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THE CYSTATHIONASE–RHODANESE SYSTEM

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SUMMARY

Cystathionase (L-homoserine hydrolase (deaminating), EC 4.2.1.15) and rhodanese** (thiosulfate:cyanide sulfurtransferase, EC 2.8.1.1) have been combined to form a coupled enzyme system which is capable of utilizing cysteine sulfur for transsulfuration. The initial product of the action of cystathionase on cystine, thiocysteine, is, at least in part, converted to bis-(2-amino-2-carboxyethyl) trisulfide, "thiocystine". Rhodanese accepts the products of the action of cystathionase, thiocystine, and possibly thiocysteine, and transfers sulfur to cyanide, sulfite, or other acceptors. In an isolated system, rhodanese utilizes thiocystine as a substrate more efficiently than it does thiosulfate.

INTRODUCTION

In recent years evidence has accumulated[1–5] to establish the enzyme, cysteine desulfhydrase (L-cysteine hydrogensulfide-lyase (deaminating), EC 4.4.1.1) to be identical with cystathionase (L-homoserine hydrolase (deaminating), EC 4.2.1.15) as first suggested by BINKLEY[6]. CAVALLINI *et al.*[3] came to the conclusion that cystathionase acts not on cysteine but on cystine, decomposing this disulfide to produce thiocysteine (alanine hydrogendisulfide, CySSH), pyruvate, and ammonia. Thiocysteine is rather unstable but FLAVIN[7] found that it is formed by the action of microbial cystathionase on cystine and survives in the incubation medium sufficiently to be converted to a mixed disulfide by reaction with iodoacetate. RAO AND GORIN[8] indicated that thiocysteine is formed in strong alkaline solution from cystine and sulfide ion. CAVALLINI *et al.*[9] found that the reaction of cysteine with elemental sulfur forms thiocysteine which subsequently is changed to other compounds of more stability.

Abbreviations: Cy-, cysteine *minus* -SH group; EDTA, ethylenediaminetetraacetic acid.

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** It appears that the approved name, thiosulfate:cyanide sulfurtransferase, will need revision. Not only is thiocystine shown below to be a more efficient substrate than thiosulfate but so also are other forms of polysulfide sulfur. Pending clarification of the physiological substrates for the enzyme, we propose to retain the classical name, rhodanese.

Thiocysteine has not been isolated in pure form but FLETCHER AND ROBSON[10] have separated crystalline bis-(2-amino-2-carboxyethyl) trisulfide from the reaction of cysteine with sulfur followed by acid treatment. In analogy to the term, thiocysteine, we will refer to this compound as thiocystine.

This paper will present evidence that cystathionase and rhodanese (thiosulfate: cyanide sulfurtransferase, EC 2.8.1.1) can form a coupled enzyme system which provides sulfur for transsulfuration originating with cystine. Also, thiocystine may be formed from thiocysteine during the action of cystathionase on cystine; and thiocystine serves as a substrate for rhodanese.

MATERIALS AND METHODS

Crystalline rhodanese, form III from beef liver, was purchased from the Sigma Chemical Company as a suspension of enzyme in 1 M ammonium sulfate, 0.1 M glycine buffer and 0.01 M thiosulfate. Activity of the preparation was 400 units* per mg (200 Sörbo units). To remove thiosulfate, the crystalline enzyme was sedimented by centrifugation, the pellet resuspended in 1 M ammonium sulfate, 0.2 M phosphate buffer, pH 7.4, containing 10^{-3} M EDTA, sedimented again and again resuspended. The product was now free of thiosulfate and no significant loss of activity was observed. Cystathionase was prepared according to MATSUO AND GREENBERG[11] as described by GREENBERG[12] from rat livers, the crystallization being omitted. (A preparation of cystathionase purified 150-fold was kindly provided by Dr. CHRISTINE BROWN and Mr. ROBERT HUDGINS.) The activity of the preparation was 5 units per ml (300 units per ml in terms of units employed by MATSUO AND GREENBERG) with homoserine as substrate. Thiocyanate was estimated according to the method of SÖRBO[13]. Thiocystine was prepared according to FLETCHER AND ROBSON[10] by the action of sulfur dichloride on *N*-acetylcysteine and by the reaction of cysteine with sulfur. The preparations were found to be pure when tested by cold cyanolysis and by paper chromatography. Thiocystine did not yield a positive sulfhydryl test with nitroprusside or with the ELLMAN reagent, 5,5′-dithiobis-(2-nitrobenzoic acid) (ref. 14). Keto acids were estimated by the method of FRIEDEMANN AND HAUGEN[15] as described by GREENBERG[12]. Thiosulfate ion was determined after precipitation with tri-(ethylenediamine)-nickel nitrate[16]. Radioactivity measurements were carried out with a Packard Tri-Carb liquid scintillation spectrometer. Spectral measurements were done with a Cary Model 14 or a Beckman Model DB spectrophotometer.

