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Purification and Characterization of Corrinoid Compounds from a Japanese Fish Sauce

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ABSTRACT

A Japanese fish sauce "Ishiru," which was made from squid by a traditional food manufacturing process, contained the highest amounts ($5.5 \pm 2.3\,\mu g/100\,g$) of B_{12} among various fish sauces tested. Two corrinoid compounds were purified from the fish sauce Ishiru and partially characterized. TLC and HPLC patterns of the main red-colored compound, purified from the fish sauce, were identical to those of authentic vitamin B_{12}, but minor compounds could not be identified. Fish sauce may not be suitable for use as a good vitamin B_{12} source, judging from the low daily intake of the sauce and occurrence of the unknown corrinoid-compound.

Key Words: TLC; HPLC; Fish sauce; Fermented foods; Vitamin B_{12}.

INTRODUCTION

Various kinds of fish sauces, traditional food supplements in the diet, are widely used in the world as condiments, as flavoring material, and sometimes as a substitute for soy-bean sauce. A fish sauce (Nam-pla) appears to contribute a major source of vitamin B_{12} (B_{12}) in Thailand, since it contains considerable amounts of B_{12}.[1,2] Although our previous paper[3] has demonstrated that the amounts of B_{12} were several-fold greater in Japanese fish sauces than in some kinds of Nam-pla, thin layer chromatography (TLC) analysis indicated that most B_{12} found in the Japanese fish sauces were derived from unidentified corrinoid compounds. Our unpublished study indicated that a Japanese fish sauce "Ishiru," which was made from squid by a traditional food manufacturing process, contained the highest amounts of B_{12} among various fish sauces tested. It is, however, not clear whether B_{12} found in the Japanese fish sauce Ishiru is actual B_{12} or inactive corrinoids for humans.

Thus, corrinoid compounds found in the fish sauce Ishiru, were characterized by the use of TLC on silica gel as an important purification and analytical tool.

EXPERIMENTAL

Materials

B_{12} and a reversed-phase high pressure liquid chromatography (HPLC) column (Wakosil-II 5C18RS, ϕ 4.6 × 150 mm^2; particle size, 5 μm) were

obtained from Wako Pure Chemical Industries (Osaka, Japan). Cosmosil 140C180-OPN was obtained from Nacakai Tesque (Kyoto, Japan). A B_{12} assay medium for *Lactobacillus delbrueckii* subsp. *lactis* (formerly *L. leichmannii*) ATCC7830 was obtained from Nissui (Tokyo, Japan). Silica gel 60 (TLC) aluminum sheets were obtained from Merck (Darmstadt, Germany). Amberlite XAD-4 was obtained from Japan Organo Co. (Tokyo, Japan). Cyanocobamides (5-hydroxybenzimidazolylcyanocobamide, benzimidazolylcyanocobamide, and 7-adenylcyanocobamide) isolated from bacteria, were kindly provided by Dr. E. Stupperich, Ulm University, Germany. All other reagents used were of the highest purity commercially available. The Japanese fish sauce Ishiru used in the experiments was provided from a local market in Kanazawa-city, Ishikawa-prefecture, Japan.

A Shimadzu (Kyoto, Japan) UV-visible spectrophotometer (UV-1600) was used for measuring turbidity of *L. delbrueckii* test culture in the microbiological B_{12} assay method. A fully automated chemiluminescence B_{12} analyzer ACS 180 (Chiron Diagnostics, East Walpole, MA) was used for B_{12} assay.

Methods

Assay of Vitamin B_{12}

B_{12} was assayed by the microbiological method with *L. delbrueckii* ATCC 7830 and a B_{12} assay medium (Nissui, Tokyo, Japan), and by the fully automated chemiluminescence B_{12} analyzer ACS 180 (IF-chemiluminescence) as described previously.[4]

Purification of Corrinoid Compounds from the Fish Sauce Ishiru

One liter of the fish sauce Ishiru was added to 1 L of 0.1 mol/L acetate buffer, pH 4.8, containing 10 mmol/L KCN. Total B_{12} was extracted from the solution by boiling for 30 min, in the dark, at 98°C. The extraction procedures were done in a Dalton (Tokyo, Japan) draught chamber with fume hood. The boiled solution was cooled to room temperature and used for purification of corrinoid compounds. Amberlite XAD-4 resin (500 g), washed with 5 L of methanol and equilibrated with distilled water, was added to the boiled solution and stirred for 3 hr at room temperature in the dark. The resin suspension was passed through a glass funnel (Buchner type) with a glass filter (type 25G1, Iwaki, Tokyo, Japan) and the resin was washed with 5 L of distilled water. The washed resin was added to 1 L of 80% (v/v) methanol solution, and stirred for 3 hr at room temperature in the dark. The resin suspension was passed through the glass funnel.

