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Hepatocyte-Catalysed Detoxification of Cyanide by L- and D-Cysteine

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ABSTRACT. The hepatocyte metabolic pathways involved in the detoxification of cyanide by cysteine have been investigated *in vitro* using hepatocytes isolated from Sprague–Dawley rats. Cyanide toxicity towards isolated hepatocytes could be prevented by the addition of L- or D-cysteine, cystine, or the cysteine metabolites thiosulfate and mercaptopyruvate, which markedly increased thiocyanate formation. Prior depletion of hepatocyte GSH markedly increased thiosulfate formation from L- or D-cysteine without affecting thiocyanate formation from L- or D-cysteine. This suggested that the major metabolic pathway for thiocyanate formation did not involve thiosulfate. Mercaptopyruvate was a more likely metabolic intermediate, as thiocyanate formation from L-cysteine but not thiosulfate was inhibited markedly by aminooxyacetate, a cysteine aminotransferase inhibitor, and propargylglycine, a γ-cystathionase inhibitor. Furthermore, propargylglycine prevented L-cysteine cytoprotection against cyanide toxicity. Thiocyanate formation from D-cysteine likely also involved mercaptopyruvate, as thiocyanate formation from D-cysteine but not L-cysteine was inhibited by benzoate, an inhibitor of D-amino acid oxidase. Furthermore, benzoate prevented D-cysteine cytoprotection against cyanide toxicity. Cystine may also be an intermediate, as hepatocyte thiocyanate formation from added L-cysteine was inhibited when L-cysteine autoxidation was prevented with the copper chelator bathocuproine disulfonate. Furthermore, thiocyanate formation by rat liver homogenates with L-cystine was far more rapid than that with L-cysteine. Hepatocyte thiocyanate metabolic intermediates of β-mercaptopyruvate and thiocystine were proposed for L-cysteine, and β-mercaptopyruvate was proposed for D-cysteine. BIOCHEM PHARMACOL **55**;12:1983–1990, 1998.

KEY WORDS. cysteine; thiosulfate; β-mercaptopyruvate; thiocystine; thiocyanate

Cyanide is a potent toxicant that is in the environment, in tobacco smoke, and in dietary cyanogenic glycosides. It is generally believed that the primary pathway for cyanide detoxification is its metabolic oxidation to thiocyanate catalysed by rhodanese, which utilizes sulfur from a sulfane-sulfur source, such as thiosulfate, or catalysed by β-mercaptopyruvate sulfurtransferase, which utilizes β-mercaptopyruvate [1, 2] (Scheme 1). An intravenous injection of the sulfur amino acids cysteine and methionine or their metabolites, thiosulfate or β-mercaptopyruvate, prevents cyanide-induced lethality [3]. Cyanide-induced lethality was also shown recently to be prevented by propargylglycine, an inhibitor of the enzyme cystathionase, which catalyses cystine cleavage to thiocystine, another sulfane-sulfur source for rhodanese [4]. The cyanogen acrylonitrile (ACN) is used extensively in the plastics and synthetic fiber industries [5]. The lethality of ACN in rats can be prevented by decreasing the levels of blood cyanide by administering L- and D-cysteine or thiosulfate [6, 7].

Thiosulfate is the standard clinical antidote for cyanide poisoning and is used together with methemoglobinemia-inducing agents to nonenzymically trap the cyanide. Recently, this laboratory showed that a more effective and less hazardous trap in mice was dihydroxyacetone, which likely traps cyanide as a cyanohydrin and restores glycolytic ATP formation [8, 9].

It is generally assumed that the cysteine metabolite thiosulfate is responsible for thiocyanate formation *in vivo*, although there have been no studies on the thiocyanate formation pathways from cysteine metabolites with intact cells or *in vivo*. In the following report, it is shown for the first time that prior GSH depletion of hepatocytes did not affect thiocyanate formation even though thiosulfate levels were increased markedly. Thiocyanate formation from L-cysteine was also prevented by inhibitors of cysteine aminotransferase or γ-cystathionase, whereas thiocyanate formation from D-cysteine was also prevented by benzoate, a D-amino acid oxidase inhibitor. Therefore, the cysteine metabolites β-mercaptopyruvate and thiocystine may contribute more to the intracellular detoxification of cyanide by cysteine than thiosulfate.

