て血中濃度が増加することは生理的に不都合である。しかし、3-ヒドロキシキヌレニンが、運動前後でほとんど変化しなかったことから、キヌレニン3-モノオキシゲナーゼ活性は運動によって影響を受けないことが示唆された。さらに、αアミノβカルボキシムコン酸εセミアルデヒドを代謝するACMSDase（EC 4.1.1.45）活性の亢進はトリプトファンを糖新生へ誘導するが、この酵素は一過性の運動の影響を受けないことが報告されている[14]。これらのことから、トリプトファンからエネルギー産生系への代謝は、一過性の運動の影響を受けない可能性が高いことが示された。しかしながら、運動前後の血中のトリプトファン、キヌレニンおよびNADの変化は運動習慣のある人と運動習慣の無い人で異なっていた[6]。したがって、継続する運動習慣はトリプトファンの代謝やニコチンアミドの代謝に影響を与える可能性があると考えられる。また、血中キヌレニン濃度と気分との間に相関がある理由として次の２つの原因が推測できる。まず、運動習慣によってトリプトファンからグルコースやNADが産生されやすくなり、骨格筋や肝臓にエネルギー源が多く保存され、運動による末梢疲労が減少する。もうひとつは、運動によりキヌレニナーゼ（EC 3.7.1.3）が活性化されてキヌレニンの分解が亢進し、キヌレニンから代謝されたアントラニル酸や神経伝達物質であるキヌレン酸が増加する。これらの代謝産物が知覚神経系に何らかの影響を及ぼしたことが考えられる。どちらもNewsholmeらの仮説とは異なり、今後の検討を要する問題である。

## 文献


1）EA. Newsholme, IN. Acworth and E. Blomstrand: Amino acids, brain neurotransmitters and a functional link between muscle and brain that is important in sustained exercise. Advances in myochemistry, 127-133, John Libbey Eurotext, London, (1987)

2）Davis JM, Alderson NL and Welsh RS: Serotonin and central nervous system fatigue: nutritional considerations. Am. J. Clin. Nutr., 72: 573s-578s, 2000

3）Davis JM and Bailey SP: Possible mechanisms of central nervous system fatigue during exercise. Med. Sci. Sports Exerc., 29: 45-57, 1996

4）Costill DL, Bowers R, Branam G and Sarks K: Muscle glycogen utilization during prolonged exercise on succesive days. J. Appl. Physiol., 31: 834-838. 1971

5）Ito Y, Saito K, Maruta K, Nakagami Y, Koike T, Oguri Y and Nagamura Y: Kynurenine concentration of serum was increased by exercise. Adv. exp. med. biol., 467: 717-722, 1999

6）米倉麗子、伊藤康宏、柴田克己、福渡　努、齋藤邦明、小栗誼人、朝山正己、長村洋一: 走行運動負荷がTrp-NAD経路に及ぼす影響. 健康創造研究, 1: 57-64, 2002

7）伊藤康宏、小栗誼人、中上　寧、丸田一皓、古池隆明、米倉麗子、山田幸恵、橋本朋久、横田千穂、内藤純子、長村洋一：適度な運動の指標としてのキヌレニンの血中動態. 藤田学園医学会誌, 24: 121-124, 2000

8）Fukuwatari T, Shibata K, Ishihara K, Fushiki T and Sugimoto E: Elevation of blood NAD level after moderate exercise in young women and mice. J. Nutr. Sci. Vitaminol., 47: 177-179, 2001

9）Shibata K and Murata K: Blood NAD as an index of niacin nutrition. Nutr. Int., 2: 177-181, 1986

10) Saito K, Crowley JS, Markey SP and Heyes MP: A mechanism for increased quinolinic acid formation following acute systemic immune stimulation. J. Biol. Chem., 21: 15496-15503, 1993

11) Naito J, Ishiguro I, Murazumi T and Morimoto M: Determination of kynurenine in serum by high-performance liquid chromatography after enzymatic conversion to 3-hydroxykynurenine. Anal. Biochem., 161: 16-19, 1987

12) McNair DM, Lorr M and Droppleman LF: Profile of Mood States. Educational and Industrial Testing Service, San Diego,USA (1992)

13) Ito Y, Yonekura R, Maruta K, Koike T, Nakagami Y, Shibata K, Saito K and Nagamura Y: Tryptophan metabolism was accelerated by exercise in rat. Developments in Tryptophan and Serotonin Metabolism, Kluwer Academic/Plenum Publishers, New York, USA (in press)

14) Shibata K and Toda S: Effects of thyroxine on the conversion ratio of tryptophan to nicotinamide in rats. Biosci. Biotech. Biochem., 58: 1787-1762, 1994

Journal of Creative Approach for Health Vol. 3, No 1 (2004)

〈Proceeding of JSTRY Meeting〉

# Tryptophan metabolism was estimated by tryptophan metabolites in blood during exercise

Yasuhiro Ito[1)], Reiko Yonekura[1)], Kuniaki Saito[2)], Katsumi Shibata[3)], Junko Naito[1)] and Yoichi Nagamura[1)]

1) Department of Clinical Chemistry, School of Health Sciences, Fujita Health University, Toyoake, Aichi 470-1192, Japan
2) Department of Laboratory Medicine, Gifu University School of Medicine, Gifu 500-8705, Japan
3) Department of Life Style Studies, School of Human Cultures, The University of Shiga Prefecture, Hikone, Shiga 522-8533, Japan

**Summary** The kynurenine pathway is the major metabolic pathway for tryptophan, and metabolites such as kynurenate, anthranilate and NAD possess important physiological activities. We previously reported that exercise alters serum levels of kynurenine, an intermediate metabolite in the kynurenine pathway, in an intensity-dependent manner. However, serum levels of kynurenine increase or decrease depending on exercise habits and subjective exercise intensity. This is because exercise affects the enzymatic activity of the kynurenine pathway, which accounts for the majority of the tryptophan metabolism. In other words, peripheral tryptophan metabolism may be involved in exercise performance or post-exercise euphoria and fatigue. We have been conducting research to determine whether these physiological phenomena are only involved with energy metabolism or the central nervous system via the sensory nervous system.

**Key words:** Exercise, Tryptophan metabolism, Kynurenine pathway

*Kidney International, Vol. 65 (2004), pp. 1099–1104*

# Nicotinamide suppresses hyperphosphatemia in hemodialysis patients

YUTAKA TAKAHASHI, ARAKI TANAKA, TSUKASA NAKAMURA, TSUTOMU FUKUWATARI, KATSUMI SHIBATA, NORIAKI SHIMADA, ISAO EBIHARA, and HIKARU KOIDE

*Division of Nephrology, Department of Medicine, Koto Hospital, Tokyo, Japan; Mizue Dialysis Center, Tokyo, Japan; Department of Medicine, Shinmatsudo Central General Hospital, Chiba, Japan; and Department of Life Style Studies, University of Shiga Prefecture, Shiga, Japan*

**Nicotinamide suppresses hyperphosphatemia in hemodialysis patients.**

*Background.* The use of calcium- or aluminum-based phosphate binders against hyperphosphatemia is limited by the adverse effects of hypercalcemia or aluminum toxicity in long-term hemodialysis. Because nicotinamide is an inhibitor of sodium-dependent phosphate cotransport in rat renal tubule and small intestine, we examined whether nicotinamide reduces serum levels of phosphorus and intact parathyroid hormone (iPTH) in patients undergoing hemodialysis.

*Methods.* Sixty-five hemodialysis patients with a serum phosphorus level of more than 6.0 mg/dL after a 2-week washout of calcium carbonate were enrolled in this study. Nicotinamide was administered for 12 weeks. The starting dose was 500 mg/day, and the dose was increased by 250 mg/day every 2 weeks until serum phosphorus levels were well controlled at less than 6.0 mg/dL. A 2-week posttreatment washout period followed the cessation of nicotinamide. Blood samples were collected every week for measurement of serum calcium, phosphorus, lipids, iPTH, and blood nicotinamide adenine dinucleotide (NAD).

*Results.* The mean dose of nicotinamide was 1080 mg/day. The mean blood NAD concentration increased from 9.3 ± 1.9 nmol/$10^5$ erythrocytes before treatment to 13.2 ± 5.3 nmol/$10^5$ erythrocytes after treatment ($P < 0.01$). The serum phosphorus concentration increased from 5.4 ± 1.5 mg/dL to 6.9 ± 1.5 mg/dL with the pretreatment washout, then decreased to 5.4 ± 1.3 mg/dL after the 12-week nicotinamide treatment ($P < 0.0001$), and rose again to 6.7 ± 1.6 mg/dL after the posttreatment washout. Serum calcium levels decreased during the pretreatment washout from 9.1 ± 0.8 mg/dL to 8.7 ± 0.7 mg/dL with the cessation of calcium carbonate. No significant changes in serum calcium levels were observed during nicotinamide treatment. Median serum iPTH levels increased with pretreatment washout from 130.0 (32.8 to 394.0) pg/mL to 200.0 (92.5 to 535.0) pg/mL and then decreased from the maximum 230.0 (90.8 to 582.0) pg/mL to 150.0 (57.6 to 518.0) pg/mL after the 12-week nicotinamide treatment ($P < 0.05$). With nicotinamide, serum high-density lipoprotein (HDL) cholesterol concentrations increased from 47.4 ± 14.9 mg/dL to 67.2 ± 22.3 mg/dL ($P < 0.0001$) and serum low-density lipoprotein (LDL) cholesterol concentrations decreased from 78.9 ± 18.8 mg/dL to 70.1 ± 25.3 mg/dL ($P < 0.01$); serum triglyceride levels did not change significantly.

*Conclusion.* Nicotinamide may provide an alternative for controlling hyperphosphatemia and hyperparathyroidism without inducing hypercalcemia in hemodialysis patients.

**Key words:** nicotinamide, hyperphosphatemia, hemodialysis patients, nicotinamide adenine dinucleotide, parathyroid hormone, serum lipids.

Received for publication April 8, 2003
and in revised form September 8, 2003
Accepted for publication October 21, 2003



End-stage renal disease (ESRD) is associated with calcium and phosphate metabolism abnormalities that can result in severe bone disease and ectopic calcification of cardiovascular tissues [1, 2]. Phosphorus-restricted diets are essential for the prevention of these deleterious complications in ESRD patients. Weekly dietary absorption of phosphate is approximately 4200 mg, assuming fractional absorption of phosphate is 60% [3], whereas phosphate efflux is approximately 1057 mg per 4-hour dialysis session or 3171 mg per week [4], suggesting a positive phosphorus balance in dialysis patients. The common phosphate binders contain aluminum or calcium. Aluminum accumulates in the tissues and causes neurologic, skeletal, and hematologic toxicities [5, 6]. Ingestion of calcium carbonate, an effective phosphate binder, leads to hypercalcemia and increases the risk of vascular calcification in ESRD patients [7, 8].

Nicotinamide is a circulating form of nicotinic acid. The biologic function of nicotinamide derives from its active form, nicotinamide adenine dinucleotide (NAD). Administration of nicotinamide is reported to increase the concentration of renal cortical tissue NAD, which was shown to inhibit phosphate uptake by brush border membrane vesicles obtained from rat proximal tubules [9, 10]. A similar effect of nicotinamide has been reported on phosphate uptake by brush border membrane vesicles isolated from the rat small intestine [11], suggesting that nicotinamide is probably an effective inhibitor of phosphorus absorption in the intestine.

**Table 1.** Baseline characteristics of study patients ($N = 65$)

| | |
|---|---|
| Gender *males/females* | 38/27 |
| Age *years* | 57.0 ± 11.5 |
| Vitamin D *users/nonusers* | 17/48 |
| Time on dialysis *years* | 6.5 ± 5.2 |
| Etiology of end-stage renal disease | |
| Chronic glomerulonephritis | 31 |
| Diabetes mellitus | 24 |
| Hypertension | 5 |
| Polycystic kidney disease | 3 |
| Other | 2 |
| Laboratory values | |
| Serum phosphorus *mg/dL* | 5.4 ± 1.5 |
| Serum calcium *mg/dL* | 9.1 ± 0.8 |
| Serum calcium-phosphorus product $mg^2/dL^2$ | 48.4 ± 13.6 |
| Serum intact parathyroid hormone *pg/mL* | 187.3 ± 204.4 |

Numbers are number of patients unless otherwise indicated.

The aim of the present study was to examine whether nicotinamide lowers serum levels of phosphorus and intact parathyroid hormone (iPTH) in long-term hemodialysis patients.