The 80% (v/v) methanol eluant (about 1 L) containing corrinoid compounds was pooled, evaporated to dryness under reduced pressure, and dissolved in 30 mL of distilled water.

After a column (24 × 120 mm^2) of Cosmosil 140C18-OPN (Nacalai Tesque, Kyoto, Japan) was washed with 75% (v/v) ethanol solution and equilibrated with distilled water, the solution was put on the column and eluted with a linear gradient (0–90% v/v) of ethanol. The B_{12}-active fractions were assayed by the IF-chemiluminescence method, pooled, evaporated to dryness under reduced pressure, and dissolved with a small amount of distilled water. The concentrated solution was purified by silica gel 60 TLC, which was developed with 2-propanol/NH_4OH (28%)/water (7 : 1 : 2 v/v/v) as a solvent, in the dark, at room temperature. The dried TLC sheets were fractionated by cutting them into small pieces. Corrinoid compounds were extracted from the pieces with 80% (v/v) methanol, evaporated to dryness under reduced pressure, and dissolved in a small amount of distilled water. The B_{12}-active fractions were assayed by the IF-chemiluminescence method. The concentrated solution was further purified by HPLC, using a Shimadzu HPLC apparatus (LC-6A Pump, SPD-6A Spectrophotometer, CTO-6A column oven, C-R6A Chromatopac). The sample (100 μL) was put on a reversed-phase HPLC column (Wakosil-II 5C18RS, ϕ 4.6 × 150 mm^2; particle size, 5 μm) equilibrated with 20% (v/v) methanol solution containing 1% (v/v) acetic acid at 35°C. The flow rate was 1 mL/min. The corrinoid compounds were isocratically eluted with the same solution, monitored by measuring absorbance at 278 nm, and collected at 1 mL with a Bio-Rad Laboratories fraction collector (Model 2110). The B_{12}-active fractions were assayed by both microbiological and IF-chemiluminescence methods. B_{12}-active fractions were separated as two peaks. Each peak was pooled, evaporated to dryness under reduced pressure, and dissolved in 0.1 mL of distilled water. Each concentrated solution was put on a silica gel 60 TLC sheet and developed with 2-propanol/NH_4OH (28%)/water (7 : 1 : 2 v/v/v) as the mobile phase, in the dark, at 25°C. Each pink-colored spot on the dried TLC sheet was collected, extracted with 80% (v/v) methanol, evaporated to dryness under reduced pressure, and dissolved in 20 μL of distilled water, and used as a purified corrinoid compound.

Analytical TLC and HPLC

The concentrated solutions (2 μL) of each corrinoid compound purified from the fish sauce, and cyanocobamides (benzimidazolyl-, 5-hydroxybenzimidazolyl-, and 7-adenyl-cyanocobamides) were spotted on the silica gel 60 TLC sheet and developed with 2-propanol/NH_4OH (28%)/water (7 : 1 : 2 v/v/v) as the mobile phase, in the dark, at 25°C. The TLC sheet was dried and R_f values of the pink-colored spots of the corrinoids were determined.

In the case of HPLC, the concentrated solutions (2 μL) of each purified corrinoid compound and these cyanocobamides, were analyzed with the reversed-phase HPLC column (Wakosil-II 5C18RS, ϕ 4.6 × 150 mm^2; particle size, 5 μm) and the Shimadzu HPLC apparatus. The corrinoids were isocratically eluted with 20% (v/v) methanol solution containing 1% (v/v) acetic acid at 35°C, and monitored by measuring absorbance at 278 nm. The retention times of these corrinoids were determined at the flow rate of 1 mL/min.

RESULTS AND DISCUSSION

The Japanese fish sauce Ishiru, which was made from squid by a traditional food manufacturing process, contained the highest amount of B_{12} (5.5 ± 2.3 μg/100 g) among various fish sauces tested using the IF-chemiluminescence method.

To determine whether the corrinoid compounds found in the fish sauce "Ishiru" are true B_{12} or inactive corrinoid compounds for humans, corrinoid compounds were purified and characterized. Figure 1 shows elution profiles of corrinoid compounds from the fish sauce Ishiru on a reversed-phase HPLC during purification. Corrinoid compounds were eluted as two peaks (main and minor) when assayed by both microbiological and IF-chemiluminescence methods. Each final purified preparation gave a single pink-colored spot by TLC on silica gel 60 (Fig. 2).

The purified corrinoid compounds, authentic B_{12}, and cyanocobamides (7-adenyl-, 5-hydroxybenzimidazolyl-, and benzimidazolyl-cyanocobamides), which occur in bacteria, were analyzed by silica gel 60 TLC and reversed-phase HPLC (Table 1). The R_f value (0:61) of the main corrinoid compound I was identical to the value of authentic B_{12}, of which the retention time (9.4 min) was also identical to that of the main corrinoid compound in reversed-phase HPLC. R_f value and retention time of the minor corrinoid compound II were not identical to those of any authentic corrinoids tested.

