Metabolic Fate of Cysteine Sulfur in Growing Rats at Various Dietary Protein Levels

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Summary The metabolic fate of L-[³⁵S]cysteine was investigated in growing rats fed on diets containing graded levels of protein calorie percentages (0, 5, 10, 15, and 30 PC%) by use of purified whole egg protein at 4,100 kcal of metabolizable energy per kilogram of diet. Incorporation of radioactivity into body protein during the 12 h period after intraperitoneal injection of ³⁵S-cysteine was about 70% of the dose in the 0 to 15 PC% group, but it decreased significantly in the 30 PC% group, showing a break point at around 15 PC% in the diet. A considerable amount of the radioactivity was recovered in the carcass soluble fraction in which the label in the taurine fraction gradually increased with increasing dietary protein levels from 0 to 30 PC%. Urinary excretion of total ³⁵S during the 12 h period was depressed in the lower PC% groups, but it increased in the 30 PC% group, about 23% of the dose being recovered. More than 50% of the urinary radioactivity was present in the inorganic sulfate fraction, but less than 10% was in the taurine fraction. These results indicate that cysteine sulfur is preferentially utilized for body protein synthesis, especially in dietary protein depletion, and that the oxidation of cysteine sulfur to taurine and inorganic sulfate is elevated with higher PC% in the diet. The response pattern of cysteine sulfur metabolism to dietary protein intake resembled that of cysteine carbon metabolism which was previously reported.

Key Words amino acid metabolism, cysteine sulfur, taurine, urinary sulfate

Cyst(e)ine is nonessential for the growth of rats and other mammals, since this amino acid can be synthesized *de novo* where cysteine sulfur is derived from methionine and its carbon skeleton from serine (1). Rose and Wixom (2) have reported that about one-third of the methionine requirement of the growing rat can be satisfied by cystine. Cysteine sulfur is catabolized mainly by two pathways, a) to cysteine sulfinic acid in a reaction catalyzed by cysteine dioxygenase and b) to reduced inorganic sulfur catalyzed by cysteine desulfhydrase (1,3). The dietary

modulation of these cysteine catabolic pathways was extensively investigated by Stipanuk *et al.* (4-6) and Yamaguchi *et al.* (7,8). Daniels and Stipanuk (5) reported that the cysteine sulfinic pathway plays a major regulatory role in cysteine degradation.

In the previous experiments (9-19), we reported the metabolic fates of carbon skeletons of individual amino acids in growing rats fed on diets with graded levels of purified whole egg protein in relation to the protein and energy metabolism. It was found that the carbon skeleton of ^{14}C -cysteine was preferentially utilized for body protein synthesis especially in dietary protein depletion, and that a significant amount of cysteine carbon was converted to taurine, a part of which was excreted into urine when excess dietary protein was given (17). In this paper, we studied the effect of dietary protein levels on the response pattern of cysteine sulfur metabolism in growing rats by measuring the distribution of ^{35}S -cysteine in body components and urine.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats were preliminarily fed a stock diet (MF, Oriental Yeast Co.) until mean body weight had reached around 80 g. Animals were housed in individual stainless wire-mesh cages at $23 \pm 1^\circ\text{C}$ in a temperature-controlled room with a 12 h light, 12 h dark cycle, the light cycle being from 6:00 to 18:00. Food and water were given *ad libitum* for 10 days. The experimental diets contained 0, 55, 111, 166, and 332 g of purified whole egg protein (20) per kilogram, as the 0, 5, 10, 15, and 30 PC% diets, respectively. All diets contained 100 g of lard, 50 g of cellulose powder, 40 g of salt mixture, 10 g of vitamin mixture, and enough corn starch to complete 1 kg. The protein calorie percentage (PC%) means the proportion of metabolizable energy of dietary protein to the total metabolizable energy of the diet (21), i.e. 4,100 kcal per kilogram of diet in this experiment.