## METHODS

Seventy-two hemodialysis patients were originally enrolled in the present study. All patients had been dialyzed three times weekly with a bicarbonate dialysate for 6.5 ± 5.2 years. The dialysate calcium concentration was 3.0 mEq/L. Patients with a history of serious gastrointestinal disease, malignancy, total parathyroidectomy, vasculitis, dementia, or poorly controlled diabetes mellitus were excluded. Inclusion criteria were stable dosage of calcium carbonate (2.9 ± 1.7 g/day) for at least 1 month and avoidance of intentional changes in diet throughout the study. Seventeen patients were being given vitamin D (0.4 ± 0.2 μg/day) at the start of the study. No changes in the dosage of vitamin D were made during the study.

We obtained written informed consent from each participant, and the study protocol was approved by the Human Research Ethics Committee of the Koto Hospital. Administration of calcium carbonate was discontinued during a 2-week pretreatment washout period (weeks −2 to 0). Sixty-five hemodialysis patients (38 men and 27 women; mean age, 57.0 years) with serum phosphorus levels of more than 6.0 mg/dL during the pretreatment washout period were eligible for nicotinamide treatment. Characteristics of these patients are shown in Table 1. The 65 patients received nicotinamide (Nippon Roche K.K., Tokyo, Japan) for 12 weeks (weeks 1 to 12). The starting dose of nicotinamide was 500 mg/day and was increased by 250 mg/day every 2 weeks if necessary to control the serum phosphorus concentration at less than 6.0 mg/dL. The nicotinamide was given twice daily in powder form immediately after meals. After 12 weeks of treatment, the patients were taken off nicotinamide (weeks 13 to 14, posttreatment washout period).

Blood samples were collected weekly prior to a hemodialysis session, and serum concentrations of phosphorus, calcium, and lipids were determined by standard clinical laboratory methods. The serum iPTH concentration was determined by immunochemilumetric assay (Intact PTH Kit) (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) (upper limit of normal, 65 pg/mL). The blood NAD concentration was determined according to the Shibata and Murata–modified [12] Nisselbaum and Green method [13]. Blood NAD concentrations were expressed as NAD per $10^5$ erythrocytes (nmol/$10^5$ erythrocytes) because NAD was not detected in serum. Dietary intake was estimated every 2 weeks as the protein catabolic rate (PCR) [14]. We judged the avoidance of intentional changes in diet by no change in PCR. Compliance was confirmed by face-to-face interview.

Data are expressed as mean ± standard deviation (SD) or as median values when the data were highly skewed. The Wilcoxon signed-rank test was used to analyze differences in paired group data. The effects of nicotinamide administration on the serum phosphorus concentrations and other laboratory values were assessed by comparing the serum levels at the end of the pretreatment washout period to those at the end of the treatment period. Spearman's rank correlation coefficient was calculated to assess association between the changes in serum phosphorus and other laboratory values. Statistical analyses were based on two-tailed Student $t$ test. iPTH levels were shown as median ($10^{th}$ to 90th percentile) and expressed as box and whisker plots. The analysis of PTH data was based on Welch $t$ test. A $P$ value of less than 0.05 was considered statistically significant.

## RESULTS

The mean dose of nicotinamide at the end of the 12-week treatment period was 1080 ± 370 mg/day. The minimum dose of nicotinamide was 500 mg/day (7.9 mg/kg body weight/day) and the maximum was 1750 mg/day (33.3 mg/kg body weight/day). Compliance was confirmed in all cases. No significant changes were observed in PCR with nicotinamide treatment (before treatment, 1.1 ± 0.2 g/kg/day vs. after treatment, 1.1 ± 0.2 g/kg/day).

The blood NAD concentration increased from 9.3 ± 1.9 nmol/$10^5$ erythrocytes to 13.2 ± 5.3 nmol/$10^5$ erythrocytes with nicotinamide treatment ($P < 0.01$) (Fig. 1). The doses of nicotinamide were significantly correlated with blood NAD concentrations ($r = 0.805$, $P < 0.0001$). After the posttreatment washout period, the blood NAD level decreased significantly to 8.4 ± 2.7 nmol/$10^5$ erythrocytes ($P < 0.005$). There was no significant difference in the NAD concentration between the pretreatment washout period and the posttreatment washout period.

Serum phosphorus levels changed significantly with nicotinamide treatment (Fig. 2). Serum phosphorus levels

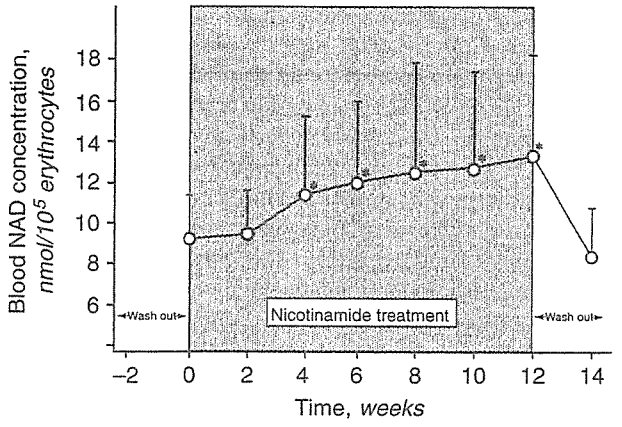


**Fig. 1. Effect of nicotinamide on blood nicotinamide adenine dinucleotide (NAD) concentration in hemodialysis patients.** −2 weeks indicates the start of pretreatment washout. *vs. 0 week, $P < 0.01$.

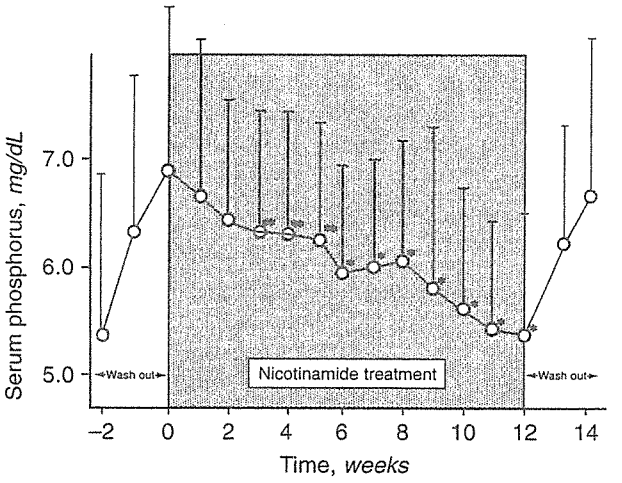


**Fig. 2. Changes in serum phosphorus levels with nicotinamide treatment in hemodialysis patients.** *vs. 0 week, $P < 0.001$; **vs. 0 week, $P < 0.01$.

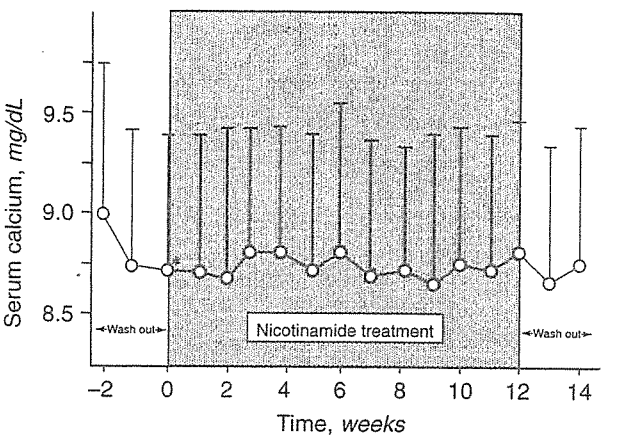


**Fig. 3. Serum calcium levels in relation to nicotinamide treatment in hemodialysis patients.**

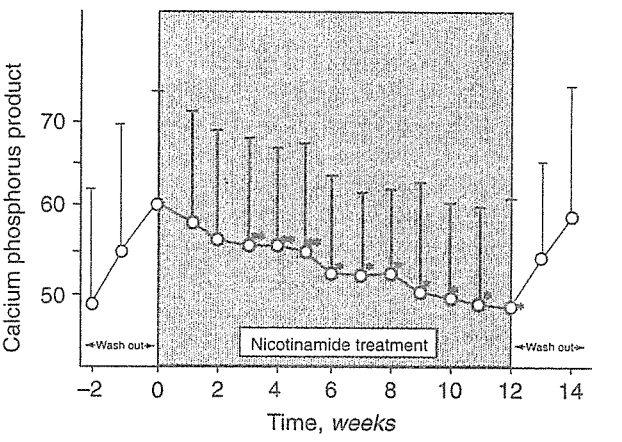


**Fig. 4. Effect of nicotinamide treatment on calcium-phosphorus product in hemodialysis patients.** *vs. 0 week, $P < 0.0001$; ** vs. 0 week, $P < 0.01$.

increased from 5.4 ± 1.5 mg/dL before the pretreatment washout period to 6.9 ± 1.5 mg/dL after the pretreatment washout period. Serum phosphorus levels decreased immediately after the start of nicotinamide treatment and continued to decrease until the cessation of treatment. Serum phosphorus levels decreased from 6.9 ± 1.5 mg/dL to 5.4 ± 1.3 mg/dL during the 12 weeks of nicotinamide treatment ($P < 0.0001$). After the posttreatment washout, serum phosphorus levels increased significantly to 6.7 ± 1.6 mg/dL ($P < 0.0001$). Reductions in serum phosphorus levels were comparable between vitamin D users and nonvitamin D users.

Serum calcium levels decreased from 9.1 ± 0.8 mg/dL before the pretreatment washout period to 8.7 ± 0.7 mg/dL after the pretreatment washout period ($P < 0.0001$) (Fig. 3). Serum calcium levels were unchanged during the 12 weeks of nicotinamide treatment (8.8 ± 0.7 mg/dL, $P = 0.4230$) and remained the same after the 2-week posttreatment washout period (8.8 ± 0.7 mg/dL). Serum calcium levels were similar between vitamin D users and nonvitamin D users.

The calcium-phosphate product increased significantly from 48.4 ± 13.6 $mg^2/dL^2$ to 59.8 ± 14.5 $mg^2/dL^2$ at the end of the pretreatment washout period ($P < 0.0001$) (Fig. 4). A calcium-phosphorus product decreased immediately and significantly to 47.3 ± 13.4 $mg^2/dL^2$ during the 12 weeks of nicotinamide treatment ($P < 0.0001$). With the cessation of nicotinamide, the serum calcium-phosphorus product increased gradually, reaching 58.7 ± 16.1 $mg^2/dL^2$ after the 2-week posttreatment washout period ($P < 0.0001$).

Median serum iPTH levels over the course of the study are shown in Figure 5. Median serum iPTH levels increased with the pretreatment washout from 130.0 (32.8 to 394.0) pg/mL to 200.0 (92.5 to 535.0) pg/mL ($P < 0.05$).

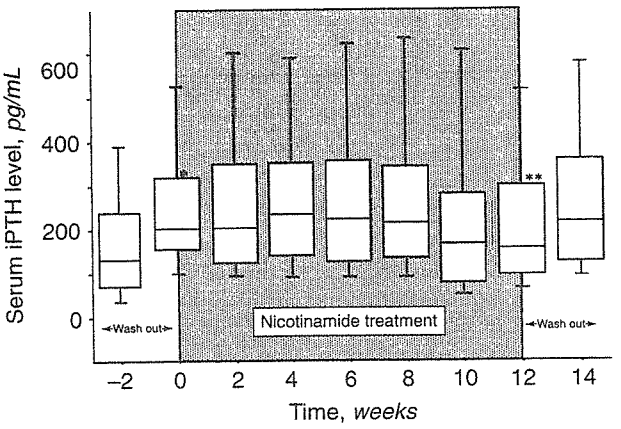


**Fig. 5. Box and whisker plots showing serum intact parathyroid hormone (iPTH) levels in nicotinamide-treated hemodialysis patients.** *vs. −2 weeks, $P < 0.05$; **vs. 4 weeks, $P < 0.05$.

With nicotinamide treatment, median serum iPTH levels reached 230.0 (90.8 to 582.0) pg/mL on week 4 and decreased, reaching 150.0 (57.6 to 518.0) pg/mL by the end of the 12 weeks of treatment ($P < 0.05$). After the 2-week posttreatment washout period, median serum iPTH levels were again increased to 220.0 (97.2 to 570.0) pg/mL. Median iPTH levels decreased with nicotinamide treatment in both vitamin D users and nonvitamin D users. Median serum iPTH levels after nicotinamide treatment did not differ significantly from those before the pretreatment washout. No changes were observed in serum magnesium concentrations with nicotinamide treatment. Serum alkaline phosphatase levels decreased significantly from 173.8 ± 79.3 IU/L to 159.3 ± 68.4 IU/L ($P < 0.01$) at 6 weeks and to 159.2 ± 58.6 IU/L ($P < 0.01$) at 12 weeks after the start of nicotinamide treatment and then increased significantly to 166.1 ± 59.8 IU/L ($P < 0.05$) at 2 weeks after the cessation of nicotinamide.

