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Abstract

The effects of a low protein diet on the excretion of sulfate and taurine, major metabolites of L-cysteine in mammals, were studied in rats fed with synthetic 10% (group A) and 25% (group B) casein diets. The average excretions of total taurine (taurine plus hypotaurine) and total sulfate (free plus ester sulfate) ($\mu\text{mol/kg}$ of body weight per day after the adaptation to the synthetic diet) in group A were 14.2 ± 13.4 and 122.3 ± 39.6 , respectively, which were very low compared with 280.4 ± 93.8 and 943.2 ± 144.8 , respectively, in group B. The taurine/sulfate ratio in group A was 0.12 ± 0.11 , which was significantly lower than that (0.30 ± 0.08) in group B. A single intraperitoneal injection of 5 mmol of L-cysteine per kg of body weight in group A resulted in an increase in average taurine and sulfate excretion to 693.4 ± 195.6 and 2440.6 ± 270.0 , respectively, and thus the average taurine/sulfate ratio increased to 0.29. These increases were transient and low taurine excretion resumed again 24 h after the L-cysteine administration. L-Cysteine injection in group B resulted in a similar increase in taurine and sulfate excretion, but the ratio changed only slightly (0.28). The present results suggest that in vivo production of taurine is reduced preferentially over sulfate production when sulfur amino acid supply is limited.

KEYWORDS: low protein diet, taurine, sulfate, crstein metabolism

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The effects of a low protein diet on the excretion of sulfate and taurine, major metabolites of L-cysteine in mammals, were studied in rats fed with synthetic 10% (group A) and 25% (group B) casein diets. The average excretions of total taurine (taurine plus hypotaurine) and total sulfate (free plus ester sulfate) ($\mu\text{mol}/\text{kg}$ of body weight per day after the adaptation to the synthetic diet) in group A were 14.2 ± 13.4 and 122.3 ± 39.6 , respectively, which were very low compared with 280.4 ± 93.8 and 943.2 ± 144.8 , respectively, in group B. The taurine/sulfate ratio in group A was 0.12 ± 0.11 , which was significantly lower than that (0.30 ± 0.08) in group B. A single intraperitoneal injection of 5 mmol of L-cysteine per kg of body weight in group A resulted in an increase in average taurine and sulfate excretion to 693.4 ± 195.6 and 2440.6 ± 270.0 , respectively, and thus the average taurine/sulfate ratio increased to 0.29. These increases were transient and low taurine excretion resumed again 24h after the L-cysteine administration. L-Cysteine injection in group B resulted in a similar increase in taurine and sulfate excretion, but the ratio changed only slightly (0.28). The present results suggest that *in vivo* production of taurine is reduced preferentially over sulfate production when sulfur amino acid supply is limited.

Key words: low protein diet, taurine, sulfate, cysteine metabolism

Sulfate is a major metabolite of L-cysteine in mammals (1, 2) and plays various important roles such as a constituent of proteoglycans (3) and as a detoxicating agent (4). Taurine is another major metabolite of L-cysteine in mammals (5) and is involved in the production of taurobile acids (6) and membrane protection

(7). Sulfate and taurine are finally excreted in urine, and the sum of these metabolites constitutes more than 90% of total urinary sulfur when cysteine is administered to mammals (8, 9). L-Cysteine is supplied to mammals as a component of dietary proteins and is produced as a metabolite of L-methionine which is also supplied as a component of dietary proteins. It has been suggested that intake and excretion of sulfur is held in a state of sulfur equilibrium (9, 10) and that excretion of taurine and sulfate are closely related each other (10). We have studied the effect of a high protein diet (11) on the excretion of taurine and sulfate in rats and found that the taurine/sulfate ratio was approximately 0.3 when sufficient sulfur amino acids were available and that further increases in protein content result in a decrease in the above ratio due to the increased excretion of sulfate. In the present study, we performed further experiments on the effects of a low protein diet on the excretion of these metabolites and compared the results with those of the standard 25% casein diet (11). Furthermore, the *in vivo* production of these metabolites was discussed in comparison with the results of experiments with the high protein diet (11).

Materials and Methods

Materials. Male Wistar rats (7 weeks of age) weighing 180-200g were used in this study. Rats were fed *ad libitum* with a laboratory diet MF (Oriental Yeast Co., Ltd., Tokyo, Japan) and then with synthetic diets as described below.

