In Vivo Studies of Cysteine Metabolism

USE OF D-CYSTEINESULFINATE, A NOVEL CYSTEINESULFINATE DECARBOXYLASE INHIBITOR, TO PROBE TAURINE AND PYRUVATE SYNTHESIS*

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Catherine L. Weinstein, Rudy H. Haschemeyer, and Owen W. Griffith‡
From the Department of Biochemistry, Cornell University Medical College, New York, New York 10021

Although several pathways contribute to the catabolism of L-cysteine, the products formed are few—taurine + CO₂ and pyruvate + ammonia + sulfate. L-Cysteinesulfinate is a key intermediate that is either decarboxylated to ultimately yield taurine or transaminated to yield pyruvate. There is strong evidence that pyruvate is also formed by several cysteinesulfinate-independent pathways collectively referred to as “cysteine desulphydrase.” The quantitative importance of cysteinesulfinate-independent pathways of taurine synthesis is less clear, but it has been suggested that taurine synthesis from the cysteamine released during phosphopantetheine and CoASH turnover accounts for decreases from 63 to 51 to 42% of the contribution of taurine synthesis to total catabolism in animal muscle and heart). In the present studies, the metabolic flux through each of these pathways was quantitated in vivo by monitoring the formation of respiratory ¹⁴CO₂ in mice administered L-[¹⁴C]- or L-[3-¹⁴C]cysteine. Mice given 0.05 mmol/kg of L-cysteine or 0.5 or 2.5 mmol/kg of L-cysteine catabolized 35%, 51%, and 72% of the dose, respectively, in 6 h; the relative contribution of taurine synthesis to total catabolism decreases from 63 to 61 to 42% as the L-cyst(e)ine dose is increased. To evaluate the role of L-cysteinesulfinate in taurine synthesis, D-cysteinesulfinate was characterized and used as a metabolism-resistant, potent, and specific inhibitor of cysteinesulfinate decarboxylase. Studies with L-[¹⁴C]- and L-[3-¹⁴C]cysteine in the presence of inhibitor indicate that 85–93% of taurine synthesis occurs from L-cysteinesulfinate; the calculated contribution of the phosphopantetheine pathway is small and may approximate zero. L-Cysteinesulfinate transamination accounts for 25% of pyruvate synthesis from L-[¹⁴C]cysteine (0.05 mmol/kg) but only 11% of pyruvate synthesis from L-[¹⁴C]cysteine (2.5 mmol/kg). Cysteine desulphydrase reactions account for most of the pyruvate synthesis.

Cysteine is among the most toxic of the protein amino acids both as a dietary constituent (1) and as a component of tissue culture media (2, 3). Although the basis of cysteine toxicity is not fully defined, formation of reactive oxygen species during the auto-oxidation of cysteine (4) and nonenzymatic formation of stable cysteine—pyridoxal 5’-phosphate adducts (5) are thought to be important factors. The rates of these and other potentially toxic, nonenzymatic reactions of cysteine are minimal in mammalian tissues because intracellular cysteine levels are very low, typically 30–250 μM (6–10). Although advantageous in terms of toxicity, maintenance of very low cysteine levels might, in principle, compromise the availability of cysteine for necessary metabolic processes. In practice, cysteine availability is supported by a much larger (0.8–8 mM) intracellular pool of glutathione (GSH, γ-glutamylcysteinylglycine). In vivo studies clearly show that GSH turnover plays an important role both in interorgan cysteine transport and in the short-term regulation and maintenance of the cellular pool of free cysteine (6, 7, 12, 13).

Mammals obtain cyst(e)ine directly from the diet and by synthesis via the transsulfuration pathway from methionine sulfur and serine carbon. Since neither the diet nor transsulfuration is specifically regulated with respect to cysteine accumulation (8, 10), cellular cysteine levels are controlled mainly by the reactions using cysteine—protein and GSH synthesis, and cyst(e)ine catabolism. In general, the synthetic processes contribute little to the net change in cysteine stores. Cysteine used for protein synthesis is balanced by cyst(e)ine released by protein degradation. In normally nourished GSH-replete animals, net synthesis of GSH, as distinct from GSH catabolism, is limited by feedback inhibition of γ-glutamylcysteine synthetase (14). Thus, under most conditions, tissue levels of free cysteine and cysteine equivalents (i.e., GSH) are ultimately regulated and limited by the reactions of cysteine catabolism (8).

Although several pathways contribute to cysteine catabolism, only two sets of products are formed—pyruvate + sulfate + ammonia and taurine + CO₂; one intermediate, cysteinesulfinate, is central to both pyruvate and taurine pathways. Thus, pyruvate is formed by decomposition of β-sulfynpyruvate, the product of cysteinesulfinate transamination, and also by the cysteine desulphydrase reaction (actually a composite of several activities (15)). Taurine is formed by oxidation of hypotaurine, an intermediate formed both by cysteinesulfinate decarboxylation and by cysteamine oxidation. Whereas cysteinesulfinate is the major taurine precursor in liver, it has been suggested that cysteamine, formed from cysteine during phosphopantetheine and CoASH turnover, is the major hypotaurine precursor in skeletal muscle and heart (16–18). The latter tissues exhibit high taurine levels but low rates of cysteinesulfinate decarboxylation.

Studies with L-[¹⁴C]- and L-[³⁵S]cysteine indicate that taurine and pyruvate + sulfate synthesis account for 70–85% and 15–30% of cysteine catabolism, respectively, in the male rat (19–21). Other studies with L-[³⁵S]cysteine suggest that >70% of cysteine is metabolized to sulfate (19, 22, 23). Although liver contains high levels of the enzymes forming and decarboxylating cysteinesulfinate, some (24), but not all (25), recent studies with rat hepatocytes support pyruvate + sulfate rather
than taurine as the major product of cysteine catabolism in that tissue. In the present studies, L-[1-14C]cysteine was used to quantitate taurine and pyruvate formation in the intact tissue. In the present studies, ~[1-14C]cysteine was used as substrates and inhibitors of rat liver cysteine sulfinate decarboxylase; d-cysteine sulfinate was the most potent inhibitor identified (28). As shown in Fig. 1 (Miniprint), inhibition by d-cysteinesulfinate is competitive with respect to L-cysteine sulfinate (A) and is characterized by a $K_i$ of 0.32 mM (B). Similar studies indicate that d-cysteinesulfonate is also a competitive inhibitor but with a $K_i$ of 2.3 mM (data not shown). Neither D-cysteine sulfinate nor d-cysteinesulfinate is decarboxylated by the enzyme (28).

