Comparison of the Response of Serum Ceruloplasmin and Cholesterol, and of Tissue Ascorbic Acid, Metallothionein, and Nonprotein Sulphydryl in Rats to the Dietary Level of Cystine and Cysteine

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The effects were compared of the addition of graded levels of l-cystine and of l-cysteine (0.3, 3, or 5%) to a 10% casein diet on several metabolic parameters in rats. The growth-promoting effect of cystine was equivalent to that of cysteine. Supplementation of these two amino acids elevated serum cholesterol, liver ascorbic acid, liver nonprotein sulphydryl (SH) and kidney metallothionein, and reduced the activity of serum ceruloplasmin. The responses of serum cholesterol, liver nonprotein SH, and serum ceruloplasmin to cystine were greater than those to cysteine. When the basal diet was supplemented with 0.3% of these amino acids, the elevation of liver ascorbic acid by cystine supplementation was less than that by cysteine supplementation. However, when supplemented with 5% of these amino acids, the elevation of liver ascorbic acid by cystine was greater than that by cysteine. There was no difference in the influence of cystine and cysteine on kidney metallothionein. This study demonstrates that dietary cystine and cysteine had the same influence on growth, but had a differential influence on such metabolic parameters as liver nonprotein SH, serum ceruloplasmin, serum cholesterol, and tissue ascorbic acid.

Key words: l-cystine; l-cysteine; metabolic response; nonprotein sulphydryl; rat

Evidence is accumulating that a dietary supplementation of cystine has several metabolic influences on animals. Supplementation of cystine has elevated kidney metallothionein in rats, whereas supplementation of methionine did not.11 Our recent studies have indicated that supplemental cystine enhanced the intestinal absorption of zinc, which in turn caused an accumulation of kidney zinc and promoted gene expression of kidney metallothionein.12 Dietary supplementation of cystine has been shown to reduce the activity of serum ceruloplasmin (a copper-metalloenzyme),21 while feeding excess cystine exaggerated the defects of dietary copper deficiency in rats.31 There is also evidence that a high level of dietary cystine was associated with a high concentration of ascorbic acid in the tissues of rats.23,24 An excessive level of cystine can induce hypercholesterolemia in rats by a mechanism that enhances the synthesis of cholesterol.25,26 While an excessive level of methionine does not (Yang and Kato, unpublished data). Hitomi and Yoshida have recently found that cystine was a more effective amino acid than methionine for maintaining the serum level of albumin and the hepatic level of albumin mRNA in rats fed on a low soybean diet.27 These facts imply the nutritional significance of dietary cystine for regulating several aspects of metabolism.

The major metabolic fate of cystine in mammals is its conversion to cysteine, and the catabolism of cystine then merges with that of cysteine.3 It has been reported that there was no difference in the growth-promoting effect of supplemental cystine and cysteine to a diet low in sulfur-containing amino acids.9,10 From these facts, it seems that these two amino acids are nutritionally equivalent. However, in a preliminary study, we observed that the dietary addition of 2% l-cystine to a 20% casein diet depressed serum ceruloplasmin activity and elevated serum cholesterol, whereas the addition of 2% l-cysteine did not.11 These findings provide the first evidence indicating a differential metabolic response to dietary cystine and cysteine. In the present study, we compared the effects of supplementing with graded levels of cystine and cysteine on several metabolic parameters, including serum ceruloplasmin and cholesterol, tissue ascorbic acid, metallothionein, and nonprotein sulphydryl (SH) in rats. In our previous study already mentioned,11 cystine or cysteine (2%) was supplemented to a basal diet containing an adequate level of sulfur-containing amino acids for the growth of rats. In the present study, l-cystine or l-cysteine (0.3, 3, or 5%) was supplemented to a 10% casein diet which contained an inadequate level of sulfur-containing amino acids for growth.