Further detailed information on the fish sauce corrinoid compounds was not available because large amounts of the purified samples were not obtained for NMR study.

Although some (5-hydroxybenzimidazolyl- and benzimidazolyl-cyanocobamides) naturally occurring corrinoid compounds are fully active for the binding of IF[5] and growth of *L. delbrueckii* ATCC7830,[6] 7-adenylcyanocobamide reveals moderate affinity to IF[5] and is inactive for pernicious anemia.[6] Although corrinoid compounds inactive for the binding of IF are probably not absorbed in mammalian intestine by the IF-mediated system, the minor corrinoid compound II was capable of binding to IF. We have no

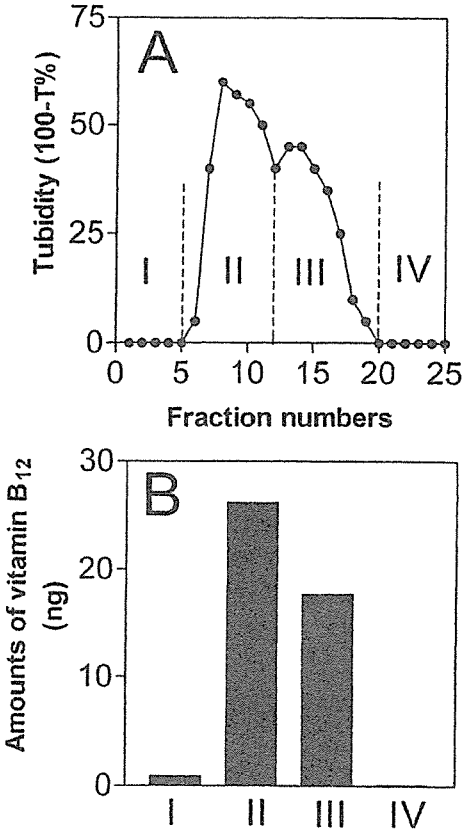

Figure 1. Elution profiles of corrinoid compounds from a Japanese traditional fish sauce "Ishiru," during a reversed-phase HPLC in the purification steps. Corrinoid compounds were determined by the microbiological method. (A) Fractions 1–5 (I), 6–12 (II), 13–20 (III), and 21–25 (IV) were combined and assayed for corrinoid compounds by the IF-chemiluminescence method. (B) Data present a typical elution pattern of corrinoid compounds by HPLC from three experiments.

information available on whether the minor corrinoid compound II is active or inactive for humans.

Areekul et al.[1] have reported that a human would obtain 0.1–0.4 μg of B_{12} per day from fish sauce in Thailand. Fish sauce may not be suitable for use as a good source of B_{12}, judging from the low daily intake [4.2–16.7% of

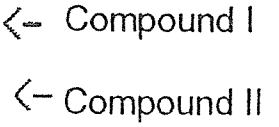

Figure 2. Silica gel 60 TLC pattern of the purified corrinoid compounds. Data present a typical migration pattern of the purified corrinoid compounds by TLC from three experiments.

Table 1. R_f values and retention times of the purified corrinoid compounds, authentic B_{12}, and cyanocobamides on TLC and HPLC.

	TLC (R_f)	HPLC (min)
Main compound I	0.61	9.4
Minor compound II	0.55	14.5
B_{12}	0.61	9.4
Benzimidazolylcyanocobamide	0.57	7.3
5-Hydroxybenzimidazolylcyanocobamide	0.49	7.0
7-Adenylcyanocobamide	0.48	7.7

the recommended dietary allowance for adults (2.4 μg/day)] and the possibility that the unidentified corrinoid compounds generally occur in various fish sauces.[3]

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ノ ー ト

日本人の母乳中ビタミンB_6含量

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The Vitamin B_6 Content in Milk of Japanese Women

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The recommended dietary allowance (RDA) of vitamin B_6 (B_6) for Japanese was formulated for the first time in the 6th revised National Reference Intake in Japan. The RDA of infant is 0.1mg/day in the 6th revision. Milk intake of infant was calculated to be 850 ml/day in the 5th revised RDA, however it was set at 750 ml/day based on investigation in the 6th revised RDA. The B_6 intake of infant deeply depended on the content of B_6 in breast milk. Thus we determined the B_6 contents in breast milk of Japanese women.

Milk samples were collected from 25 healthy nursing women whose infants were 2 ～ 5 ages in month. Total B_6 content in milk was quantified by HPLC. The average content of B_6 was 0.25 mg/*l* breast milk. This value was not different from those of other studies and the datum in 6th revised RDA.

