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Excretion of 3-Mercaptolactate-Cysteine Disulfide, Sulfate and Taurine in human Urine before and after Oral Administration of Sulfur-containing Amino Acids.

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Abstract

The excretion of 3-mercaptolactate-cysteine mixed disulfide [S-(2-hydroxy-2-carboxyethylthio)-L-cysteine, HCETC], sulfate and taurine in the urine of normal adults was investigated before and after oral administration of L-cysteine and related sulfur-containing amino acids. Before the loading of amino acids, the excretion (mean $\pm$ SD) per kg of body weight per day of HCETC, free sulfate and taurine was 0.096 $\pm$ 0.042, 305.7 $\pm$ 66.1 and 31.9 $\pm$ 8.7 μ mol, respectively. After the loading of L-cysteine (800 μ mol/kg of body weight), the average excretion in the 24-h urine of HCETC increased 2-fold and that of taurine increased 1.6-fold. The average excretion of free sulfate after the L-cysteine loading was 989.4 $\pm$ 145.1 and 388.8 $\pm$ 51.6 μ mol/kg per day in the first and second 24-h urine, respectively, indicating that the sulfur corresponding to 85% of the L-cysteine loaded was excreted as free sulfate in 24 h. Administration of L-cystine (400 μ mol/kg) resulted in similar results. The increase in HCETC after L-cysteine or L-cystine administration indicates that L-cysteine is metabolized in part through the transamination pathway (3-mercaptopyruvate pathway) and that an equilibrium exists between the intake and excretion of sulfur in humans.

KEYWORDS: 3-mercaptolactate-cysteine mixed disulfide, cysteine metabolism, 3-mercaptopyruvate pathway, sulfur amino acid, sulfate excretion

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Excretion of 3-Mercaptolactate-Cysteine Mixed Disulfide, Sulfate and Taurine in Human Urine before and after Oral Administration of Sulfur-Containing Amino Acids

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The excretion of 3-mercaptolactate-cysteine mixed disulfide [S-(2-hydroxy-2-carboxyethylthio)-L-cysteine, HCETC], sulfate and taurine in the urine of normal adults was investigated before and after oral administration of L-cysteine and related sulfur-containing amino acids. Before the loading of amino acids, the excretion (mean $\pm$ SD) per kg of body weight per day of HCETC, free sulfate and taurine was 0.096 ± 0.042 , 305.7 ± 66.1 and $31.9 \pm 8.7 \mu\text{mol}$, respectively. After the loading of L-cysteine ($800 \mu\text{mol/kg}$ of body weight), the average excretion in the 24-h urine of HCETC increased 2-fold and that of taurine increased 1.6-fold. The average excretion of free sulfate after the L-cysteine loading was 989.4 ± 145.1 and $388.8 \pm 51.6 \mu\text{mol/kg}$ per day in the first and second 24-h urine, respectively, indicating that the sulfur corresponding to 85 % of the L-cysteine loaded was excreted as free sulfate in 24 h. Administration of L-cystine ($400 \mu\text{mol/kg}$) resulted in similar results. The increase in HCETC after L-cysteine or L-cystine administration indicates that L-cysteine is metabolized in part through the transamination pathway (3-mercaptopyruvate pathway) and that an equilibrium exists between the intake and excretion of sulfur in humans.

Key words : 3-mercaptolactate-cysteine mixed disulfide, cysteine metabolism, 3-mercaptopyruvate pathway, sulfur amino acid, sulfate excretion

3-Mercaptolactate-cysteine mixed disulfide [S-(2-hydroxy-2-carboxyethylthio)-L-cysteine, HCETC] is excreted as one of the minor constituents of normal human urine (1). Patients with β -mercaptolactate-cysteine disulfiduria, an inborn error of cysteine metabolism, excrete a

large amount of 3-mercaptolactate-cysteine mixed disulfide in the urine (2-5). It is considered from experiments using tissues of rats and guinea pigs that HCETC is formed from L-cysteine through 3-mercaptopyruvate and 3-mercaptolactate in the transamination pathway of L-cysteine metabolism (6, 7). The pathway may be referred to as the 3-mercaptopyruvate pathway (8) because it proceeds through 3-mercaptopyruvate. In the present study, the effect of the oral administration of sulfur-containing amino acids on the excretion of

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HCETC in normal humans was investigated.