Materials and Methods
Animals and diets. Male rats of the Wistar strain weighing 90-120 g (Hiroshima Laboratory Animal Center, Hiroshima) were used. They were initially fed on a commercial stock diet (MF; Oriental Yeast Co., Ltd, Tokyo) for 3 d and were given free access to the experimental diet and deionized water for 1 d. The room temperature was kept at 24°C with a 12 h light-dark cycle (08:00 h-20:00 h and 20:00 h-08:00 h, respectively). The animals were cared for in accordance with the guidelines established by the Japanese Society of Nutrition and Food Science. The composition of the basal diet was as follows (as% of total): casein, 10; α-corn starch, 50; sucrose, 25; cellulose powder, 4; corn oil, 5; vitamin mixture,12 2; salt mixture,12.4 This basal diet contained 0.3% of sulfur-containing amino acids.11 The basal diet was supplemented with L-cysteine (purity > 99%) or L-cystine (purity > 98%) (Katayama Chemical Ind., Osaka) at the level of 0.3, 3, or 5% of the diet (Table I). Dietary additions of these amino acids were made at the expense of carbohydrate as one part of sucrose and two parts of corn starch. These experimental diets contained 37.5-39.6 mg of Zn/kg of diet as determined by atomic absorption spectrophotometry. To prevent its auto-oxidation in the diet, cysteine was
mixed every day with the basal diet in the period 19:00-20:00h and immediately given to the animals. After feeding the experimental diets (6 animals in each group) for 10 d, the feed was removed from the cages at 8:00 h, and the animals were lightly anesthetized with diethyl ether before being killed between 13:00 h and 15:00 h. Blood was collected by heart puncture, serum samples were obtained by centrifugation, and tissues were immediately excised and weighed.

**Analytical procedures.** Ceruloplasmin activity was measured by the method of Schöinsky et al.15 one unit of ceruloplasmin activity being defined as the volume of serum that oxidized 1 μmol of o-dianisidine/min. Serum cholesterol was determined according to the method of Pearson et al.17 The tissue concentration of nonprotein SH was measured by the method of Sedlak and Lindsay.17) and the tissue concentration of ascorbic acid was measured as previously described.16) The serum activity of alanine aminotransferase (EC 2.6.1.2) was measured with a kit (GPT UV Test Wako, Wako Pure Chemical, Osaka).17) Hepatic lipid peroxidation was evaluated by determining the concentration of thiobarbituric acid-reactive substances (TBARS) by the method of Ohkawa et al.18) An analysis of zinc in the diets, serum and tissues was performed by atomic absorption spectrophotometry as described previously.19) The tissue level of metallothionein was measured by the method of Onosaka and Cherian.20) The statistical significance of differences between values was analyzed by one-way ANOVA and Duncan’s multiple-range test.21) Differences in mean values with p<0.05 being considered significant.

**Results**
As shown in Table I, elevated food intake and growth were observed after the addition of 0.3% cystine or 0.3% cysteine to the basal diet. The addition of 3% and 5% of either of these amino acids caused lower food intake and growth than the addition of 0.3% of these amino acids. The effects of dietary cystine on the food intake and growth were equivalent to those of dietary cysteine. A significant elevation of liver weight (% of body weight) was observed with the addition of either 3% cystine or 0.3% cysteine to the basal diet. The addition of either cystine or cysteine (0.3–5%) caused a higher concentration of liver nonprotein SH, the elevation of nonprotein SH by supplemental cystine being greater than that by supplemental cysteine in the 0.3% and 3% groups. Supplementation of either cystine or cysteine significantly reduced the liver concentration of TBARS, although there was no difference in the effects of these amino acids on the TBARS level. The serum activity of alanine aminotransferase was unaffected by dietary manipulation (data not shown)

As shown in Table II, a higher level of dietary cystine was associated with lower activity of ceruloplasmin. Although cysteine supplementation also caused lower activity of ceruloplasmin, there was no difference in activity among the cysteine (0.3–5%)-supplemented groups. A higher level of serum cholesterol was observed with the supplementation of 3% and 5% cystine and of 5% cysteine as compared with the basal diet alone. Higher level of dietary cystine was associated with a higher concentration of

### Table I. Effects of a Dietary Addition of Cystine or Cysteine on Food Intake, Growth, Liver Non-protein SH, and Liver Thiobarbituric Acid Reactive Substances (TBARS)

<table>
<thead>
<tr>
<th>Dietary addition (% of diet)</th>
<th>Food intake (g/10d)</th>
<th>Gain in body wt. (g/10d)</th>
<th>Relative weight (% of body wt.)</th>
<th>Non-protein SH (μmol/g of tissue)</th>
<th>TBARS (nmol/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>108±3d</td>
<td>8±5</td>
<td>4.92±0.17</td>
<td>2.6±0.2</td>
<td>132±10</td>
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<tr>
<td>Cystine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>202±6</td>
<td>66±5</td>
<td>4.42±0.13</td>
<td>7.4±0.6</td>
<td>109±9</td>
</tr>
<tr>
<td>3</td>
<td>147±10b</td>
<td>35±6</td>
<td>5.7±0.26</td>
<td>10.5±0.3</td>
<td>92±7</td>
</tr>
<tr>
<td>5</td>
<td>95±10d</td>
<td>2±5</td>
<td>4.82±0.15</td>
<td>10.1±0.3</td>
<td>99±6</td>
</tr>
<tr>
<td>Cysteine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>201±10c</td>
<td>61±3</td>
<td>5.78±0.13</td>
<td>5.9±0.4</td>
<td>97±5</td>
</tr>
<tr>
<td>3</td>
<td>124±13c</td>
<td>29±5</td>
<td>5.40±0.23</td>
<td>8.1±0.4</td>
<td>101±3</td>
</tr>
<tr>
<td>5</td>
<td>85±3a</td>
<td>10±2</td>
<td>5.0±0.16</td>
<td>10.0±0.6</td>
<td>100±6</td>
</tr>
</tbody>
</table>