Key Words: vitamin B_6, human milk, Japanese women, infant, RDA

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緒 言

日本人の栄養所要量は，昭和45年に初回の策定が施行されて以来，日本人の体格，生活習慣などに合わせて5年ごとに改定されている．平成12年度(2000年)から実施されている第6次改定[1]では，食事摂取基準が設けられ，さらにビタミン6項目，ミネラル7項目が新規に追加された．ビタミンB_6(B_6)は第6次改定により初めて策定されたビタミンのひとつである．成人の所要量は，疫学調査を基に求められているが乳児の所要量は摂取する母乳に依存する．母乳は3大栄養素の

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略語：PCA, Perchloric acid; PL, Pyridoxal; PLP, Pyridoxal 5'-phosphate.

ほかビタミン，ミネラルなどバランスよく含む食品であり[2]，乳児の成育には最も適した栄養源である．

第6次改定日本人の栄養所要量での乳児の B_6 所要量は，0.1 mg/日と定められている[1]．これは 2.5 mg/日以下の B_6 摂取量の健康な母親の母乳中の B_6 含量が0.13 mg/*l* であるという West と Kirksey の報告[3]を基に設定されている．また第5次改定までは乳児の哺乳量を 850 ml/日として所要量を算定していたが，第6次改定においてこれまでの哺乳量の調査に基づいて，乳児の哺乳量は 750 ml/日と 100 ml 削減された．

乳児の所要量は母乳に含まれるビタミン B_6 含量と哺乳量に依存するため，これらを明らかにすることにより乳児の所要量を明確にできると考えられる．また，これまでのところ母乳中の B_6 含量については諸外国のデータがほとんどであり，食習慣や体格の違いを考慮すると日本人を対象にしたデータを用いて十分に検討する必要があると考えられる．そこで今回は，わが国の授乳婦から採取した母乳中の B_6 含量を測定した．

実 験 方 法

1．被験者

被験者は妊娠並びに出産が正常な経過で満期出産し，満月齢で 2 ～ 5ヶ月の乳児を完全母乳哺育している日本人授乳婦を対象にした．対象者の摂取している食事組成については不明であるが，栄養に対する興味も高くバランスのとれた食事を摂取していることを前提とし，本趣意に同意し体調の良いボランティアから採取した母乳 25 検体を使用した．

また今回の研究は，全てヘルシンキ宣言に従って実施され，昭和女子大学における倫理委員会より承認を得て実施したものである(承認番号 01-06　平成 14 年 2 月 4 日承認)．

2．母乳採取

母乳は，ほぼ 14 時～ 16 時の授乳後に，乳房をマッサージした後の後乳を採取して，冷凍母乳パック(カネソン本舗社製)に保存し，分析に供するまで－20℃にて保存した．

3．ビタミン B_6 の分析

母乳中の PLP 濃度および B_6 ビタマー濃度は，HPLC 法を用いて測定した[4]．母乳サンプル 0.5 ml は 3*N* 過塩素酸(PCA)0.25 ml で除タンパク質処理を行った．この上清に 1 M リン酸ナトリウム緩衝液(pH 5.5)を 0.2 ml 加え，5N KOH で pH を 3.5 に調整したものを PCA 抽出液とした．PLP の検出は PCA 抽出液を pH 7.5 に調整してからシアン化カリウムで処理を行い，再び pH 3.5 に調整してから HPLC 分析に供した．また他の B_6 ビタマーは，PCA 抽出液を 0.45 μM のメンブランフィルターに通した後，HPLC 分析に供した．HPLC 分析の条件は以下の通りである．

HPLC分析条件
検出波長；
＜PLP＞励起波長 320 nm，蛍光波長 420 nm
＜PLP 以外の B_6 ビタマー＞
励起波長 305 nm，蛍光波長 390 nm
流速；0.5 ml/min
温度；30℃
移動相；CH_3CN / 0.1M $KHPO_4$ - 0.1M $NaClO_4$ (pH 3.5) = 1 : 99 (v/v)
カラム；TOSOH TSKgel ODS-120A (4.6 mm ID 25 cm)

結　　果

表 1 および図 1 は，日本人授乳婦より採取した母乳に含まれる B_6 含量の平均値と 25 検体の総 B_6 量の分布を示したものである．分析の結果，PLP と PL 以外のビタマーは検出されなかった．分析した 25 検体の母乳中 B_6 含量は，全平均で PLP が 1.15±0.09 μM，PL が 0.30± 0.02 μM であり，全 B_6 濃度として 1.45±0.09 μM (0.25 mg/*l* PN 換算量)であった．

考　　察

これまでに報告されている母乳中の B_6 含量を表 2 に示した．母乳中に含まれる B_6 濃度は，母親の B_6 摂取量に依存して変化することが報告[3]されている．Borschel ら[5]は，母親が 2.5 mg/日の B_6 摂取量であると，乳児の B_6 摂取量は 0.1 mg/日であるとしている．また West と Kirksey[3]によると 2.5 mg/日以下の B_6 摂取量の母親の母乳中の B_6 含量は，0.13 mg/*l* であるとし，Thomas ら[6]は，0.21 mg/*l* であると報告している．第6次改定日本人の栄養所要量では，2.5 mg/日以下の B_6 摂取をしている母親

表 1．Content of Vitamin B_6 in Human Milk.