Serum high-density lipoprotein (HDL) cholesterol levels increased significantly from 47.4 ± 14.9 mg/dL before the pretreatment washout period to 67.2 ± 22.3 mg/dL ($P < 0.0001$) after the 12 weeks of nicotinamide treatment. In contrast, serum low-density lipoprotein (LDL) cholesterol levels decreased from 78.9 ± 18.8 mg/dL to 70.1 ± 25.3 mg/dL ($P < 0.01$). There were no changes in serum total cholesterol levels (before treatment, 160.9 ± 30.3 mg/dL vs. after treatment, 161.6 ± 33.9 mg/dL) or in serum triglyceride levels (before treatment, 145.7 ± 82.2 mg/dL vs. after treatment, 131.4 ± 69.6 mg/dL) throughout the study period. Changes in serum HDL cholesterol levels correlated significantly with changes in NAD concentrations ($r = 0.512$, $P < 0.01$).

There were no significant changes in other laboratory values during nicotinamide treatment. Serum albumin and total serum proteins did not change significantly during the study. Adverse events possibly related to treatment included diarrhea (five patients, 7.8%) and thrombocytopenia (one patient, 1.6%). The platelet count of the patient with thrombocytopenia decreased from 16.8 × $10^4$/μL to 8.3 × $10^4$/μL. Two weeks after discontinuance of nicotinamide, the platelet count increased to 18.0 × $10^4$/μL. There were no changes in his erythrocyte or leucocyte count during nicotinamide treatment. All six patients with adverse effects had received more than 1500 mg/day of nicotinamide. The diarrhea and thrombocytopenia disappeared when the nicotinamide was reduced or discontinued.

## DISCUSSION

Hyperphosphatemia is an important risk factor for the development of ectopic calcification and cardiovascular changes in patients undergoing hemodialysis. Although calcium- or aluminum-based phosphate binders are usually essential for avoiding hyperphosphatemia in long-term hemodialysis patients, certain adverse effects associated with the absorption of calcium and/or aluminum are inevitable. We showed in the present study that nicotinamide controls serum phosphorus levels in hemodialysis patients at levels similar to those achieved with currently available calcium- or aluminum-based phosphate binders.

A novel calcium- and aluminum-free phosphate binder, poly[allylamine hydrochloride] (RenaGel, GelTex Pharmaceuticals, Inc., Waltham, MA, USA), was recently reported to reduce serum phosphorus and iPTH concentrations without significant changes in serum calcium levels [15–17]. Total serum cholesterol and LDL cholesterol levels were also shown to be significantly reduced in RenaGel-treated patients without a reduction in HDL cholesterol [15–17].

Nicotinamide, a metabolite of nicotinic acid, stimulates biosynthesis of NAD, inhibits catabolism of NAD, and increases the ratio of NAD (oxidized) to NADH (reduced) [10]. The mechanism by which nicotinamide lowers serum phosphorus levels remains unknown. NAD is proposed to be an intracellular regulator of sodium-dependent phosphate transport [10]. Nicotinamide has been shown in rats to increase the renal cortical NAD concentration, inhibit phosphate uptake by brush border membrane vesicles of the renal proximal tubules in the rat kidney, and increase phosphate excretion in thyroparathyroidectomized rats [9, 10]. Intestinal phosphate transport is reported to occur by a sodium-independent, nonsaturable process and an active, sodium-dependent component of phosphate absorption in the duodenum and jejunum [18]. Katai et al [11] showed that nicotinamide inhibits phosphate uptake in the brush border membrane of rat small intestine. Furthermore, nicotinamide, apart from its inhibitory effect on poly(ADP-ribose) polymerase (PARP)-1 and its ability to restore intracellular $NAD^+$ pools, has recently been

suggested to act against the pathogenic process leading to insulin-dependent diabetes mellitus (IDDM). A recent meta-analysis showed nicotinamide, when given at the time of IDDM diagnosis, to have a protective effect on residual cell function of the pancreas as assessed by C-peptide secretion [19]. It is probable that nicotinamide improves insulin secretion and consequently decreases serum phosphorus levels by shifting phosphorus from the extracellular to the intracellular space.

Nicotinamide significantly reduced serum phosphorus levels in hemodialysis patients in the present study. Mean serum phosphorus levels decreased significantly during the 12 weeks of nicotinamide treatment and increased significantly to pretreatment levels after the 2-week post-treatment washout, suggesting the serum phosphorus-lowering effect to be due to nicotinamide. The onset of nicotinamide action was relatively rapid; the substantial reduction in serum phosphorus levels occurred within 2 weeks. This study proved that the serum phosphorus–lowering ability of nicotinamide is nearly equivalent to that of calcium-based phosphate binders.

Serum calcium levels declined after the pretreatment washout in our study, probably due to the removal of calcium carbonate. Nicotinamide treatment did not change serum calcium levels during the 12 weeks. The risk of vascular calcification increases with increases in serum calcium-phosphorus product. The mean increase in serum calcium-phosphorus product we observed after the pretreatment washout along with the remarkable reduction to below prewashout levels indicates that nicotinamide can reduce the risk of vascular calcification in hemodialysis patients.

Several investigators have shown that increased serum phosphorus levels increase the synthesis and secretion of PTH [20–22]. Evidence exists for a direct role of serum phosphorus as a regulator of parathyroid gland function [23]. In the present study, median serum iPTH levels gradually decreased after the start of nicotinamide treatment and showed significant reduction after 12 weeks of treatment. The increase in serum phosphorus and the decrease in serum calcium with pretreatment washout stimulated a corresponding increase in median serum iPTH levels. The decline in serum iPTH levels during the second half of nicotinamide treatment was associated with the decline in serum phosphorus.

Nicotinamide treatment significantly increased serum HDL cholesterol and decreased LDL cholesterol in our subjects. Shepherd et al [24] reported that nicotinic acid elevates the $HDL_2$-to-$HDL_3$ ratio because of a great increase in the absolute level of circulating $HDL_2$ and a small absolute decrease in circulating $HDL_3$. Cardiovascular diseases, including myocardial infarction, sudden death, and stroke, collectively account for approximately 50% of the mortality of ESRD patients [25, 26]. There are multiple abnormalities in the lipid profile of ESRD patients, and these may contribute to the high incidence of atherosclerosis [27]. Controlled clinical trials will ultimately determine whether an increase in HDL cholesterol will be of benefit to ESRD patients with atherosclerosis.

Nicotinamide treatment has a few adverse effects, including the possible occurrence of gastrointestinal disorders such as diarrhea. One patient showed a statistically significant decrease in platelet count during nicotinamide treatment. After the washout period, however, the platelet count returned to the pretreatment level. Nicotinamide has been used at 1500 mg/day to 3000 mg/day without adverse effects for protection of beta cells from end-stage destruction in patients with recent-onset IDDM [28]. However, we used a mean dose of 1080 mg/day. Rutkowski et al [29] reported recently that serum *N*-methyl-2-pyridine-5-carboxamide (2-PY), an end product of NAD degradation, was elevated in hemodialysis patients and that nicotinamide inhibited PARP-1 activity in vitro. In the present study, however, intracellular NAD levels in hemodialysis patients (9.3 nmol/$10^5$ erythrocytes) were nearly the same as levels in healthy subjects (9.0 nmol/$10^5$ erythrocytes) [12], suggesting that intracellular 2-PY concentrations may not be increased in chronic renal failure patients. Nicotinamide is a well-known inhibitor of PARP-1. Activation of PARP-1 has been implicated in the pathogenesis of stroke, myocardial ischemia, diabetes, cardiovascular dysfunction, shock, central nervous system injury, and various other forms of inflammation. Therefore, inhibition of PARP-1 by pharmacological agents may prove useful in the treatment of these diseases. Although blood NAD concentrations were increased up to 13.2 nmol/$10^5$ erythrocytes after nicotinamide administration in our study, it is not clear whether this concentration of intracellular NAD can inhibit PARP-1 activity. Further studies on adverse effects of long-term administration of nicotinamide are needed.

## CONCLUSION

Nicotinamide may provide an alternative for controlling hyperphosphatemia and hyperparathyroidism in hemodialysis patients.

*Reprint requests to Hikaru Koide, M.D., Division of Nephrology, Department of Medicine, Koto Hospital, 6-8-5 Ojima, Koto-ku, Tokyo 136-0072, Japan.*
*E-mail: hkoide@koto-hospital.or.jp*

## REFERENCES

1. SLATOPOLSKY E, BRICKER NS: The role of phosphate restriction in the prevention of secondary hyperparathyroidism in chronic renal disease. *Kidney Int* 4:141–145, 1973
2. DELMEZ JA, SLATOPOLSKY E: Hyperphosphatemia: Its consequences and treatment in patients with chronic renal disease. *Am J Kidney Dis* 19:303–317, 1992

3. Ramirez JA, Emmett M, White MG, *et al*: The absorption of dietary phosphorus and calcium in hemodialysis patients. *Kidney Int* 30:753–759, 1986
4. Hou SH, Zhao J, Ellman CF, *et al*: Calcium and phosphorus fluxes during hemodialysis with low calcium dialysate. *Am J Kidney Dis* 18:217–224, 1991
5. Alfrey AC, Legendre GR, Kaehny WD: The dialysis encephalopathy syndrome. Possible aluminum intoxication. *N Engl J Med* 294:184–188, 1976
6. Ott SM, Maloney NA, Coburn JW, *et al*: The prevalence of bone aluminum deposition in renal osteodystrophy and its relation to the response to calcitriol therapy. *N Engl J Med* 307:709–713, 1982
7. Slatopolsky E, Weerts C, Lopez-Hilker S, *et al*: Calcium carbonate as a phosphate binder in patients with chronic renal failure undergoing dialysis. *N Engl J Med* 315:157–161, 1986
8. Meric F, Yap P, Bia MJ: Etiology of hypercalcemia in hemodialysis patients on calcium carbonate therapy. *Am J Kidney Dis* 5:459–464, 1990
9. Berndt TJ, Pfeifer JD, Knox FG, *et al*: Nicotinamide restores phosphaturic effect of PTH and calcitonin in phosphate deprivation. *Am J Physiol* 242:F447–F452, 1982
10. Kempson SA, Colon-Otero G, Ou SY, *et al*: Possible role of nicotinamide adenine dinucleotide as an intracellular regulator of renal transport of phosphate in the rat. *J Clin Invest* 67:1347–1360, 1981
11. Katai K, Tanaka H, Tatsumi S, *et al*: Nicotinamide inhibits sodium-dependent phosphate cotransport activity in rat small intestine. *Nephrol Dial Transplant* 14:1195–1201, 1999
12. Siibata K, Murata K: Blood NAD as an index of niacin nutrition. *Nutr Int* 2:177–181, 1986
13. Nisselbaum JS, Green S: A simple ultramicro method for determination of pyridine nucleotides in tissues. *Anal Biochem* 27:212–217, 1969
14. Shinzato T, Nakai S, Miwa M, *et al*: New method to calculate creatinine generation rate using pre- and postdialysis creatinine concentrations. *Artif Organs* 21:864–872, 1997
15. Chertow GM, Burke SK, Lazarus JM, *et al*: Poly[allylamine hydrochloride] (RenaGel): A noncalcemic phosphate binder for the treatment of hyperphosphatemia in chronic renal failure. *Am J Kidney Dis* 29:66–71, 1997
16. Goldberg DI, Dillon MA, Slatopolsky EA, *et al*: Effect of RenaGel®, a non-absorbed, calcium- and alminium-free phosphate binder, on serum phosphorus, calcium, and intact parathyroid hormone in end-stage renal disease patients. *Nephrol Dial Transplant* 13:2303–2310, 1998
17. Slatopolsky EA, Burke SK, Dillon MA: Rena Gel®, a non-absorbed calcium- and aluminum-free phosphate binder, lowers serum phosphorus and parathyroid hormone. *Kidney Int* 55:299–307, 1999
18. Bleyer AJ, Burke SK, Dillon M, *et al*: A comparison of the calcium-free phosphate binder sevelamer hydrochloride with calcium acetate in the treatment of hyperphosphatemia in hemodialysis patients. *Am J Kidney Dis* 33:694–701, 1999
19. Pozzilli P, Browne PD, Kolb H: Meta-analysis of nicotinamide treatment in patients with recent-onset IDDM. *Diabetes Care* 19:1357–1363, 1996
20. Lopez-Hilker S, Dusso AS, Rapp NS, *et al*: Phosphorus restriction reverses hyperparathyroidism in uremia independent of changes in calcium and calcitriol. *Am J Physiol* 259:F432–F437, 1990
21. Yi H, Fukagawa M, Yamato H, *et al*: Prevention of enhanced parathyroid hormone secretion, synthesis and hyperplasia by mild dietary phosphorus restriction in early chronic renal failure in rats: Possible direct role of phosphorus. *Nephron* 70:242–248, 1995
22. Slatopolsky E, Finch J, Denda M, *et al*: Phosphorus restriction prevents parathyroid gland growth. High phosphorus directly stimulates PTH secretion in vitro. *J Clin Invest* 97:2534–2540, 1996
23. Nielsen PK, Feldt-Rasmussen U, Olgaard K: A direct effect in vitro of phosphate on PTH release from bovine parathyroid tissue slices but not from dispersed parathyroid cells. *Nephrol Dial Transplant* 11:1762–1768, 1996
24. Shepherd J, Packard CJ, Patsch JR, *et al*: Effects of nicotinic acid therapy on plasma high density lipoprotein subfraction distribution and composition and on apolipoprotein A metabolism. *J Clin Invest* 63:858–867, 1979
25. Wheeler DC: Should hyperlipidaemia in dialysis patients be treated? *Nephrol Dial Transplant* 12:19–21, 1997
26. Bommer J, Strohbeck E, Goerich J, *et al*: Arteriosclerosis in dialysis patients. *Int J Artif Organs* 19:638–644, 1996
27. O'Neal D, Lee P, Murphy B, Best J: Low-density lipoprotein particle size distribution in end-stage renal disease treated with hemodialysis or peritoneal dialysis. *Am J Kidney Dis* 27:84–91, 1996
28. Pozzilli P, Visalli N, Signore A, *et al*: Double blind trial of nicotinamide in recent-onset IDDM (the IMDIAB III study). *Diabetologia* 38:848–852, 1995
29. Rutkowski B, Slominska E, Szolkiewicz M, *et al*: N-methyl-2-pyridone-5-carboxamide: A novel uremic toxin? *Kidney Int* 63 (Suppl 84):S19–S21, 2003