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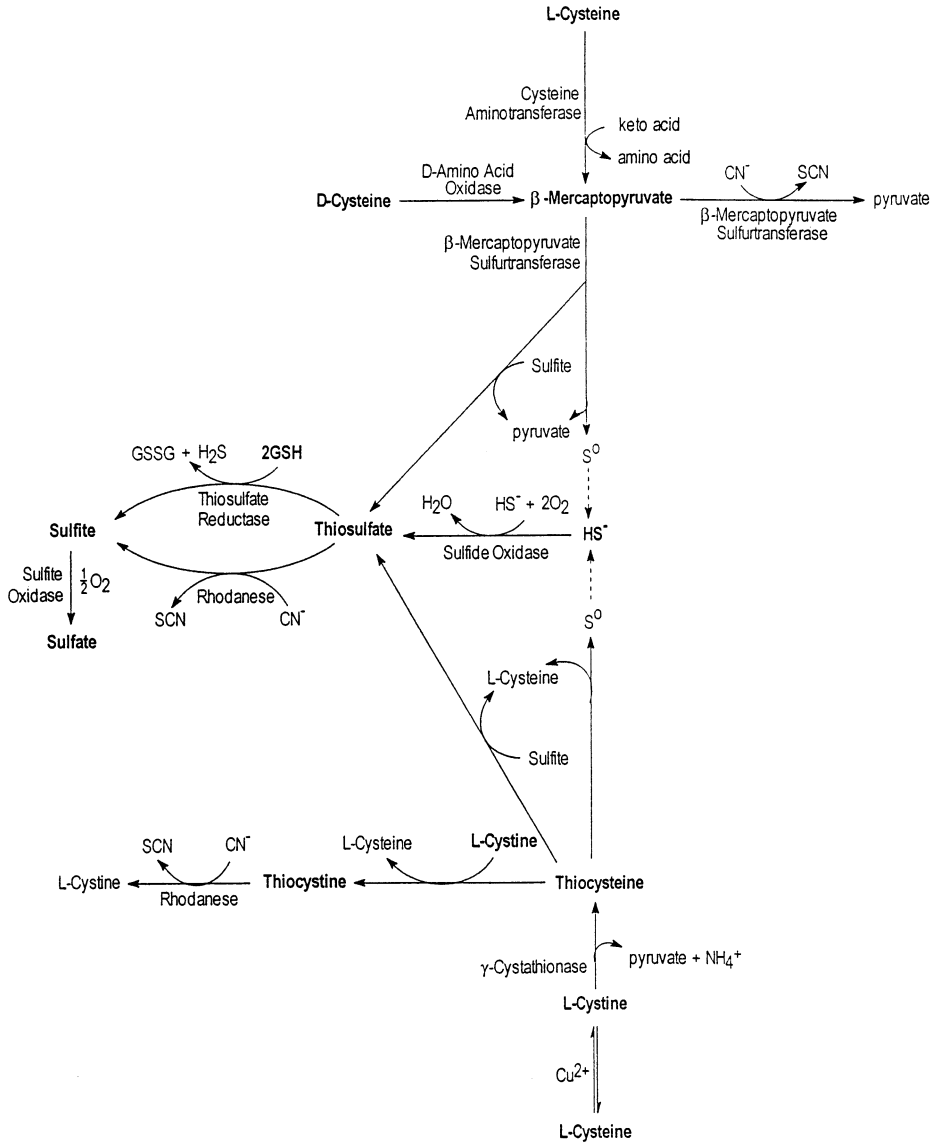

SCHEME 1. Formation of thiocyanate from cysteine.

MATERIALS AND METHODS

Materials

L-Cysteine, D-cysteine, reduced glutathione, β-mercaptopyruvate, BSO,† AOA, PPG, BCS, sodium benzoate, and ferric nitrate were purchased from the Sigma Chemical Co. 1-Bromoheptane was purchased from the Aldrich Chemical Co. Potassium cyanide was purchased from Fisher Scientific. Sodium thiosulfate was purchased from the J. T. Baker Chemical Co. Collagenase (from *Clostridium histolyticum*), HEPES, and bovine serum albumin were obtained from Boehringer–Mannheim. All other chemicals used were of analytical grade.