Sulfate and taurine are two major metabolites of sulfur compounds in mammals, and they are excreted in the urine (9, 10). In a previous study using rats, we observed that the oral administration of L-cysteine resulted in the rapid excretion of sulfate and taurine, which suggested the existence of an equilibrium of sulfur metabolism (11). This paper also reports the existence of such a sulfur balance in humans.

Materials and Methods

Materials. L-Cysteine (free base) was purchased from Sigma Chemical Co., St. Louis, MO, USA. L-Cystine, L-methionine and barium perchlorate were obtained from Wako Pure Chemical Ind., Osaka, Japan. Thorin [2-(2-hydroxy-3, 6-disulfo-1-naphthylazo)-benzenearsenic acid, sodium salt] was obtained from Dojindo Labs., Kumamoto, Japan. Magnesium hydroxide carbonate was from E. Merck, Darmstadt, FRG. A weakly basic anion-exchange resin, AG-3 (100–200 mesh), was obtained from Bio-Rad Labs., Richmond, CA, USA. HCETC was synthesized according to the method of Kobayashi (12).

Administration of sulfur-containing amino acids and collection of urine. Sulfur-containing amino acids were administered as follows to 3 or 4 healthy male adults in this department ranging from 31 to 44 years of age and from 65 to 74 kg of body weight, whose informed consent was obtained. Food and water intake was not restricted during this experiment except for avoiding food with a high taurine content. L-Cysteine (free base, 0.8 mmol/kg of body weight) was divided into three portions and each portion was administered at 9:30, 14:30 and 20:30 o'clock. The urine collected for 24 h before the administration of the first portion of L-cysteine was taken as the control. The urine collected for 24 h after the start of L-cysteine administration was taken as the urine of the first day of L-cysteine loading, and the second and third day urine was collected likewise. The 24-h urine was collected in a bottle containing 10 ml of toluene and 10 ml of 4M HCl, and stored at 4°C until the analysis was carried out. L-Cystine and L-methionine were administered at doses of 0.40 and 0.27 mmol/kg of body weight, respectively, and urine was collected as above. Basal levels of urinary amino acids and free sulfate of the above

human subjects were determined repeatedly at intervals of one week, and the numbers of determinations include these repeated determinations.

Determination of HCETC. Because of the low concentration of HCETC in normal human urine, the amino acid fraction containing HCETC was concentrated using a column of ion-exchange resin as follows. Fifty ml of urine was adjusted to pH 3.1 and applied to a column (2 × 13 cm) of AG-3 equilibrated with 0.2M sodium acetate buffer, pH 3.1. The column was then washed with 200 ml of the same buffer, followed by 200 ml of 0.2M acetic acid. Thereafter, the column was eluted with 250 ml of 2M acetic acid, and the eluate was evaporated to dryness under reduced pressure in a flash evaporator. The residue was dissolved in 1.5 ml of water, and 0.5 ml of the solution was applied to a Hitachi KLA-5 amino acid analyzer (Hitachi Seisakusho, Tokyo, Japan) equipped with a column (0.9 × 55 cm) of Hitachi custom ion-exchange resin 2613. Amino acid analysis was carried out at 55°C at a flow rate of 60 ml/h using 0.2N sodium citrate buffer (pH 3.19). HCETC was eluted at 67 min under these conditions as shown in Fig. 1. HCETC was identified by co-chromatography with authentic HCETC. The HCETC peak disappeared upon treatment of the sample with dithiothreitol, indicating that the peak was a disulfide. Other peaks in the chromatogram were not identified in the present study.

Determination of taurine. Taurine was determined with the same amino acid analyzer as above, which was equipped with a column (0.9 × 55 cm) of Hitachi custom ion-exchange resin 2614 as previously reported (13). Five-tenths ml of the 24-h urine was analyzed.

