**Effects of Dietary Fat and Protein on the Activity of
 α -Amino- β -Carboxymuconate- ϵ -Semialdehyde
Decarboxylase and the Urinary Excretion
of Niacin Metabolites in Rats**

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Summary The effects of dietary protein and soybean oil on the metabolism of L-tryptophan through the NAD pathway and the activity of α -amino- β -carboxymuconate- ϵ -semialdehyde decarboxylase (ACMSD) [EC 4.1.1.45] in rat liver and kidney were investigated. The animals were fed on the following niacin-free diets for 11 days: group 1, a fat-free 20% casein diet (20% Cas); group 2, a fat-free 40% casein diet (40% Cas); group 3, a 40% casein diet containing 20% soybean oil (40% Cas+20% F). After feeding on each diet for 7 days, the urine was collected during 48 h. On day 9, L-tryptophan was force-fed and the urine was collected during 48 h. Niacin metabolites such as *N*-methylnicotinamide (MNA), *N*-methyl-2-pyridone-5-carboxamide (2-Py) and *N*-methyl-4-pyridone-3-carboxamide (4-Py) in the urine samples were analyzed by chromatography. The activity of ACMSD in the liver and kidney was assayed immediately after the last urine collection. The results indicate that the urinary excretion of the niacin metabolites in the 40% Cas group was lower than that of the 20% Cas group in spite of the more intake of tryptophan in the former group. On the other hand, in the 40% Cas+20% F group, tryptophan intake was lower and the excretion of the metabolites was significantly higher than that in the 40% Cas group. The hepatic ACMSD activity in the 40% Cas group was 5.8 times that of the 20% Cas group and that in the 40% Cas+20% F group was one tenth that of the 20% Cas group. These results indicate that the ratio of the excreted metabolites to the tryptophan intake was reduced by the high dietary protein level, but increased by the addition of high soybean oil. The data analysis shows that the amount of urinary total niacin metabolites ([TNM]: MNA+2-Py+4-Py) could be expressed in the following equation in the rats fed on each diet:

$$[\text{TNM}] = 0.090([\text{Trp}] - 22.5 \cdot \Delta w) \{1/(1 + 2.4[\text{ACMSD}])\},$$

where [ACMSD] is the hepatic ACMSD activity, [Trp] the tryptophan intake, and Δw the body weight gain.

Key Words α -amino- β -carboxymuconate- ϵ -semialdehyde decarboxylase, niacin metabolites, triptophan-niacin metabolism

Conversion ratio of tryptophan to niacin under various physiological conditions is important in mammalian nutrition. α -Amino- β -carboxymuconate- ϵ -semialdehyde (ACMS), a key intermediate of tryptophan-NAD and tryptophan-acetyl-CoA pathways, enters the NAD pathway through its nonenzymatic cyclization to quinolinate (1). α -Amino- β -carboxymuconate- ϵ -semialdehyde, however, is decarboxylated by α -amino- β -carboxymuconate- ϵ -semialdehyde decarboxylase (ACMSD) [EC 4.1.1.45] to α -aminomuconate- ϵ -semialdehyde, and catabolized into tricarboxylic acid cycle (2,3). Thus L-tryptophan is hard to transform to NAD when the ACMSD activity is elevated in the liver. Such observation was found in diabetic (4,5) and hypophysectomized rats (6,7).

In our previous papers, the hepatic ACMSD activity was elevated by a high-protein diet (8) and suppressed by a diet contained unsaturated fatty acids (9). However, it is not clear whether these dietary factors could affect the metabolism of L-tryptophan *via* the NAD pathway. In the present study, we investigated the effects of dietary protein and soybean oil, which contains about 85% unsaturated fatty acids, on urinary niacin metabolites such as *N*-methylnicotinamide (MNA), *N*-methyl-2-pyridone-5-carboxamide (2-Py), and *N*-methyl-4-pyridone-3-carboxamide (4-Py) and the ACMSD activity in liver and kidney. Further, the data was analyzed to elucidate the relation among tryptophan intake, urinary niacin metabolites, hepatic ACMSD activity and body weight gain in the rats.