D-Cysteinesulfinate is not an effective inhibitor of either cytoplasmic or mitochondrial aspartate aminotransferase (Table 1, Miniprint). This finding is consistent with the observation that aspartate aminotransferase is only weakly inhibited by D-aspartate (Table 1) and with previous reports that aspartate aminotransferase interacts either not at all (29) or only very weakly (30) with D-amino acids. Since L-cysteine sulfinate transamination is catalyzed by aspartate aminotransferase (31, 32), the absence of significant aspartate aminotransferase inhibition supports the view that D-cysteinesulfinate is a specific inhibitor of L-cysteine sulfinate decarboxylation.

In Vivo Inhibition of Cysteine Sulfinate Decarboxylase by D-Cysteinesulfonate—In vitro inhibition of cysteine sulfinate decarboxylase activity can be determined in vivo by comparing the extent of $^{14}CO_2$ formation from L-[1-14C]- and L-[3-14C]cysteine sulfinated (33) or by quantitating the rate of $^{14}CO_2$ formation from L-[1-14C]cysteine sulfonate (34). The latter approach, which is both direct and technically simple, is based on the observation that (i) L-cysteine sulfonate is rapidly decarboxylated by cysteine sulfinate decarboxylase in vivo and (ii) that L-cysteine sulfonate transamination, in contrast to L-cysteine sulfinate transamination, yields metabolites ($\beta$-sulfo-lactate and $\beta$-sulfopyruvate) that are thermodynamically stable and not catabolized to $CO_2$ (34).

Mice administered L-[1-14C]cysteine sulfonate (0.25 mmol/kg) metabolize about 72% of the dose to $^{14}CO_2$ in 6 h; the maximum rate of metabolism occurs in the first hour and corresponds to a rate of 95 $\mu$mol/h/kg (Fig. 2). Mice given D-cysteine sulfonate (10 mmol/kg) 60 min prior to administration of L-[1-14C]cysteine sulfonate metabolize only about 16% of the dose to $^{14}CO_2$ in 6 h. The maximum rate of metabolism is decreased to 12 $\mu$mol/h/kg, an inhibition of 87%. In the first hour, metabolism averages only 8 $\mu$mol/h/kg, an inhibition of 92% (Fig. 2).

D-Cysteinesulfinate-mediated inhibition of cysteine sulfinate decarboxylase persists for several hours. When the interval between D-cysteinesulfinate administration and L-[1-14C]cysteine sulfonate injection is extended from 1 to 5 h, the maximum rate of $^{14}CO_2$ formation is decreased by 81% relative to uninhibited controls (Fig. 3A, Miniprint). The extent of inhibition at 5 h is thus only slightly less than seen at 1 h. If L-[1-14C]cysteinesulfonate is given 10 or 15 h following D-cysteinesulfinate administration, the maximum rates of $^{14}CO_2$ formation are decreased by 72 and 32%, respectively (Fig. 3B and C, Miniprint). Note that the data at 15 h shows considerable scatter, suggesting excretion or metabolism has reduced tissue D-cysteinesulfinate concentrations to nearly noninhibitory levels in some but not all animals.

It is probable that the anionic amino acid transport system mediates both D-cysteinesulfonate and L-[1-14C]cysteinesulfinate uptake (35, 36). It is thus possible that inhibition of L-[1-14C]cysteinesulfinate metabolism by D-cysteinesulfinate reflects inhibition of transport into tissues rather than specific inhibition of cysteinesulfinate decarboxylase. In vivo studies comparing the metabolic effects of D-cysteinesulfinate with D-aspartate, a more effective transport inhibitor, indicate, however, that little, if any, of the D-cysteinesulfinate-mediated inhibition of $^{14}CO_2$ formation is due to reduced L-[1-14C]cysteinesulfonate uptake by tissues (studies described in Miniprint). In addition, the finding that urinary excretion of $\beta$-sulfolactate, a L-[1-14C]cysteinesulfonate metabolite formed by the transamination pathway (34), is greatly increased by D-cysteinesulfinate administration indicates that D-cysteinesulfinate alters the metabolic partitioning of radiolabeled L-cysteinesulfonate without significantly changing the overall extent of its metabolism (studies described in Miniprint).

Effect of D-Cysteinesulfinate on $\beta$-Sulfo-Lactate and $\beta$-Sulfo-Pyruvate Metabolism—Although neither $\beta$-sulfo-lactate nor $\beta$-sulfo-pyruvate is directly metabolized to $CO_2$ at a significant
rate, both are precursors of L-cysteinesulfonate by reversal of the transamination pathway of cysteinesulfonate and cysteinesulfinate metabolism (34). As shown in Fig. 5, A and B, rate, both are precursors of L-cysteinesulfonate by reversal of the transamination pathway of cysteinesulfonate and cysteinesulfinate decarboxylase. The results shown for each compound represent averages ± S.D. of data from 8 to 16 mice. Where indicated, d-cysteinesulfinate was given by intraperitoneal injection 1 h prior to the administration of the labeled compound.