Determination of cystine and methionine. Cystine and methionine were determined with the same amino acid analyzer and column which were used for the determination of HCETC, except for the elution buffers. Five-tenths ml of the 24-h urine was applied, and elution was carried out using 0.2N sodium citrate buffer, pH 3.25, for 90 min, and thereafter with 0.2N sodium citrate buffer, pH 4.25.

Determination of sulfate. Free sulfate in the urine was determined by microtitration as reported elsewhere (11).

Statistical significance was examined with a paired *t*-test.

Results and Discussion

The average excretion of HCETC in normal

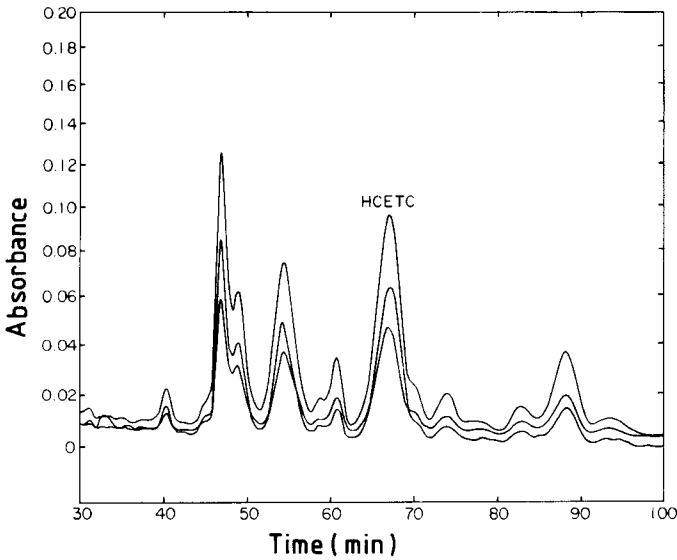


Fig. 1 An elution profile of urinary 3-mercaptolactate-cysteine mixed disulfide (HCETC). HCETC-containing fraction was concentrated using an AG-3 column and was analyzed with an amino acid analyzer. Upper and middle traces, absorbance at 570 nm; lower trace, at 440nm. Experimental details are described under Materials and Methods.

Table 1 Excretion of 3-mercaptolactate-cysteine mixed disulfide [S-(2-hydroxy-2-carboxyethylthio)-L-cysteine, HCETC] in normal human urine before and after the oral administration of L-cysteine (800 $\mu\text{mol/kg}$ of body weight), L-cystine (400 $\mu\text{mol/kg}$) or L-methionine (270 $\mu\text{mol/kg}$)^a

Amino acid administered	HCETC ($\mu\text{mol/kg}$ of body weight per day, n = 4)			
	Days after administration			
	Control	1	2	3
L-Cysteine	0.122 $\pm$ 0.043	0.251 $\pm$ 0.093*	0.093 $\pm$ 0.043	0.099 $\pm$ 0.037
L-Cystine	0.064 $\pm$ 0.009	0.178 $\pm$ 0.034*	0.089 $\pm$ 0.024	0.063 $\pm$ 0.028
L-Methionine	0.101 $\pm$ 0.049	0.074 $\pm$ 0.027	0.088 $\pm$ 0.025	0.074 $\pm$ 0.023

^a: Amino acid was divided into 3 portions, and each portion was administered to 3 normal adult volunteers at 9:30, 14:30 and 20:30 o'clock. One of them underwent the same experiment twice. Urine was collected for 24 h after the first administration. The 24-h urine before the administration was taken as the control. HCETC was determined with an amino acid analyzer after concentrating the HCETC-containing fraction with an ion-exchange resin column. Experimental details are described under Materials and Methods. * : Significantly different from the control ($p < 0.025$).

human subjects was $0.096 \pm 0.042 \mu\text{mol/kg}$ of body weight per day ($8.2 \pm 2.4 \mu\text{mol/person}$ per day) (n = 9). The HCETC excretion in the 24-h urine after the oral administration of L-cysteine (800 $\mu\text{mol/kg}$ of body weight) increased about 2-fold as shown in Table 1. On the second day, the HCETC excretion returned to the basal level. These results are in accordance with our previous report (14). When L-cystine (400 $\mu\text{mol/kg}$) was loaded, a similar manner of HCETC excretion to

that after L-cysteine loading was observed, but HCETC excretion did not increase when L-methionine (270 $\mu\text{mol/kg}$) was loaded in the present study.