RESULTS

Preliminary experiments showed that rhodanese greatly enhanced the formation of thiocyanate when added to an incubation medium containing cystathionase, cystine, and cyanide. Under the conditions employed, only minute amounts of thiocyanate were formed in the absence of rhodanese, whereas in the presence of the enzyme, one μmole of thiocyanate was formed from one μmole of cystine. Typical results are recorded in Table I.

* Units of enzyme activity are expressed as recommended by the Commission on Enzymes of the International Union of Biochemistry as μmoles of product produced per min at 25°. The assay of cystathionase with homoserine as a substrate is customarily performed at 37°. No correction for the temperature difference has been applied in calculating international units.

TABLE I

FORMATION OF THIOCYANATE FROM CYSTINE AND CYANIDE IN THE CYSTATHIONASE–RHODANESE SYSTEM

The complete incubation medium was 0.1 M phosphate buffer, pH 7.4, 1 mM in EDTA and 10 μM in pyridoxal phosphate and contained: cystathionase, 0.25 units; rhodanese, 16 units; cystine, 1 μmole; KCN, 2 μmoles in a total volume of 1.08 ml. Cystathionase activity was 5 units/ml estimated against homoserine. Cystathionase and rhodanese were denatured by heating at 90° for 10 min. Rhodanese was a thiosulfate-free suspension of 4 mg of enzyme per ml. Incubations were for 150 min at 25°. After incubation, water was added to a final volume of 2 ml, then 0.5 ml of 38% formaldehyde and 2.5 ml of ferric nitrate reagent according to SÖRBO[13]. Absorbance was measured with the Beckman Model DB spectrometer after centrifugation of the tubes.

Modifications	*SCN^- formed (μmoles)*
None	1
Rhodanese omitted	0.05
Rhodanese denatured	0.05
Cystathionase denatured	<0.03

Fig. 1 shows the effect of rhodanese concentration on the formation of thiocyanate in the cystathionase–cyanide system. It is evident from these data that optimal formation of thiocyanate occurred at a rhodanese concentration of about 80 μg (32 units) in the incubation medium. Activity of cystathionase present in the solution was 0.25 units estimated with homoserine*. Thus, it was probable that the product

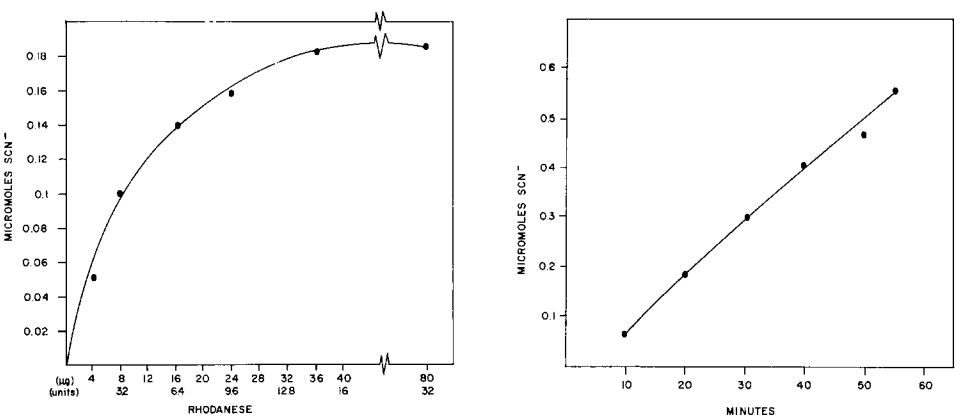


Fig. 1. Effect of rhodanese concentration on thiocyanate formation in the cystathionase–rhodanese system. Incubation mixtures contained per tube: 1 ml modified phosphate buffer, 0.1 M, pH 7.4, 1 mM in EDTA and 10 μM in pyridoxal phosphate, 0.25 units of cystathionase, 1 μmole of cystine, 1 μmole of KCN and amounts of rhodanese from 4–80 μg in a total volume of 1.07 ml. The incubation time was 20 min at 25°. The reaction was stopped by addition of 38% formaldehyde and the thiocyanate present was estimated by the method of SÖRBO[13].