Initial body wt. (g): 128±1 (n=42). Each value is the mean±SE (n=6). Means in a column not followed by the same letter are significantly different (p<0.05).

### Table II. Effects of a Dietary Addition of Cystine or Cysteine on Serum Ceruloplasmin, Serum Cholesterol, and Tissue Ascorbic Acid

<table>
<thead>
<tr>
<th>Dietary addition (% of diet)</th>
<th>Serum ceruloplasmin (U/liter)</th>
<th>Serum cholesterol (nmol/liter)</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>None</td>
<td>196±8</td>
<td>2.8±0.2</td>
<td>0.79±0.02</td>
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<tr>
<td>Cystine</td>
<td></td>
<td></td>
<td>0.61±0.01</td>
</tr>
<tr>
<td>0.3</td>
<td>118±4</td>
<td>2.7±0.1</td>
<td>0.85±0.06</td>
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<tr>
<td>3</td>
<td>87±6</td>
<td>3.6±0.1</td>
<td>1.25±0.07</td>
</tr>
<tr>
<td>5</td>
<td>68±5</td>
<td>3.9±0.3</td>
<td>1.44±0.03</td>
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<tr>
<td>Cysteine</td>
<td></td>
<td></td>
<td>1.14±0.03</td>
</tr>
<tr>
<td>0.3</td>
<td>142±10c</td>
<td>2.6±0.2</td>
<td>1.22±0.05</td>
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<tr>
<td>3</td>
<td>137±9</td>
<td>2.9±0.2</td>
<td>1.28±0.03</td>
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<tr>
<td>5</td>
<td>152±11b</td>
<td>3.5±0.2</td>
<td>1.08±0.04</td>
</tr>
</tbody>
</table>

Each value is the mean±SE (n=6). Means in a column not followed by the same letter are significantly different (p<0.05).
ascorbic acid in the liver, kidney, and spleen. Supplementation of cysteine (0.3–5%) elevated the concentration of ascorbic acid in these tissues. However, the difference in concentration of liver and kidney ascorbic acid was only slight among the cysteine-supplemented groups. When the basal diet was supplemented with 0.3% of these amino acids, the elevation of liver ascorbic acid by cystine supplementation was less than that by cysteine supplementation. However, when supplemented with 5% of the amino acids, the elevation of liver ascorbic acid by cysteine was greater than that by cysteine.

An elevation in serum zinc was observed with 0.3–5% of these amino acids (Table III), although there was no difference in serum zinc between the cystine and cysteine groups. Supplementation by either of these two amino acids caused a higher concentration of liver zinc. When compared with the effect from the basal diet, a slight elevation of liver metallothionein was observed with a 5% cystine addition, while a lower level of liver metallothionein was observed with a 5% cysteine addition. Elevated concentrations of kidney zinc and metallothionein were observed with the supplementation of 3% and 5% of either of these amino acids, the effect of cystine on kidney metallothionein being equivalent to that of cysteine.

**Discussion**

We have reported that a higher concentration of cholesterol and lower activity of ceruloplasmin in the serum of rats were brought about by adding 2% cystine to a diet containing 20% casein. However, the addition of 2% cystine to the same diet had no influence on these parameters. These findings led us to the present study to compare the influence of graded levels of these amino acids in the diet on several metabolic parameters. A higher level of dietary cystine is known to be associated with higher concentrations of ascorbic acid and nonprotein SH (predominantly reduced glutathione) in the liver, and with lower activity of ceruloplasmin. It has also been reported that an excessive level of dietary cystine elevated serum cholesterol, and kidney metallothionein and zinc. The present study confirmed these findings. While a differential response of food intake, growth, zinc status, and tissue metallothionein to these amino acids was hardly or only slightly apparent, differential response of cholesterol, ceruloplasmin, ascorbic acid, and nonprotein SH to dietary cysteine and cystine was clearly demonstrated. These differences seemed not to be mediated through any differential liver damage or oxidative stress since there was no difference in the serum activity of alanine aminotransferase and liver TBARS between the cystine- and cysteine-supplemented groups. In general, the response of cholesterol, ceruloplasmin, and nonprotein SH, but not of ascorbic acid, to dietary cystine was greater than to dietary cysteine.

