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Nutritional and Protective Properties of Thiocystine (41006)

JOHN L. WOOD

Department of Biochemistry, University of Tennessee, Center for the Health Sciences, Memphis, Tennessee 38163

Abstract. Some biological properties of thiocystine (bis[2-amino-2-carboxyethyl]trisulfide), a persulfide analog of cystine (1), have been investigated. Thiocystine replaced cystine in supporting growth of young rats fed a low-fat, low-methionine diet. When injected intravenously into adult rats, thiocystine protected against two to three times an LD_{50} dose of intraperitoneal sodium cyanide. Thiocystine exhibited marginal activity for protecting mice against lethal X-ray irradiation. The biological properties of thiocystine can be attributed to its conversion *in vivo* to cystine and a persulfide sulfur moiety.

Biological properties of thiocystine. Thiocystine (bis[2-amino-2-carboxyethyl]-trisulfide) is the trisulfide analog of cystine (1). The compound has been isolated from *Rhodopseudomonas spheroides* (2). It can be produced by the action of cystathionase (EC 4.4.1.1) on cystine (3).

The biological reactions of thiocystine are of interest because it behaves like a persulfide compound in transferring sulfur to available thiophilic receptors (4). Such transfers can be catalyzed by rhodanese (thiosulfate:cyanide sulfurtransferase, EC 2.8.1.1) (5). Thiocystine has been shown to function as an activator for aminolevulinate synthetase (2). It probably can serve as donor or activator in a number of biological systems which react with elemental sulfur.

Some other biological properties of thiocystine have been investigated. In this report it is shown that thiocystine supports the growth of young rats *in lieu* of cystine. Thiocystine also protects rats against two to three times the LD_{50} dosage of sodium cyanide. It has a marginal activity as a radiation protector.

Materials and methods. L-Thiocystine was synthesized from L-cystine monosulfoxide and sulfide ion by the method of Savige *et al.* (6). The compound was assayed for persulfide sulfur by a slight modification of the cold cyanolysis method of Fletcher and Robson (1).

For growth support studies, weanling Wistar strain rats were placed on a basal, low-fat diet as follows:

(g) amino acid mixture 165, sucrose 150, dextrin 592, salt mixture (Jones-Foster, (7)) 40, corn oil 20, Vitamin A and D (8000 and 650 IU/ml, respectively 1.5 ml, inositol 1, choline 2, vitamin mixture 10, Celluflour 20. The vitamin mixture was in milligrams: thiamin 5, riboflavin 10, pyridoxine hydrochloride 5, nicotinic acid 5, calcium pantothenate 25, α-tocopherol 25, menadione 2, biotin 0.1, folic acid 0.1, *p*-aminobenzoic acid 300, cobalamin 0.0015, and dextrin to make 10 g.

The amino acid mixture was number XXIII of Rose *et al.* (8) excepting that the DL-methionine content was reduced from 0.8 to 0.2% and hydroxyproline and cystine were omitted. For tests of growth-supporting qualities, 0.4% cystine or 0.45% thiocystine was added to the basal diet in place of an equivalent weight of dextrin.

Animals in Group I were placed initially on a basal diet containing 0.4% DL-methionine. After 4 days the methionine content of the diet was reduced to 0.2% and after another 4 days the diet was supplemented with thiocystine or cystine. Group II animals were carried for 8 days on a 0.2% methionine diet before it was supplemented with a sulfur amino acid.

Cyanide and thiocystine administration. Adult male and female rats were used to determine the effect of thiocystine on cyanide toxicity. The LD_{50} for cyanide injected subcutaneously was found by Sörbo and co-workers (9) to be 3 mg/kg. This value was approximated for intraperitoneal injection. In the first experiments thiocystine was administered in a solution of 60

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mg/ml of dilute HCl, pH 3.5. The animals were lightly anesthetized with ether and injected iv with 120 mg of thiocystine/kg of body wt by means of the tail vein. The only reaction to the solution was rapid respiration. In subsequent tests thiocystine was dispersed in 1% gum tragacanth by means of a Potter–Elvejhem homogenizer and injected by tail vein at a concentration of 62.5 mg/ml over a period of 1–3 min. A sodium cyanide solution was prepared to contain 3 mg of cyanide ion/ml buffered to pH 6.6 with phosphoric acid. This was administered ip immediately after the thiocystine. The animals were observed for 40 min and scored for survival.

