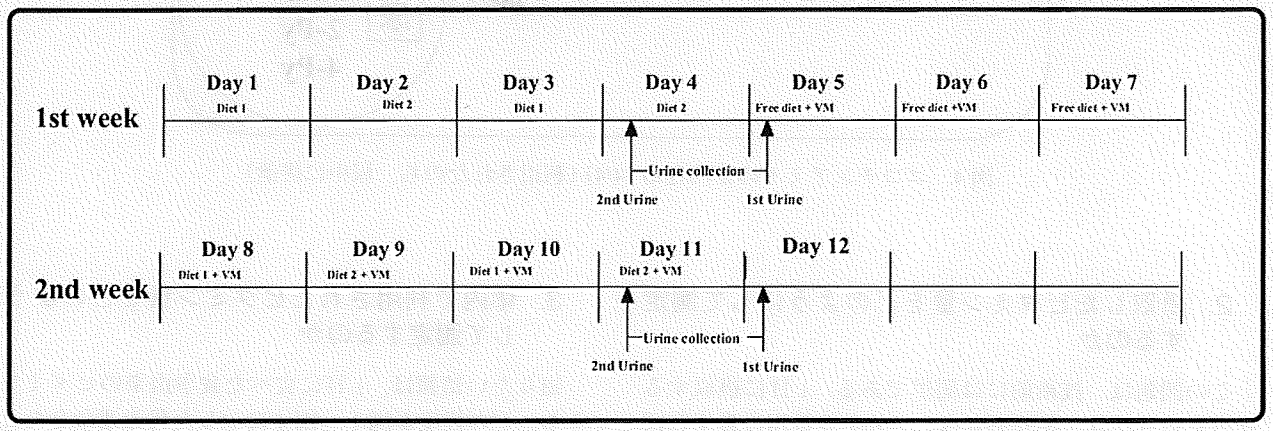

図5．著者らが提案する1日食事由来のB群ビタミンの生体利用率の実験計画の概略

和量を超えるような過剰な摂取量の場合は，排泄系が能動的にはたらいて，積極的に尿中に排泄を行っている．そして，必要量から体内飽和量の間の摂取量の時は，再吸収系も排泄系も積極的にはたらいておらず，ただ単純拡散によって尿中にもれでているだけであると考えている．模式的に書くと図7のようになる．著者らが提案する生体利用率を求める時は，摂取量と排泄量との関係が正比例の関係にある範囲内で行わなければならない．