Materials for the preparation of synthetic diets were obtained from Oriental Yeast Co., except for the mineral mixture (11). The composition (% in weight) of the 10% casein diet (low protein diet) was as follows: casein, 10; corn starch (α), 53; potato starch (α), 10; cellulose powder, 8; corn oil, 6; sucrose, 5; a mixture of vitamins (Oriental Yeast formula) (12), 2; and a mixture of

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minerals, 6. Casein content in the 25 % casein diet (standard diet) was 25 % and corn starch was reduced to 38 %. The mineral mixture was prepared using sulfate-free compounds obtained from Wako Pure Chemical Ind., Osaka, Japan (11). L-Cysteine was obtained from Sigma Chemical Co., St. Louis, MO, USA.

In vivo experiments. All the rats in groups A and B (5 rats per group) were housed separately in metabolic cages. After feeding with the MF diet for one week, rats of groups A and B were fed with the 10 and 25 % casein diet, respectively, and water *ad libitum*. At two and three weeks after the start of feeding with the synthetic diets, 5 mmol of L-cysteine per kg of body weight was administered intraperitoneally. The 24 h urine was collected every day in a 100-ml Erlenmeyer flask containing 5 ml of 50 % acetic acid and one ml of toluene. After centrifugation at $1,200 \times g$ for 10 min, urine samples were filtered through filter paper and used for the determination of sulfate and taurine.

Determination of sulfate in the urine. As urinary sulfate is composed of free (inorganic) and bound (ester) sulfate, total sulfate (free + bound) was determined after hydrolysis of urine samples using an ion chromatograph (11, 13).

Determination of taurine and hypotaurine in the urine. Hypotaurine is the immediate precursor of taurine synthesis in the oxidation pathway of L-cysteine in mammals, and its urinary concentration in rats is usually very low. However, substantial amounts of hypotaurine were excreted when cysteine was administered to rats (14). Therefore, taurine and hypotaurine were determined separately and the sum of taurine and hypotaurine was expressed as total taurine. Rat urine (10 μ l) was treated with 4-dimethylaminoazobenzene-4'-sulfonyl (dabsyl) chloride and dabsyl-amino acids formed were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) according to the previously reported method (11, 14).

Statistical analysis was performed with the Student's *t*-test. Atypical data obtained from rats in which cysteine injection seemed to have been unsuccessful were omitted from statistical analyses.

Results and Discussion

Fig. 1 shows the growth curves and dietary intake of rats fed with 10 % (group A) and 25 % (group B) casein diets. Upon change to the synthetic 10 % casein diet from

the laboratory diet, the body weight gain of group A rats stopped and body weights actually decreased. This seems to be due mainly to decreases in dietary intake. After adaptation to the synthetic diet, namely, the dietary intake of group A rats returned to the level of group B, the growth rate of group A became similar to that of group B, although the average body weight of the former group was significantly lower than that of the latter group ($P < 0.001$).

Fig. 2 shows excretion of total taurine. Average excretions of total taurine during days 6-14 after the adaptation to the synthetic diets were 14.2 ± 13.4 and 280.4 ± 93.8 in groups A and B, respectively. Thus, the taurine excretion of group A during this period was only 5.1 % of that of group B. When 5 mmol of L-cysteine/kg of body weight was injected into these rats, a sharp increase in taurine excretion was observed, as shown in Fig. 2 (total excretions in groups A and B were 693.4 ± 195.6 and 1127.5 ± 120.2 , $n = 9$ and 8 , respectively). The average increases compared to the excretions of the previous day were 684.6 ± 192.3 ($n = 9$) and 818.6 ± 117.9 ($n = 8$) in groups A and B, respectively, which corresponded to 13.7 and 16.4 %, respectively, of total L-cysteine loaded.

Fig. 3 shows the urinary excretion of total sulfate. Average sulfate excretions in groups A and B during days 6-14 after the adaptation to the synthetic diets were 122.3 ± 39.6 and $943.2 \pm 144.8 \mu\text{mol/kg}$ of body weight, respectively. Although the dietary intake of group A during this period was variable within a wide range, as shown in Fig. 1, the excretion of total sulfate stayed at a rather constant level. This fact seems to suggest the presence of steady-state sulfur metabolism in rats. When 5 mmol of L-cysteine/kg of body weight was administered by intraperitoneal injection, the sulfate excretion increased sharply in both groups as shown in Fig. 3 (total excretions in groups A and B were 2440.6 ± 270.0 and 4043.0 ± 305.6 , and $n = 9$ and 8 , respectively). The average increases compared to the excretions of the previous day were 2318.5 ± 164.0 and 2910.4 ± 107.1 in groups A and B, respectively, which corresponded to 46.4 and 58.2 %, respectively, of total L-cysteine loaded.