Isolation and Incubation of Hepatocytes

Freshly isolated hepatocytes were prepared from adult male Sprague–Dawley rats (280–300 g) fed *ad lib*. The cells were isolated by collagenase perfusion of the liver as described by Moldéus *et al.* [10]. The cells (1×10^6 cells/mL) were preincubated in Krebs–Henseleit bicarbonate buffer (pH 7.4) supplemented with 12.5 mM of HEPES for 30 min in an atmosphere of 20% O_2/75% N_2/5% CO_2 or 1% O_2/94% N_2/5% CO_2 in continuously rotating 50-mL round-bottom flasks before the addition of chemicals. Stock solutions of cyanide or cysteine were dissolved in incubation buffer, and enzyme inhibitors were dissolved in DMSO. Cyanide and cysteine were added to the hepatocyte suspensions as indicated. Enzyme inhibitors were added to the hepatocytes 30 min prior to the addition of substrates. GSH-depleted hepatocytes were prepared by preincubating the hepatocytes with 1-bromoheptane for 30 min [11]. Glutamyl-

† *Abbreviations:* AOA, aminooxyacetic acid; BCS, bathocuproine disulfonate; BSO, buthionine sulfoximine; PPG, DL-propargylglycine; and TCA, trichloroacetic acid.

TABLE 1. Prevention of cyanide-induced hepatocyte cytotoxicity by cysteine or metabolites

	% Cells taking up trypan blue			
	30 min	60 min	120 min	180 min
Control hepatocytes	18 ± 2	19 ± 2	20 ± 2	21 ± 2
+Cyanide (400 μM)	42 ± 4*	62 ± 6*	75 ± 7*	90 ± 9*
+L-Cysteine (2 mM)	38 ± 4*	42 ± 4*†	46 ± 6*†	42 ± 6*†
+PPG (0.1 mM)	40 ± 3*	56 ± 6*	77 ± 8*	94 ± 7*
+Benzoate (0.5 mM)	32 ± 4*	43 ± 4*†	47 ± 5*†	51 ± 5*†
+D-Cysteine (2 mM)	40 ± 4*	42 ± 5*†	45 ± 6*†	42 ± 6*†
+Benzoate (0.5 mM)	39 ± 4*	57 ± 6*	73 ± 7*	92 ± 8*
+L-Cystine (1 mM)	38 ± 5*	45 ± 4*†	41 ± 5*†	39 ± 5*†
+PPG (0.1 mM)	36 ± 4*	58 ± 5*	63 ± 6*	71 ± 7*
+Thiosulfate (0.4 mM)	34 ± 3*	37 ± 3*†	35 ± 3*†	40 ± 4*†
+Mercaptopyruvate (0.4 mM)	36 ± 5*	36 ± 4*†	41 ± 5*†	38 ± 4*†
+PPG (0.1 mM)	39 ± 4*	61 ± 6*	77 ± 7*	92 ± 9*
+Benzoate (0.5 mM)	37 ± 4*	63 ± 6*	79 ± 7*	90 ± 8*

Rat hepatocytes (1×10^6 cells/mL) were maintained at 37°, pH 7.4, under an atmosphere of 1% O_2/5% CO_2/94% N_2. Hepatocyte cytotoxicity was estimated by determining the fraction of cells taking up trypan blue at the time points specified after the addition of cyanide to hepatocytes as described in Materials and Methods. Each value represents the mean ± SEM of 3 incubations.

*Significantly different from control ($P < 0.05$).

†Significantly different from cyanide alone ($P < 0.05$).

cysteine synthelase inhibited hepatocytes were prepared by incubating the hepatocytes with 0.5 M of B50 for 30 min.

Measurement of Cytotoxicity

Immediately after isolation, hepatocyte viability was assessed by counting the number of cells that excluded trypan blue and the number of cells that did not exclude trypan blue. The fraction of cells that excluded trypan blue ranged between 85 and 90%, and there was a little cell debris and occasional nonparenchymal cells [10]. Hepatocyte cytotoxicity was estimated as the fraction of cells that did not exclude trypan blue and was determined at 30, 60, 120, and 180 min after the addition of cyanide.

Thiocyanate Determination

The concentration of thiocyanate formed in isolated hepatocytes or homogenates was measured in deproteinized samples (5% TCA) by the colorimetric procedure described by Westley [12]. Hepatocyte samples for thiocyanate determination were carried out at 30, 60, 120, and 180 min after the addition of cyanide.

Thiosulfate Determination

The concentration of thiosulfate formed in isolated hepatocytes (4×10^6 cells/mL) was measured in deproteinized samples (5% TCA) by the colorimetric procedure described by Westley [12].