Fig. 2. Rate of thiocyanate formation in the cystathionase–rhodanese system. Incubation mixtures contained per tube: 1 ml of phosphate buffer, 0.1 M, pH 7.4, 1 mM in EDTA and 10 μM in pyridoxal phosphate, 0.25 units cystathionase, 1 μmole cystine, 2 μmoles KCN, 16 units (40 μg) rhodanese in a total volume of 1.09 ml. The incubations were stopped at various times by addition of 0.5 ml of 38% formaldehyde and the thiocyanate formed was estimated by the method of SÖRBO[13].

* The true activity of cystathionase was lower in the incubation medium due to partial inhibition by cyanide.

TABLE II

THIOSULFATE FORMATION IN THE CYSTATHIONASE–RHODANESE STYSEM

The incubation medium contained 0.1 M phosphate buffer, pH 7.4 (1 mM in EDTA, 10 μM in pyridoxal phosphate), 0.5 ml; cystathionase, 0.25 units; rhodanese, 16 units; and [^{35}S]cystine, 1 μmole; all in a volume of 0.62 ml. The specific activity of the cystine was 466 counts/min per μmole. After the incubations, the samples were treated with 0.5 ml of 0.1 M sodium thiosulfate added as carrier and then with 60 mg of tri-(ethyl enediamine)-nickel nitrate *in substantia*. The complex with thiosulfate was sedimented by centrifugation, resuspended in 50% ethanol and collected on a disk of glass paper for radioactivity measurements. The thiosulfate formed was calculated from radioactivity measurements after suitable blank correction.

Modifications	*Thiosulfate formed (μmoles)*
None	0.2
Rhodanese omitted	<0.03
Rhodanese and $S_2O_3^-$ omitted	<0.03
Rhodanese denatured	<0.03
Cystathionase omitted	blank

formed by cystathionase was released to the medium before serving as a substrate for rhodanese.

The rate of reaction of the complete system is shown in Fig. 2. Although the kinetics appear to be rather complex, a plot of the results generally yields a straight line function.

Since sulfite may accept sulfur from rhodanese[17,18] thiosulfate formation in the cystathionase–rhodanese system was investigated also. Sulfite rapidly inactivates rhodanese as shown by SÖRBO[17]. To avoid this inactivation, cystathionase was incubated first with cystine for 15 min and then rhodanese and sulfite were added. Incubation in the presence of sulfite was for 5 min. To confirm the formation of thiosulfate from precursor cystine and sulfite, [^{35}S]cystine was used. It is apparent from

TABLE III

EFFECT OF ACID ON THE STABILITY OF THE SUBSTRATE FOR RHODANESE PRODUCED BY ACTION OF CYSTATHIONASE ON CYSTINE

The incubation mixture contained: phosphate buffer, 0.1 M, pH 7.4, 1 mM in EDTA, 10 μM in pyridoxal phosphate, 1 ml; cystathionase, 0.25 units; cystine, 1 μmole; KCN, 2 μmoles; all in a volume of 1.08 ml. Nos. 1, 2 and 4 were incubated 20 min before supplementary additions; No. 3 was incubated 30 min then HCL was added, sample kept acid for 5 min, and an additional 5 min used for neutralization (cystathionase was activated in acid solution).

Expt. No.	*Modifications*		*SCN^- formed (μmoles)*
	Initial	*After 20 min incubation*	
1	rhodanese, 16 units	none	0.21 (0.18–0.23)
2	none	rhodanese, 16 units	0.21 (0.19–0.22)
3	none	rhodanese, 16 units, *plus* 0.4 mmole HCl then 0.4 mmole NaOH	0.20 (0.16–0.22)
4	none	none	<0.03

the data of Table II that the system formed thiosulfate from cystine and sulfite ion.

Two stages occurred in the course of cystine decomposition by cystathionase when rhodanese and cyanide were omitted from the incubation medium. Initially, and usually when no more than half of the cystine had reacted, the solution remained clear. Subsequently, a turbidity developed and hydrogen sulfide was evolved. This course of events did not change when the incubation was performed in the presence of 2 μmoles of KCN per μmole of cystine. However, if rhodanese were added to the clear incubation mixture together with cyanide, no turbidity was observed. Experiments were performed with rhodanese added at various times, all before any turbidity was observed. The results recorded in Table III show that there was no difference in the amount of thiocyanate formed whether rhodanese was present from the beginning of the incubation or was added 20 min later. Furthermore, acidification of the cystathionase incubation medium to a low pH followed by neutralization did not destroy the reactivity of the intermediate products toward rhodanese. The amount of thiocyanate found was approximately the same as observed in unacidified samples.