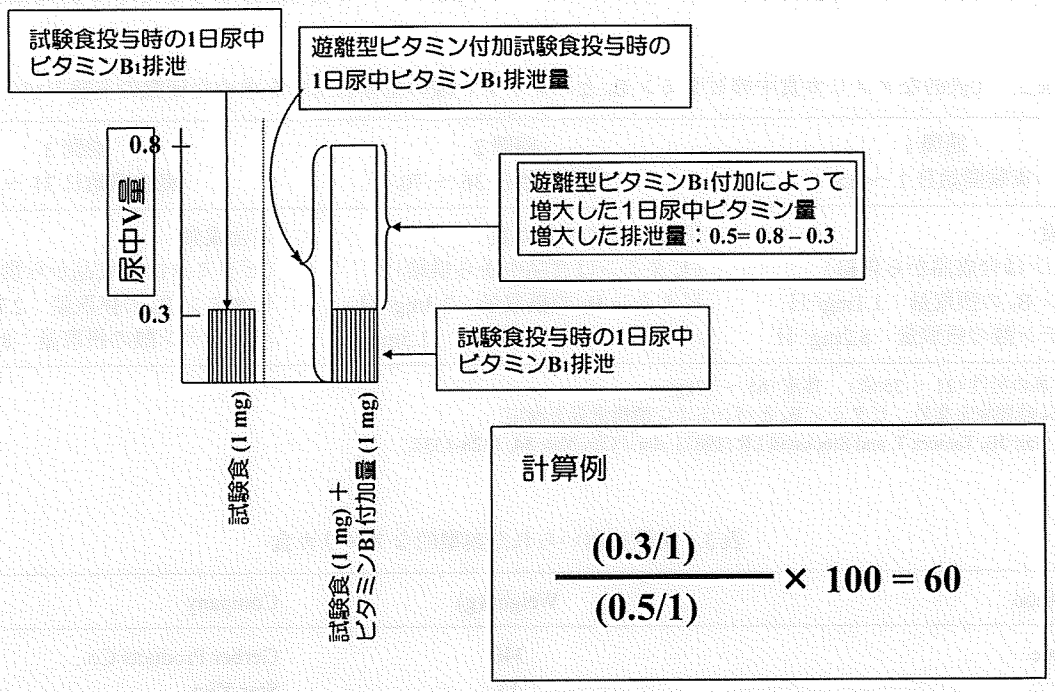

図6．著者らが提案する1日食事由来のB群ビタミンの生体利用率の求め方

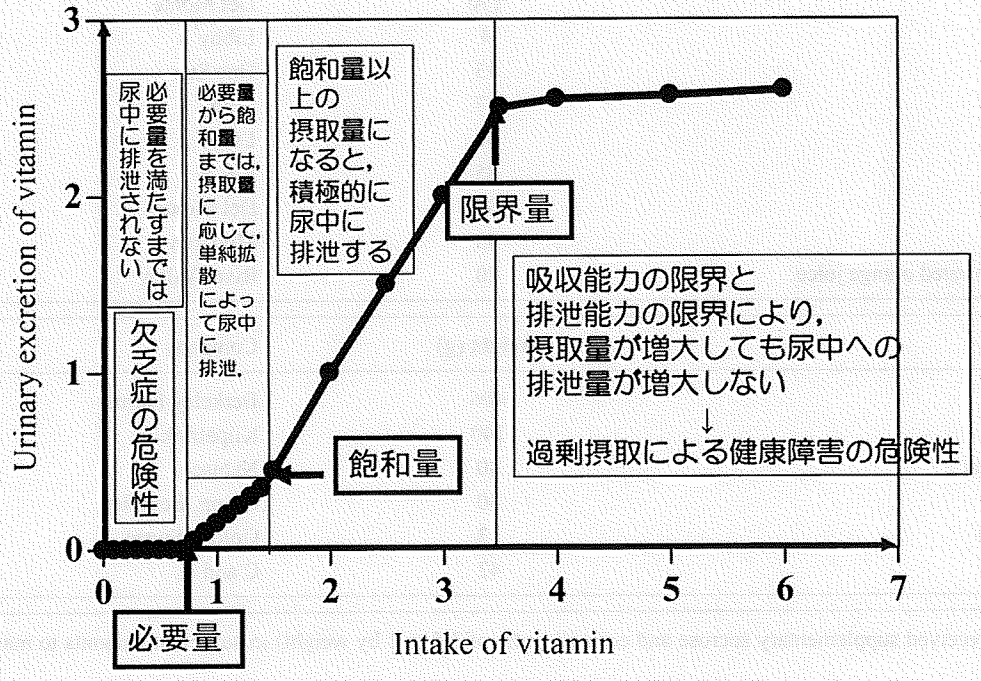

図7．ビタミンの尿中への排泄量への調節（推測）
縦軸と横軸の数値はともに，相対的な数値である．

5. 1日食事由来のビタミンの生体利用率はどの程度か

ここで提案したような方法を駆使している論文を検索した結果，類似した方法を駆使した論文が一報のみ検索された．詳しく紹介する．代表的なアメリカ食中のビタミンB_6とパントテン酸の生体利用率[33]という論文である．

彼らの実験方法の概略を表2に示した．期間1と期間3は合成ビタミンを投与した期間で，この時に尿中に排泄されたビタミンB_6量とパントテン酸量を100%の生体利用率と考えたものである．そして，期間2では，代表的なアメリカ食(表3)を与えた時に24時間尿中に排泄さ

表2．代表的なアメリカ食中のビタミンB_6とパントテン酸の生体利用率を求めた研究の実験方法の概略

期間1 実験開始日1～35	期間2 実験開始日36～70	期間3 実験開始日71～91
半合成食 (ビタミンは合成品から供給) ビタミンB_6の摂取量：1.1mg/日 パントテン酸の摂取量：8.2mg/日	代表的なアメリカ食 (ビタミンは食品中から供給) ビタミンB_6の摂取量：2.3mg/日 パントテン酸の摂取量：11.5mg/日	半合成食 (ビタミンは合成品から供給) ビタミンB_6の摂取量：2.7mg/日 パントテン酸の摂取量：8.2mg/日

被験者：6名の男性(21～35歳)，体重(65～72kg)．
定期的に24時間尿を集め，ビタミンB_6とパントテン酸排泄量を測定．
引用文献：Tarr JB, Tamura T, and Stockstad LR (1981) *Am J Clin Nutr* **34**, 1328-1337.