### Table 4

<table>
<thead>
<tr>
<th>Compound administered (dose in mmol/kg)</th>
<th>[1-14C]Pyruvate or precursor</th>
<th>% of dose recovered as 14CO2</th>
<th>t½</th>
<th>% of dose recovered in the urine</th>
<th>[3-14C]Pyruvate or precursor</th>
<th>% of dose recovered as 14CO2</th>
<th>t½</th>
<th>% of dose recovered in the urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate (0.1)</td>
<td></td>
<td>81.8 ± 3.1</td>
<td>21 ± 4</td>
<td>5.6 ± 2.3</td>
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<td>66.5 ± 1.4</td>
<td>42 ± 4</td>
<td>7.4 ± 1.5</td>
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<tr>
<td>Pyruvate (0.1) + d-cysteinesulfinate (10)</td>
<td></td>
<td>80.7 ± 4.5</td>
<td>19 ± 4</td>
<td>6.4 ± 1.3</td>
<td></td>
<td>63.5 ± 1.4</td>
<td>41 ± 4</td>
<td>7.3 ± 1.4</td>
</tr>
<tr>
<td>Pyruvate (0.5)</td>
<td></td>
<td>77.5 ± 5.6</td>
<td>27 ± 4</td>
<td>6.7 ± 1.1</td>
<td></td>
<td>66.5 ± 1.8</td>
<td>33 ± 6</td>
<td>7.2 ± 1.1</td>
</tr>
<tr>
<td>Pyruvate (0.5) + d-cysteinesulfinate (10)</td>
<td></td>
<td>81.7 ± 0.9</td>
<td>21 ± 2</td>
<td>6.8 ± 0.8</td>
<td></td>
<td>66.0 ± 2.8</td>
<td>37 ± 5</td>
<td>8.4 ± 2.3</td>
</tr>
<tr>
<td>Pyruvate (2.5)</td>
<td></td>
<td>81.1 ± 4.0</td>
<td>22 ± 2</td>
<td>5.3 ± 2.5</td>
<td></td>
<td>62.8 ± 3.3</td>
<td>38 ± 8</td>
<td>7.4 ± 0.7</td>
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<tr>
<td>Pyruvate (2.5) + d-cysteinesulfinate (10)</td>
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<td>81.6 ± 2.4</td>
<td>21 ± 3</td>
<td>6.2 ± 0.5</td>
<td></td>
<td>63.1 ± 1.6</td>
<td>41 ± 4</td>
<td>8.5 ± 1.3</td>
</tr>
<tr>
<td>D-Alanine (0.5)</td>
<td></td>
<td>81.1 ± 2.7</td>
<td>63 ± 2</td>
<td>3.1 ± 1.2</td>
<td></td>
<td>64.1 ± 1.6</td>
<td>0.1 ± 1</td>
<td>4.9 ± 1.9</td>
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<tr>
<td>D-Alanine (0.5) + d-cysteinesulfinate (10)</td>
<td></td>
<td>80.2 ± 3.8</td>
<td>60 ± 9</td>
<td>3.6 ± 0.7</td>
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<td>67.4 ± 1.1</td>
<td>70 ± 5</td>
<td>5.6 ± 0.4</td>
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<td>L-Alanine (0.5)</td>
<td></td>
<td>77.3 ± 2.5</td>
<td>30 ± 4</td>
<td>2.1 ± 0.2</td>
<td></td>
<td>62.2 ± 2.0</td>
<td>44 ± 2</td>
<td>3.1 ± 0.3</td>
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<tr>
<td>L-Alanine (0.5) + d-cysteinesulfinate (10)</td>
<td></td>
<td>77.1 ± 1.6</td>
<td>30 ± 4</td>
<td>2.6 ± 0.6</td>
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<td>59.3 ± 0.7</td>
<td>47 ± 5</td>
<td>2.1 ± 0.2</td>
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<tr>
<td>Glucose (0.5)</td>
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<td>72.2 ± 1.5</td>
<td>49 ± 4</td>
<td>2.8 ± 0.8</td>
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<td>65.5 ± 3.6</td>
<td>58 ± 8</td>
<td>2.2 ± 0.8</td>
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<tr>
<td>Glucose (0.5) + d-cysteinesulfinate (10)</td>
<td></td>
<td>72.9 ± 2.2</td>
<td>51 ± 8</td>
<td>2.8 ± 0.4</td>
<td></td>
<td>63.3 ± 4.4</td>
<td>63 ± 4</td>
<td>3.3 ± 0.4</td>
</tr>
</tbody>
</table>

The urine of d-cysteinesulfinate was determined 6 and 24 h after administration of the inhibitor at doses of 0.5 and 10 mmol/kg (Table 3). Since pyruvate is rapidly metabolized to CO2 (see below), the rate of formation of 14CO2 from D-[1-14C]cysteinesulfinate reflects the metabolism of d-cysteinesulfinate by this pathway (and possibly others) in vivo. As anticipated from the finding that cysteinesulfinate metabolism is inhibited for several hours following D-cysteinesulfinate administration, metabolism of the inhibitor is slow (Fig. 6, Miniprint). In 6 h only 7.5% of an intraperitoneal dose of D-[1-14C]cysteinesulfinate (10 mmol/kg) is metabolized to 14CO2 during that period the rate of metabolism is essentially constant at 0.130 ± 0.007 μmol/h · kg. This rate may be maximal for d-cysteinesulfinate metabolism since decreasing the dose by 95% to 0.5 mmol/kg reduces the rate of 14CO2 formation only 54% (i.e. to 0.06 μmol/h · kg). The central role of d-aspartate oxidase in D-[1-14C]cysteinesulfinate metabolism is supported by the observation that the maximum rate of 14CO2 formation is inhibited about 70% in animals given d-aspartate, a preferred oxidase substrate (Fig. 6, Miniprint), and inhibited about 35% in animals given meso tartarate (10 mmol/kg), a competitive inhibitor of the oxidase (39) (results not shown). It may be noted that d-[4-14C]aspartate is metabolized to 14CO2 much more rapidly than is d-cysteinesulfinate; maximum rates observed following intraperitoneal injection at doses of 10 and 0.5 mmol/kg are 2.24 and 0.25 μmol/h · kg, respectively.

Tissue, plasma, and urine levels of d-cysteinesulfinate were determined 6 and 24 h after administration of the inhibitor at doses of 0.5 and 10 mmol/kg (Table 3, Miniprint). At the lower dose, the tissue level of d-cysteinesulfinate determined 6 h after injection was reproducibly above the Kᵢ for cysteinesulfinate decarboxylase inhibition only in the kidney; after 24 h, tissue levels were uniformly below 0.1 mmol/kg and were often undetectable. At the higher dose, the concentration of d-cysteinesulfinate 6 h after injection averaged 14 and 16 μmol/g in kidney and liver, respectively. Concentrations above 1 μmol/g were found in skeletal and heart muscle, whereas brain contained 0.42 μmol/g. In most mice, kidney and liver levels of d-cysteinesulfinate remained relatively high (>2 μmol/g) even 24 h after injection, although the range of concentrations observed was large. Interestingly, the brain d-cysteinesulfinate concentration decreased only 19% between 6 and 24 h; by 24 h, skeletal and heart muscle levels decreased >90% to levels below the Kᵢ for decarboxylase inhibition. Chromatography of portions of the kidney and liver homogenates indicated that cysteinesulfinate accounted for >95% of the radiolabeled material present. Plasma levels of d-cysteinesulfinate were low (<0.3 mM) at both 6 and 24 h. Urine levels of d-cysteinesulfinate were high even at 6 h and, when 10 mmol/kg of inhibitor was given, accounted for about 60% of the dose. Chromatography of the urine indicated that about 94% of the radiolabeled material was cysteinesulfinate; the remaining 6% co-eluted with cysteinesulfonate.

**Effect of D-Cysteinesulfinate on the Metabolism of Pyruvate and Pyruvate Precursors**—In vivo inhibition of cysteinesulfinate decarboxylase is expected to alter the partitioning of L-
cysteine between metabolism to taurine and metabolism to pyruvate plus sulfate (see below). Prior to quantitating those changes, it was necessary to determine the extent to which [1-14C]- and [3-14C]pyruvate are metabolized to 14CO2 and to establish that d-cysteinesulfinate did not affect pyruvate metabolism. As shown in Table 4, mice given [1-14C]pyruvate by subcutaneous injection at doses of 0.1, 0.5, and 2.5 mmol/kg metabolize about 80% of the dose to 14CO2 in 6 h; the rate of metabolism (reflected in the t 1/2 values), and the fraction of the dose excreted in the urine are unchanged over the range of pyruvate doses tested. D-cysteinesulfinate (10 mmol/kg) affects neither the rate nor extent of [1-14C]pyruvate metabolism. As observed previously (20, 33), [3-14C]pyruvate is less extensively metabolized to 14CO2. At all doses tested, about 65% of the administered [3-14C]pyruvate is metabolized to 14CO2 within 6 h; d-cysteinesulfinate is again without significant effect on the rate or extent of metabolism.