MATERIALS AND METHODS

Materials. Reagents were obtained as follows: *N*-methylnicotinamide (MNA) from Tokyo Kasei Kogyo, L-tryptophan and Nuchar C-190N (charcoal) from Wako Pure Chemical, Dowex 50 $\times$ 8 (200–400 mesh) and Dowex 1 $\times$ 8 (200–400 mesh) from Muromachi Kagaku Kogyo, cellulose powder CF11 from Whatman. Other chemicals were of the highest purity commercially available.

Preparation of 4-Py and 2-Py. 4-Py and 2-Py were purified from the urine of rats fed on a purified diet containing 0.1% nicotinamide. 4-Py was purified according to the methods of Chang and Johnson (10) with a slight modification by the authors. A paper chromatographic separation was performed by *n*-buthanol saturated with 3% aqueous ammonia as a developing solvent and Dowex 50 $\times$ 8 was used instead of IRA 400 ion-exchange resin. 2-Py was purified from the mixture containing 2-Py and 4-Py, which was prepared from 10% pyridine-extract from

Fig. 1. Separation of *N*-methyl-2-pyridone-5-carboxamide (2-Py) and *N*-methyl-4-pyridone-5-carboxamide (4-Py) from urine. *Rat urine samples diluted as described in METHODS were used.

charcoal (Nucher C-190N) column by Chang. 2-Py was separated from 4-Py by passing through Dowex 50×8 (H⁺) column and washing with distilled water. The passed solution and washings were collected, and evaporated to dryness at 45°C under vacuum. The residue was extracted with hot acetone. After concentrating the acetone solution, 2-Py was further purified by paper chromatography, the same as the procedure for 4-Py purification (10). 2-Py was extracted from the paper with hot acetone and crystallized at 4°C from the solution.

Animals and diets. Male Sprague Dawley rats (3 weeks of age) were purchased from Clea Japan (Tokyo). The compositions of the experimental diets are shown in Table 1. All diets were free of niacin. The diets were supplied as fresh puddings prepared by adding appropriate amount of water to the powdery diets (25 g dry weight per day per rat). After 24 h, the residue of the diet for each rat was dried at 105–110°C for 3 h and weighed. Then the consumptions of diets were calculated. They were housed in an air-conditioned room with a 12-h light and dark cycle.

Experimental design. When they were 4 weeks of age, they were assigned to three groups and were fed *ad libitum* on a fat-free 20% casein diet (20% Cas), a fat-free 40% casein diet (40% Cas) and a 20% fat-40% casein diet (40% Cas + 20% F) for 11 days. After animals were fed one week on the experimental diets, the urine was collected during 48 h in conical beakers containing a small amount of toluene. After the ending of the first urine collection, an L-tryptophan solution, 100

Table 1. Composition of experimental diet.

Ingredients	20% Cas (%)	40% Cas (%)	40% Cas+20% F (%)
Casein	20.0	40.0	40.0
Cornstarch	73.8	53.8	33.8
Soybean oil	0	0	20.0
Mineral mixture*	4.0	4.0	4.0
Vitamin mixture* (niacin-free)	1.0	1.0	1.0
Cellulose powder	1.2	1.2	1.2

*The composition of mineral mixture and vitamin mixture is the same as that described by Harper (15) and Sanada *et al.* (5), respectively.

mg dissolved in 4.5 ml of 1% acetic acid, was force-fed to each rat by a stomach tube. Then, the urine was collected during the following 48 h. All rats were decapitated at the end of the experiment, and their liver and kidney were removed then stored at -20°C until the enzyme assay. Urine samples were diluted to 120 ml with distilled water, and stored at -20°C until use for the analysis (Fig. 1) of niacin metabolites. The L-tryptophan intake was estimated from its content in casein according to the Tables of Amino Acid Composition of Foods (11).

The activities of ACMSD in liver and kidney were assayed as described previously (5).

RESULTS

Effect of dietary composition on body weight

No significant difference was observed in the body weights among the three groups before and after tryptophan force-feeding as shown in Fig. 2. However, the 20% Cas group was slightly heavier than the other groups during the whole experimental period as shown in Table 2.