報 文

# 代謝撹乱物質ビスフェノールAのトリプトファン–ニコチンアミド転換経路の撹乱作用部位

(平成16年3月24日受理)

福渡 努* 鈴浦千絵* 佐々木隆造* 柴田克己†,*

# Action Site of Bisphenol A as Metabolic Disruptor Lies in the Tryptophan–Nicotinamide Conversion Pathway

Tsutomu FUKUWATARI, Chie SUZUURA, Ryuzo SASAKI and Katsumi SHIBATA†

(Laboratories of Food Science and Nutrition, Department of Life Style Studies, School of Human Cultures, The University of Shiga Prefecture: 2500 Hassaka-cho, Hikone 522-8533, Japan; † Corresponding author)

We have reported that the administration of bisphenol A to rats reduces the conversion ratio of tryptophan to nicotinamide. In the present paper, we show that bisphenol A, a monomer of polycarbonate plastics, inhibits the enzyme activity of kynurenine 3-hydroxylase. Namely, the conversion ratio of tryptophan to nicotinamide is reduced through the inhibition of kynurenine 3-hydroxylase activity by bisphenol A.

(Received March 24, 2004)

**Key words:** 内分泌撹乱物質 endocrine disruptor; ビスフェノールA bisphenol A; 代謝撹乱物質 metabolic disruptor; トリプトファン tryptophan; ニコチンアミド nicotinamide; キヌレニン3-ヒドロキシラーゼ kynurenine 3-hydroxylase

## 緒 言

我々は，内分泌撹乱物質候補に挙げられているビスフェノールAがトリプトファン–ニコチンアミド転換率を顕著に阻害することを報告した[1]．ヒトを含む哺乳動物はB群ビタミンの中で最も必要量の多いナイアシン（ビタミン$B_3$ともいう）をすでにビタミン体となっているニコチンアミドとして摂取しているが，トリプトファンからもニコチンアミドを生合成する経路を有している[2)~4)]．日本人が一般的な食事をしている場合，ナイアシンの約50%はトリプトファンから供給されている[5]．したがって，ビスフェノールAの摂取によって，本転換経路が阻害されるという事実は公衆栄養学上重要な問題である．前報[1]では，ラットの飼料中に終濃度1%レベルでの影響を調べたのみであった．本研究は，本転換率に影響を及ぼす最低濃度とその作用部位の解明，さらに他のビタミン代謝に対する影響を調べることを目的として行い，成果を得たので報告する．

† 連絡先
* 滋賀県立大学人間文化学部生活文化学科食生活専攻：〒522-8533 滋賀県彦根市八坂町2500

## 実験方法

### 1. 試 薬

飼料に使用したカゼイン，L-メチオニン，ショ糖は和光純薬工業(株)より購入した．ミネラル混合（AIN93配合；AIN-93M），ビタミン混合（AIN93配合；AIN-93VX，重酒石酸コリン添加）はオリエンタル酵母工業(株)より購入した．

尿中代謝産物の定量用標準品として使用したアンスラニル酸，キヌレン酸，キサンツレン酸，3-ヒドロキシアンスラニル酸，$N^1$-メチルニコチンアミド(MNA)は東京化成工業(株)より，キノリン酸，ニコチンアミド，チアミン塩酸塩，リボフラビン，アスコルビン酸，ビスフェノールAは和光純薬工業(株)より購入した．$N^1$-メチル-2-ピリドン-5-カルボキサミド(2-Py)はPullmanとColowickの方法[3]により，$N^1$-メチル-4-ピリドン-3-カルボキサミド(4-Py)は柴田らの方法[4]により合成した．4-ピリドキシン酸(PIC)はシグマケミカル(株)より購入した．

### 2. 動物の飼育方法

本実験は滋賀県立大学動物実験委員会で承認を受けた．

飼育室の温度は22℃前後に，湿度は60%前後に調節した．明暗サイクルは，午前6時～午後6時を明，午後6時～午前6時までを暗とした．

**Table 1.** Composition of the Diet

| | Control diet (%) | Test diet 0.1% BPA (%) | Test diet 0.5% BPA (%) |
|---|---|---|---|
| Casein | 20 | 20 | 20 |
| L-Methionine | 0.2 | 0.2 | 0.2 |
| Gelatinized cornstarch | 45.9 | 45.8 | 45.4 |
| Sucrose | 22.9 | 22.9 | 22.9 |
| Corn oil | 5 | 5 | 5 |
| Mineral mixure[a] (AIN-93M) | 5 | 5 | 5 |
| Vitamin mixture (NiA-free)[a] (AIN-93-VX containing 25 choline bitartrate) | 1 | 1 | 1 |
| Bisphenol A | 0 | 0.1 | 0.5 |

[a] AIN 93 was used [Reeves, P. G., Components of the AIN-93 diets as improvements in the AIN-76A diet. *J. Nutr.*, **127**, 838S–841S (1997)].

3週齢の Wistar 系雄ラットを日本クレア(株)より購入し，平均体重がほぼ等しくなるよう5匹ずつ3群（0, 0.1, 0.5% ビスフェノールA含有食）に分け，ラット用代謝ケージ（CT-10，日本クレア(株)製）に入れた．飼料は Table 1 に示す 20% カゼイン食をコントロール食とした．試験食は，終濃度で 0.1%, 0.5% を含む飼料を投与した．飼育期間は 22 日間で，飼料と水は自由摂取とし，1日ないし2日おきの午前9～10時に新しいものと交換した．また，その時に体重と飼料摂取量を測定した．

実験最終日の1日尿（午前10時～翌日午前10時：24時間）を集めた．トリプトファン代謝産物，ニコチンアミドおよびその代謝産物，チアミン，リボフラビン，PIC を測定するための尿は分析するまで塩酸酸性下，−20℃ で保存した．アスコルビン酸とその代謝産物（デヒドロアスコルビン酸，2,3-ジケトグロン酸）を測定するための尿は10%メタリン酸で2倍希釈した後，−20℃ で保存した．

実験最終日の採尿後にラットを断頭と殺し各種臓器を取り出し，重量を測定した．尿はトリプトファン–ニコチンアミド転換経路代謝産物量の測定に使用した．なお，対照群の肝臓は，キヌレニン 3-ヒドロキシラーゼ活性の測定に使用した．

### 3. 分析方法

#### 3.1 トリプトファン–ニコチンアミド代謝産物の測定方法

尿を 0.45 μm のミクロフィルターでろ過した後，アンスラニル酸[8]，キヌレン酸[9]，キサンツレン酸[10]，3-ヒドロキシアンスラニル酸[10] およびキノリン酸[11] をそれぞれ文献に示した HPLC 法で直接測定した．

尿中の MNA の定量は，強アルカリ性下でアセトフェノンと縮合させることにより蛍光物質に変換し，これを HPLC にて測定した[12]．

尿中のニコチンアミド，2-Py および 4-Py の定量は，尿に炭酸カリウムを飽和量加えた後，ジエチルエーテルで抽出し，乾固させた抽出物を水に溶解し，その液を HPLC にて測定した[7]．

#### 3.2 キヌレニン 3-ヒドロキシラーゼ (EC 2.1.3.1) 活性の測定方法

ラットから単離した直後の肝臓を材料として，De Duve ら[13] の報告した遠心分画法に従ってミトコンドリア画分を得，タンパク質濃度が 10 mg/mL 程度になるように適当量の 50 mM リン酸カリウム緩衝液 (pH 7.0) に懸濁したものを本酵素活性源とした．酵素反応は柴田・戸田[14] が報告した方法に従った．また，反応産物の 3-ヒドロキシキヌレニンの測定も柴田・戸田[14] が報告した HPLC 法に従った．簡単に説明すると，標準の酵素反応組成（全容量 500 μL）は次のとおりである．50 μL の 0.5 M Tris–HCl 緩衝液 (pH 8.0), 15 μL の 10 mM KCN, 50 μL の 100 mM KCl, 10 μL の 10 mM NADPH, 10 μL の 10 mM 硫酸 L-キヌレニン，10 μL のエタノール，255 μL の水，100 μL のミトコンドリア懸濁液．ミトコンドリア懸濁液を添加することで反応を開始し，37℃ で 10 分間行った．停止は 70% 過塩素酸を 40 μL 添加することで行った．停止させた酵素反応液を室温で5分間放置後，10,000×*g*, 3分間遠心分離することで，上清を得た．沈殿には 500 μL の水を加え，5分間混合後，10,000×*g*, 3分間遠心分離することで，上清を得た．合わせた上清中の 3-ヒドロキシキヌレニンを HPLC を用いて測定した[14]．

ビスフェノールAの本酵素活性に及ぼす影響を調べるために，5 mM, 25 mM, 50 mM, 150 mM 濃度のビスフェノールAエタノール溶液を作製した．これらのビスフェノールAエタノール溶液を標準反応組成液のエタノール（10 μL 添加）の代わりに添加した．したがって，反応組成液中の終濃度は 0.1 mM, 0.5 mM, 1 mM, 3 mM となる（Fig. 5 参照）．

#### 3.3 尿中のチアミン（ビタミン $B_1$）の測定方法

基本的には，木村らが[15] 報告した血液中のチアミン測定方法に従った．HPLC 注入用試料は，集めた尿を 0.45 μm のミクロフィルターでろ過した液とする．木村らの方法では，チアミンをカラムで分離した後，反応液としてフェリシアン化カリウムと水酸化ナトリウム混合液を送液しているが，再現性が低かったため，Fig. 1 に示したように，始めにフェリシアン化カリウム溶液を送液し，次に水酸化ナトリウム溶液を送液した．この改良により，再現性を高めることに成功した．分析条件を以下に示した．移動相は 0.2 M $NaH_2PO_4$ を用い，流速 1.0 mL/min で流した．反応液1は 0.01% $K_3Fe(CN)_6$ で流速 0.15 mL/min で流した．反応液2は 15% NaOH で流速 0.15 mL/min で流した．反応コイルは PEEK チューブ（外径，1.80 mm; 内径，0.50 mm）で長さは 1,200 mm，カラムは Shodex Rs-pak NN-614 (150 mm×6.0 mm i.d.) を使用し，カラム温度は 40℃ に維持し，検出は励起波長 365 nm，蛍光波長 435 nm で行った．