(μM)	PLP	PL	Total B_6
	1.15±0.09	0.30±0.02	1.45±0.09

Mean ± S.E. (n=25)

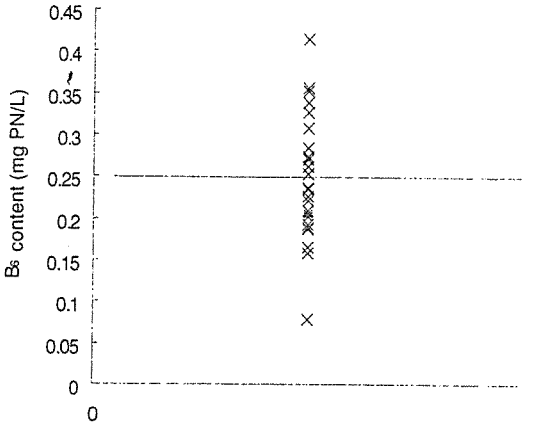

図 1. Distribution of B_6 content in human milk. ※ Line shows mean value (n=25).

表 2. Content of Vitamin B_6 in Human Milk.

Literature	Year	B_6 content in human milk (mg/*l*)	Methods of analysis
West and Kirksey[3]	1976	0.13	microbiological assay
Thomas *et al.*[10]	1979	0.204	microbiological assay
Thomas *et al.*[6]	1980	0.21	microbiological assay
Börschel *et al.*[5]	1986	0.11～0.33	microbiological assay
Andon *et al.*[7]	1989	0.124※	microbiological assay
Morrison and Driskell[8]	1985	0.162※	HPLC
Present study	2004	0.25	HPLC

※文献では nM 表記であったが，PN 換算の数値として示した．

の母乳中の B_6 含量は 0.13 mg/*l* であるという報告に基づき，一日の哺乳量が 750 ml として 0～5ヶ月齢の乳児の所要量は 0.1 mg/日と設定されている．

一般に食品中の B_6 含量を測定する方法は，*Saccharomyces cerevisiae* ATCC9080 を用いた微生物定量法が主である．この方法は全 B_6 化合物を一括して定量することができるため簡便である．しかし，*S. cerevisiae* はリン酸エステル型を含めた結合型誘導体を利用できないため，定量に先立ち試料を加水分解し遊離型に変換するための前処理操作が必要である．最近では HPLC 法による B_6 定量法が用いられており，微生物定量法を用いた Andon ら[7]の報告と，HPLC 法を用いた Morrison と Driskell[8]の測定法による差異は認められない．本研究では Tsuge[4]の方法による HPLC 法を用いて母乳中の B_6 含量を測定した．

West と Kirksey[3]は産後の日数の違いによる母乳中 B_6 含量についても報告している．これによると 2.5 mg/日あるいはそれ以上の B_6 摂取をしている母親において，産後 3ヶ月以内の母乳中 B_6 含量は，0.26 mg/*l*，3～7 ヶ月では 0.29 mg/*l*，7 ヶ月以上では 0.25 mg/*l* と，産後の日数の違いによる変動は見られていない．今回の母乳は産後 2～5ヶ月であり，母乳中の B_6 含量は平均で 0.25 mg/*l* であった．これは，これまでに報告されている同時期の母乳中の含量とほぼ同値であった．

わが国の調製粉乳に含まれる B_6 量[9]は，一般調製粉乳で製品 100 g 当たり 0.3～0.6 mg(60～120 μg/100 kcal，あるいは 14 % 調製乳液 100 ml 当たり 42～84 μg)である．この人工乳を 1 日 750 ml 与えると，0.32～0.63 mg/日の B_6 を摂取できることになる．この値は，第 6 次改定日本人の栄養所要量に示されている 0.1 mg/日を十分に満たしていることになる．Borschel ら[5]の報告によると，人工乳により 0.45～0.58 mg/日の B_6 を摂取している乳児と，B_6 の摂取量が 2.5 mg/日以下の母親の母乳により 0.11～0.33 mg/日の B_6 量を摂取している乳児の成

育は，6ヶ月齢までは身長，体重共に有意な差は見られなかったと報告している．これより第6次改定日本人の栄養所要量で定められている乳児の所要量 0.1 mg/日は成育には適していると考えられる．今回の調査は日本人の乳児の B_6 所要量に対する基礎データとして有用であると考えられる．

謝　辞

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

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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.