Effect of dietary composition on food consumption

The food intake and tryptophan intake by the 40% Cas+20% F group were less than that of the 40% Cas group before and after tryptophan force-feeding (Table 2).

Analysis of niacin metabolites

The concentration of MNA, 2-Py, and 4-Py in urine was measured basically by the methods described by Price *et al.* (12) and Chang and Johnson (13). To separate niacin metabolites in urine, five glass columns were prepared: a Dowex 1 $\times$ 8 (acetate form) (14 $\times$ 30 mm height) column, a Dowex 50W $\times$ 8 (H^+) (14 $\times$ 25 mm height) column, a column containing a mixture (2 g in dry weight) of charcoal (Nuchar C, 1 g in dry weight) and cellulose powder (Whatman CF11, 1 g in dry

Fig. 2. Body weight gain of rats fed casein and soybean oil. Tryptophan (100 mg) was force-fed on day 9, and urine was collected during 48 h before and after the force-feeding. 20% Cas group (○), 40% Cas group (△), 40% Cas+20% F group (□).

Table 2. Effects of experimental diets on the body weight gain, food- and tryptophan-intake by rat.

Group	Body weight gain (g/2 days)	Food intake (g/2 days)	Tryptophan intake (μ mol/2 days)
[before force-fed tryptophan]			
20% Cas	17.0 $\pm$ 0.5 ^a	44.6 $\pm$ 2.0 ^a	480 $\pm$ 21 ^a
40% Cas	16.8 $\pm$ 1.0 ^a	36.1 $\pm$ 2.0 ^b	777 $\pm$ 44 ^b
40% Cas+20% F	13.5 $\pm$ 0.9 ^b	28.1 $\pm$ 0.8 ^c	606 $\pm$ 17 ^c
[after force-fed tryptophan]			
20% Cas	15.1 $\pm$ 2.1	42.3 $\pm$ 2.8 ^a	945 $\pm$ 31 ^a
40% Cas	12.9 $\pm$ 1.3	33.8 $\pm$ 2.0 ^a	1,218 $\pm$ 44 ^b
40% Cas+20% F	13.6 $\pm$ 0.7	25.7 $\pm$ 0.4 ^b	1,044 $\pm$ 8 ^c

Values represent mean $\pm$ SE of 4 rats. Means in the same column not followed by the same superscript letter differ significantly ($p < 0.05$). Student's *t*-test was used to determine the significance (20). Tryptophan intake ([Trp]) was calculated from food intake ([Food]) and Tables of Amino Acid Composition of Foods (11) as follows: [Trp]=[Food] $\times$ (0.2 or 0.4) $\times$ 11.0 $\times$ 1,000/204.22+(0 or 489.7): in the first parenthesis, 0.2 for 20% Cas and 0.4 for 40% Cas and 40% Cas+20% F; in the second parenthesis, 0 before force-fed tryptophan and 489.7 after force-fed tryptophan; tryptophan contents in casein, 11.0 mg/g edible portion; molecular weight of tryptophan, 204.22; and the amount of tryptophan force-fed, 489.7 μ mol.

weight) (14 $\times$ ca. 35 mm height) and a Dowex 1 $\times$ 8 (OH⁻) (14 $\times$ 25 mm height) column, and a Dowex 50W $\times$ 8 (H⁺) (8 $\times$ 30 mm height) column. The first 3 series of columns were set in this order to allow the effluent from the Dowex 1 (acetate form) column to run through a Dowex 50 (H⁺) (14 mm diameter) column and