Isotope experiments. On the last day of feeding, the rats were starved for 9 h, from 12:00 to 21:00, and then given the same experimental diets for 3 h, from 21:00 to 24:00. After this feeding, L- ^{35}S -cysteine in a saline solution (1.70 GBq/mmol, Amersham Japan) was administered by intraperitoneal injection. The standard dosage was 153.9 kBq/0.4 ml/100 g of body weight. Immediately following the injection, each animal was placed in a glass metabolism apparatus, and offered only water. Expired gas was washed at 2 h intervals in 0.6% Zn-acetate solution, and urine was collected in 5% acetic acid during the 12 h period of the isotope experiment. Radioactivity was measured in a liquid scintillation spectrometer (Aloka LSC-900) using an NT (nonionone-toluene) scintillation solution containing 4 g of DPO per liter of 3 parts of nonylphenoxy polyethoxy ethanol and 7 parts of toluene (22). The efficiency of the counting system was estimated for each sample by an external standard ratio method, and ^{35}S decay (half-life: 87.4 days) was corrected in each assay. The radioactivities in total ^{35}S , ^{35}S -taurine and inorganic

^{35}S -sulfate of the urine were measured by a slight modification of the method of Stipanuk (4). Non-labeled taurine (about 0.5 mg) was added as a carrier to each sample of urine, and then the mixture was treated with 3% sulfosalicylic acid overnight and centrifuged at 3,000 rpm for 15 min. The deproteinized urine supernatant was put on the top of the combined columns of Dowex 50×8 (200–400 mesh, H^+ form, 1.5×2 cm) and Dowex 1×2 (200–400 mesh, acetate form, 1.5×2 cm) which were assembled in a series. The columns were washed with 30 ml of 0.1 N acetic acid, the eluate being called the taurine fraction. The amino acid fraction containing small peptides was obtained by eluting the Dowex 50×8 column with 15 ml of 3 N ammonia. To measure the amount of label incorporated into urinary inorganic sulfate, duplicate tubes containing 2 ml of urine plus 1 ml of 2% Na_2SO_4 (as a carrier) were prepared; 1 ml of 10% BaCl_2 was added to one tube, and 1 ml of water was added to the other. After centrifugation at 3,000 rpm for 10 min, 1 ml of the supernatant from each tube was counted. The urinary $^{35}\text{SO}_4$ that was precipitated as $\text{Ba}^{35}\text{SO}_4$ was assumed to be the difference in radioactivity in the supernatant of the tubes to which BaCl_2 was and was not added.

Tissue analyses. Animals were killed by decapitation 12 h after administration of the isotopes. The liver was removed, and shed blood was combined with the carcass. The carcass and liver were homogenized and analyzed for radioactivity in their major components such as the protein, lipid, and soluble fractions, details of which were described previously (9,14). A portion of the homogenate was extracted with chloroform-methanol mixture (2:1, v/v) to obtain the lipid fraction, and then the residue was extracted with cold 10% trichloroacetic acid (TCA). The extract was combined with the upper phase solution by Folch's washing method (23) to obtain the soluble fraction of the sample. The TCA insoluble residue was dissolved in 2 N NaOH solution as the protein fraction. The radioactivity in each fraction was measured as described above.

To examine the distribution of ^{35}S in the soluble fraction, a sample of the carcass soluble fraction was evaporated, extracted with diethyl ether to remove TCA, and then chromatographed by the Dowex 50×8 and Dowex 1×2 columns as described previously (17). The assembled columns were washed with 0.1 N acetic acid, the eluate being the taurine fraction. After washing the columns, they were taken to pieces and then the Dowex 50×8 column was eluted with 3 N ammonia, the eluate being the amino acid fraction, and the Dowex 1×2 column was eluted with 2 N HCl, the eluate being the acidic fraction. The radioactivity of each fraction was measured in a NT scintillation solution.