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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.

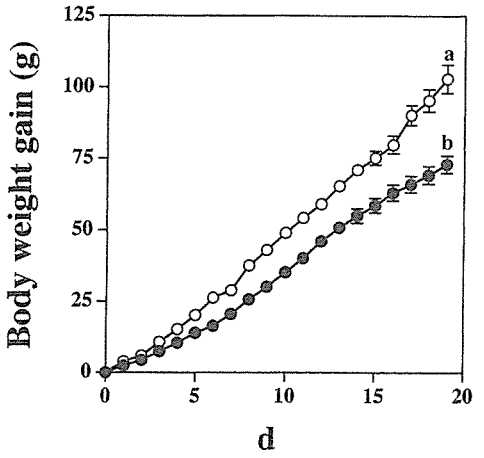

Fig. 1. Effects of Feeding the 70% Casein Diets with or without NiA on Body Weight Gain (Experiment 1).

○, +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).

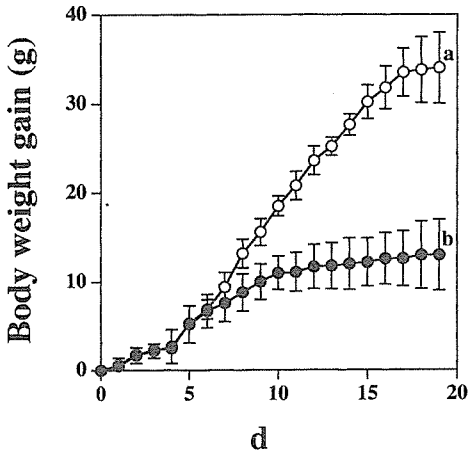

Fig. 2. Effects of Feeding the Vitamin B_6-Free and 70% Casein Diets with or without NiA on Body Weight Gain (Experiment 2).
○, +NiA & $-B_6$; ●, −NiA & $-B_6$. Each point represents the mean ± SEM for five rats. Values with different superscript letters are statistically significantly different at $p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

Table 9. Effects of NiA Addition to the 20%, 40%, and 60% Casein Diets on Body Weight Gain, Food Intake, and Food Efficiency Ratio (Experiment 3)

	20% Casein diet[1]		40% Casein diet		60% Casein diet	
	+NiA	−NiA	+NiA	−NiA	+NiA	−NiA
Initial body weight (g)	103 ± 2	106 ± 2	102 ± 2	102 ± 2	102 ± 1	102 ± 1
Final body weight (g)	216 ± 4	216 ± 4	217 ± 5	223 ± 5	213 ± 4	212 ± 4
Body weight gain (g/19 d)	113 ± 4	110 ± 3	115 ± 4	121 ± 4	111 ± 3	110 ± 4
Food intake (g/19 d)	273 ± 5	287 ± 5	284 ± 5	294 ± 6	262 ± 6	260 ± 3
Food efficiency ratio*	0.41 ± 0.01	0.38 ± 0.01	0.40 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.42 ± 0.02

*FER, body weight gain (g/19 d)/food intake (g/19 d).
Each value is the mean ± SEM for five rats.
[1]Data were drawn from reference 1.

Table 10. Effects of Feeding the 20%, 40%, and 60% 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 3)

	20% Casein diet[1]		40% Casein diet		60% Casein diet	
	+NiA	−NiA	+NiA	−NiA	+NiA	−NiA
Food intake (g/day)	16.7 ± 0.6	18.0 ± 0.8	17.9 ± 0.6	18.5 ± 0.7	17.9 ± 0.6	16.5 ± 0.4
KA	35 ± 2.1	40 ± 5.6	105 ± 7.5	126 ± 20.5	156 ± 26.7	191 ± 8.7
XA	28 ± 2.5	36 ± 4.7	55 ± 6.4	51 ± 4.2	67 ± 7.8	81 ± 3.1
Nam	16 ± 0.8	13 ± 1.3	17 ± 1.4	9 ± 0.4	9 ± 1.4	7 ± 0.3
MNA	55 ± 1.1	18 ± 1.8*	57 ± 4.9	22 ± 2.2*	84 ± 8.1	24 ± 1.1*
2-Py	61 ± 2.4	16 ± 2.7*	55 ± 5.3	20 ± 1.9	66 ± 4.1	21 ± 0.9*
4-Py	484 ± 19	161 ± 21*	449 ± 53	199 ± 14*	257 ± 30	149 ± 19*
Sum[1]	616 ± 18	208 ± 27*	578 ± 57	250 ± 17*	416 ± 35	201 ± 20*
(2-Py + 4-Py)/MNA	9.9 ± 0.4	9.8 ± 0.5	8.8 ± 1.5	10.0 ± 1.1	3.8 ± 0.6	7.1 ± 0.7
NiA intake	487 ± 0	0	487 ± 0	0	487 ± 0	0
Trp intake	11081 ± 0	11081 ± 0	22162 ± 0	22162 ± 0	33242 ± 0	33242 ± 0
Conversion ratio of Trp to niacin (%)	Not calculated	1.90 ± 0.25	Not calculated	1.13 ± 0.07	Not calculated	0.60 ± 0.08

[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 respective the +NiA group, as evaluated by Student's t test.
[1]Data were drawn from reference 1.