then pour into a charcoal-cellulose column as shown in Fig. 1. After adding 20 ml of distilled water to 30 ml of each urine sample which was pretreated as described in METHODS, the solution was passed through the above three series of columns. Then the columns were washed once with 120 ml of distilled water. After removing upper Dowex 1 column, 140 ml of 0.1 N sodium acetate in 0.01 N HCl was passed through the series of the Dowex 50 and charcoal-cellulose columns. After washing the columns with 50 ml of distilled water, the Dowex 50 column was removed. MNA was eluted from this column by passing 150 ml of 1 M NaCl in 0.1 N HCl. The remaining Nucher-cellulose column was then washed with 50 ml of distilled water. 2-Py and 4-Py were eluted by 180 ml of 2% pyridine in 50% ethanol. The eluate was dried up *in vacuo* at 35°C and the residue was dissolved in 5.5 ml of distilled water. A 5 ml portion of this solution was then passed successively through Dowex 1 (OH⁻) (14×25 mm height) and Dowex 50W (H⁺) (8×30 mm height) columns in this order. A UV-monitor was set to measure the absorbance at 280 nm of eluate from the last Dowex 50 column and 5 ml portions of eluate were collected by a fraction collector. The columns were washed with 10 ml of distilled water. After removing the Dowex 1 column, the remaining Dowex 50 column was further washed with 50 ml of distilled water. The fractions eluted with water as a single absorbance peak were collected and used for the determination of 2-Py. The Dowex 50 column was further washed with 30 ml of 0.1 N HCl, 20 ml of 0.1 N sodium acetate in 0.01 N HCl in this order. 4-Py was eluted with 40 ml of 0.3 N sodium acetate in 0.01 N HCl. The fractions eluted with a single absorbance peak were collected and used for the determination of 4-Py. To a portion of each 2-Py and 4-Py fraction, the equal amount of ethyl acetate saturated with water was added and mixed vigorously. A few minutes later, the upper layer was discarded. This procedure was repeated. The aqueous layer was washed twice with water-saturated diethyl ether. After removing ether layer, the remaining aqueous layer was warmed at 40°C for 3 min to evaporate the contaminated ethyl ether. The concentration of 2-Py and 4-Py was determined photometrically at 258 nm based on the molar extinction of 4-Py ($1.31 \times 10^4 \text{ mol}^{-1} \cdot \text{cm}^{-1}$) (13) and 2-Py ($1.43 \times 10^4 \text{ mol}^{-1} \cdot \text{cm}^{-1}$) (14). The recovery of MNA, 2-Py, and 4-Py from this procedure was $96.2 \pm 2.9\%$ ($n=5$), $71.2 \pm 5.1\%$ ($n=5$), and $88.5 \pm 5.6\%$ ($n=5$), respectively. The urinary excretions of MNA, 2-Py, and 4-Py are demonstrated in Table 3. On day 9 before tryptophan administration, the excreted amount of MNA, 2-Py and 4-Py in the 40% Cas group did not differ from that in the 20% Cas group, although the tryptophan intake in the former group was higher, as shown in Table 2. Moreover, the amount of any one of the metabolites in urine was greater in the 40% Cas+20% F group than that in the 40% Cas group, although the tryptophan intake was smaller in the former group. After tryptophan force-feeding, the excretion of any one of the niacin metabolites in the 40% Cas group was lower than that in the 20% Cas group. Although the amount of MNA in the 40% Cas+20% F group was greater than that of the 40% Cas group, those of 2-Py and 4-Py did not differ significantly. The sum of the urinary metabolites (MNA+2-Py+4-Py) in

Table 3. Effects of dietary protein and soybean oil on the urinary excretion of niacin metabolites.

Group	Niacin metabolites ($\mu\text{mol/day}$)			Ratio $\{(2\text{-Py} + 4\text{-Py})/\text{MNA}\}$
	MNA	2-Py	4-Py	
20% Cas	1.38 ± 0.47^a	[before force-fed tryptophan]		1.24 ± 0.41
40% Cas	0.95 ± 0.65^a	0.26 ± 0.06^a	0.89 ± 0.14	2.25 ± 0.79
40% Cas + 20% F	7.08 ± 1.95^b	0.15 ± 0.01^a	0.59 ± 0.11	0.55 ± 0.25
		1.07 ± 0.20^b	1.48 ± 0.38	
20% Cas	7.53 ± 2.23^{ab}	[after force-fed tryptophan]		0.87 ± 0.44
40% Cas	2.73 ± 1.96^a	1.06 ± 0.26^a	2.74 ± 0.36^a	1.62 ± 0.83
40% Cas + 20% F	15.60 ± 3.01^b	0.34 ± 0.12^b	0.99 ± 0.34^b	0.35 ± 0.18
		2.46 ± 0.96^{ab}	1.71 ± 0.68^{ab}	

Values represent mean $\pm$ SE of 4 rats. Means in the same column not followed by the same superscript letter differ significantly ($p < 0.05$). Student's t -test was used to determine the significance (20). *MNA + 2-Py + 4-Py.