Statistics

Statistically significant differences between control and experimental groups were obtained using Student's *t*-test. The minimal level of significance chosen was $P < 0.05$.

RESULTS

Prevention of Cyanide-induced Cytotoxicity by L- or D-Cysteine and Metabolites

As shown in Table 1, hepatocyte cytotoxicity induced by cyanide was prevented by D-cysteine, L-cysteine or L-cystine. The cysteine metabolites mercaptopyruvate and thiosulfate at lower concentrations were also cytoprotective. As shown in Table 2, the cytoprotective effectiveness of L- and D-cysteine and metabolites was associated with their effectiveness at enhancing thiocyanate formation. The much more rapid thiocyanate formation with thiosulfate suggests that thiosulfate is readily transported into mitochondria [13], whereas the slower thiocyanate formation with L-cystine presumably reflects the slow transport rate of cystine into hepatocytes compared with cysteine [14]. The nonenzymic reaction between cystine and cyanide to form cysteine and the neurotoxin 2-imino-4-thiazolidine-4-carboxylic acid [15] was insignificant at the low cyanide concentrations used here (results not shown). The low levels of thiocyanate formation in the absence of added L-cysteine presumably reflect the depletion of the cysteine and/or sulfane-sulfur pool following hepatocyte isolation (Table 2).

Hepatocyte-catalysed Thiocyanate and Thiosulfate Formation from L-Cysteine

At 20% O_2, hepatocytes are much more resistant to cyanide than at 1% O_2 [16] so that cysteine metabolism can be studied without interference by the cytotoxic process. As

TABLE 2. Hepatocyte-catalysed thiocyanate formation from cyanide and cysteine or metabolites

	Thiocyanate formed (nmol/1 × 10^6 cells)			
	Incubation time (min)			
	30	60	120	180
Control hepatocytes	ND*	ND	ND	ND
+Cyanide (400 μM)	ND	ND	ND	ND
+L-Cysteine (2 mM)	53.1 ± 5†	79.3 ± 7†	124.2 ± 11†	171.4 ± 15†
+D-Cysteine (2 mM)	56.7 ± 9†	99.5 ± 17†	157.2 ± 29†	184.2 ± 33†
+L-Cystine (1 mM)	14.7 ± 4†	38.0 ± 10†	88.3 ± 6†	132.3 ± 28†
+Thiosulfate (0.4 mM)	288.3 ± 19†	348.3 ± 10†	390.7 ± 7†	391.3 ± 30†
+Mercaptopyruvate (0.4 mM)	133.3 ± 15†	150.2 ± 12†	180.1 ± 15†	201.3 ± 22†

Rat hepatocytes (1 × 10^6 cells/mL) were maintained at 37°, pH 7.4, under an atmosphere of 1% O_2/5% CO_2/94% N_2. Thiocyanate levels were measured at the time points specified after the addition of cyanide to hepatocytes preincubated for 30 min with cysteine, cystine, and thiosulfate, as described in Materials and Methods. Each value represents the mean ± SEM of 3 incubations.

*ND: not detectable.

†Significantly different from cyanide alone ($P < 0.05$).

shown in Fig. 1, thiocyanate formation from L-cysteine continued to occur over the 3 hr incubation period (Fig. 1A), whereas thiocyanate formation from thiosulfate was nearly complete in 1 hr (Fig. 1B). Hepatocyte thiocyanate formation was also slower at 20% O_2 (Fig. 1A) than at 1% O_2 (Table 2), as previously described [16]. Depletion of hepatocyte GSH, however, did not affect thiocyanate formation from L-cysteine or thiosulfate. As shown in Fig. 1C, thiosulfate was formed rapidly without a lag period when rat hepatocytes were incubated with 10 mM of L-cysteine. Furthermore, the initial rate of thiosulfate formation in 1 hr was increased two-fold in GSH-depleted hepatocytes. The total amount of thiosulfate formed at 2 and 3 hr was also doubled in GSH-depleted hepatocytes provided GSH resynthesis was prevented with BSO, a γ-glutamyl-cysteine synthetase inhibitor.