Table 11. Effects of Feeding the 20%, 40%, and 60% Casein Diets with or without NiA on the ACMSDase Activity in the Liver (Experiment 3)

	20% Casein diet[1]		40% Casein diet		60% Casein diet	
	+NiA	−NiA	+NiA	−NiA	+NiA	−NiA
ACMSDase (μmol/h/g of liver)	2.4 ± 0.6	2.3 ± 0.6	3.81 ± 0.37	3.69 ± 0.57	8.85 ± 0.36	7.90 ± 1.16

[1]Data were drawn from reference 1.

Discussion

Sanada[15] and our group[5,16] have reported that the ACMSDase activity increases with dietary protein levels. We also found that the conversion ratio of Trp to niacin decreases with dietary protein levels.[5,16] Hence we thought that the ACMSDase controls niacin formation to shunt the excessive niacin supply. However, from the present experimental findings, we learned that the view of the Trp–niacin relationship that the ACMSDase controls the niacin formation is not right.

In a previous report,[1] we found that the body weight gains between the rats fed on a diet containing NiA and those fed on the diet minus only NiA were exactly the same. That is, when rats were fed on the 20% casein diet, they do not need niacin. Vitamin B_6 is important in the Trp–niacin metabolism, especially in the metabolism of kynurenine.[17] The body weight gain in young rats was significantly lower in the group fed on the 20% casein diet without vitamin B_6 than in the group fed on the 20% casein diet with vitamin B_6.[1] The lower body weight gain in the vitamin B_6-free group was due to a

deficiency of vitamin B_6.[1] In fact, the urinary excretion of XA, which is an indicator of vitamin B_6 deficiency,[18] was significantly higher in the vitamin B_6-free group than in the vitamin B_6-containing group.[1] However, no necessity of niacin on the 20% casein diet was observed in the diet without vitamin B_6.[1]

Feeding the NiA-free 70% casein diets rats caused a decrease in body weight gain as compared with the 70% casein diets containing NiA (Fig. 1).

In the groups fed on the NiA-free 20%, 40%, and 60% casein diets, the urinary excretion of the sum were 200–250 nmol/g of diet (Table 10), while it was around 130 nmol/g of diet in the groups on the NiA-free 70% casein diet (Tables 4 and 7). These results indicate that the rats fed on the 70% casein diet without NiA were niacin deficient. So the supplementation of NiA to the rats fed on the 70% casein diet caused the growth promoting action. These findings are very curious, since niacin is believed to be supplied from dietary Trp.[19] Under the 20% casein diet conditions, about 2% of Trp is converted to niacin.[1] On the contrary, when rats were fed on 70% protein diets, the conversion ratio was very low, about 0.3% (Table 4), and the rats, therefore, needed niacin for normal growth. The intake of Trp in the group fed on the 70% protein diet increased by 7/2 in comparison with the 20% casein diet, while the conversion decreased by 2/0.3. Therefore, the absolute formation of niacin was about half ($7/2 \times 0.3/2 = 0.525$) that of the rats fed on the 20% casein diet.

The conversion ratio was not affected by the presence or absence of vitamin B_6 on the 70% casein diets (Tables 4 and 7), although it was severely affected by the presence or absence of vitamin B_6 on the 20% casein diets.[1] In the experiment with the 70% casein diets, the urinary excretion of XA was much more increased by feeding the vitamin B_6-free diets (Table 7) than by feeding the B_6-containing diets (Table 4). That is, in Experiment 1, the rats were not vitamin B_6 deficient even when they were fed the 70% casein diet. Therefore, the necessity of niacin in the 70% casein diet was not associated with the nutritional state of vitamin B_6.[20]

The excretion of KA was almost the same among the four groups (Tables 4 and 7). The formations of KA and XA are catalyzed by the same enzyme, kynurenine aminotransferase, which is a PLP-dependent enzyme. This enzyme activity was much lower in the groups fed on the vitamin B_6-free diets (Table 8) than in those fed on the vitamin B_6-containing diets (Table 5). Nevertheless, the flux of Trp to XA increased greatly (Tables 4 and 7). The mechanism in the case of increased XA only can be explained as follows: Kynurenine was more efficiently converted to 3-hydroxykynurenine in the vitamin B_6-deficient rats than in the normal rats because the activity of kynurenine 3-hydroxylase was increased on the vitamin B_6-free diets (Tables 5 and 8), and 3-hydroxykynurenine, therefore, accumulates because the activity of kynureninase, which catalyzes the reaction of 3-hydroxykynurenine to 3-hydroxyanthranilic acid, decreased on the vitamin B_6-free diets (Tables 5 and 8). The accumulated 3-hydroxykynurenine in the group fed on two vitamin B_6-free diets in Experiment 2 was converted to XA by kynurenine aminotransferase. The reason the urinary excretion of KA did not increase might be increased kynurenine 3-hydroxylase.