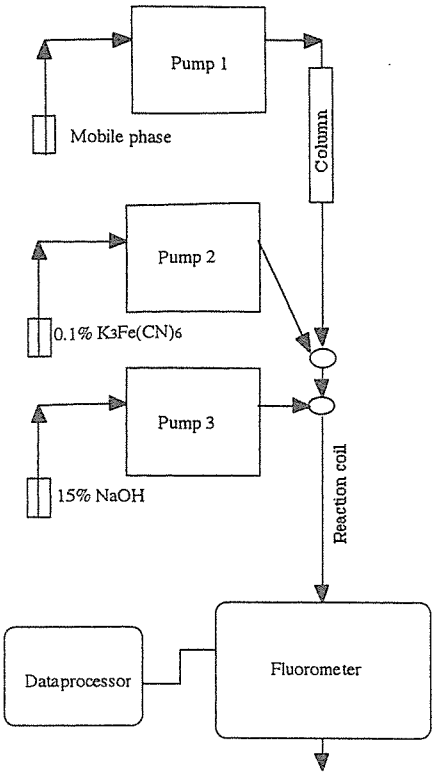


**Fig. 1.** Diagram of HPLC system for measurement of thiamin

### 3.4　尿中のリボフラビンの測定方法

尿中リボフラビンは，リボフラビン自体が発する蛍光を蛍光検出器付きのHPLCで測定した[16]．HPLC注入用試料は，集めた尿を0.45 μmのミクロフィルターでろ過した液である．カラムはTosoh TSKgel ODS-80Ts (250×4.6 mm i.d.)を用い，移動相としては10 mM $NaH_2PO_4$ (pH 5.5; 6 M NaOHでpHを調整)-メタノール(7 : 3)を使用した．流速は0.8 mL/minで，カラム温度は40℃に維持した．検出は，励起波長445 nm，蛍光波長530 nmで行った．

### 3.5　尿中のPICの測定方法

ビタミン$B_6$の異化代謝産物であるPICは，PIC自体が発する蛍光を蛍光検出器付きのHPLCで測定した[17]．HPLC注入用試料は，集めた尿を0.45 μmのミクロフィルターでろ過した液である．移動相として500 mLの超純水に85%リン酸を2.3 mL添加し，50% KOHでpH 2.2に調製後，900 mLに定容した後，100 mLのメタノールを加えたものを使用した．カラムはTosoh TSKgel ODS-120A (250 mm×4.6 mm i.d.)を使用し，流速1.0 mL/minで流した．カラム温度は30℃に維持した．測定は励起波長355 nm，蛍光波長436 nmで行った．

### 3.6　尿中のアスコルビン酸（還元型アスコルビン酸＋酸化型アスコルビン酸＋2,3-ジケトグロン酸）の測定方法

メタリン酸酸性下で保存した尿をKishidaらの方法[18]に従って，オサゾン誘導体に変換した後測定を行った．カラムはWaters μBondasphere 5 μ$C_{18}$-100A (150 mm×3.9 mm i.d.)を用い，移動相として，アセトニトリル500 mLに終濃度が0.1%になるようにトリエチルアミン(pH 3.0)溶液を加えた後，水で1,000 mLにしたものを使用した．カラム温度は40℃に維持し，流速1.0 mL/minで，検出は505 nmで行った．

## 結　　果

### 1.　体重と飼料摂取量への影響

幼若ラットの体重増加量 (Fig. 2A) と飼料摂取量 (Fig. 2B) は，0.1% ビスフェノールA添加食では対照群と比較

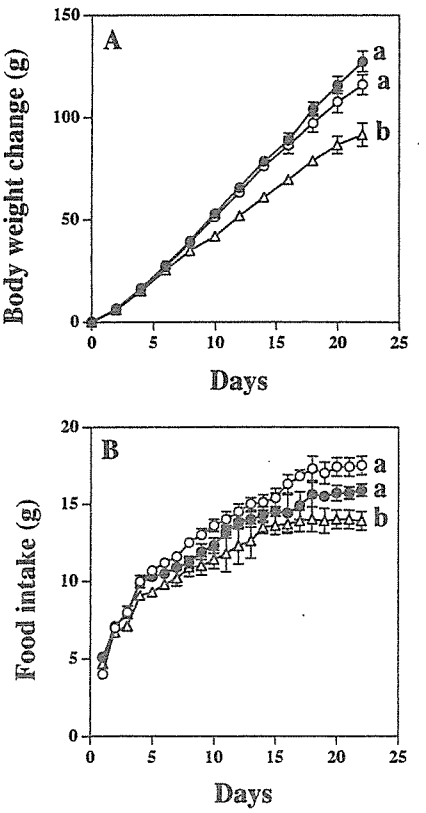


**Fig. 2.** Effects of bisphenol A on the body weight gain (A) and food intake (B) of rats.
Male rats of the Wistar strain (3 weeks old) were obtained and immediately placed in individual metabolic cages (CT-10; Clea Japan). They were fed *ad libitum* (Table 1) for 22 days. ●, 0% bisphenol A (control) group; ○, 0.1% bisphenol A group; △, 0.5% bisphenol group. Values are means±SEM for five rats; different superscript letters indicate significant differences at $p<0.05$ in the Student-Newman-Keuels multiple comparison test.

**Table 2.** Effect of Dietary BPA on the Organ Weights

| | Control | 0.1% BPA | 0.5% BPA |
|---|---|---|---|
| Liver (g/rat) | 7.77±0.55 | 7.24±0.32 | 6.27±0.17 |
| Kidney (g/rat) | 1.62±0.07[a] | 1.54±0.07[a] | 1.29±0.07[b] |
| Heart (g/rat) | 0.71±0.03 | 0.66±0.02 | 0.59±0.04 |
| Lung (g/rat) | 0.92±0.04 | 0.89±0.04 | 0.80±0.03 |
| Spleen (g/rat) | 0.61±0.04[a] | 0.57±0.04[a, c] | 0.44±0.05[b, c] |
| Brain (g/rat) | 1.10±0.04 | 1.09±0.02 | 1.00±0.03 |
| Testis (g/rat) | 1.58±0.08[a] | 1.50±0.08[a] | 0.65±0.03[b] |
| Liver (g/100 g of b.w.*) | 4.71±0.16 | 4.73±0.16 | 4.91±0.14 |
| Kidney (g/100 g of b.w.) | 0.99±0.03 | 1.01±0.05 | 1.00±0.02 |
| Heart (g/100 g of b.w.) | 0.43±0.01 | 0.43±0.01 | 0.46±0.02 |
| Lung (g/100 g of b.w.) | 0.56±0.01 | 0.58±0.02 | 0.63±0.03 |
| Spleen (g/100 g of b.w.) | 0.37±0.01 | 0.37±0.02 | 0.34±0.03 |
| Brain (g/100 g of b.w.) | 0.67±0.04 | 0.72±0.02 | 0.79±0.03 |
| Testis (g/100 g of b.w.) | 0.96±0.03[a] | 0.98±0.03[a] | 0.48±0.02[b] |

* b.w.=body weight

Male rats of the Wistar strain (3 weeks old) were obtained from Clea Japan (Tokyo, Japan) and immediately placed in individual metabolic cages (CT-10; Clea Japan). They were then divided into three groups, and fed *ad libitum* for 22 days (Table 1).

Values are means±SEM for five rats; different superscript letters indicate significant differences at $p<0.05$ in the Student-Newman-Keuels multiple comparison test.

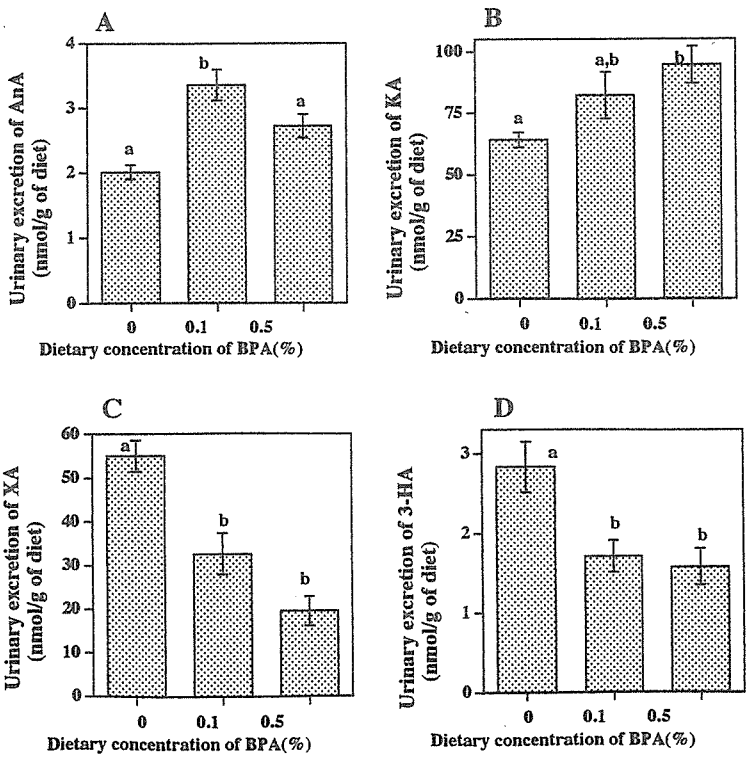


**Fig. 3.** Effects of bisphenol A on the urinary excretion of AnA (A), KA (B), XA (C) and 3-HA (D).
Twenty-four-hour urine samples were collected on the last day of the experiment. Values are means± SEM for five rats; different superscript letters indicate significant differences at $p<0.05$ in the Student-Newman-Keuels multiple comparison test. AnA=anthranilic acid, KA=kynurenic acid, XA=xanthurenic acid, 3-HA=3-hydroxyanthranilic acid.

して，有意な低下は認められなかった．0.5% 添加食の投与によっては，体重増加量も飼料摂取量も対照群と比較して有意に低下した．

### 2. 臓器重量への影響

0.1% ビスフェノール A 添加食の投与は，Table 2 に示したように，肝臓，腎臓，心臓，肺臓，脾臓，脳，精巣の

各重量に，全く影響を与えなかった．0.5% 添加食では，肝臓，腎臓および脾臓がラット当たりでは減少傾向を示したが，100 g 体重当たりの値に換算した値では，差異を認めなかった．有意な差異が認められたのは，精巣のみであった．他の臓器重量には差異は認められなかった．

### 3. トリプトファン-3-ヒドロキシアンスラニル酸代謝系への影響

Fig. 3 に示した化合物はトリプトファン-ニコチンアミド転換経路の上流部分に位置する中間代謝産物である．これらは，体内にほとんど検出されないことから，尿中への排泄量がほぼ生成量を反映する．0.1% ビスフェノールA添加食の摂取により，アンスラニル酸の生成量は増加し，キヌレン酸は増加傾向を示した．0.5% 添加食では，アンスラニル酸は増加傾向を示し，キヌレン酸は有意に増加した．一方，キサンツレン酸および3-ヒドロキシアンスラニル酸の生成量は，0.1% 添加食でも 0.5% 添加食でも対照群の 1/2 程度にまで低下した．

### 4. キノリン酸の生成量とトリプトファン-ニコチンアミド転換率への影響

キノリン酸 (Fig. 4A) の生成量は飼料中のビスフェノールA量に応じて低下した．全く同じ結果が，トリプトファン-ニコチンアミド転換率 (Fig. 4B) においても見られた．

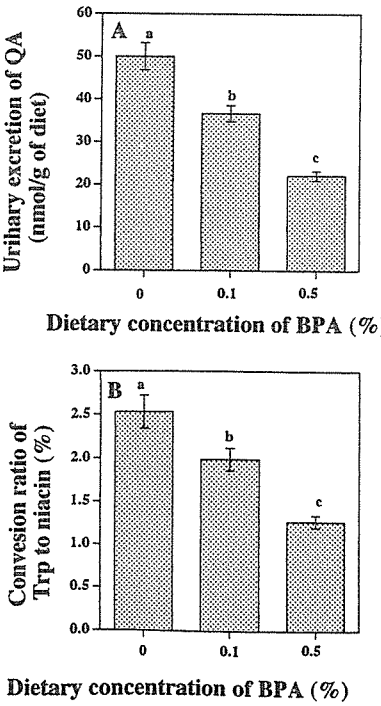


**Fig. 4.** Effects of bisphenol A on the urinary excretion of QA (A) and the conversion ratio of Trp to Nam (B).
Twenty-four-hour urine samples were collected on the last day of the experiment. Values are means ±SEM for five rats; different superscript letters indicate significant differences at $p<0.05$ in the Student-Newman-Keuels multiple comparison test. QA=quinolinic acid, Trp=tryptophan, Nam =nicotinamide.