Statistical analysis. The results were tested by an analysis of variance, and Tukey's procedure for multiple comparisons was used to evaluate the statistical significance with a probability level of 0.05 (24).

RESULTS

The growth curves of the 10, 15, and 30 PC% groups were almost linear, while

Fig. 1. Growth curves of rats fed on diets of various protein calories percentages.

that of the 5 PC% group was retarded slightly during the early days of the experimental period (Fig. 1). The body weight gain reached its approximate maximum with around 10 PC% in the diet. The results of the growth experiment were essentially identical to those obtained in our previous experiments, in which body protein retention reached a plateau and body lipid retention attained a maximum with around 10 PC% in the diet (10). As has been pointed out, no drastic change in pool size of tissue amino acid was induced by the injection of the isotope, since only a trace of labeled amino acid was used (17). The total recovery percentages of the ^{35}S in the 0, 5, 10, 15, and 30 PC% groups were 96.2, 96.3, 98.2, 97.9, and 98.5%, respectively (the means of three to four rats in each group).

No radioactivity was detected in expired gas which was washed in 0.6% Zn-acetate solution during the 12 h period after intraperitoneal injection of L- ^{35}S -cysteine. Urinary excretion of the radioactivity from ^{35}S -cysteine was low in the 0 to 15 PC% groups and it increased to the great extent in the 30 PC% group, about 23% of the dose being recovered (Table 1). To characterize the radioactive compounds excreted in the urine after injection of labeled cysteine, we examined the distribution of radioactivity in their metabolites as described in MATERIALS AND METHODS. More than 50% of the ^{35}S excreted in the urine was present in the inorganic sulfate fraction over the whole range of dietary protein calorie percentages. The values increased with increasing dietary protein levels and about 80% of urinary label was excreted as sulfate in the 30 PC% group. The recovery of radioactivity in the taurine fraction was reduced in the lower PC% groups and increased with higher PC%, about 10% of the urinary ^{35}S being recovered in the 30 PC% group. The radioactivity in the amino acid and peptide fraction was promptly examined by ion-exchange chromatography, and about 35 and 16% of the urinary ^{35}S were accounted for in the 0 and 5 PC% groups, respectively (data not

Table 1. Urinary excretion of total ^{35}S , [^{35}S]sulfate, and [^{35}S]taurine after injecting L-[^{35}S]cysteine in rats fed on diets of various dietary protein calorie percentages.

Dietary group (PC%)	Urinary excretion		
	Total ^{35}S (% of the dose)	[^{35}S]Sulfate (% of total ^{35}S)	[^{35}S]Taurine (% of total ^{35}S)
0	6.4 $\pm$ 2.4	52.8 $\pm$ 6.6	3.2 $\pm$ 0.8
5	8.6 $\pm$ 1.3	70.9 $\pm$ 5.2	2.6 $\pm$ 0.5
10	8.0 $\pm$ 1.7	73.4 $\pm$ 3.1	4.2 $\pm$ 1.2
15	8.7 $\pm$ 0.9	69.9 $\pm$ 3.5	6.5 $\pm$ 1.9
30	23.4 $\pm$ 1.9	84.4 $\pm$ 4.1	9.8 $\pm$ 1.7
Difference ($p < 0.05$)	9.1	4.4	1.0

The values are $M \pm \text{SE}$ for three to four rats. Tukey's test was taken as the criterion of statistical significance with a probability level of 0.05.

Table 2. Distribution of radioactivity in the protein, lipid, and soluble fractions of carcass and liver from L-[^{35}S]cysteine in rats fed on diets of various dietary protein calorie percentages.