The side flux of Trp increased in the groups fed on the two vitamin B_6-free diets in experiment 2, but the conversion ratio of Trp to niacin did not change between the groups fed on the diets with or without vitamin B_6 (Tables 4 and 7). This phenomenon has not been explained. The decreased conversion ratio in the high protein diets was due to the increased activity of ACMSDase as compared with that of the 20% protein diets (Tables 5 and 8 and Reference 1). It is a question why the activity of ACMSDase so increased on the high protein diets. High protein diets mean low carbohydrate diets, so that under the conditions, amino acids can be catabolized into energy formation pathways but not into protein synthesis and other biofactors. But in the present experiments it was clearly shown that the conversion ratio of Trp to niacin is subjected to the reaction α-amino-β-carboxymuconate-ε-semialdehyde $\rightarrow$ α-amino muconate-ε-semialdehyde, which is catalyzed by ACMSDase, and α-aminomuconate-ε-semialdehyde is then catabolized into acetyl-CoA, but not into niacin. On the contrary, the reaction α-amino-β-carboxymuconate-ε-semialdehyde $\rightarrow$ quinolinic acid is non-enzymatic, and quinolinic acid is then metabolized into niacin. Accordingly, quinolinic acid formation from Trp is subjected to the activity of ACMSDase. The administration of an inhibitor of ACMSDase causes the greatly increased conversion ratio of Trp to niacin.[21]

In conclusion, we found that rats need dietary niacin when they are fed a 70% casein diet for maximum growth, while they do not need it when they are fed 20%, 40%, or 60% diets. This phenomenon is attributed to changes in the Trp–niacin conversion ratio due to the amount of protein intake. Therefore, when evaluating niacin requirements or status, protein intake must be considered.

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ノート

カツオ由来ナイアシン高濃度含有パウダーのナイアシンとしての生物有効性

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Bioavailability of Nicotinamide-Rich Powder Obtained from Bonito as a Niacin Source in Humans and Rats

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The bioavailability of niacin in bonito powder high in nicotinamide content was investigated in female college students. They were given a semi-purified diet based on the Japanese Dietary Reference Intakes for 4 days. On days 1, 2, they were given only the required diet for obtaining controlled urinary excretion of the nicotinamide metabolites. On day 3, 15 g of the bonito powder (5 g of the powder after each meal) containing 51 mg of nicotinamide was administered. The urinary excretion of the nicotinamide metabolites was significantly increased, with 52% being excreted on day 3. On day 4, they were given only the required diet, however, the urinary excretion of the nicotinamide metabolites was still higher than the scores for days 1 and 2. These findings indicate that the bioavailability of nicotinamide in the bonito powder was high and provided an excellent source of niacin.

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Keywords: bonito カツオ，niacin ナイアシン，bioavailability 生物有効性，nicotinamide-rich powder ナイアシン高濃度含有パウダー，vitamin ビタミン．

1. 緒 言

ナイアシンにはニコチン酸とニコチンアミドの他に糖やタンパク質と結合した結合型ナイアシンも存在する．結合型ナイアシンは穀類中に多く存在し，消化されにくいため，哺乳動物には利用されにくい[1]．したがって，ある食品をナイアシンの供給源として評価するためには，化学的な方法によって測定されたナイアシン量のみならず，生物有効性も重要な因子となる．

世界的にみると，ビタミンの欠乏症で最も頻繁にみられるのは，ペラグラである[2]．米国では，20世紀初頭に大発生し，公衆衛生上の大きな問題となり[3)~6)]，結果的に有効物質であるナイアシンの発見[7]に繋がったという経緯がある．Millerは[8]，1978年に米国におけるペラグラによる死者をまとめている．日本ではペラグラの発生と時を同じくしてビタミンB_1欠乏である脚気が流行したが[9]，ペラグラの発生は報告されていない．これは，ナイアシンの豊富な魚を食していたことと無関係ではないと思われる．カツオは特にナイアシン含量の高い魚であるが，保存の点で扱いにくい魚であった．しかし，最近，我々は，新しい手法で，保存性の優れたカツオ由来の高濃度ナイアシン含有パウダーを開発することに成功した（後述の実験方法の項を参照）．そこで，本実験では，ヒトを用いて，ナイアシン高含有カツオパウダー（カツオパウダー）に