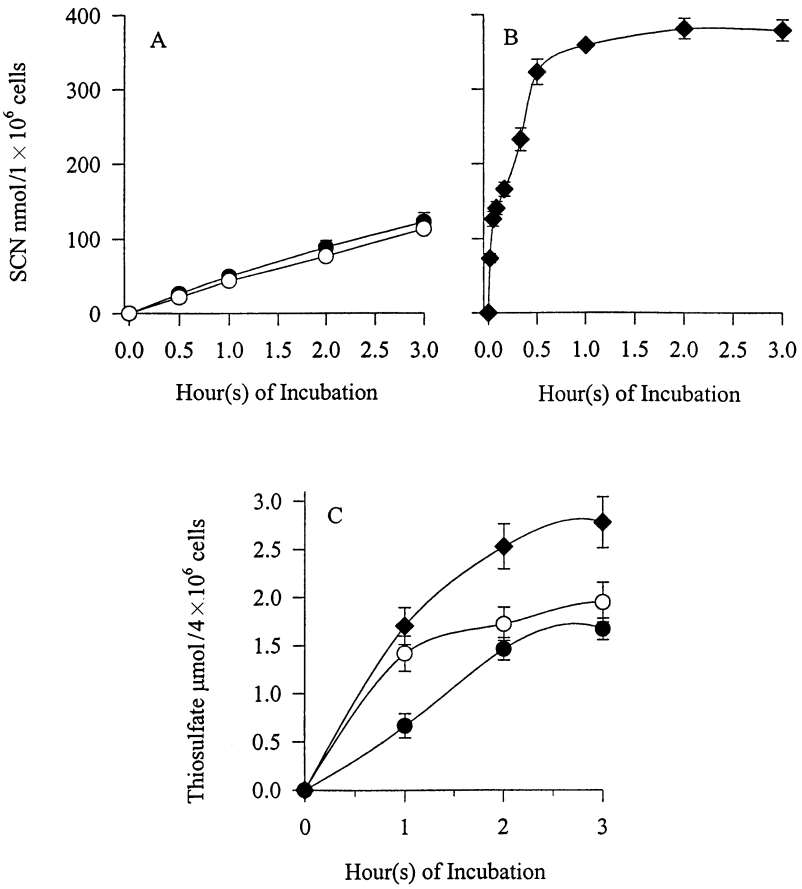

FIG. 1. Dependence of thiocyanate (SCN) formation from L-cysteine versus thiosulfate on hepatocyte GSH levels. Rat hepatocytes (1 × 10^6 cells/mL) were incubated at 37°, pH 7.4, and 20% O_2/75% N_2/5% CO_2. GSH-depleted or GSH-depleted/γ-glutamyl-cysteine synthetase-inhibited hepatocytes were prepared as described in Materials and Methods. Reactions were stopped by the addition of 5% (w/v) TCA to hepatocyte samples taken at 30, 60, 90, 120, and 180 min after the addition of L-cysteine. The concentrations of SCN and thiosufate were determined as described under Materials and Methods. Each symbol represents the mean ± SEM of 3–9 incubations. (A) SCN formation from L-cysteine. Key: (●) 2 mM of L-cysteine + control hepatocytes; and (○) 2 mM of L-cysteine + GSH-depleted/γ-glutamyl-cysteine synthetase-inhibited hepatocytes. (B) SCN formation from thiosulfate. Key: (◆) 0.5 mM of thiosulfate + control hepatocytes. (C) Thiosulfate formation from L-cysteine. Key: (●) 10 mM of L-cysteine + control hepatocytes; (○) 10 mM of L-cysteine + GSH-depleted hepatocytes; and (◆) 10 mM of L-cysteine + GSH-depleted/γ-glutamyl-cysteine synthetase-inhibited hepatocytes. Cyanide (400 μM) was added to all incubations at time zero.

TABLE 3. Involvement of aminotransferase, γ-cystathionase, and D-amino acid oxidase in hepatocyte-catalysed thiocyanate formation from cyanide

Enzyme inhibitors	Thiocyanate formed (nmol/1 × 10^6 cells in 3 hr)		
	L-Cysteine (2 mM)	L-Cystine (1 mM)	D-Cysteine (2 mM)
None	122.7 ± 12.4	132.0 ± 19.6	180.1 ± 32.8
AOA (25 μM)	40.2 ± 2.9*	2.2 ± 0.5†	176.5 ± 29.4
PPG (100 μM)	34.1 ± 2.3*	16.4 ± 1.4†	178.3 ± 28.2
Benzoate (500 μM)	118.6 ± 10.2	130.3 ± 17.8	4.6 ± 0.9‡