Evidence for thiocystine formation

Thiocystine exhibits a plateau in its spectrum between 240 and 250 mμ, resembling in this respect cystine but with enhanced molar absorption. The molar absorption for cystine at 245 mμ in 0.1 M phosphate buffer, pH 7.4, is 310 whereas the corresponding absorption for thiocystine is 1550. It may be seen in Fig. 3 that spectral

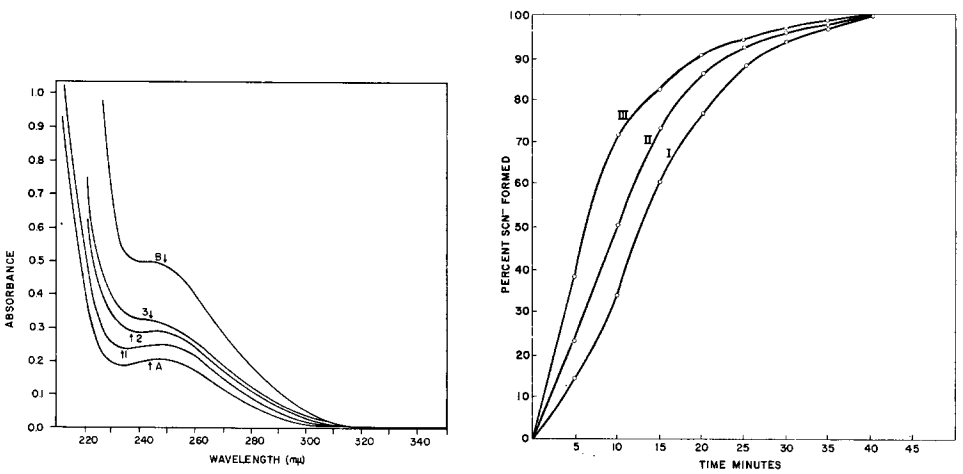


Fig. 3. Spectral evidence for the formation of thiocystine. Incubations were performed in the Cary Model 14 recording spectrometer in 1 cm cells maintained at 25°. Both unknown and reference cells were filled with 3 ml of phosphate buffer, 0.1 M, pH 7.4, and 1 mM in EDTA and 0.01 ml (0.05 units) of cystathionase solution, 10^{-3} M in pyridoxal phosphate. To one of the cells were added 2 μmoles of cystine (20 μl) of 0.1 M solution in 0.5 M HCl and to the reference cell were added 20 μl of 0.5 M HCl. The spectrum was recorded at various times: Curve 1, 7 min, Curve 2, 17 min; Curve 3, 29 min. The spectrum of cystine (Curve A) and of thiocystine (Curve B) are given for comparison purposes. The concentration of cystine was 2 μmoles/3.02 ml and that of thiocystine 1 μmole/3.02 ml.

Fig. 4. Curve I, the rate of cyanolysis of thiocystine. Curve II, the rate of cyanolysis of the product formed during the action of cystathionase on cystine. Curve III, the rate of cyanolysis of thiocystine in the presence of cysteine. Conditions of cyanolysis are described in the text.

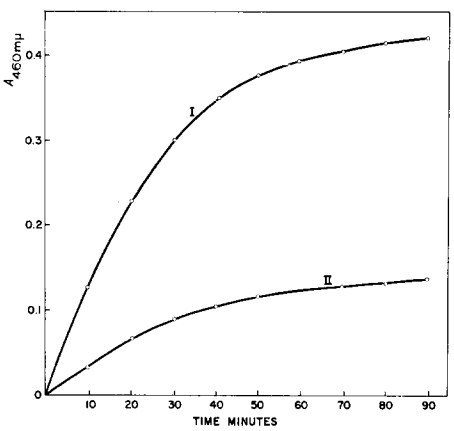
changes strongly suggest that formation of thiocystine had occurred in the incubation medium of the cystathionase-cystine system. After a prolonged period of incubation, the shape of the absorption curve changed indicating the formation of other products.