As shown above, the level of HCETC excretion and the increase in the HCETC excretion after L-cysteine or L-cystine loading to normal humans was low among urinary sulfur compounds (0.02–0.05 % of the sulfur loaded), but the increase was statistically significant ($p <$

0.025). These results indicate that L-cysteine was partly metabolized through the transamination pathway, although its proportion in the total sulfur metabolism seems to be small in comparison with the oxidative metabolism of L-cysteine. As the activity of 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2) has been found to be high in human erythrocytes and blood platelets (15), 3-mercaptopyruvate formed from L-cysteine by transamination may be further metabolized by the transsulfuration reaction. Therefore, the proportion of 3-mercaptopyruvate used for the formation of HCETC may be quite small in normal humans.

Patients with β -mercaptolactate-cysteine disulfiduria excrete in the urine 20-50 times as much HCETC as normal subjects do (2-5). In a patient whose 3-mercaptopyruvate sulfurtransferase was confirmed to be deficient (16), the HCETC excretion increased after the oral loading of L-cysteine ($2.36 \text{ g} \times 3$ per day), reaching more than 5 % of the sulfur loaded as L-cysteine (17). These facts suggest that the participation of the 3-mercaptopyruvate pathway in the overall metabolism of L-cysteine in normal humans is much greater than that assumed from the excretion of HCETC in normal human subjects.

The average excretion of free sulfate in the normal humans in the present study was $305.7 \pm 66.1 \mu\text{mol/kg}$ of body weight per day ($26.0 \pm 5.7 \text{ mmol/person}$ per day) ($n = 11$). As shown in Fig. 2, a large increase in sulfate excretion was observed after the L-cysteine loading, reaching about 3 times the basal excretion level. The excess portion of the sulfate excreted in the first and second 24-h urine samples corresponded to 82.3 % and 7.3 %, respectively, of the sulfur loaded as L-cysteine.

The taurine excretion in these subjects before L-cysteine loading was $31.9 \pm 8.7 \mu\text{mol/kg}$ of body weight per day ($2.4 \pm 0.4 \text{ mmol/person}$ per day) ($n = 11$). The level of the taurine excretion in the first 24-h urine of each human subject after L-cysteine loading ranged from 1.4 to 2 times the basal level. The increased portion corresponded to about 2 % of the sulfur loaded as L-cysteine.

Cystine and methionine excretion before L-cysteine loading was 1.32 ± 0.89 and $0.53 \pm 0.13 \mu\text{mol/kg}$ of body weight per day (87.7 ± 56.1 and $36.9 \pm 9.7 \mu\text{mol/person}$ per day) ($n = 9$), respectively. About a 2-fold increase in these amino acids was observed after L-cysteine loading.

The increased portion in the total excretion of free sulfate and these amino acids in the first and second 24-h urine after the L-cysteine loading corresponded to 85 % and 8 % of the sulfur administered as L-cysteine. On the third day after L-cysteine loading, the excretion of these sulfur compounds returned to the basal level.

When L-cystine ($400 \mu\text{mol/kg}$ of body weight) was administered, the patterns of the excretion of free sulfate and sulfur-containing amino acids were similar to those after L-cysteine loading as shown in Fig. 3.

When L-methionine ($270 \mu\text{mol/kg}$ of body weight) was administered, the sulfate excretion increased significantly, but HCETC, taurine and