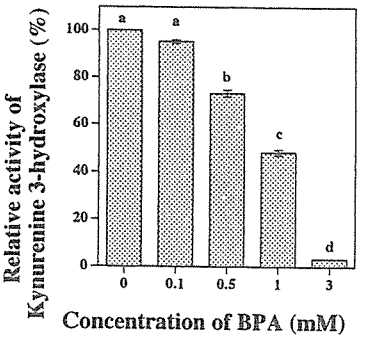


**Fig. 5.** Inhibition of kynurenine 3-hydroxylase activity by bisphenol A *in vitro*.
Values are means±SEM for three separate experiments; different superscript letters indicate significant differences at $p<0.05$ in the Student-Newman-Keuels multiple comparison test.

### 5. キヌレニン3-ヒドロキシラーゼ活性に及ぼす影響 (*in vitro*)

トリプトファン-ニコチンアミド代謝系の中間代謝産物の測定から，ビスフェノールAの作用部位がキヌレニン3-ヒドロキシラーゼであると推定されたので，本酵素活性に及ぼす影響を *in vitro* で調べた．Fig. 5 に示したように，本酵素活性は，反応液に添加するビスフェノールAの添加量に応じて阻害された．

### 6. 尿中へのチアミン，リボフラビン，PICおよびアスコルビン酸の排泄量に及ぼす影響

ビスフェノールAの摂取により，尿中へのチアミン (Fig. 6A)，リボフラビン (Fig. 6B)，アスコルビン酸（この場合はアスコルビン酸＋デヒドロアスコルビン酸＋2,3-ジケトグロン酸）(Fig. 6D) の排泄量は有意に増大した．一方，ビタミン $B_6$ の異化代謝産物である PIC はビスフェノールAの摂取により有意に低下した (Fig. 6C).

## 考　察

我々は，ラットに 1% ビスフェノールA含有食を添加すると，トリプトファン-ニコチンアミド転換率が顕著に低下することを報告した[1]．1% ビスフェノールA群のラット1匹当たりの1日の摂取量は約 200 mg であり，体重 1 kg 当たりでは，800 mg となる．この量は雌性ホルモンの撹乱作用が報告されている 400 mg/kg 体重/日[19] の倍量に相当した．そこで，0.1% ビスフェノール含有食，0.5% 含有食の投与がトリプトファン-ニコチンアミド転換率に及ぼす影響を調べた．前報[1] では，6週齢のラットを用いたが，一般的に毒性の現れやすいと考えられている離乳したての3週齢のラットを今回は使用した．幼若ラットの飼料摂取量と体重増加に及ぼす影響は，0.1% 群では認められなかったが，0.5% 群では認められた (Fig. 2)．また，各種臓器重量に及ぼす影響の結果も，

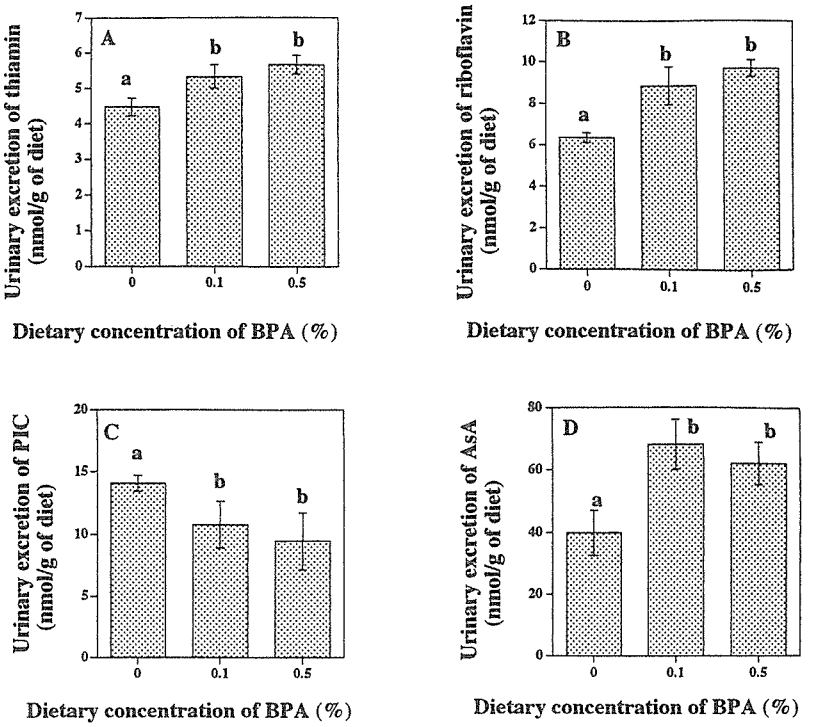


**Fig. 6.** Effects of bisphenol A on the urinary excretion of thiamin (A), riboflavin (B), 4-pyridoxic acid (PIC) (C) and ascorbic acid (D).
Twenty-four-hour urine samples were collected on the last day of the experiment. Values are means± SEM for five rats; different superscript letters indicate significant differences at $p<0.05$ in the Student-Newman-Keuels multiple comparison test.

0.1% 含有食では全く影響を及ぼさなかったが，0.5% 含有食の投与は精巣重量の低下をもたらした (Table 2). すなわち，0.1% ビスフェノールA含有食の投与はラットに見かけ上の影響を全く与えなかった．しかしながら，0.1% ビスフェノールA含有食の投与は，対照群である0% 含有食群と比較して，トリプトファン-ニコチンアミド転換経路の代謝産物であるアンスラニル酸とキヌレン酸の産生量をそれぞれ有意に増大あるいは増大傾向にさせ，一方，キサンツレン酸と3-ヒドロキシアンスラニル酸の産生量を有意に低下させた (Fig. 3). さらに，キノリン酸の産生量を有意に低下させ，この結果と連動してトリプトファン-ニコチンアミド転換率を有意に低下させた (Fig. 4). すなわち，広く家庭用品に使用されているビスフェノールAの生物学的暴露量を推定するには，これらの代謝産物量を測定することが有効であることが明らかとなった．

次に，前報[1]で推測したビスフェノールAの作用点を調べた．本経路の代謝産物の変動から，ビスフェノールAの作用点をキヌレニン3-ヒドロキシゲナーゼと推定した[1]. 今回の実験結果でも，全く同じ現象が認められた (Fig. 2, 3). そこで，本酵素活性に及ぼすビスフェノールAの影響を *in vitro* で調べた．その結果は，Fig. 5 に示したように，濃度依存的に活性が阻害された．本酵素は多くの化合物によって活性が変動することが知られている．例えば，Nishimoto ら[21]は，反応系にリン脂質を添加すると活性が有意に増大することを，Shin ら[22]は分岐鎖 $\alpha$-ケト酸が阻害することを，Mayer ら[23]は $Cu^{2+}$ や Dicumarol による阻害を，Okamoto ら[24]は甲状腺機能亢進，すなわちチロキシンによる阻害を，Bender と Smith[25]はある種の芳香族化合物による阻害を報告している．Müller[26]は「Flavin-dependent hydroxylases」と題する論文の中で，多くの芳香族化合物が Flavin-dependent hydroxylases によって水酸化される反応を紹介している．

キヌレニン3-ヒドロキシラーゼはミトコンドリア外膜に存在するFAD酵素であり，補酵素としてNADPHを要求する[20]. そこで，トリプトファン-ニコチンアミド転換経路に関与するB群ビタミンの代謝に及ぼす影響を調べた．その結果は Fig. 6 に示したように，チアミン（ビタミン $B_1$）とリボフラビン（ビタミン $B_2$）の排泄量がビスフェノールAの摂取により有意に増大し，ビタミン $B_6$ の異化代謝産物である PIC は減少した．摂取量が等しいときは（Fig. 6 の値は1gの飼料を摂取したときの値），尿中へのビタミンの排泄量の増大は，一般的に，体内での必要度の低下を意味するものと考えられる．チアミンとリボフラビンの排泄量の増大は，ビスフェノールAが体内において，これらの必要とする酵素反応を阻害している可能性を示唆している．なお，リボフラビンとビスフェノールAとの接点は多くのフラビン酵素が芳香族化合物の水酸化反応に関与している[26]という事実から推定されるが，

チアミンとビスフェノールAとの接点は不明である．一方，PIC排泄量の減少は，ビタミン$B_6$が関与する反応，例えば，アミノ酸の異化代謝をビスフェノールAが亢進させていることを示唆している．

アスコルビン酸はラットでは，ビタミンではない．ラットは生体異物を摂取するとアスコルビン酸の産生量を増大させることが知られている[27]．この増大は解毒に関係する薬物代謝系に関連する応答である．本実験においても，Fig. 6Dに示したように，ビスフェノールAの摂取により増大したことから，ラットはビスフェノールAを生体異物として認識し，水酸基を付加させて，脂溶性から水溶性物質に変化させて，積極的にビスフェノールAを尿中に排泄されているものと思われる．

## 謝　辞

本研究は滋賀県立大学重点研究費の補助を受けた．関係各位に謝意を表する．

## 文　献

1) Fukuwatari, T., Toriochi, M., Ohta, M., Sasaki, R., Shibata, K., Metabolic disturbance of tryptophan–nicotinamide conversion pathway by putative endocrine disruptors, bisphenol A and styrene monomer. Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Japan), **45**, 1–7 (2004).
2) Goldberger, J., Wheeler, G. A., The experimental production of pellagra in human subjects by means of diet. U.S. Pub. Health Serv. Hygienic Lab. Bull., No. 120 (1920).
3) Krehl, W. A., Teply, L. J., Sarma, P. S., Elvehjem, C. A., Growth-retarding effect of corn in nicotinic acid-low rations and its counteraction by tryptophane. Science, **101**, 489–490 (1945).
4) Perlzweig, W. A., Rosen, F., Levitas, N., Robinson, J., The excretion of nicotinic acid derivatives after ingestion of tryptophane by man. J. Biol. Chem., **167**, 511–514 (1947).
5) Fukuwatari, T., Shibata, K., Hayakawa, F., Sugimoto, E., Niacin intakes and niacin coenzymes levels in blood of female college students. Vitamins (Japan), **74**, 137–141 (2000).
6) Pullman, M. P., Colowic, S. P., Preparation of 2- and 6-pyridones of $N^1$-methylnicotinamide. J. Biol. Chem., **206**, 121–127 (1954).
7) Shibata, K., Kawada, T., Iwai, K., Simultaneous microdetermination of nicotinamide and its major metabolites, $N^1$-methyl-2-pyridone-5-carboxamide and $N^1$-methyl-4-pyridone-3-carboxamide, by high-performance liquid chromatography. J. Chromatogr., **424**, 23–28 (1988).
8) Shibata, K., Onodera, M., Measurement of 3-hydroxyanthranilic acid and anthranilic acid in urine by high-performance liquid chromatography. Agric. Biol. Chem., **55**, 143–148 (1991).
9) Shibata, K., Fluorimetric micro-determination of kynurenic acid, an endogenous blocker of neurotoxicity, by high-performance liquid chromatography. J. Chromatogr., **430**, 376–380 (1988).
10) Shibata, K., Onodera, M., Simultaneous high-performance liquid chromatographic measurement of xanthurenic acid and 3-hydroxyanthranilic acid in urine. Biosci. Biotechnol. Biochem., **56**, 974, (1992).
11) Mawatari, K., Oshida, K., Iinuma, F., Watanabe, M., Determination of quinolinic acid in human urine by liquid chromatography with fluorimetric detection. Anal. Chim. Acta, **302**, 179–183 (1995).
12) Shibata, K., Ultramicro-determinantion of $N^1$-methylnicotinamide in urine by high-performance liquid chromatography. Vitamins (Japan), **61**, 599–604 (1987).
13) de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., Appelmans, F., Biochem. J. Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. **60**, 604–617 (1955).
14) Shibata, K., Toda, S., Effects of thyroxine on the conversion ratio of tryptophan to nicotinamide in rats. Biosci. Biotechnol. Biochem., **58**, 1,757–1,762 (1994).
15) Kimura, M., Fujita, T., Itokawa, Y., Liquid chromatographic determination of the total thiamin content of blood. Clin. Chem., **28**, 29–31 (1982).
16) Roughead Z. K., McCormick, D. B., Urinary riboflavin and its metabolites: effects of riboflavin supplementation in healthy residents of rural Georgia (USA). Eur. J. Clin. Nutr., **45**, 299–307 (1991).
17) Gregory, J. F., Kirk, J. R., Determination of urinary 4-pyridoxic acid using high performance liquid chromatography. Am. J. Clin. Nutr., **32**, 879–883 (1979).
18) Kishida, K., Nishimoto, Y., Kojo, S., Specific determination of ascorbic acid with chemical derivatization and high-performamce liquid chromatography. Anal. Chem., **64**, 1,505–1,507 (1992).
19) Ashby, J., Tinwell, H., Uterotrophic activity of bisphenol A in the immature rat. Environ Health Perspect., **106**, 719–720 (1998).
20) Okamoto, H., Hayaishi, O., Solubilization and partial purification of kynurenine hydroxylase from mitochondria outer membrane and its electron donors. Arch. Biochem. Biophys., **131**, 603–608 (1969).
21) Nishimoto, Y., Takeuchi, F., Shibata, Y., Isolation of L-kynurenine 3-hydroxylase from the mitochondrial outer membrane of rat liver. J. Biochem., **78**, 573–581 (1975).
22) Shin, M., Yamada, O., Sano, K., Umezawa, C., Effect of branched chain $\alpha$-keto acids on kynurenine 3-hydroxylase from rat liver. J. Nutr. Sci. Vitaminol., **28**, 203–207 (1982).
23) Mayer, G., Ullrich, V., Staudinger, H., Possible involvement of cytochrome b5 in L-kynurenine-3-hydroxylase of rat liver mitochondria. Hoppe-Seyler's Physiol. Chem., **349**, 459–464 (1968).
24) Okamoto, H., Okada, F., Hayaishi, O., Kynurenine metabolism in hyperthyroidism. A biochemical basis for the low NAD(P) level in hyperthyroid rat liver. J. Biol. Chem., **246**, 7,759–7,763 (1971).