Table 4. Effect of dietary protein and soybean oil on the hepatic and renal ACMSD activities.

Group	Liver		Kidney	
	Weight (g)	ACMSD activity ($\mu\text{mol/h/g liver}$)	Weight (g)	ACMSD activity ($\mu\text{mol/h/g kidney}$)
20% Cas	9.23 ± 0.74	0.68 ± 0.25^a	1.73 ± 0.07	15.31 ± 1.07^a
40% Cas	8.50 ± 0.31	4.05 ± 0.69^b	1.81 ± 0.04	22.83 ± 2.21^b
40% Cas + 20% F	9.13 ± 0.21	0.07 ± 0.03^a	1.84 ± 0.06	19.84 ± 1.40^b

Values represent mean $\pm$ SE of 4 rats. Means in the same column not followed by the same superscript letter differ significantly ($p < 0.05$). Student's t -test was used to determine the significance (20).

the 40% Cas group was significantly lower than those of the 20% Cas and 40% Cas+20% F groups. Therefore, this clearly indicates that excess dietary protein reduces the formation of niacin metabolites from tryptophan, but excess soybean oil increases it.

ACMSD activity in liver and kidney

The hepatic ACMSD activity of the 40% Cas group was extremely higher than those of the 20% Cas and 40% Cas+20% F groups (Table 4). The hepatic enzyme activity in the 40% Cas+20% F group was significantly lower than those of the 40% Cas and 20% Cas groups. These facts show that hepatic ACMSD activity is increased by the dietary high protein but suppressed by the dietary high protein+soybean oil. On the other hand, the renal ACMSD activity was slightly increased by the dietary protein, but was not affected by the dietary high protein+soybean oil.

Relation between ACMSD activity and niacin metabolites

The relation between hepatic ACMSD activities and the reciprocal of total amounts of the niacin metabolites (MNA+2-Py+4-Py) was investigated for each group of rats (Fig. 3). A direct proportional correlation between the hepatic ACMSD activity and the reciprocal of total amounts of niacin metabolites was observed ($r = -0.720$, $p < 0.005$). The increase of urinary niacin metabolites induced by dietary high protein+soybean oil was correlated with the extreme reduction of the hepatic ACMSD activity. These data were analyzed according to

Fig. 3. The relation between the reciprocal of [TNM] (the amounts of urinary total niacin metabolites, MNA+2-Py+4-Py) and [ACMSD] (the hepatic ACMSD activity). Each point represents reciprocal of [TNM] before and after tryptophan force-feeding (100 mg per rat) and [ACMSD] (the hepatic ACMSD activity) in each animal. 20% Cas group (○), 40% Cas group (△), 40% Cas+20% F group (□), 20% Cas group with tryptophan (●), 40% Cas group with tryptophan (▲), 40% Cas+20% F group with tryptophan (■).

the following equation (7):

$$[\text{TNM}] = a \{ [\text{Trp}] - b \cdot \Delta w \} \{ 1 / (1 + n \cdot [\text{ACMSD}]) \}, \quad (\text{Eq. 1})$$

where $[\text{TNM}]$ is the amount of metabolites (MNA + 2-Py + 4-Py) in urine (μmol per day) which is corrected for their recovery; $[\text{Trp}]$ is the amount of tryptophan intake (μmol per day); a , b , and n are coefficients; Δw is body weight gain (g per day) and $[\text{ACMSD}]$ is the activity of hepatic ACMSD (μmol per h per g).

a) *Estimation of a coefficient n in the Eq. 1 by graphical methods.* If $b \cdot \Delta w$ is assumed to be negligible, i.e. $b \cdot \Delta w = 0$, Eq. 1 can be rewritten as follows:

$$[\text{ACMSD}] = (a/n) ([\text{Trp}]/[\text{TNM}]) - 1/n,$$

where $[\text{TNM}]$ is not zero. When the value of $[\text{Trp}]/[\text{TNM}]$ is extrapolated to 0, $[\text{ACMSD}] = -1/n$. Therefore, we can obtain the value 2.44 of n from Fig. 4a in rats before tryptophan force-feeding (regression line: $y = 0.0154x - 0.41$). The correlation coefficients (r) between $[\text{Trp}]/[\text{TNM}]$ and $[\text{ACMSD}]$ are 0.96 and 0.64 in rats before and after tryptophan force-feeding, respectively.