To examine the possibility that injected pyruvate is metabolized differently than pyruvate formed metabolically, the formation of 14CO2 from several precursors of [1-14C]- and [3-14C]pyruvate was also quantitated. In mammals, d-alanine is metabolized efficiently and uniquely to pyruvate by d-alanine oxidase; the yield of 14CO2 from D-[1-14C]alanine and D-[3-14C]alanine is comparable to that from pyruvate in the presence and absence of d-cysteinesulfinate (Table 4). The averaged results from the pyruvate and d-alanine studies indicate that the yield of 14CO2 from C-1 of pyruvate is 80.4 ± 1.9% and 81.1 ± 0.7% in the absence and presence of d-cysteinesulfinate, respectively. The yield of 14CO2 from C-3 of pyruvate is 65.0 ± 1.8% and 65.0 ± 2.1% in the absence and presence of d-cysteinesulfinate, respectively. It is noted that these values are not corrected for the urinary loss of a few percent of the administered radioactivity. If the 14CO2 data are expressed as a percentage of the dose not excreted in the urine, the yields in the absence and presence of inhibitor from C-1 and C-3 of pyruvate are 84.8 ± 1.7%, 86.0 ± 2.0%, 69.7 ± 2.4%, and 70.2 ± 1.8%, respectively.

L-Alanine and glucose are also efficiently metabolized to pyruvate but are subject to alternative metabolisms as well. Although the overall yields are consequently a few percent lower, it is notable that the yield of 14CO2 from precursors of [3-14C]pyruvate continues to be about 80% of that from [1-14C]pyruvate precursors (Table 4). The results support the conclusions that the extent of pyruvate metabolism to CO2 is not sensitive to the source of the pyruvate and that the rate symbols) were given d-cysteinesulfinate (10 mmol/kg) by intraperitoneal injection 1 h prior to administration of radioisotope. The mice were placed in metabolic chambers immediately after L-[14C]cystine injection, and respiratory 14CO2 was monitored as described under "Experimental Procedures" (Miniprint). The points shown on each line represent averages ± S.D. for six to seven animals. Urine was collected for 24 h. For mice given L-[14C]cystine, the amount of radioisotope in the urine was 6.7 ± 2.7 and 7.0 ± 1.8% of the dose administered for control and inhibitor-treated mice, respectively. For mice given L-[3-14C]cystine, the corresponding values were 10.1 ± 1.2 and 8.6 ± 2.2%. B, mice were treated as in A except that the radioisotope given was L-[14C]cysteine (six mice per group) or L-[3-14C]cysteine (four mice per group); the dose was 0.5 mmol/kg. In mice given L-[14C]cysteine, the amount of radioisotope in the urine at 24 h was 3.1 ± 1.3 and 3.5 ± 0.5% of the dose administered in control and inhibitor-treated mice, respectively. Urine data for mice receiving L-[3-14C]cysteine is not available. C, mice were treated as in A except that the radioisotope given was L-[1-14C]cysteine (six mice per group) or L-[3-14C]cysteine (six mice per group); the dose was 2.5 mmol/kg. In mice given L-[1-14C]cysteine, the amount of radioisotope in the urine at 24 h was 2.9 ± 0.4 and 3.5 ± 0.4% of the dose administered in control and inhibitor-treated mice, respectively. For mice given L-[3-14C]cysteine, the corresponding values were 10.0 ± 1.2 and 8.9 ± 1.1%.

![Fig. 7. Effect of d-cysteinesulfinate on the metabolism of L-[14C]cysteine to 14CO2.](image)
Cysteine Metabolism

Effect of D-cysteinesulfinate on radiolabeled L-cyst(e)ine metabolism

Experimental conditions were as shown in Fig. 7. Data are shown for percent of dose recovered as $^{14}$CO$_2$ at 6 h. These data and inhibition data from Table 6 were used to calculate the metabolic fluxes for each dose of L-[14C] cyst(e)ine. The calculated metabolic flux values for reaction 8 were tested statistically to determine if they were significantly greater than zero; at L-[14C]cyst(e)ine doses of 0.05, 0.5, and 2.5 mmol/kg, the corresponding p values are 0.019, 0.066, and 0.042, respectively (see “Numerical Methods” (Miniprint) and the “Discussion”). CYSO$_2$, cysteinesulfinate.

<table>
<thead>
<tr>
<th>Compound administered (dose in mmol/kg)</th>
<th>% of dose recovered as $^{14}$CO$_2$ Without D-cysteinesulfinate</th>
<th>With D-cysteinesulfinate</th>
<th>Calculated metabolic flux as % of dose*</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-[14C]Cys (0.05)</td>
<td>32.32 ± 0.98</td>
<td>40.80 ± 2.27</td>
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<tr>
<td>L-[13C]Cys (0.05)</td>
<td>10.69 ± 0.88</td>
<td>25.85 ± 1.58</td>
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<tr>
<td>L-[14C]Cys (0.5)</td>
<td>45.81 ± 0.67</td>
<td>51.30 ± 1.16</td>
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<tr>
<td>L-[13C]Cys (0.5)</td>
<td>19.89 ± 2.38</td>
<td>35.00 ± 0.64</td>
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<tr>
<td>L-[14C]Cys (2.5)</td>
<td>63.86 ± 0.97</td>
<td>66.45 ± 1.66</td>
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<tr>
<td>L-[13C]Cys (2.5)</td>
<td>30.01 ± 2.05</td>
<td>44.82 ± 1.93</td>
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</tbody>
</table>

* Numbers inside parentheses represent % of observed catabolism.

and extent of metabolism are not affected by D-cysteinesulfinate.

Effect of D-Cysteinesulfinate on L-Cyst(e)ine Metabolism—In vivo inhibition of cysteinesulfinate decarboxylase alters the metabolic partitioning of cysteinesulfinate in favor of transamination. The formation of pyruvate and sulfate is increased, whereas the formation of taurine by the cysteinesulfinate decarboxylase pathway, but not by other pathways, is decreased. Since taurine is not metabolized to CO$_2$ (33), decreased formation of taurine results in increased formation of pyruvate and sulfate (Fig. 7B). In vivo inhibition of cysteinesulfinate decarboxylase is evidenced by the present or previous studies as follows: (i) the catabolism of cysteinesulfinate to CO$_2$ or cysteinesulfinate, the product of cysteine dioxygenase (reaction 1), is not significantly inhibited. The finding that D-cysteinesulfinate increases the yield of 14CO$_2$ from L-[14C]cystine (0.05 mmol/kg) by subcutaneous injection, the extent of 14CO$_2$ formation at 6 h is increased from 10.7 to 25.9% by previous treatment with D-cysteinesulfinate (10 mmol/kg) (Fig. 7A). Formation of 14CO$_2$ from L-[14C]cystine is decreased to a lesser extent (see “Discussion”). Qualitatively similar results were obtained in studies with L-cysteine at doses of 0.5 mmol/kg (Fig. 7B) and 2.5 mmol/kg (Fig. 7C). The results are summarized in Table 5.