25) Bender, D. A., Smith, W. R., Inhibition of kynurenine hydrolase by benserazide, cardidopa and other aromatic hydrazine derivatives: evidence for sub-clinical iatrogenic niacin deficiency. Biochem. Soc. Trans., **6**, 120–122 (1978).

26) Müller, F., Flavin-dependent hydroxylases. Biochem. Soc. Trans., **13**, 443–445 (1985).

27) Horio, F., Yoshida, A., Effects of some xenobiotics on ascorbic acid metabolism in rats. J. Nutr., **112**, 416–425 (1982).

*Biosci. Biotechnol. Biochem.*, **69** (2), 273–279, 2005

# The Necessity of Niacin in Rats Fed on a High Protein Diet

Naoko KIMURA, Tsutomu FUKUWATARI, Ryuzo SASAKI, and Katsumi SHIBATA†

*Laboratories of Food Science and Nutrition, Department of Life Style Studies, School of Human Cultures, The University of Shiga Prefecture, 2500 Hassakacho, Hikone, Shiga 522-8533, Japan*

Received June 29, 2004; Accepted November 4, 2004

**It is known that niacin itself is not necessary in rats when tryptophan is given in adequate amounts, because rats can biosynthesize niacin from tryptophan. In our experiment, young rats were fed on a 20%, 40%, 60%, or 70% casein diet with or without niacin. The rats fed on the 20%, 40%, and 60% casein diets did not require niacin for growth, but the rats fed on the 70% casein diet needed it. This phenomenon was attributed to the supposition that liver aminocarboxymuconate–semialdehyde decarboxylase activities increased according with the dietary casein levels. The conversion ratio of tryptophan–niacin in rats fed on the 70% casein diet became extremely low, and then the rats needed niacin.**

**Key words:** tryptophan; necessity of niacin; high protein diet; conversion ratio of tryptophan–niacin

Because more than 500 enzymes need niacin coenzymes, it is important to determine the control mechanisms of the coenzyme supply *in vivo*. Mammals including humans can biosynthesize niacin from an indispensable amino acid Trp. Therefore, many nutritionists including our group claim that niacin itself is not necessary when Trp is taken suitably. In fact, it lacks any influence on the growth of young rats even if they are given niacin-free diets containing a suitable amount of protein, such as 20% casein diets.[1] However, we found that rats needed niacin for maximum growth when they are fed a 70% casein diet. Our paper explains our methods and results.[1]

## Materials and Methods

*Chemicals.* Vitamin-free milk casein, sucrose, L-methionine, Nam, and L-Trp were purchased from Wako Pure Chemical Industries (Osaka, Japan). Kynurenine sulfate, KA, and MNA chloride were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). 2-Py and 4-Py were synthesized by the methods of Pullman and Colowick[2] and Shibata *et al.*[3] respectively. Corn oil was purchased from Ajinomoto (Tokyo, Japan). The mineral (AIN-93M-MX) and vitamin (AIN-93-VX) mixtures were obtained from Oriental Yeast Kogyo (Tokyo, Japan), all the other chemicals used being 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.

*Experiment 1* (70% casein diets with or without NiA in the presence of vitamin $B_6$). Male rats of the Wistar strain (4 weeks old with a body weight of around 60 g) were obtained from CLEA Japan (Tokyo, Japan) and immediately placed in individual metabolic cages (CT-10; CLEA Japan). To acclimatize the rats to these conditions, they were initially fed *ad libitum* for 7 d with a complete 20% casein diet[1] and water. They were then divided into the two groups and fed *ad libitum* for 19 d, with a 70% casein diet with or without NiA in the presence of vitamin $B_6$ (Table 1).

The room temperature was kept at 22 ± 2 °C at about 60% humidity, and a 12-h light/12-h dark cycle was maintained. Body weight and food intake were measured periodically, usually every other day at 9:00–10:00 a.m. Urine samples (24-h; 9:00 a.m.–9:00 a.m.) were collected for the last day of the experiment in amber bottles containing 1 ml of 1 M HCl, and were stored at −25 °C until needed. The rats were killed by decapitation after the collection of urine samples. The liver of each animal was removed, and a portion of it (approximately 1 g) was treated as described in the literature[4,5] to measure the enzyme activities involved in the metabolism of Trp to niacin.

*Experiment 2* (70% casein diets with or without NiA in the absence of vitamin $B_6$). The same procedure was performed as with Experiment 1 except for the diet, from which was removed only vitamin $B_6$, as shown in Table 1.

† To whom correspondence should be addressed. Tel: +81-749-28-8439; Fax: +81-749-28-8601; E-mail: kshibata@shc.usp.ac.jp

*Abbreviations*: Trp, L-tryptophan; XA, xanthurenic acid; KA, kynurenic acid; 3-HK, 3-hydroxyanthranilic acid; Nam, nicotinamide; MNA, $N^1$-methylnicotinamide; 2-Py, $N^1$-methyl-2-pyridone-5-carboxamide; 4-Py, $N^1$-methyl-4-pyridone-3-carboxamide; ACMSDase, aminocarboxymuconate-semialdehyde decarboxylase

**Table 1.** Composition of the 70% Casein Diets

| | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | +NiA & +$B_6$ % | −NiA & +$B_6$ % | +NiA & −$B_6$ % | −NiA & −$B_6$ % |
| Vitamin-free milk casein | 70 | 70 | 70 | 70 |
| L-Methionine | 0.5 | 0.5 | 0.5 | 0.5 |
| Sucrose | 18.5 | 18.5 | 18.5 | 18.5 |
| Corn oil | 5 | 5 | 5 | 5 |
| Mineral mixture* | 5 | 5 | 5 | 5 |
| Vitamin mixture* | 1 | 0 | 0 | 0 |
| NiA-free vitamin mixture* | 0 | 1 | 0 | 0 |
| $B_6$-free vitamin mixture* | 0 | 0 | 1 | 0 |
| NiA and $B_6$-free vitamin mixture* | 0 | 0 | 0 | 1 |

*AIN 93 was used (Reeves, P.G., Components of the AIN-93 diets as improvements in AIN-76A diet. *J. Nutr.*, **127**, 838S–841S (1997)). The diet (+NiA & +$B_6$) contained 6 mg NiA and 0.8 mg of pyridoxine-HCl per 100 g of diet.

**Table 2.** Composition of the 40% and 60% Casein Diets

| | 40% Casein diet | | 60% Casein diet | |
|---|---|---|---|---|
| | +NiA % | −NiA % | +NiA % | −NiA % |
| Vitamin-free milk casein | 40 | 40 | 60 | 60 |
| L-Methionine | 0.4 | 0.4 | 0.6 | 0.6 |
| Sucrose | 48.6 | 48.6 | 28.4 | 28.4 |
| Corn oil | 5 | 5 | 5 | 5 |
| Mineral mixture* | 5 | 5 | 5 | 5 |
| Vitamin mixture* | 1 | 0 | 1 | 0 |
| NiA-free vitamin mixture | 0 | 1 | 0 | 1 |

*AIN 93 was used (Reeves, P.G., Components of the AIN-93 diets as improvements in AIN-76A diet. *J. Nutr.*, **127**, 838S–841S (1997)). The diet (+NiA & +$B_6$) contained 6 mg NiA and 0.8 mg of pyridoxine-HCl per 100 g of diet.

*Experiment 3* (40% and 60% casein diets with or without NiA in the presence of vitamin $B_6$). The same procedure was performed as with Experiment 1 except for the diets was done. The composition of the diets used in Experiment 3 is shown in Table 2.

*Analyses.* To measure the conversion ratio of Trp to niacin, the urinary contents of Nam and its metabolites MNA, 2-Py, and 4-Py were measured. This method does not take account of the increased body store of Nam during growth, and the value does not, therefore, represent the net conversion ratio. However, this value is useful for the assessment of the apparent conversion ratio. The conversion ratio was calculated as the sum of the urinary excretions of {Nam + MNA + 2-Py + 4-Py(μmol/day)} × 100/Trp intake during urine collection (μmol/day). The contents of Nam, 2-Py, and 4-Py in the urine were simultaneously measured by the HPLC method of Shibata *et al.*,[3] while the content of MNA in the urine was measured by the HPLC method of Shibata.[6]

The contents of KA[7] and XA[8] in the urine were measured by HPLC.

Trp oxygenase (EC 1.13.11.11),[9] kynureninase (EC 3.7.1.3: the reaction was done in the absence of added pyridoxal 5′-phosphate),[7] kynurenine aminotransferase (EC 2.6.1.7: the reaction was done in the absence of added pyridoxal 5′-phosphate),[10] 3-HA oxygenase (EC 1.13.1.1),[9] kynurenine 3-hydroxylase (EC 1.14.13.9: the reaction was done in the presence of added NADPH),[11] ACMSDase (EC 4.1.1.45),[12] NMN adenylyltransferase (EC 6.3.5.1),[13] Nam methyltransferase (EC 2.1.1.1),[14] 2-Py-forming MNA oxidase (EC 1.2.3.1),[14] and 4-Py-forming MNA oxidase (EC number not identified)[12] were measured as described in the literature.

## Results

*Experiment 1* (70% casein diets with or without NiA in the presence of vitamin $B_6$)

Table 3 shows the effects of feeding the 70% casein diet with or without NiA on the body weight gain, food intake, and food efficiency ratio. The food intake was almost the same between the two groups, but the body weight gain was significantly lower in the group fed on the NiA-free diet, as shown in Fig. 1. As a result, the food efficiency ratio was significantly lower in the −NiA group than in the +NiA group. That is, the necessity of niacin itself was observed in the 70% casein diet, even when a sufficient amount of Trp was taken.

The urinary excretion of KA and XA in terms of nmol/g of diet is shown in Table 4. The urinary

**Table 3.** Effects of Feeding the 70% Casein Diet with or without NiA on Body Weight Gain, Food Intake, and Food Efficiency Ratio (Experiment 1)

| | +NiA & +$B_6$ | −NiA & +$B_6$ |
|---|---|---|
| Initial body weight (g) | 102 ± 2 | 105 ± 1 |
| Final body weight (g) | 205 ± 5 | 178 ± 4* |
| Body weight gain (g/19 days) | 103 ± 5 | 73 ± 3* |
| Food intake (g/19 days) | 231 ± 6 | 223 ± 3 |
| FER[1] | 0.45 ± 0.02 | 0.33 ± 0.01* |

[1]FER, Food Efficiency Ratio.
*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's $t$ test.