b) *Estimation of coefficients a and b in the Eq. 1 by graphical methods.* Equation 1 can be rewritten as follows:

Fig. 4. The analysis of the experimental data according to the Eq. 1.

$$[\text{TNM}] = a \{ [\text{Trp}] - b \cdot \Delta w \} \{ 1 / (1 + n \cdot [\text{ACMSD}]) \}.$$

20% Cas group (○), 40% Cas group (△), 40% Cas+20% F group (□), 20% Cas group with tryptophan (●), 40% Cas group with tryptophan (▲), 40% Cas+20% F group with tryptophan (■). $[\text{TNM}]$ is total niacin metabolites (MNA + 2-Py + 4-Py) in urine; $[\text{Trp}]$ is tryptophan intake; $[\text{ACMSD}]$ is hepatic ACMSD activity; a , b , and n are coefficients and Δw is body weight gain. a) Estimation of a coefficient n . Each point represents $[\text{Trp}]/[\text{TNM}]$ and $[\text{ACMSD}]$ in each rat. b) Estimation of coefficients a and b . Each point represents $[\text{TNM}]/\Delta w \{ 1 + n[\text{ACMSD}] \}$ and $[\text{Trp}]/\Delta w$ in each rat ($n = 2.44$).

$$[\text{Trp}]/\Delta w = a^{-1}([\text{TNM}]/\Delta w)(1+n[\text{ACMSD}]) + b,$$

where Δw is not zero. When $n=2.44$, we can obtain the value 0.090 of a and 22.5 of b from Fig. 4b in rats before tryptophan force-feeding (regression line: $y=11.11x+22.5$). The correlation coefficients (r) between $[\text{Trp}]/\Delta w$ and $[\text{TNM}] \cdot (1+n \cdot [\text{ACMSD}])/\Delta w$ are 0.91 and 0.62 in rats before and after tryptophan force-feeding, respectively. Then, the Eq. 1 was as follows:

$$[\text{TNM}] = 0.090([\text{Trp}] - 22.5 \cdot \Delta w) \{1/(1+2.4[\text{ACMSD}])\}. \quad (\text{Eq. 2})$$

DISCUSSION

Our previous reports show that the ACMSD activity of rat liver was elevated by feeding on a high-protein diet and depressed by dietary fatty acids (8,9). Unsaturated fatty acids reduced the hepatic ACMSD activity in rats fed on a normal or high-protein diet (9). However, in the present experiments, soybean oil was added to only a 40% protein diet, because the addition of soybean oil to a 20% protein diet would result in too low ACMSD activity to analyze the relation between the activity and niacin metabolites. The present study clarified that NAD metabolites and ACMSD activity were affected not only by high-protein diet but also by dietary soybean oil. Especially, the effect of dietary soybean oil is more important, because activity of the hepatic ACMSD was quite sensitive to dietary unsaturated fatty acid levels as the activity decreased by about 50% by every 4% increase of dietary soybean oil contents (9). The increase of urinary niacin metabolites by fed on a high-fat diet was also reported by Shibata and Murata (16). The tryptophan influx into NAD pathway is primarily elucidated by an alteration of hepatic ACMSD activity (2). In the present experiments, however, renal ACMSD activity was higher than hepatic one, and not affected by dietary high protein+soybean oil but slightly increased by the high-protein diet. The no change of renal ACMSD activity was also reported in a low-protein feeding experiment (17). These results suggest that the renal ACMSD activity in the niacin metabolism might not be affected by dietary protein and oil.