Effect of D-Cysteinesulfinate on L-Cysteinesulfinate Decarboxylation—Since D-cysteinesulfinate is a competitive inhibitor, the extent of cysteinesulfinate decarboxylase inhibition in vivo will depend on the concentration of both the inhibitor and the substrate, L-cysteinesulfinate; the intracellular concentration of the latter may increase with increased doses of L-cyst(e)ine. To quantitate the extent of inhibition directly, mice were administered a tracer dose of L-[35S] cysteinesulfinate along with unlabeled L-cyst(e)ine in doses identical to the L-[14C]cyst(e)ine doses used in the studies of Fig. 7. After 6 h, the mice were killed, and formation of [35S] taurine was quantitated in the urine, liver, and remaining carcass (Table 6). As anticipated, the extent of inhibition decreased slightly with increasing doses of unlabeled L-cyst(e)ine. The percent inhibition values, with S.D. values calculated as described under “Experimental Procedures,” are 78.6 ± 1.7%, 74.0 ± 3.1%, and 72.1 ± 2.7% for animals given 0.05 mmol/kg of L-cyst(e)ine, 0.5 or 2.5 mmol/kg of L-cyst(e)ine, respectively.

**Discussion**

Several pathways and reactions contribute to the catabolism of L-cyst(e)ine (Fig. 8). Although a variety of intermediates are formed, the final products are few, taurine + CO$_2$ or pyruvate + sulfate + “ammonia.” In mammals, taurine is not metabolized further (33), but pyruvate is rapidly metabolized to CO$_2$ and pyruvate is rapidly metabolized to CO$_2$ in moderate to high yield as shown in Table 4. Pathways yielding only pyruvate + sulfate (progressed as reaction 6), pathways yielding only taurine (initiated by reaction 8), and pathways yielding both taurine and pyruvate + sulfate (initiated by reaction 1) have been proposed. The present studies, in concert with previous studies defining the partitioning of L-cysteinesulfinate (33), allow the relative metabolic flux through the indicated pathways to be quantitated in vivo.

As examined here, the metabolism of radiolabeled L-cyst(e)ine to $^{14}$CO$_2$ is described quantitatively by four equations in four unknowns (Fig. 9, Equations 1–4). Since R4 and R6, the effect of pyruvate, the amino group of L-cyst(e)ine is either lost directly as ammonia (e.g. by action of γ-cystathionase on cysteine) or released in various forms by the cysteine desulfhydrase and thus part of cysteine desulfhydrase, normally interacts with a substrate structurally similar to D-cysteinesulfinate. Of the enzymes catalyzing reactions 1, 6, and 8 is not likely. Inhibition of reaction 2 does not influence the partitioning of L-cyst(e)ine between reactions 1, 6, and 8. As noted, direct inhibition of the enzymes catalyzing reactions 1, 6, and 8 is not likely. Inhibition of reaction 2 may, however, increase intracellular levels of L-cyst(e)inesulfinate, the product of cysteine dioxygenase (reaction 1). Fortunately, in vitro studies indicate that the cysteine dioxygenase reaction is irreversible and not inhibited by L-cysteinesulfinate (40); partitioning of L-cyst(e)ine, as opposed to L-cysteinesulfinate, is thus unlikely to be affected. In fact, since L-cysteinesulfinate is irreversibly transaminated by aspartate aminotransferase (reaction 4), significant...

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The cytoplasmic GSH pools of kidney, liver, and pancreas turn over too rapidly to contribute to the 6-h sequesterization of L-cysteine in the present context.

These results support the view that excess L-cyst(e)ine is quickly catabolized (see Introduction) and are quantitatively similar to findings in previous studies with male rats (19). In those studies, total catabolism at 8 h increased from 30 to 74% as the amount of L-[35S]cysteine given was increased from about 0.1 pmol/kg to 8 mmol/kg (19). However, in contrast to the present results with mice, the relative contribution of taurine synthesis to total cysteine catabolism in rats studied with tracer doses of L-[35S]cysteine (19, 20).
In the absence of D-cysteinesulfinate:

\[
\text{eq.1: } {^{14}}\text{CO}_2 \text{ from } (1-^{14}\text{C})\text{Cys} = 0.8(R_6) + (R_8) + 0.8(R_4) + (R_2)
\]

In the presence of D-cysteinesulfinate:

\[
\text{eq.3: } {^{14}}\text{CO}_2 \text{ from } (1-^{14}\text{C})\text{Cys} = 0.8(R_6) + (R_8) + 0.8(R_4+I(R_2)) + (1-I)(R_2)
\]

\[
\text{eq.4: } {^{14}}\text{CO}_2 \text{ from } (3-^{14}\text{C})\text{Cys} = 0.65(R_6) + 0.1(R_8) + 0.65(R_4+I(R_2)) + 0.1(1-I)(R_2)
\]

**FIG. 9.** Contribution of various reactions to the formation of \( {^{14}}\text{CO}_2 \) from L-[\(^{14}\text{C}\)]cysteine. The relative flux through reactions 2, 4, 6, 8, of Fig. 8, typically expressed as percent of dose, are represented as \( R_2, R_4, R_6, \) and \( R_8 \), respectively. The extent of \( {^{14}}\text{CO}_2 \) formation at the time of interest (6 h in the present context) is expressed in the same units (e.g. percent of dose). The value 0.8 at 0.65 represent the fractional yield of \( {^{14}}\text{CO}_2 \) in the complete metabolism of L-[\(^{14}\text{C}\)]pyruvate and [3-\(^{14}\text{C}\)]pyruvate through reaction 7, respectively. The value 0.1 represents the yield of \( {^{14}}\text{CO}_2 \) in the metabolism of \( \beta-[^{14}\text{C}] \) hypotaurine through reaction 3 (see Fig. 8 and Ref. 33). The fractional inhibition effected by D-cysteinesulfinate is represented as \( I \).