Results and discussion. Growth of young rats was used as an assay for the capacity of thiocystine to replace cystine in the diet. A low-fat, low-methionine amino acid diet which had been used in sulfur metabolism studies in this laboratory provided a means for the test.

As shown in Table I, animals in Group I were preconditioned for 4 days on a 0.4% DL-methionine diet. Such animals were able to maintain a growth rate of 0.69 ± 0.02 g/day for 28 days when placed on the basal diet. The Group II control animals were placed directly on the basal diet at weaning and failed to survive the period of the test. When the diet was supplemented with L-cystine all animals grew adequately for the test although not at an optimum rate. When the cystine supplement was replaced by 0.45% L-thiocystine the growth rate was not significantly different. Because of the small number of animals and the special nature of the diet one can only conclude from this test that L-thiocystine is approximately equivalent to L-cystine in supporting the growth of the young rat.

Although thiocystine results from the action of cystathionase on cystine its properties suggest that it does not accumulate in the body. The metabolism of thiocystine

TABLE I. GROWTH-SUPPORTING PROPERTY OF THIOCYSTINE

	Animal (sex)	Weight (g)	Weight gain (g/28 days)[a]	Food consumption (g/28 days)	Diet supplement
Group I	F	55	20	198	None (basal diet)
	M	66	19	179	None (basal diet)
	F	58	73	316	0.4% L-Cystine
	M	55	97	320	0.4% L-Cystine
	M	68	114	361	0.45% L-Thiocystine
	M	58	78	282	0.45% L-Thiocystine
	M	60	88	312	0.45% L-Thiocystine
Group II	M	47	−17	7	None (basal diet)
	M	49	−21	8	None (basal diet)
	M	48	86	292	0.4% L-Cystine
	M	31	73	257	0.4% L-Cystine
	M	48	86	287	0.45% L-Thiocystine
	M	48	80	281	0.45% L-Thiocystine
	M	47	89	333	0.45% L-Thiocystine
	M	47	97	301	0.45% L-Thiocystine

Note. See Materials and Methods for basal diet. Animals in the first group were maintained on a basal diet plus 0.2% DL-methionine for 4 days before starting the experiment. Animals in the second group were fed the basal diet for weaning. In Group II the first control animal died on the 6th day and the second on the 10th.

[a] Mean weight change per day: Basal diet, Group I, 0.69 ± 0.02 (n = 2); Group II, −0.68 ± 0.07 (n = 2 Cystine diet, Groups I and II pooled, 2.93 ± 0.21 (n = 4). Thiocystine diet, Groups I and II pooled, 3.23 ± 0.1 (n = 7). t test of the difference of means: cystine diet vs thiocystine, not significant; cystine or thiocystine di vs basal, $P < 0.0005$.

probably occurs through intermediate formation of cystine. The process is slow in pure solutions of thiocystine,

$$CySSSCy \rightarrow CySSCy + S^0, \quad [1]$$

but is accelerated through catalysis by sulfhydryl compounds. The second product of the breakdown of thiocystine *in vitro* is elementary sulfur. This probably never occurs in the body because it appears as persulfide sulfur from reaction with an equivalent amount of sulfhydryl compound (4).

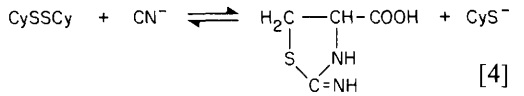
$$RSH + S^0 \rightleftharpoons RSSH. \quad [2]$$

In the mitochondrion the persulfide sulfur of thiocystine is transferred to rhodanese, a persulfide enzyme that is more stable than most persulfides (10).

Thiocystine was expected to be effective for protection against cyanide by reacting with it to form thiocyanate and cystine (3). The reaction is catalyzed by rhodanese.

$$CySSSCy + CN^- \rightarrow CySSCy + SCN^- \quad [3]$$

The cystine byproduct of reaction [4] was shown by Wood and Cooley (11) to further react with cyanide to form 2-iminothiazolidine-4-carboxylic acid. This reaction is uncatalyzed and is too slow to provide effective protection against cyanide poisoning.