表3．実験に用いられた典型的なアメリカ食

Canned food	Weight (g)	Company
Beef puree	340	Gerber Products Co.,
Tuna	13	Star-Kist
Chicken	42	Swanson, Campbell Soup Co.,
Spinach	42	Co-op, Berkeley
Potatoes	170	Del Monte
Lima beans	4	Libby's
Peas	13	One Star
String beans	17	Libby's
Tomato juice	68	Libby's
Peaches	85	F & P
Pineapple	85	Del Monte
Carrots	43	Monarch
Frozen concentrated orange juice	20	River Valley

Other foods	Weight (g)	Company
Whole milk	406	Berkeley Farms
White bread	140	Kilpatrick's
Shredded wheat	30	Nabisco
Margarine	30	Co-op, Berkeley
Cottonseed oil	13	Gold-n-Sweet
Sucrose	42	C and H

Each subject received supplementary sucrose and corn oil (ratio of 3.5 to 1 by weight) in sufficient amounts to maintain constant body weight.

引用文献：Tarr JB, Tamura T, and Stockstad LR (1981) Availability of vitamin B_6 and pantothenate in an average American diet in man. *Am J Clin Nutr* **34**, 1328-1337.

れるビタミン B_6 量とパントテン酸量を測定し，この期間に排泄された値をもとに，期間 1 と期間 3 で得られた値を基準として比較することにより，体内で利用された値を求める，つまり，摂取実測値と尿中の値から，計算によって得られた利用されたビタミン値を得る．この計算によって得られた利用値と摂取実測値の比率から，生体利用率を求めている．代表的なアメリカ食に含まれるビタミン B_6 とパントテン酸の生体利用率を表 4 と表 5 に示した．ビタミン B_6 は 80％程度，パントテン酸は 50％程度という値を彼らは報告している．他のビタミンに関する報告はみあたらない．

我々も，女子学生が普段摂取している食事中のビタミンの生体利用率を測定しているが，まだ，原著論文として報告していないので，文章として報告できないが，平成 17 年度のシンポジウム「B 群ビタミンと生理活性ミネラル」で，紹介したとおりである．

6. ビタミンの生体利用率に影響をおよぼす栄養失調（ミネラル欠乏）

6-1. 生理活性ミネラルと B 群ビタミンの生体利用率との関係

生理活性ミネラルとこのシンポジウムでは発表したが，栄養学領域では「ミネラル」だけで充分である．生体に必要なという意味合いを強めるために「生理活性」という単語をつけた．栄養学でいう「ミネラル」とは，「栄養素として生理作用に必要な無機物の称」である．日本語では，「無機質」という．無機物は，有機物に対する言葉で，無機物の中で，生体の成長・維持に不可欠な無機物を「無機質」という．

ところで，ミネラルは，B 群ビタミンの生体利用率に関して次の項目に関わっている．①結合型ビタミンの消化・吸収に関わるミネラル含有酵素，②遊離型ビタミン

表 4．代表的なアメリカ食中に含まれるビタミン B_6 の生体利用率（尿中へのビタミン B_6 排泄量を指標にした場合）

被験者番号	尿中ビタミン B_6（μg/d）			利用された見かけ上のビタミン B_6 量（mg/日）	生体利用率（%）
	期間 1（摂取量；1.1mg/日）	期間 2（摂取量；2.3mg/日）	期間 3（摂取量；2.7mg/日）		
1	39	79	134	1.77	77
2	40	87	152	1.72	75
3	53	81	109	1.82	79
4	45	73	106	1.80	78
5	51	101	124	2.11	92
6	61	105	155	1.69	73
平均値	48	87	130	1.82	79

引用文献：Tarr JB, Tamura T, and Stockstad LR (1981) Availability of vitamin B_6 and pantothenate in an average American diet in man. *Am J Clin Nutr* **34**, 1328-1337.

表 5．代表的なアメリカ食中に含まれるパントテン酸の生体利用率（尿中へのパントテン酸排泄量を指標にした場合）

被験者番号	尿中パントテン酸（mg/日）			利用された見かけ上のパントテン酸量（mg/日）	生体利用率（%）
	期間 1（摂取量；8.2mg/日）	期間 2（摂取量；11.5mg/日）	期間 3（摂取量；8.2mg/日）		
1	5.4	2.6	3.5	5.14	44
2	3.6	2.4	3.1	6.22	54
3	5.6	3.5	4.3	6.15	54
4	3.6	1.7	2.5	4.65	40
5	4.4	3.4	3.9	6.97	61
6	2.7	1.7	2.4	5.66	49
平均値	4.2	2.6	3.3	5.79	50