**TABLE 7**

*Relative importance of several reactions of L-cysteine catabolism*

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Cysteine dioxygenase, reaction 1</th>
<th>Cysteinesulfinate decarboxylase, reaction 2</th>
<th>Aspartate aminotransferase, reaction 4</th>
<th>Cysteine desulphhydrase, reaction 6</th>
<th>Phosphopantetheine pathway, reaction 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-[(^{14}\text{C})]Cyst(e)ine (0.05)</td>
<td>0.24</td>
<td>59.0</td>
<td>9.4</td>
<td>29.3</td>
<td>9.5</td>
</tr>
<tr>
<td>L-[(^{14}\text{C})]Cyst(e)ine (0.5)</td>
<td>51.8</td>
<td>44.0</td>
<td>7.8</td>
<td>44.5</td>
<td>3.3</td>
</tr>
<tr>
<td>L-[(^{14}\text{C})]Cyst(e)ine (2.5)</td>
<td>43.4</td>
<td>36.9</td>
<td>6.5</td>
<td>51.1</td>
<td>5.6</td>
</tr>
</tbody>
</table>

The values shown are calculated as described in the text.

The values shown are calculated as described in the text.

---

Cysteine metabolism in rats did not decrease with the increasing cysteine dosage (19). In considering this apparent species difference, it should be noted that in the rat studies DL-[\(^{14}\text{C}\)]cysteine was diluted with unlabeled L-cysteine before injection (19); at high L-cysteine doses, limited catabolism of undiluted D-[\(^{14}\text{C}\)]cysteine of very high specific activity may have accounted for a substantial fraction of the \( {^{14}}\text{CO}_2 \).

The relative importance of L-cysteinesulfinate-dependent and -independent pathways of taurine synthesis has not previously been determined in vivo. The question was addressed in the present studies by using D-cysteinesulfinate to specifically inhibit cysteinesulfinate decarboxylase (reaction 2). With inhibitor present, taurine synthesis via reaction 2 is decreased, and the fraction of L-[\(^{14}\text{C}\)]cysteinesulfinate metabolized by aspartate aminotransferase to pyruvate (reaction 4) is increased (observed as a markedly increased yield of \( {^{14}}\text{CO}_2 \) from L-[\(^{14}\text{C}\)]cysteine). If the extent of decarboxylase inhibition is known, the relative contributions of reaction 2 and the phosphopantetheine pathway (initiated by reaction 8) to total taurine synthesis can be calculated from data on \( {^{14}}\text{CO}_2 \) formation from either [1-\(^{14}\text{C}\)]- or [3-\(^{14}\text{C}\)]cysteine in the presence and absence of D-cysteinesulfinate. Using extent of inhibition values determined in separate studies with L-[\(^{35}\text{S}\)]cysteinesulfinate (Table 6), the role of the phosphopantetheine pathway (or other L-cysteinesulfinate-independent pathways) in taurine biosynthesis was quantitated in the intact mouse (Table 5). At all doses of L-[\(^{14}\text{C}\)]cysteine tested, the contribution of the phosphopantetheine pathway was small (range 7-15% of taurine synthesis or 3.3-7.5% of total cysteine catabolism). Although all of the values are within one S.D. of zero, it is noted that two of the three metabolic flux values for reaction 8 (Table 5) are significantly greater than zero by the usual statistical criteria. In view of the complexity of the studies and calculations, the present data should be interpreted conservatively; the contribution of the phosphopantetheine pathway to taurine synthesis is small and may approximate zero.

In previous studies, we showed that hypotaurine and pyruvate synthesis account for about 85 and 15%, respectively, of the metabolism of an intraperitoneal dose of L-cysteinesulfinate (1.0 mmol/kg) (33). Applying this ratio to the present results, the relative metabolic flux through reactions 2, 4, 6, and 8 of Fig. 8 was estimated from data in Table 5; the flux through reaction 1 is the sum of reactions 2 and 4. The results, collected in Table 7, indicate that the cysteine dioxygenase-cysteinesulfinate decarboxylase pathway accounts for virtually all (85-93%) taurine synthesis and that the cysteine desulphhydrase pathways account for 75-89% of total pyruvate synthesis (i.e. the contribution of the aspartate aminotransferase pathways). The values shown in Table 6 (only those values are affected). In the absence of a specific explanation for the increased metabolism, a more exact correction of the calculations cannot be made. It is noted that the D-cysteinesulfinate-mediated apparent increase in total catabolism is relatively large at low doses of L-cyst(e)ine (38 and 21% at dose of 0.1 and 0.5 mmol/kg, respectively) and is small at the highest doses tested (11% at 2.5 mmol/kg). Since the calculated flux through reaction 8 is low at all doses and does not show a similar trend, it is unlikely that the results have been significantly skewed by the assumption that cysteine catabolism is uniformly increased by D-cysteinesulfinate administration.
ferase reaction to pyruvate synthesis drops from 25% at a low
dose of L-[14C]cysteine to 11% at a high dose of L-[14C]cys-
teine. It may be noted that the amount of L-[14C]cysteine
administered in the low dose studies approximates the size of
the endogenous L-cysteine pool (i.e. >0.11 mmol/kg) and is
a small fraction of the average daily cysteine intake of the mice
(>2-5 mmol/kg). Since the metabolically active GSH pool
(>0.3 mmol/kg in liver, kidney, and pancreas (11)) can readily
incorporate a dose of this size, the metabolic partitioning
observed with 0.05 mmol/kg of L-[14C]cysteine offers a reason-
able estimate of normal catabolism of L-cyst(e)ine. The meta-
abolic partitioning in studies with 0.5 and 2.5 mmol/kg of L-
[14C]cysteine provide an estimate of cysteine catabolism fol-
lowing an average and a very large meal, respectively (0.5
mmol/kg of L-cysteine corresponds to a 30-gm mouse ingest-
ing 0.5 gm of a standard amino acid-based mouse diet (42).
The present studies thus reflect the range of L-cysteine me-
tabolism likely to occur in mice on normal diets. As shown in
Table 7, the relative contribution of cysteine dioxygenase to
L-cysteine catabolism is diminished by 30% at the highest L-
[14C]cysteine dose tested, and the contribution of the cysteine
desulfhydrase reactions is greatly (81%) increased. The rela-
tive importance of the several possible "cysteine desulfhy-
drase" pathways has been examined in vitro (15), but addi-
tional studies are required to determine which of these activ-
ities is important in vivo at various L-cysteine loads.