[4]

Although Reaction [3] when catalyzed by rhodanese is rapid, the enzyme is found only in mitochondria. Cyanide is expected to diffuse into the mitochondrion faster than thiocystine and poison the cytochrome system before the substrate is present in a useful amount. Clemedson, Hultman, and Sörbo (9) have shown that if rhodanese is injected intravenously with a sulfur donor such as thiosulfate, the LD_{50} for subcutaneous cyanide was increased from 3 to 45 mg/kg. To test whether thiocystine could provide effective protection against cyanide poisoning, the trisulfide was administered intravenously followed by an intraperitoneal injection of sodium cyanide as described under Materials and methods. In these screening tests the cyanide dose was varied. The highest practicable amount of thiocystine that could be injected intravenously was used. Thiocystine is only about 10 times as soluble as cystine and had to be administered as a suspension.

Table II shows that in the first test all 10 rats survived twice the LD_{50} dose of cyanide. Under these conditions the molar ratio of thiocystine to cyanide was 4. When the dosage of cyanide was increased to 3 × LD_{50} the ratio of thiocystine to cyanide was reduced to 2.5 and 3 of 6 animals survived. In a repetition of the screening test 2 of 10 animals died when the cyanide dose was 2 × LD_{50} and 3 of 4 died at 4 × LD_{50}.

In 1926 Voegtlin *et al.* (12) gave cystine, cysteine, or oxidized glutathione to each of five rats by iv injection followed immediately by 1.5 times the MLD (5.2 mg/kg CN^-) of sodium cyanide subcutaneously. Cystine as noted above reacts with cyanide to form iminothiazolidinecarboxylic acid (11). Voegtlin *et al.* (12) found that the two disulfides gave protection to all animals when the molar ratio of cystine or glutathione to cyanide was 5:1 but only one of the animals survived when the cystine:cyanide ratio was 2.5:1. Cysteine was also described as effective at 10:1 ratio to

TABLE II. SCREENING FOR PROTECTION AGAINST CYANIDE POISONING

No. animals	Weight (g)	Thiocystine (mg/kg)	−CN (mg/kg)	Thiocystine (Cyanide)	Status (40 min)
8	325 ± 30	—	3.0	—	3L, 5D[a]
10	374 ± 40	244 ± 23	6.0	4	10L, OD
7	308 ± 80	237 ± 6	9.0	2.5	4L, 3D
10	284 ± 43	244 ± 13	6.0	4	8L, 2D
4	240 ± 10	246 ± 3	12.0	2	1L, 3D

Note. See Materials and methods for conditions of experiment.
[a] L, living after 40 min, D, dead before 40 min.

cyanide but Clemedson *et al.* (9) did not confirm this in their experiments. In our experiments we used a 4:1 ratio of thiocystine to cyanide (two times the LD_{50} dosage, 6 mg/kg CN^-) which gave nearly complete protection to the animals. Thus, thiocystine was scarcely more effective than cystine as a prophylaxis against cyanide poisoning and was not considered to be a particularly useful compound for this purpose.

Szczepkowski *et al.* (13) found bis[2-hydroxypropyl]trisulfide to provide a slight protection to rats and mice exposed to large amounts of X ray. A screening test of the radioprotective effect of thiocystine in mice was carried out for us by Dr. David Doherty at the Biology Division, Oak Ridge National Laboratory. Male mice of the C_3H strain, 10 to 12 weeks old and weighing 20–24 g were injected intraperitoneally with 100 mg/kg of thiocystine 15 min prior to irradiation with 900 rad at about 50 rad/min as described by Doherty *et al.* (14). There was a 20% survival (8/40, 30 days) with thiocystine. All of the animals injected only with saline died while 100% of mice injected with 150 mg/kg of 2-aminoethylisothiourea hydrobromide (AET) survived. Thus, thiocystine provided only marginal protection against lethal amounts of X-ray irradiation.

Since cystine is without any protective effect against radiation (15) the small effect observed with thiocystine may be ascribed to the trisulfide group.

At present it appears thiocystine is a naturally occurring compound which serves both as a source of cystine and persulfide sulfur in metabolic reactions.

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