Fig. 4 shows the ratio of taurine to sulfate excretion in groups A and B. In group B, the ratio remained at a constant level of 0.30 ± 0.08 after the initial adaptation to the synthetic diet. In contrast to group B, the ratio in group A decreased strikingly after day 6 to 0.12 ± 0.11 , a significantly lower value ($P < 0.001$) than the ratio in

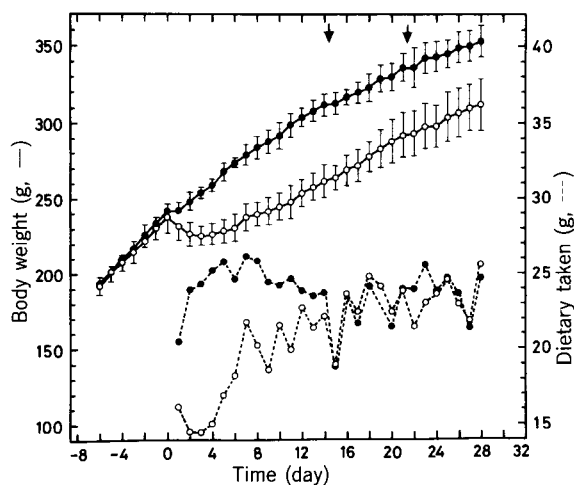


Fig. 1 Growth curve and food intake of rats. Two groups of rats (5 animals per group) were fed with synthetic 10% (○) or 25% (●) casein diet. Mean ± SD of body weight and mean of diet intake are plotted. Day 0 is the start of feeding with synthetic diets. Arrows indicate injection of L-cysteine.

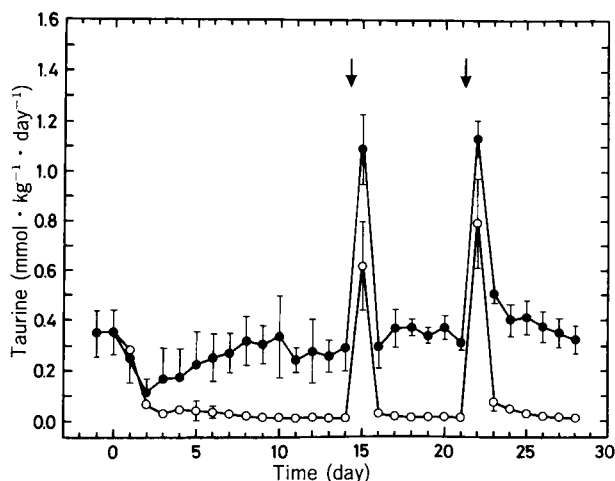


Fig. 2 Urinary excretion of total taurine (taurine + hypotaurine) in rats. The rats (the same rats as those in Fig. 1) were fed with synthetic 10% (○) or 25% (●) casein diet. See legend to Fig. 1. Mean ± SD is plotted.

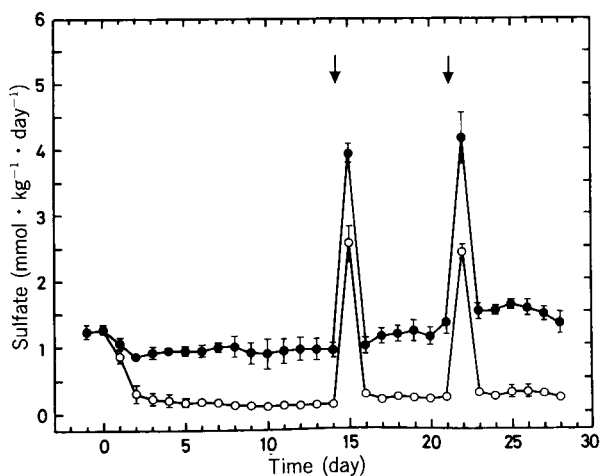


Fig. 3 Urinary excretion of total (free + ester) sulfate in rats. The rats (the same rats as those in Fig. 1) were fed with synthetic 10% (○) or 25% (●) casein diet. See legend to Fig. 1. Mean ± SD is plotted.