Although most previous in vitro and in vivo studies have
addressed cyst(e)ine metabolism in the rat, metabolism in the
mouse and rat are similar, and it is thus of interest to compare
the present results to earlier work with the rat. The present
mouse studies confirm the quantitative importance of cysteine
dioxygenase in cysteine catabolism (19) but indicate that in
short-term studies the relative flux through the dioxygenase
pathway is diminished at higher cysteine loads. The finding
that cysteine dioxygenase activity is increased several-fold in
rats fed a high cysteine diet for 5-7 days (8) suggests that the
high metabolic flux through the dioxygenase is ultimately
restored as an adaptive response to continued high cysteine
loads. The present results are also in accord with perfused rat
liver studies indicating that 25-30% of pyruvate synthesis is
cysteinesulfinate-dependent (43); this partitioning has not
previously been determined in vivo. The present findings do
not support studies indicating that pyruvate + sulfate rather
than taurine is the major cysteine metabolite under normal
conditions (19, 22-24); as shown in Table 5, taurine synthesis
accounts for about 50% of the cysteine catabolized even at
high cysteine loads. Since radiolabeled taurine is diluted into
a large body pool and therefore excreted slowly, [35S]taurine
synthesis was probably underestimated in previous studies
showing [35S]sulfate as the predominant product. Although we
have not observed significant metabolism of taurine to
sulfate, taurine formed from orally administered cyst(e)ine
may be metabolized to sulfate (20).

The present studies do not support a quantitatively impor-
tant role for phosphopantetheine turnover in taurine synthe-
sis. Although taurine is formed more rapidly from phospho-
pantethenyl-L-cysteine than from L-cysteine by tissue ho-
logenates (17), synthesis of phosphopantetheine from
cysteine in vivo is shown here to be slow. Since we have
confirmed by immunological and enzymatic assays that
cysteinesulfinate decarboxylase activity is negligible in skeletal
muscle and heart (28), the large taurine pools of these tissues
is presumably maintained mainly by uptake of plasma taurine.
Plasma taurine can be maintained by cysteinesulfinate decar-
boxylase-mediated taurine synthesis in other tissues. Cys-
teinesulfinate decarboxylase levels are high in liver and mod-
erate in kidney and brain (28). Studies by Huxtable and
Lippincott in mice (44) and rats (45) show that both dietary
and biosynthetic taurine are actively exchanged among tis-

Finally, it is noted that d-cysteinesulfinate is the most
potent and specific inhibitor of cysteinesulfinate decarboxy-
lase reported to date. Whereas d-cysteinesulfinate inhibits the
metabolism of L-[14C]cysteinesulfinate to [14C]CO2 by 90%, β-
methylene-BL-aspartate, an irreversible, mechanism-based
inhibitor reported previously (33), inhibits the same metabo-
ligism by only 45% at maximum tolerated doses (34). In contrast
and cysteinesulfinate does not significantly inhibit aspartate aminotransferase. Although in-
hibition of the decarboxylase by d-cysteinesulfinate is com-
petitive with L-cysteinesulfinate and thus reversed by high L-
cysteinesulfinate levels, substantial inhibition is possible in
vivo because (i) L-cysteinesulfinate levels are normally very
low (47), (ii) significant accumulations of L-cysteinesulfinate
are prevented by its irreversible aspartate aminotransferase-
mediated transamination to β-sulfinylpyruvate, and (iii) d-
cysteinesulfinate is nontoxic and slowly metabolized. It is
thus possible to maintain high tissue levels of d-cysteinesul-
finate and favorable ratios of d- to L-cysteinesulfinate. Similar
advantages should apply to the use of d-cysteinesulfinate with
isolated cells and tissues.

Acknowledgments—We thank Michael A. Hayward and Ernest B.
Campbell for excellent technical assistance.

REFERENCES
In Vitro 12, 635-638
Invest. 76, 567-574
6. Tateishi, N., Higashi, T., Naruse, A., Nakashima, K., Shiokazi,
7. Tateishi, N., Higashi, T., Shinva, S., Naruse, A., Sakamoto, Y.
(1974) J. Biochem. (Tokyo) 75, 93-103
8. Kohashi, N., Yamaguchi, K., Hosokawa, Y., Kori, Y., Fujii, O.,
and Ueda, I. (1978) J. Biochem. (Tokyo) 84, 159-168
10. Finenstein, J. D., Ky, W. E., Harris, B. J., and Martin, J.
U. S. A. 76, 5606-5610
Biochem. (Tokyo) 82, 117-124
12, 545-551
1422-1426
267-277
D. (1980) in Natural Sulfur Compounds: Novel Biochemical and
Structural Aspects (Cavallini, D., Gaull, G. E., and Zappia, V.,
17. Scandurra, R., Politi, L., Dupre', S., Morigli, M., Barra, D.,
18. Cavallini, D., Scandurra, R., Dupre', S., Federici, G., Santoro, L.,
Ricci, G., and Barra, D. (1976) in Taurine (Huxtable, R., and
Biochim. Biophys. Acta 297, 48-59
1437
Physiol. 81B, 565-571
2141


**30. Haarhoff, K.**


The so1~flan- described above then yielded cnantiomerically pure 0-[3-14C]alanine 162% yield. Inatad with unlabeled glyclne; this material treated with 4°9 of acylase judged by shiral solvent HPLC was dissolved in Dowex 50 X 8 (200 mesh) and passed through a small column (4.0 x 20 cm). The column was washed with water to elute fractions yielded chemically and enantiomerically pure L-[3-14C]alanine as described previously (3). The radioactivity was determined by liquid scintillation counting (LSC) with a liquid scintillation spectrometer (model 2030, Beckman Instruments, Fullerton, CA). The percentages of radioactivity were calculated from the observed tissue radioactivity. The formation of [1-14C]alanine was followed by injecting alanine at the dose indicated in Table I. At the end of the experiments, the tissues of interest were immediately removed and homogenized in 1 volume of 5 N NaOH in tris(hydroxymethyl)aminomethane buffer (pH 7.4). The homogenates were submitted to LSC. For most tissues the whole organ was homogenized, only portions of plasma and ventral dura were used, and the blood was collected in heparin. A portion of the muscle account for 0.7 and 0.4 % of the body weight, respectively. The concentration of [1-14C]alanine was calculated from the observed tissue radioactivity and the hepoptic specific activity of the injected material. The results were corrected using internal standards as described for the [L-14C]alanine studies (see below).