引用文献：Tarr JB, Tamura T, and Stockstad LR (1981) Availability of vitamin B_6 and pantothenate in an average American diet in man. *Am J Clin Nutr* **34**, *1328-1337.*

から補酵素型の合成に関わるミネラル含有酵素，③ビタミンの代謝に関わるミネラル含有酵素，④ビタミンを補酵素とする過程で関わるミネラル含有酵素，である．我々が，被験者とするヒトには栄養失調はいないので，これから述べることは，疾病時においてのみみられる現象である．

結合型ビタミンの消化・吸収に関わるミネラル含有酵素として，アミノペプチダーゼ(Zn，Co)，カルボキシペプチダーゼ(Zn)，α-アミラーゼ(Ca)，γ-グルタミルヒドロラーゼ(通称：コンジュガーゼ)(Zn)などが知られている．亜鉛欠乏時患者においては，食品に含まれているポリグルタミン酸型の葉酸の消化が低下することが予想される．しかし，モノグルタミン酸型は消化される必要がないので，亜鉛欠乏患者ではモノグルタミン酸型の投与が有効である．なお，亜鉛欠乏患者で，葉酸の吸収後の体内利用率がどのようになっているかに関する情報はみあたらない．

遊離型ビタミンから補酵素型の合成に関わる酵素でミネラルを必要とする酵素に関する実験がある[33]．ニコチンアミドから$NADP^+$への生合成系においてMgを必要とする酵素がある．ニコチンアミドホスホリボシルトランスフェラーゼ，NMNアデニリルトランスフェラーゼ，NAD^+シンターゼ，NAD^+キナーゼである．表6に示したように，ミネラル欠乏ラットでは，トリプトファンからのナイアシンへの合成量が低下した．

ニコチンアミドからMNAをへて2-Pyと4-Pyへの異化代謝系において，2-Py生成MNAオキシダーゼ(Mo，Fe)と4-Py生成MNAオキシダーゼ(Mo，Fe)がミネラル酵素である．ミネラル欠乏では，表7に示したように，これら両酵素活性が低下し，代謝産物である2-Pyと4-Pyの生成量が低下し，その結果，(2-Py + 4-Py)/MNAが低下した．

ビタミンを補酵素とするミネラル含有酵素として，アルコールデヒドロゲナーゼ(ナイアシン，Zn)，プロピオニルCoAカルボキシラーゼ(パントテン酸，K)，グルタチオンペルオキシダーゼ(リボフラビン，Se)，ピルビン酸カルボキシラーゼ(ビオチン，Mn)，キサンチン酸化酵素(リボフラビン，Mo)などがある．ミネラル結合が体内のNAD含量におよぼす影響を調べたが，表8に示したように，差異は認められなかった．

表6．ミネラル欠乏がトリプトファン－ナイアシン代謝におよぼす影響(ラット)

	対照群	ミネラル欠乏群
尿中へのニコチンアミド及び異化代謝産物量の総和（nmol/g food）	638 ± 58	304 ± 27*

ニコチンアミド及び異化代謝産物量の総和＝ Nam + MNA + 2-Py + 4-Py.
* 対照群との比較において，$p < 0.05$ で有意差が認められた(Student t 検定)．
引用文献：柴田克己，才野木当糸(1993)ミネラル欠乏がトリプトファン－ニコチンアミド代謝に及ぼす影響．*ビタミン* **67**, 429-434

表7．ミネラル欠乏がニコチンアミドの異化代謝におよぼす影響(ラット)

	対照群	ミネラル欠乏群
2-Py生成MNAオキシダーゼ（nmol/h/g肝臓）	715 ± 54	307 ± 23*
4-Py生成MNAオキシダーゼ（nmol/h/g肝臓）	1683 ± 199	628 ± 71*
尿中の（2-Py+4-Py)/MNA	9.6 ± 0.7	4.3 ± 0.5*

* 各々，対照群との比較において，$p < 0.05$ で有意差が認められた(Student t 検定)．
引用文献：柴田克己，才野木当糸(1993)ミネラル欠乏がトリプトファン－ニコチンアミド代謝に及ぼす影響．*ビタミン* **67**, 429-434

表8．ミネラル欠乏が体内のナイアシン含量におよぼす影響(ラット)

	対照群	ミネラル欠乏群
肝臓中の総ナイアシン（nmol/g）	2070 ± 60	2210 ± 80
全血中NAD（nmol/mL）	81.2 ± 2.1	78.5 ± 0.9

引用文献：柴田克己，才野木当糸(1993)ミネラル欠乏がトリプトファン－ニコチンアミド代謝に及ぼす影響．*ビタミン* **67**, 429-434