The aminotransferase inhibitor AOA, the γ-cystathionase inhibitor PPG, and the D-amino acid oxidase inhibitor benzoate were added to the incubations 30 min prior to the addition of cyanide (400 μM), L-cysteine, L-cystine, and D-cysteine. Rat hepatocytes (1 × 10^6 cells/mL) were incubated at 37°, pH 7.4, and 20% O_2/75% N_2/5% CO_2. Reactions were stopped at 3 hr after the addition of cysteine and cystine with 5% (w/v) TCA. The concentrations of thiocyanate formed in 3 hr were measured as described under Materials and Methods. Each value represents the mean ± SEM of 3–9 incubations.

*Significantly different from L-cysteine alone ($P < 0.05$).

†Significantly different from L-cystine alone ($P < 0.05$).

‡Significantly different from D-cysteine alone ($P < 0.05$).

Aminotransferase and γ-Cystathionase Involvement in Thiocyanate Formation from L-Cysteine

As shown in Table 3, the rate of thiocyanate formation from L-cysteine was inhibited markedly when hepatocytes were preincubated with the aminotransferase inhibitor AOA [17] or the γ-cystathionase inhibitor PPG [4]. The D-amino acid oxidase inhibitor sodium benzoate [18] did not affect thiocyanate formation from L-cysteine. Thiocyanate formation from L-cystine was also more susceptible to inhibition by AOA or PPG than thiocyanate formation from L-cysteine. Furthermore, as shown in Table 1, PPG overcame the cytoprotectiveness of L-cysteine but had little effect on cyanide toxicity in the absence of L-cysteine.

Hepatocyte-Catalysed Thiocyanate and Thiosulfate Formation from D-Cysteine

As shown in Fig. 2A, thiocyanate was also readily formed from cyanide when hepatocytes were incubated with D-cysteine. Depletion of hepatocyte GSH beforehand did not affect thiocyanate formation. Thiocyanate formation also was not affected when hepatocytes were preincubated with AOA or PPG. However, the D-amino acid oxidase inhibitor sodium benzoate completely prevented thiocyanate formation from D-cysteine (Table 3). Furthermore, as shown in Table 1, benzoate overcame the cytoprotectiveness of D-cysteine but had little effect on cyanide cytotoxicity in the absence of D-cysteine. On the other hand, benzoate did not affect significantly the cytoprotectiveness of L-cysteine. As shown in Fig. 2B, thiosulfate was also readily formed from D-cysteine in hepatocytes. Moreover, the initial rate of thiosulfate formation increased two-fold in GSH-depleted hepatocytes.

Rates of Thiocyanate Formation from L-Cysteine/Cystine by Liver Homogenates

As shown in Table 2, the rate of thiocyanate formation from L-cystine was similar to that formed with L-cysteine in rat hepatocytes. However, as shown in Fig. 3, the initial rate of thiocyanate formation from L-cystine was much higher with rat liver homogenate and was now much faster than that found with L-cysteine. When homogenate was preincubated with the copper chelator BCS to prevent cysteine autoxidation [19], there was a marked decrease in the rate of thiocyanate formation from L-cysteine but not from L-cystine (Table 4), suggesting that part of the thiocyanate formation from L-cysteine may involve a copper-dependent autoxidation to L-cystine.

DISCUSSION

It is generally believed that cyanide is detoxified *in vivo* by thiosulfate to form thiocyanate, catalysed by the enzyme thiosulfate sulfurtransferase [1]. The liver is the tissue with the highest levels of rhodanese and is thus considered to be a major site of cyanide detoxification [20]. We have found that both L- and D-cysteine and the metabolites L-cystine, β-mercaptopyruvate, or thiosulfate partly protected hepatocytes from cyanide toxicity and caused the metabolism of cyanide to thiocyanate. Thiocyanate was also readily formed from added thiosulfate even though rhodanese is located in the mitochondrial matrix, possibly because thiosulfate can be transported by the mitochondrial dicarboxylate carrier in exchange for phosphate and sulfite [13].