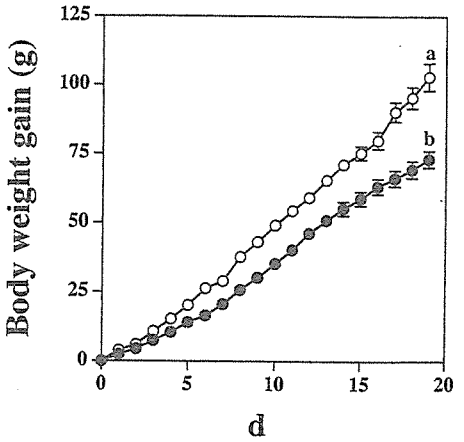


**Fig. 1.** Effects of Feeding the 70% Casein Diets with or without NiA on Body Weight Gain (Experiment 1).

○, +NiA & +$B_6$; ●, −NiA & +$B_6$. Each point represents the mean ± SEM for five rats. Values with different superscript letters are statistically significantly different at $p < 0.05$, as calculated by the Student–Newman–Keuls Multiple Comparisons test.

**Table 4.** Effects of Feeding the 70% Casein Diets with or without NiA on the Urinary Excretion of KA and XA, Nam and Its Metabolites, the Excretion Ratio of (2-Py + 4-Py)/MNA, and the Conversion Ratio of Trp to Niacin (Experiment 1)

| | +NiA & +$B_6$ | −NiA & +$B_6$ |
|---|---|---|
| Food intake (g/day) | 14.3 ± 0.3 | 15.0 ± 0.7 |
| KA | 198 ± 17 | 163 ± 15 |
| XA | 79 ± 5 | 62 ± 5 |
| Nam | 16 ± 1 | 3 ± 1* |
| MNA | 87 ± 3 | 15 ± 3* |
| 2-Py | 80 ± 3 | 11 ± 2* |
| 4-Py | 331 ± 9 | 96 ± 5* |
| Sum[1] | 514 ± 13 | 125 ± 10* |
| (2-Py + 4-Py)/MNA | 4.7 ± 0.3 | 7.1 ± 0.8 |
| NiA intake | 487 ± 0 | 0 |
| Trp intake | 38783 ± 0 | 38783 ± 0 |
| Conversion ratio of Trp to Niacin (%) | Not calculated | 0.32 ± 0.03 |

[1]Sum, Nam + MNA + 2-Py + 4-Py.
Values are means ± SEM for five rats, expressed as nmol/g of food, except for (2-Py + 4-Py)/MNA and the conversion ratio of Trp to niacin and means ± SEM for five rats.
*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's $t$ test.

excretion of KA and XA was almost the same between the two groups. The urinary excretion of Nam, MNA, 2-Py, 4-Py, and the sum of Nam + MNA + 2-Py + 4-Py in terms of g of diet respectively is also shown in Table 4. The higher values of each of these in the +NiA group than in the −NiA group was attributed to the intake of dietary NiA. The conversion ratio could not be calculated for the group fed on the +NiA diet, but it could be done on the group fed on −NiA diet by comparison with Trp intake during urine collection and the urinary excretion of sum. The value was 0.32 ± 0.03% (mean ± SEM for 5 rats), as shown in Table 4.

**Table 5.** Effects of Feeding the 70% Casein Diets with or without Niacin on the Enzyme Activities Involved in the Metabolism of Trp to Niacin (Experiment 1)

| | +NiA & +$B_6$ | −NiA & +$B_6$ |
|---|---|---|
| Trp oxygenase | 2.03 ± 0.22 | 1.77 ± 0.22 |
| Kynureninase | 1.57 ± 0.05 | 1.55 ± 0.04 |
| Kynurenine aminotransferase | 1.14 ± 0.10 | 1.03 ± 0.02 |
| Kynurenine 3-hydroxylase | 1.54 ± 0.15 | 1.96 ± 0.40 |
| 3-HA oxygenase | 551 ± 35 | 550 ± 22 |
| ACMSDase | 11.3 ± 1.4 | 12.1 ± 1.1 |
| NMN adenylyltransferase | 8.97 ± 0.71 | 8.31 ± 0.46 |
| $NAD^+$ synthetase | 0.59 ± 0.12 | 0.61 ± 0.04 |
| Nam methyltransferase | 1.85 ± 0.03 | 1.89 ± 0.04 |
| 2-Py-forming MNA oxidase | 0.68 ± 0.07 | 0.71 ± 0.06 |
| 4-Py-forming MNA oxidase | 1.70 ± 0.08 | 1.59 ± 0.03 |

Values are expressed as μmol/h/g of liver and means ± SEM for five rats.

**Table 6.** Effects of Feeding the Vitamin $B_6$-Free, and 70% Casein Diets with or without Niacin on Body Weight Gain, Food Intake, and Food Efficiency Ratio (Experiment 2)

| | +NiA & −$B_6$ | −NiA & −$B_6$ |
|---|---|---|
| Initial body weight (g) | 106 ± 1 | 102 ± 2 |
| Final body weight (g) | 139 ± 5 | 115 ± 4* |
| Body weight gain (g/19 days) | 33 ± 4 | 13 ± 4* |
| Food intake (g/19 days) | 162 ± 2 | 141 ± 5* |
| FER[1] | 0.20 ± 0.02 | 0.09 ± 0.01* |

[1]FER, Food Efficiency Ratio.
Values are means ± SEM for five rats, expressed as μmol/h/g of liver and means ± SEM for five rats.
*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's $t$ test.

The next step was done to investigate the effects of the 70% casein diet with or without NiA on the enzyme activity of Trp to niacin. As Table 5 shows, none of the enzyme activities showed a difference between the two groups.

*Experiment 2* (70% casein diets with or without NiA in the absence of vitamin $B_6$)

Table 6 shows the effects of feeding the vitamin $B_6$-free, 70% casein diet with or without NiA on the body weight gain, food intake, and food efficiency ratio. The food intake was significantly lower in the −NiA group than in the +NiA group and the body weight gain was greatly lower in the group fed on the NiA-free diet, as shown in Fig. 2. As a result, the food efficiency ratio was significantly lower in the −NiA group than in the +NiA group. That is, the necessity of niacin itself was also ascertained in the vitamin $B_6$-free and 70% casein diet.

The urinary excretion of KA and XA in terms of nmol/g of diet is shown in Table 7. The urinary excretion of KA and XA was almost the same between the two groups. But that of XA was much higher in Experiment 2 than in Experiment 1 (Tables 4 and 7).

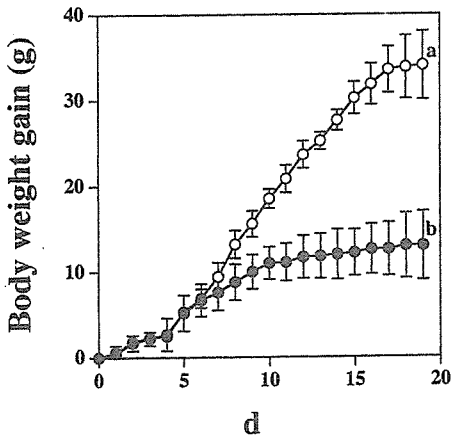


**Fig. 2.** Effects of Feeding the Vitamin $B_6$-Free and 70% Casein Diets with or without NiA on Body Weight Gain (Experiment 2).
○, +NiA & −$B_6$; ●, −NiA & −$B_6$. Each point represents the mean ± SEM for five rats. Values with different superscript letters are statistically significantly different at $p < 0.05$, as calculated by the Student–Newman–Keuls Multiple Comparisons test.

**Table 7.** Effects of Feeding the Vitamin $B_6$-Free, and 70% Casein Diet with or without NiA on the Urinary Excretion of KA and XA, Nam and Its Metabolites, the Excretion Ratio of (2-Py + 4-Py)/MNA, and the Conversion Ratio of Trp to Niacin (Experiment 2)

| | +NiA & −$B_6$ | −NiA & −$B_6$ |
|---|---|---|
| Food intake (g/day) | 7.4 ± 1.3 | 5.8 ± 1.1 |
| KA | 145 ± 5 | 144 ± 34 |
| XA | 1775 ± 173 | 2100 ± 234 |
| Nam | 16 ± 2 | 11 ± 4 |
| MNA | 295 ± 30 | 65 ± 11* |
| 2-Py | 23 ± 3 | 7 ± 1* |
| 4-Py | 137 ± 18 | 70 ± 6* |
| Sum[1] | 471 ± 48 | 153 ± 14* |
| (2-Py + 4-Py)/MNA | 0.54 ± 0.04 | 1.2 ± 0.06* |
| NiA intake | 487 ± 0 | 0 |
| Trp intake | 38783 ± 0 | 38783 ± 0 |
| Conversion ratio of Trp to Niacin (%) | Not calculated | 0.39 ± 0.05 |

[1]Sum, Nam + MNA + 2-Py + 4-Py.
Values are means ± SEM for five rats, expressed as nmol/g of food, except for (2-Py + 4-Py)/MNA and conversion ratio of Trp to niacin and means ± SEM for five rats.
*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's $t$ test.

The abnormal increase in XA means that the rats were in a vitamin $B_6$-deficient state. The urinary excretion of Nam, MNA, 2-Py, 4-Py, and the sum of Nam + MNA + 2-Py + 4-Py in terms of g of diet respectively is also shown in Table 7. The higher values of each of these in the +NiA group than in the −NiA group was attributed the intake of dietary NiA. The conversion ratio could not be calculated on the group fed on +NiA diet, but it could be done on the group fed on −NiA group by comparison with Trp intake during urine collection and the urinary excretion of sum. The value was 0.39 ± 0.05%, as shown in Table 7.

**Table 8.** Effects of Feeding Vitamin $B_6$-Free, and 70% Casein Diet with or without NiA on the Enzyme Activities Involved in the Metabolism of Trp to Niacin (Experiment 2)

| | +NiA & −$B_6$ | −NiA & −$B_6$ |
|---|---|---|
| Trp oxygenase | 1.82 ± 0.09 | 1.81 ± 0.14 |
| Kynureninase | 0.39 ± 0.09 | 0.32 ± 0.03 |
| Kynurenine aminotransferase | 0.38 ± 0.04 | 0.35 ± 0.04 |
| Kynurenine 3-hydroxylase | 3.07 ± 0.17 | 2.79 ± 0.18 |
| 3-HA oxygenase | 511 ± 39 | 565 ± 43 |
| ACMSDase | 11.1 ± 1.6 | 12.6 ± 1.5 |
| NMN adenylyltransferase | 8.16 ± 0.27 | 8.75 ± 0.57 |
| $NAD^+$ synthetase | 0.56 ± 0.07 | 0.56 ± 0.06 |
| Nam methyltransferase | 1.79 ± 0.02 | 1.82 ± 0.03 |
| 2-Py-forming MNA oxidase | 0.05 ± 0.02 | 0.10 ± 0.04 |
| 4-Py-forming MNA oxidase | 0.18 ± 0.09 | 0.74 ± 0.10* |

Values are expressed as μmol/h/g of liver and means ± SEM for five rats.
* Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's $t$ test.

The next step was done to investigate the effects of the 70% casein diet with or without NiA on the enzyme activity of Trp to niacin. As Table 8 shows, none of the enzyme activities except for 2-Py- and 4-Py-forming MNA oxidases showed a difference between the two groups.

*Experiment 3* (40% and 60% casein diets with or without NiA in the presence of vitamin $B_6$)

The body weight gain, food intake, and food efficiency ratio in the rats fed the 20%,[1)] 40%, and 60% casein diets with or without NiA are shown in Table 9. These values are almost the same among all of the groups irrespective of dietary protein levels and NiA intake.

Table 10 shows the urinary excretions of Trp–niacin metabolites in the groups of 20%,[1)] 40%, and 60% casein diets with and without NiA. The urinary excretions of KA and XA in terms of nmol/g of diet increased with dietary protein intake irrespective of the presence or absence of vitamin $B_6$. The higher values of Nam, MNA, 2-Py, and 4-Py in the +NiA group than in the −NiA group between each protein level were attributed the intake of dietary NiA. The conversion ratio could not be calculated on the group fed on +NiA diet, but it could be done on the group fed on −NiA diet by comparison with Trp intake during urine collection and the urinary excretion of sum. The value was 1.90 ± 0.25% for the 20% casein diet,[1)] 1.13 ± 0.07 for the 40% casein diet, and 0.60 ± 0.08% for the 60% casein diet.

Table 11 shows the activities of ACMSDase in the liver. The activities were not different irrespective of presence or absence of vitamin $B_6$ between the same protein levels, while the activities increased with dietary protein levels.