The urinary MNA in 40% Cas+20% F group was higher than that of 40% Cas group by about 7 times (before tryptophan force-fed) or 5 times (after tryptophan force-fed). The urinary 2-Py+4-Py of the former group was also higher than that of the latter group by 3 times (before and after tryptophan force-fed). Moreover, the ratio (2-Py+4-Py)/MNA of the 40% Cas+20% F group tends to be smaller than that of the 40% Cas group before and after tryptophan force-feeding. This is seen between the ratios of these two dietary groups only when the data before and after tryptophan force-feeding are combined, *i.e.* 0.45 ± 0.15 for 40% Cas+20% F group and 1.94 ± 0.54 for 40% Cas group (significant difference: $p < 0.05$) (Table 3). These results indicate that the increase of MNA formation by soybean oil was not accompanied by the MNA oxidation in

the same rate. The ratio (2-Py+4-Py)/MNA of 20% Cas group was not significantly different from that of the 40% Cas group even when the data before and after tryptophan force-feeding are combined. The similar results were reported for rats fed on a 20 and 40% casein diets containing 5% corn oil (18,19). Tryptophan force-feeding, however, would not alter the hepatic ACMSD activity in the rats, as our previous experiments demonstrate that the excess addition of tryptophan to normal diet does not change the activity.

Figure 4a shows the relations between the ratios $[Trp]/[TNM]$ and the hepatic ACMSD activities in individual rats. They were roughly proportional. Then, the present data were analyzed using the hypothetical equation (Eq. 1) according to our previous report (7) to estimate the amount of tryptophan metabolized through NAD pathway. Tryptophan is metabolized according to the following simplified pathway (7):

$$[TNM] = a([Trp] - b \cdot \Delta w) \{V_1 / (V_1 + V_2)\}, \quad (\text{Eq. 3})$$

where V_1 and V_2 are the velocity of spontaneous nonenzymatic quinolinate formation ($\mu\text{mol/h}$) and of decarboxylation of ACMS by ACMSD ($\mu\text{mol/h}$), respectively. The meaning of other symbols is described in RESULTS. In this equation, we assumed the following 5 matters: 1, a part of tryptophan ($b \cdot \Delta w$) is used for biosynthesis of protein and other substances for body weight gain and released from body protein according as the body weight loss; 2, most of the tryptophan is metabolized through the alternative pathways, such as NAD pathway and glutarate pathway; 3, quinolinate is formed through a nonenzymatic first order reaction ($V_1 = k_1 S$), where k_1 is rate constant for quinolinate formation and S is concentration of ACMS. The enzymatic reaction of ACMSD, however, proceeds according to the Michaelis-Menten equation ($V_2 = (V_{\max} \cdot S) / (K_m + S)$), where S is concentration of ACMS, $V_{\max}$ is a maximum velocity of ACMSD reaction, K_m is a Michaelis constant, then

$$V_1 / (V_1 + V_2) = k_1 S / \{k_1 S + V_{\max} \cdot S / (K_m + S)\} = 1 / (1 + n[ACMSD]),$$

where $n = c / \{k_1 (K_m + S)\}$ and $V_{\max} = c \cdot [ACMSD]$ (c , proportionality constant). As ACMSD activity was assayed under about 20 μM of substrate, which is several

times higher than K_m of ACMSD, [ACMSD] was almost the same value as $V_{\max}$ under *in vitro* assay conditions. So, [ACMSD] might also be directly proportional to $V_{\max}$ under *in vivo* conditions. In addition, no efficient inhibitors or activators of ACMSD have been found in a rat liver; 4, [ACMSD] and S do not change significantly during *in vivo* experiment; 5, the specific activity of renal ACMSD is so high that S metabolized through NAD pathway in the kidney would be negligible.

Equation 2 was obtained from the data before tryptophan force-feeding. In the experiment after tryptophan force-feeding, the coefficients (a and b) in Eq. 1 could not be estimated, because the assumption 4 described above is not adopted in this case, especially S would be altered gradually during *in vivo* experiments.

In conclusion, the amounts of urinary niacin metabolites (MNA, 2-Py, and 4-Py) were increased by high-protein + soybean oil diet. This increase was caused by the depression of hepatic ACMSD activity with the soybean oil feeding. On the contrary, by feeding experiment using higher protein without soybean oil, the amount of niacin metabolites did not increase because of the elevation of hepatic ACMSD activity by higher protein level.

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