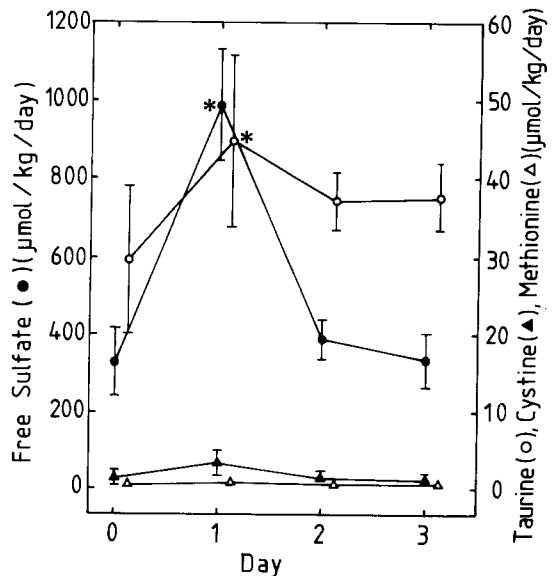


Fig. 2 Urinary excretion of free sulfate, taurine, cystine and methionine after the oral administration of L-cysteine ($800 \mu\text{mol/kg}$ of body weight). The administration of L-cysteine and collection of urine were performed as described in the legend to Table 1 and under Materials and Methods. *: Significantly different from the control (day 0), $p < 0.005$; $n = 5$.

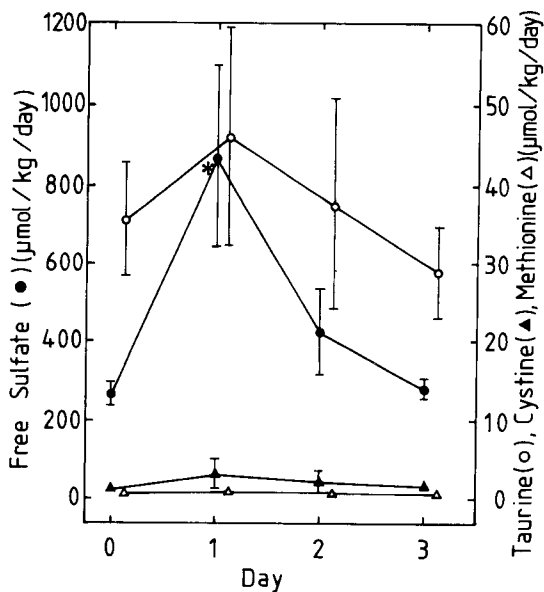


Fig. 3 Urinary excretion of free sulfate, taurine, cystine and methionine after the oral administration of L-cysteine ($400 \mu\text{mol/kg}$ of body weight). For details, see the legend to Fig. 2. *: Significantly different from the control (day 0), $p < 0.05$; $n = 3$.

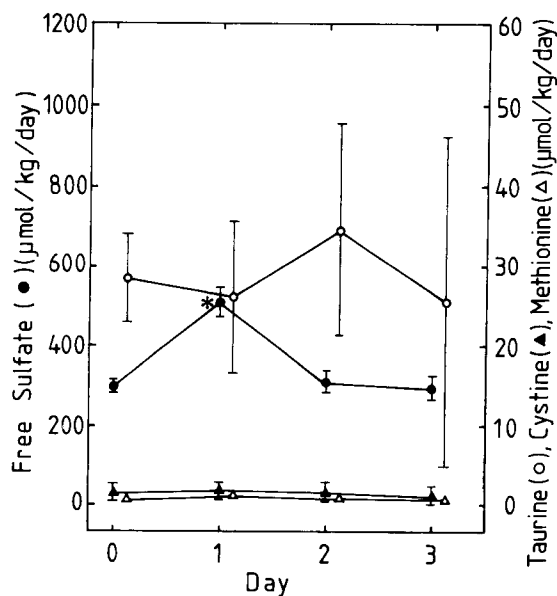


Fig. 4 Urinary excretion of free sulfate, taurine, cystine and methionine after the oral administration of L-methionine ($270 \mu\text{mol/kg}$ of body weight). For details, see the legend to Fig. 2. *: Significantly different from the control (day 0), $p < 0.005$; $n = 3$.

cystine excretion did not increase as shown in Table 1 and Fig. 4. These results seem to reflect the low dose of L-methionine and/or the metabolism of L-methionine which does not proceed through L-cysteine (18).

As described above, the excretion of sulfate and taurine, two major metabolites of sulfur compounds in mammals, increased significantly after L-cysteine or L-cysteine loading. The excess excretion of sulfur in the first 24-h urine after the administration of these two amino acids corresponded to about 85 % of the sulfur. These results seem to indicate that an equilibrium exists between the intake and excretion of sulfur in the human body as it has been shown in the rat (11).

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