Dietary group (PC%)	Radioactivity (% of the injected dose)					
	Protein		Lipid		Soluble fraction	
	Carcass	Liver	Carcass	Liver	Carcass	Liver
0	62.7 $\pm$ 3.4	6.6 $\pm$ 1.1	1.1 $\pm$ 0.05	0.07 $\pm$ 0.01	17.9 $\pm$ 1.7	1.4 $\pm$ 0.2
5	65.8 $\pm$ 2.5	5.6 $\pm$ 0.7	0.9 $\pm$ 0.08	0.06 $\pm$ 0.01	13.8 $\pm$ 1.2	1.5 $\pm$ 0.1
10	69.7 $\pm$ 3.1	4.4 $\pm$ 0.6	0.8 $\pm$ 0.08	0.03 $\pm$ 0.01	14.5 $\pm$ 0.8	0.8 $\pm$ 0.3
15	64.3 $\pm$ 1.6	2.6 $\pm$ 0.1	0.6 $\pm$ 0.02	0.02 $\pm$ 0.00	20.2 $\pm$ 0.3	1.5 $\pm$ 0.5
30	48.7 $\pm$ 0.9	2.0 $\pm$ 0.2	0.5 $\pm$ 0.01	0.03 $\pm$ 0.00	21.7 $\pm$ 0.7	2.2 $\pm$ 0.7
Difference ($p < 0.05$)	14.1	3.5	0.3	0.04	6.5	NS

The values are $M \pm \text{SE}$ for three to four rats. Tukey's test was taken as the criterion of statistical significance with a probability level of 0.05. NS: not significant.

shown).

Incorporation of ^{35}S -cysteine into the carcass protein was more than 60% of the dose in the 0 to 15 PC% groups but decreased in the 30 PC% group (Table 2). ^{35}S incorporation into the liver protein was high in the 0 PC% group and the value decreased gradually with increasing dietary protein levels, showing a somewhat different response pattern to that of the carcass protein. The incorporation of cysteine sulfur into the carcass and liver lipid fractions was negligible. The recovery of radioactivity in the carcass soluble fraction was about 15% of the dose in both 5 and 10 PC% groups, and increased in the 15 and 30 PC% groups, more

Fig. 2. Distribution of radioactivity in the taurine, amino acid, and organic acid fractions from the carcass soluble fraction after injecting L-[^{35}S]cysteine. A pooled sample of the carcass soluble fraction from each group was analyzed by the ion-exchange chromatography. ■, amino acid fraction; ▤, acidic fraction; □, taurine fraction.

than 20% of the dose being recovered. Small amount of ^{35}S was recovered in the liver soluble fraction, and no significant differences were observed between dietary groups.

Because of the large amount of radioactivity in the carcass soluble fraction, this fraction from a pooled sample of each dietary group was further analyzed by ion-exchange chromatography on Dowex 50 and Dowex 1 columns. The distribution of radioactivity in the amino acid fraction (adsorbed by Dowex 50 resin), the acidic fraction (adsorbed by Dowex 1 resin), and the taurine fraction (non-adsorbed by either Dowex 50 or Dowex 1 resins) was examined (Fig. 2). In the amino acid fraction, more than 70% of the ^{35}S recovered in the carcass soluble fraction was recovered in the 0PC% group, but the values in the higher PC% groups gradually decreased with increasing PC%. Radioactivity in the taurine fraction was reduced at 0PC%, but increased linearly with increasing dietary protein levels, about 60% of the ^{35}S being recovered in the 30PC% group. No significant difference in radioactivity was observed in the acidic fraction, where radioactive components were unidentified further.

DISCUSSION

In this experiment, the metabolic fate of cysteine sulfur was examined in growing rats at various dietary protein calorie percentages, and its metabolic response to dietary protein intake was compared to that of cysteine carbon, obtained in our previous experiment (17). The gross distributions of radioactivity in body protein, body lipid, and body soluble fractions from ^{35}S -cysteine and ^{14}C -cysteine are summarized in Fig. 3. The value for each body component was calculated as the total of carcass and liver. By using purified whole egg protein as a dietary protein source, it was possible to investigate the metabolic fate of the