The present study demonstrates that the addition of either 3% cystine or 5% cysteine was required for the maximum elevation of nonprotein SH. The differential response of liver nonprotein SH to an addition of 0.3% cystine or 0.3% cysteine was also clearly demonstrated. This finding implies that such a differential response can be observed not only with a high or excessive amount of either of these supplemental amino acids, but also occurred with lower supplemental levels. Dietary sulfur-containing amino acids are utilized for the biosynthesis of glutathione, the dietary level of sulfur-containing amino acids being known to be closely associated with the liver concentrations of nonprotein SH or glutathione. From the data for liver nonprotein SH (Table I), the availability of dietary cystine seems to be higher than that of dietary cysteine. In addition, the dietary level of either of these amino acids that is required for maximum growth seems to be lower than that required for maintaining the maximum level of liver nonprotein SH. Takada and Banai have reported that the rat hepatocyte has a transport system for the selective uptake of cystine but not of cysteine. A higher uptake of cystine by this transport system may account for the higher response of liver nonprotein SH to dietary cystine.

Hypercholesterolemia due to a dietary excess of cystine is mediated through the induction of hepatic cholesterol biosynthesis. Reduced glutathione and other thiols can activate the activity of 3-hydroxy-3-methylglutaryl CoA reductase, an enzyme that catalyzes the rate-limiting step in the pathway of cholesterol synthesis. Therefore, it seems that a higher concentration of hepatic glutathione by a dietary excess of cystine would lead to enhanced cholesterol biosynthesis. The higher response of serum cholesterol to dietary cystine than to dietary cysteine might possibly be at least in part ascribed to the higher response of liver nonprotein SH to dietary cysteine.

A high level of dietary cystine was associated with the
reduced activity of ceruloplasmin (Table II). Although supplemental cysteine also caused lower ceruloplasmin activity, confirming in large part the findings of Grimbale et al., the effect of cysteine on ceruloplasmin was not strong as described in their report. The change in ceruloplasmin by dietary cystine may be ascribable to the altered liver nonprotein SH, and the different level of response of ceruloplasmin to cystine and cysteine also seems to be partially related to the difference in response of hepatic nonprotein SH. However, among the cysteine-supplemented groups, there was no difference in ceruloplasmin activity, while the hepatic nonprotein SH level was related to the dietary level of cysteine. These results imply that the change in ceruloplasmin activity by dietary cystine and cysteine cannot be explained solely by the change in hepatic nonprotein SH. A further study on the relationship between ceruloplasmin activity and the tissue concentration of several metabolites (reduced glutathione, oxidized glutathione, etc.) of these amino acids is now in progress.

A high level of dietary cystine was associated with an elevated concentration of tissue ascorbic acid (Table II), while supplementation with cysteine also caused a higher concentration of tissue ascorbic acid. However, the response of liver and kidney ascorbic acid to dietary cysteine was clearly different from that to dietary cystine. With a low level of supplementation of the amino acids (0.3%), dietary cysteine seems to have had a greater influence on tissue ascorbic acid, while, with a high level of supplementation (5%), dietary cystine seems to have greater influence. These findings also indicate the differential response of ascorbate metabolism to these two amino acids. Further study is necessary to elucidate the reason of this difference in response.

The elevation of kidney metallothionein and serum zinc by supplemental cystine is mediated through the enhanced intestinal absorption of zinc. Our recent study has also demonstrated a higher intestinal absorption of zinc and serum zinc in rats by supplementation with 3% cysteine to a 10% casein diet (Yang et al., unpublished data). The elevation of kidney metallothionein by supplemental cysteine (Table III) may also have been mediated through the enhanced absorption of zinc. Other responses to cystine such as those of ceruloplasmin, cholesterol, and ascorbic acid to cystine seem to have resulted from metabolic changes in the liver, since this is the major organ responsible for the biosynthesis of ceruloplasmin, sterols, and ascorbic acid. The reason for no difference in the response of kidney metallothionein between cystine and cysteine may be related to the effects of these two amino acids on the intestinal absorption of zinc, and not to the effects on hepatic zinc metabolism.

In summary, the present study has demonstrated that dietary cystine and cysteine have a differential metabolic influence. Subsequent to this study, we found that a dietary excess of cystine or cysteine enhanced the defects of dietary copper deficiency by a differential mechanism involving altering iron metabolism. Taken together, our findings imply that these two amino acids are not always nutritionally equivalent, and that we should pay attention to the nutritional differences that do exist.

References