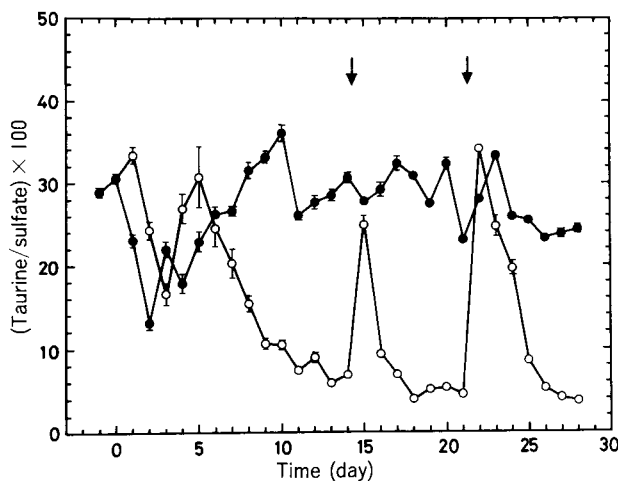


Fig. 4 Ratio of urinary excretion of total taurine (taurine + hypotaurine) and total (free + ester) sulfate in rats. The rats (the same rats as those in Fig. 1) were fed with synthetic 10% (○) or 25% (●) casein diet. See legend to Fig. 1. Mean ± SD is plotted.

group B. This decrease seems to be due to the low intake of sulfur amino acids in the synthetic diet. When L-cysteine was administered, the ratio in group B changed only slightly (0.28 ± 0.05). However, the ratio in group A increased to 0.29 ± 0.10 (the mean of two experiments), which is close to the level of group B. These results suggest that the ratio of taurine to sulfate excretion is approximately 0.3 when sufficient sulfur amino acids are supplied by dietary protein and/or L-cysteine, and that

the ratio of *in vivo* production of taurine to sulfate is about 0.3 in rats when sufficient sulfur amino acids are supplied by dietary protein and/or L-cysteine as reported (11).

The present results also indicate that taurine excretion decreases preferentially over sulfate excretion when the supply of sulfur amino acids in dietary protein is limited. Thus it is suggested that the decrease in taurine production occurs preferentially over that of sulfate under such conditions.

Table 1 Intake of diet and urinary excretion of sulfate and taurine

Group	A	B ^a	C ^a
Diet	10% casein	25% casein	40% casein
Intake of diet ^b	84.3 ± 10.9	84.1 ± 8.8	81.3 ± 7.5
Intake of Met and Cys ^c	1.61	4.02	6.22
Excretion of taurine + sulfate ^d	0.14 ± 0.04	1.22 ± 0.22	3.23 ± 0.34
% of ingested sulfur as Met + Cys	8.5	30.4	51.9
Sulfur retained ^e	1.47	2.80	2.99
Taurine/sulfate	0.12 ± 0.11	0.30 ± 0.08	0.22 ± 0.07
Increased excretion ^f			
Taurine + sulfate	2.96 ± 0.27	3.74 ± 0.27	3.74 ± 0.32
(% of sulfur injected as Cys)	(59.2)	(74.8)	(74.8)

^a: Adopted from reference 11.

^b: g/kg of body weight, mean ± SD of days 6-14.

^c: Sum of L-methionine (Met) and L-cysteine (Cys), calculated from their contents in casein and corrected for water content in the diets, mmol/kg of body weight per day.

^d: mmol/kg of body weight per day, mean ± SD of days 6-14.

^e: Calculated assuming that the sulfur in L-methionine and L-cysteine was metabolized completely to sulfate and taurine, and the recoveries of these metabolites were 100%.

^f: Increased excretions after L-cysteine injection, mean ± SD of days 15 and 22.

Table 1 compares the dietary intake and excretions of taurine and sulfate in the low protein diet group with those of groups fed with diets of sufficient protein content (11). Average excretion of sulfate plus taurine in the 10% casein group in the steady state was only 11.5% of that in the 25% casein diet group although the mean intake of methionine plus cysteine in the former group was 40.0% of the latter. This seems to reflect the difference in the retention of sulfur in the body between these groups. When 5 mmol of L-cysteine per kg of body weight was administered, the increased excretion of taurine plus sulfate in the 10% casein diet group was 79.1% of that in the 25% and 40% casein diet groups and returned to the basal level as shown in Figs. 2 and 3. These results suggest that sulfur metabolism is held in a steady state even when the intake of sulfur amino acids is limited, and that dietary intake does not increase even though there is increased metabolic capacity of proteins and the growth rate is limited.

The taurine/sulfate ratios in the urine of the 10% and 40% casein groups were both lower than that of the 25% casein group. However, the mechanisms of the decrease in the ratio of both groups were different, namely, the decrease in the ratio of the 10% casein group was due to a decrease in taurine excretion and that of the 40% casein group was due to the enhanced excretion of sulfate, and this difference seems to reflect the difference in the

production of taurine and sulfate in rats fed with low and high protein diets.

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