Additional references are found on p. 16579.
Cysteine Metabolism

RESULTS AND DISCUSSION

Inhibition of Cysteinesulfonate Decarboxylase and Aspartate Aminotransferase by D-Amino Acids

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inhibitor</th>
<th>Concentration</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteinesulfinate</td>
<td>D-Cysteinesulfinate</td>
<td>5 mM</td>
<td>90.0 ± 1.4</td>
</tr>
<tr>
<td>D-Apartate</td>
<td>5 mM</td>
<td>7.3 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>10 mM</td>
<td>16.4 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>D-Cysteinesulfonate</td>
<td>D-Apartate</td>
<td>5 mM</td>
<td>7.0 ± 1.1</td>
</tr>
<tr>
<td>Alaninotransferase</td>
<td>D-Apartate</td>
<td>5 mM</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>Aspartate</td>
<td>10 mM</td>
<td>16.4 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>10 mM</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>D-Cysteinesulfonate</td>
<td>Aspartate</td>
<td>5 mM</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Alaninotransferase</td>
<td>Aspartate</td>
<td>10 mM</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>Aspartate</td>
<td>10 mM</td>
<td>0.1 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

D-Cysteinesulfonate decarboxylase was assayed in the presence of 0.2 mM L-[1-14C]cysteinesulfinate as described in Experimental Procedures. D-Amino acids were added at the concentrations indicated. The cysteinesulfinate and aspartate uptake was significantly reduced by the addition of radioactive L-[1-14C]cysteinesulfinate.
# Cysteine Metabolism

## Effect of D-Cysteinesulfonate on the Formation of Urinary and Respiratory Metabolites from Radiolabeled Cysteinesulfonate

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Radioabeled Compound Given</th>
<th>Inhibitor Given</th>
<th>Percent of Administered Compound Recovered as Urinary Metabolites</th>
<th>CO$_2$ or ( \text{N}_2 ) ( \text{CO} ) in resp.</th>
<th>CO$_2$ or ( \text{N}_2 ) ( \text{CO} ) in resp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>L-[1-(^{14} \text{C})]Cysteinesulfonate</td>
<td>No</td>
<td>88.1 %</td>
<td>3.4 %</td>
<td>0.3 %</td>
</tr>
<tr>
<td>2.</td>
<td>L-[1-(^{14} \text{C})]Cysteinesulfonate</td>
<td>Yes</td>
<td>72.4 %</td>
<td>7.0 ± 1.4 %</td>
<td>10.0 ± 13.5 %</td>
</tr>
<tr>
<td>3.</td>
<td>L-[1-(^{14} \text{C})]Cysteinesulfonate</td>
<td>No</td>
<td>72.4 %</td>
<td>4.1 ± 0.4 %</td>
<td>15.1 ± 2.5 %</td>
</tr>
<tr>
<td>4.</td>
<td>L-[1-(^{14} \text{C})]Cysteinesulfonate</td>
<td>Yes</td>
<td>14.1 %</td>
<td>16.3 ± 1.7 %</td>
<td>59.0 ± 9.0 %</td>
</tr>
</tbody>
</table>

### Experimental Procedures
- Mice were given inhibitor by intraperitoneal injection at the dose indicated. Respiratory CO$_2$ was determined at intervals for 6 h, and the animals were killed at the time points indicated or allowed to remain in the metabolic chambers for 24 h and then killed. The tissue distribution of radioactivity and its characterization as D-cysteinesulfinate were carried out as described in Experimental Procedures.

### Table 3: Tissue Distribution of D-Cysteinesulfonate

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Dose (\text{\text{mol/g}})</th>
<th>After 6 h</th>
<th>After 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{\text{mol/g}})</td>
<td>(% of dose)</td>
<td>(% of dose)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.5</td>
<td>1.8 ± 0.38</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>19.6 ± 5.6</td>
<td>19.9 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>9.7 ± 6.5</td>
<td>9.7 ± 6.5</td>
</tr>
<tr>
<td>Liver</td>
<td>0.5</td>
<td>1.0 ± 0.5</td>
<td>8.0 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1.0 ± 0.5</td>
<td>8.0 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.0 0.05</td>
<td>0.0 ± 0.1</td>
</tr>
<tr>
<td>Heart</td>
<td>0.5</td>
<td>1.2 ± 0.5</td>
<td>0.0 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1.2 ± 0.5</td>
<td>0.0 ± 0.05</td>
</tr>
<tr>
<td>Brain</td>
<td>0.5</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>0.0 ± 0.2</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>Skeletal Muscles</td>
<td>0.5</td>
<td>0.0 0.02</td>
<td>0.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1.0 ± 0.4</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.5</td>
<td>0.0 0.02</td>
<td>0.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>0.0 0.04</td>
<td>0.0 ± 0.3</td>
</tr>
<tr>
<td>Urine</td>
<td>0.5</td>
<td>0.0 0.04</td>
<td>0.0 0.04</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>0.0 0.04</td>
<td>0.0 ± 0.3</td>
</tr>
<tr>
<td>Respiratory CO$_2$</td>
<td>0.5</td>
<td>1.5 ± 1.5</td>
<td>5.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>0.0 ± 1.7</td>
<td>5.5 ± 0.6</td>
</tr>
</tbody>
</table>

### Figure 1: Inhibition of cysteinesulfinate decarboxylase by D-cysteinesulfinate

- Enzyme was assayed as described in Experimental Procedures using L-[1-\(^{14} \text{C}\)]cysteinesulfinate at the concentrations shown. Cysteinesulfinate was present at 5 mmol (closed circles), 0.5 mmol (open circles), 0.05 mmol (closed squares), and 0.005 mmol (open squares). Panel A is a double reciprocal plot showing competitive inhibition. Panel B is a Scatchard plot indicating that the Ki for D-cysteinesulfinate is 0.32 mmol.

### Figure 2: Effect of L-cysteinesulfonate on the metabolism of D-cysteinesulfinate

- Mice were given L-[1-\(^{14} \text{C}\)]cysteinesulfonate (0.25 mmol) by intraperitoneal injection on the back of the neck. Control mice (closed circles) received no other treatment; experimental mice (open circles) received L-cysteinesulfonate (0.25 mmol) by intraperitoneal injection prior to the administration of the radiolabeled. Formation of respiratory CO$_2$ was monitored as described in Experimental Procedures. The interval between D-cysteinesulfonate and L-[1-\(^{14} \text{C}\)]cysteinesulfonate administration was 5 h. Ten animals were used in each group. For all experiments the results shown on each line represent averages ± S.D. for groups of 5 to 10 animals.
Cysteine Metabolism

Figure 5: Effect of D-cysteine sulfinic and L-cysteine sulfinic on D- and L-cysteine metabolism. Panel A: Mice were given L-lysine (1.0 mmol/kg) by intraperitoneal injection and were placed immediately in individual metabolic cages immediately after injection. The met abolites in the urine were determined as described in Experiment 3. After an interval of 4 hours, mice were given either a control injection (open circles) or D-cysteine sulfinic acid (10 mmol/kg) by intraperitoneal injection (closed circles). The percent of L-lysine recovered in the urine is shown in the figure. Panel B: The procedure was similar to that described for panel A except that L-cysteine sulfinic acid (10 mmol/kg) instead of D-cysteine sulfinic acid was given by intraperitoneal injection. The percent of L-lysine recovered in the urine is shown in the figure.

Figure 5: Metabolism of D-cysteine sulfinic. Mice were given D-[1-14C]cysteine sulfinic acid (0.1 mmol/kg) by intraperitoneal injection. The percent of L-lysine recovered in the urine is shown in the figure. See text for details.