There have been no reports on the cysteine metabolic pathways responsible for thiocyanate formation by hepatocytes. In the absence of added L-cysteine, very low levels of thiocyanate were formed from cyanide, which could suggest that the endogenous sulfane-sulfur pool is small or is depleted during hepatocyte preparation and incubation prior to use. No differences in thiocyanate formation between normal and GSH-depleted hepatocytes from added D- or L-cysteine were found. However, thiosulfate formation from added L-cysteine was increased markedly in GSH-depleted hepatocytes, presumably because thiosulfate reductase requires GSH to metabolize thiosulfate to sulfite [21, 22]. By contrast, thiosulfate sulfurtransferase does not

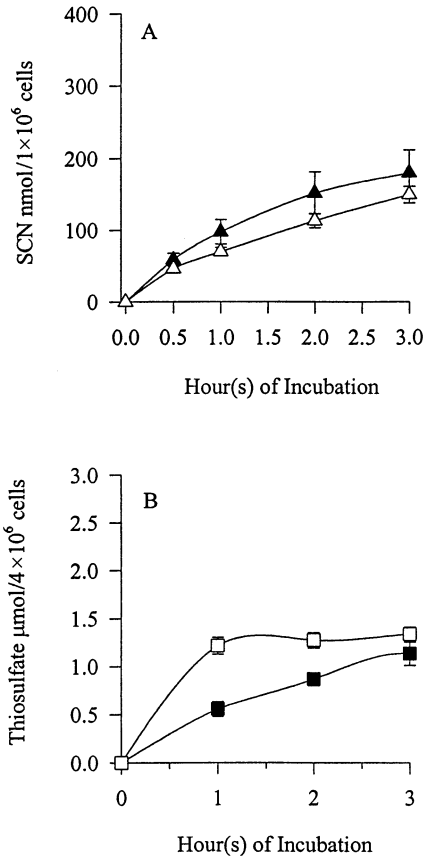

FIG. 2. Thiocyanate (SCN) formation from D-cysteine by control versus GSH-depleted rat hepatocytes. The reaction conditions used are described in the legend of Fig. 1. Each symbol represents the mean ± SEM of 3–9 incubations. (A) SCN formation from D-cysteine. Key: (▲) 2 mM of D-cysteine + control hepatocytes; and (△) 2 mM of D-cysteine + GSH-depleted hepatocytes. (B) Thiosulfate formation from D-cysteine. Key: (■) 10 mM of D-cysteine + control hepatocytes; and (□) 10 mM of D-cysteine + GSH-depleted hepatocytes. Cyanide (400 μM) was added to all incubations at time zero.

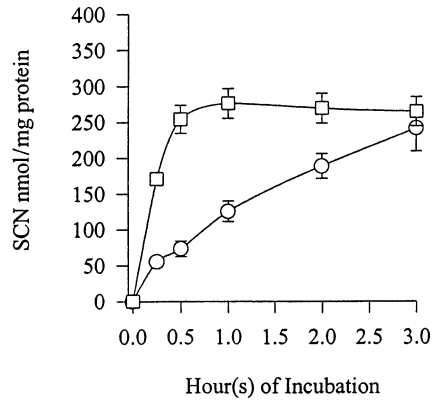

FIG. 3. Thiocyanate (SCN) formation from L-cysteine versus L-cystine in rat homogenates. The reaction conditions used are described in the legend of Fig. 1. Each symbol represents the mean ± SEM of 3–9 incubations. Key: (○) 2 mM of L-cysteine; and (□) 1 mM of L-cystine. Cyanide (400 μM) was added to all incubations at time zero.

utilize GSH [23]. Further evidence suggesting the involvement of thiosulfate reductase was that sulfate formation from added L-cysteine was prevented in GSH-depleted hepatocytes [24]. The increased thiosulfate levels in GSH-depleted hepatocytes, however, did not increase thiocyanate formation, which suggests that other D- and L-cysteine metabolites contributed more to thiocyanate formation than thiosulfate. As shown in Scheme 1, other possible routes for thiocyanate formation to be considered include: a) the trans-sulfuration of sulfur from β-mercaptopyruvate to cyanide by β-mercaptopyruvate sulfurtransferase [25–27]; b) the trans-sulfuration of sulfur from thiocystine to cyanide by thiosulfate sulfurtransferase [28, 29]; and c) the desulfuration of β-mercaptopyruvate and thiocystine to form sulfane sulfur, which could also react with cyanide to form thiocyanate [28, 29].