Fig. 3. Gross distribution of radioactivity in rats fed the diets with various protein calorie percentages 12 h after injecting L-[³⁵S] cysteine and L-[U-¹⁴C] cysteine. Each value represents the mean for three to four rats, and the vertical bar indicates standard errors of the mean. The data for L-[U-¹⁴C]cysteine is cited from our previous paper (17). ○, expired CO₂; ●, body protein; ×, body lipid; ▲, body soluble fraction; ■, urine.

amino acids without inappropriate factors such as limiting amino acid. ³⁵S-Cysteine was incorporated to a great extent into the body protein, especially at lower dietary protein calorie percentages. The response pattern of cysteine sulfur to protein intake resembled that of cysteine carbon. It should be noticed that cysteine which is one of the non-essential amino acids for the growth of rats is primarily utilized for body protein synthesis, by evaluating from both cysteine sulfur and carbon. In our previous studies (9–19), the metabolic fates of the carbon skeletons of 20 individual amino acids were examined in growing rats in relation to the balance data on protein and energy metabolism. These results showed that the carbon skeletons of the essential amino acids were preferentially utilized for body protein synthesis. Much more carbon from cysteine and tyrosine were also incorporated into the body protein and their response patterns to protein intake resembled those of the essential amino acids. In contrast, the carbon skeletons of the nonessential amino acids, alanine, aspartic acid, glutamic acid, and glutamine, were rapidly degraded to expired carbon dioxide even with dietary protein depletion, but incorporated to a lesser extent into the body protein, whereas considerable amounts of glycine, serine, proline, and asparagine were utilized for body protein synthesis rather than for energy production. Thus, the metabolic aspects of carbon skeletons of these amino acids are classified into three groups, showing a systematic pattern that is peculiar to their essentiality and catabolic activity. The conversion of cysteine sulfur to body lipid fraction was negligible in all the dietary groups, and no radioactivity was detected in the expired gases throughout the overall experimental period for 12 h. It appeared that these conversions of cysteine sulfur did not have any metabolic or nutritional significance.

On the contrary, the conversion of cysteine to taurine may be one of the important metabolic roles of this amino acid. As shown in Fig. 3, in both the experiments with ^{35}S -cysteine and ^{14}C -cysteine, considerable amounts of label were recovered in the body soluble fraction in all the dietary groups, in which the proportion of labeled taurine from cysteine sulfur or carbon was elevated extremely in the higher PC% groups. When ^{35}S -cysteine was injected intraperitoneally, about 60% of the label in the carcass soluble fraction was converted to taurine in the 30 PC% group (Fig. 2), while more than 50% of the label in the soluble fraction was also recovered as taurine when ^{14}C -cysteine was injected (17). Urinary excretion of total ^{35}S was less than 8% of the dose in the 0 to 15 PC% groups and it increased to 22% at 30 PC% in the diet, while the urinary ^{14}C excretion was 3 to 6% of the dose in all dietary groups and the expired $^{14}\text{CO}_2$ output increased to 25% of the dose at 30 PC% in the diet (17). A much higher proportion of ^{35}S excreted in the urine was recovered as the inorganic sulfate fraction, but less than 10% of urinary ^{35}S was present in the taurine fraction. Similar excretion patterns following injection of ^{35}S -cysteine were observed by Stipanuk (4), Daniels and Stipanuk (5), and Benjamin and Steele (25), who have reported that the cysteine sulfinic acid pathway plays a major regulatory role in cysteine degradation and that a greater proportion of the cysteine sulfinic acid formed by the action of cysteine dioxygenase is catabolized by the decarboxylation pathway, which yields taurine, than by the transamination pathway, which yields sulfate, ammonia, and pyruvate in rats fed a large amount of cysteine. However, the results from their experiments and others showed that the ratio of $^{35}\text{SO}_4$ to total ^{35}S was much greater than that of ^{35}S -taurine to total ^{35}S in urine. Thus, it is necessary to confirm whether cysteine sulfur is mainly catabolized by the taurine pathway or not, especially in dietary protein depletion.

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