Thiocyanate formation from L- or D-cysteine likely involves the metabolites β-mercaptopyruvate and thiocystine, as thiocyanate formation was inhibited when the formation of these cysteine metabolites was prevented by AOA or PPG. Furthermore, PPG overcame the cytoprotection by L-cysteine or L-cystine against cyanide toxicity. The aminotransferase inhibitor AOA inhibits the conversion of L-cysteine to β-mercaptopyruvate [13], and the γ-cystathionase inhibitor PPG prevents the conversion of L-cystine to thiocystine [4, 30]. However, it may be difficult to distinguish between the thiocystine and β-mercaptopyruvate pathways, as both aminotransferase and γ-cystathionase inhibitors were able to inhibit both enzymes because of their pyridoxal-phosphate dependence [31, 32]. At the PPG concentration used, however, the hepatocyte γ-cystathionase activity was inhibited 85% without affecting aminotransferase activity [31]. In fact, AOA was more efficient at inhibiting γ-cystathionase than aminotransferase, since AOA completely inhibited thiocyanate formation from L-cystine but inhibited only 67% of the thiocyanate formation from L-cysteine. This suggests that at least 33% of the thiocyanate formed from L-cysteine involves the β-mercaptopyruvate pathway.

Another way to test the thiocyanate-forming potential of β-mercaptopyruvate was by taking advantage of the fact

TABLE 4. Effect of preventing cysteine autoxidation upon thiocyanate formation by rat liver homogenates

	Thiocyanate formed (nmol/mg protein)	
Cu^{2+} chelator	L-Cysteine (2 mM)	L-Cystine (1 mM)
None	241.7 ± 31.9	264.9 ± 35
BCS (500 μM)	117.9 ± 12.3*	261.7 ± 26.4

Inhibitors were added to the incubations 30 min prior to the addition of cyanide (400 μM), L-cysteine, and L-cystine. Rat homogenates (1 mg protein/mL incubation mixture) were incubated at 37°, pH 7.4, and 20% O_2/75% N_2/5% CO_2. Reactions were stopped at 3 hr after the addition of cysteine and cystine with 5% (w/v) TCA. The concentrations of thiocyanate formed in 3 hr were measured as described under Materials and Methods. Each value represents the mean ± SEM of 3 incubations.

*Significantly different from L-cysteine alone ($P < 0.05$).

that D-cysteine cannot form thiocyanate through the thiocystine-dependent pathway, as γ-cystathionase is stereospecific for L-cystine. The formation of thiocyanate from D-cysteine should, therefore, be β-mercaptopyruvate dependent. Furthermore, it was found that thiocyanate formation from D-cysteine was inhibited completely if D-amino acid oxidase, which catalyses the conversion of D-cysteine to β-mercaptopyruvate, was inhibited by benzoate, a well-known inhibitor of D-amino acid oxidase [18]. Furthermore, benzoate overcame the cytoprotection by D-cysteine but not L-cysteine against cyanide toxicity. GSH-depleted and normal cells also showed no difference in thiocyanate formation from D-cysteine, even though thiosulfate formation was increased markedly, and suggests that β-mercaptopyruvate, a thiosulfate precursor, directly donates its sulfur to cyanide rather than donating it indirectly through thiosulfate.

This, of course, does not eliminate the possible involvement of thiocysteine in thiocyanate formation, since both aminotransferase and γ-cystathionase inhibitors were shown to be more potent at preventing thiocyanate formation from L-cystine than from L-cysteine. The rate of thiocyanate formation from L-cystine was similar to that found with L-cysteine in hepatocytes. However, with liver homogenates, the rate of thiocyanate formation from L-cystine was much faster than that with L-cysteine. This is probably because L-cystine is transported into hepatocytes much slower than L-cysteine [14].

In addition, when the autoxidation of L-cysteine to L-cystine was prevented by the copper chelator BCS [19], the rate of thiocyanate formation from L-cysteine was also inhibited. L-Cystine is believed to be metabolized to thiocystine [29, 33, 34], which converts cyanide to thiocyanate. Although BCS may affect the metabolism of other sulfur compounds, these results indicate that thiocystine may also be a crucial intermediate in the formation of thiocyanate from L-cysteine in rat hepatocytes, with the rate-limiting step in this pathway being the extracellular or intracellular autoxidation of L-cysteine.

We therefore conclude that thiosulfate is not the main intermediate in thiocyanate formation from L- or D-cysteine. The main intermediates for thiocyanate formation are more likely to be β-mercaptopyruvate from L-cysteine or D-cysteine, and/or thiocysteine from L-cysteine or L-cystine.

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