Cold cyanolysis was carried out on the products of the action of cystathionase on cystine by an adaptation of the method of FLETCHER AND ROBSON[10]. A mixture of 5 units of cystathionase in 4 ml of 0.2 M K_2HPO_4, 10^{-3} M in EDTA, and 15 μmoles of cystine in 0.5 ml of 0.2 M HCl were incubated for 30 min. Then 3.7 ml of water, 1 ml of 0.1 M KCN and 0.35 ml of 2 M ammonia were added. At suitable intervals, aliquots were removed and treated with ferric nitrate reagent[13]. The thiocyanate formed was estimated spectrophotometrically. Fig. 4 shows the course of cyanolysis It may be seen that the course of cyanolysis for thiocystine (Curve 1) is somewhat different than for the product of cystathionase on cystine (Curve II). However, a mixture of thiocystine and cysteine underwent cyanolysis (Curve III) similarly to the enzymic reaction product.

Thiocystine was isolated from the reaction of cystine in the cystathionase system by isotopic dilution. To 5 ml of 0.1 M phosphate buffer, pH 7.4, 1 mM in EDTA, was added 0.1 μmole of pyridoxal phosphate, 10 μmoles of L-[^{35}S]cystine ($5.8 \cdot 10^5$ counts/min) and 6 units of cystathionase in a total volume of 6.6 ml. After incubation at 25° for 30 min, the solution had become somewhat opalescent. Thiocystine, 2 mg, was added and, 15 min later, an additional 57 mg. The solution was adjusted to pH 4.5 with pyridine. Thiocystine, possibly contaminated with cystine, crystallized from the solution. This product, 42 mg, was dissolved in 2.5 M HCl and passed through a Dowex 50-X 4 column according to the procedure of FLETCHER AND ROBSON[10]. Thiocystine, 12.5 mg, was crystallized from the appropriate fraction and had a radioactivity count of $3 \cdot 10^3$ counts/min per mg. A chromatogram developed with butanol–acetic acid–water (12:3:5) showed the radioactivity to travel with the thiocystine spot. On the basis of the dilution factor, cystine equivalent to $1.77 \cdot 10^5$ counts/min was converted to thiocystine. This represents a 30% yield. In a similar experiment but with only 0.002 units of cystathionase in the incubation medium, the thiocystine reisolated had 152 counts/min per mg.

Stability of thiocystine

Although thiocysteine is presumed to be unstable, the trisulfide, thiocystine, is a well characterized compound[10]. It is unchanged in boiling acid and is relatively stable in weakly alkaline solution. When thiocystine was incubated at 37° in appropriate buffers, its stability was estimated by its appearance as a single spot on paper chromatograms as follows: pH 6.7 to 7.4, 6 h; 7.6 to 8.3, 3 h; 9.4, 2 h. As the intensity of the thiocystine spot diminished with successive chromatograms, cystine appeared. After prolonged incubation, only a cystine spot was detected. When the incubation was carried out in the presence of 5,5'-dithiobis-(2-nitrobenzoic acid), the color development which would have indicated formation of sulfhydryl, sulfide, or alkyl hydrogen disulfide (thiocysteine) groups did not occur. A sample of thiocystine incubated for 12 h at pH 8 deposited a sediment which was identified as free sulfur. When an equimicromolar amount of cysteine was added, sulfur was precipitated within a short time. Fig. 5 shows the rate of production of free sulfur measured turbidimetrically at pH 7.8 (Curve I). The rate of sulfur formation was inhibited by addition of cystine to the medium (Curve II). Evidently, as noted below, cysteine decreases the stability of



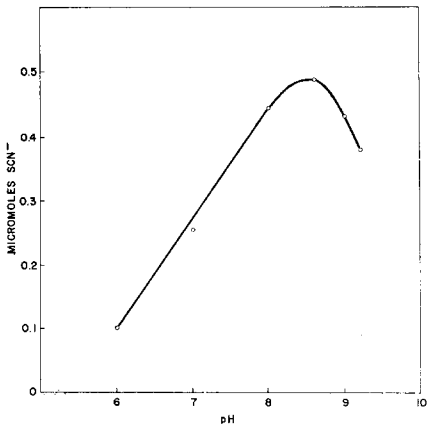


Fig. 5. The rate of sulfur formation from thiocystine as measured by absorbance at 460 mμ. Curve I, sulfur formation in the system: thiocystine, 4 μmoles; cysteine, 4 μmoles; phosphate buffer, 0.2 M, pH 7.8, 3 ml; glycerol, 1 ml; total volume, 4.44 ml. Curve II, the same system + 8 μmoles of cystine.

Fig. 6. pH dependence of rhodanese activity in the system, rhodanese–thiocystine–cyanide. For pH 6 and 7, phosphate buffers and for higher pH values, veronal buffers were used. Incubation mixtures contained per tube: 2 ml of 0.1 M buffer; thiocystine, 1 μmole; rhodanese, 2 units; KCN, 2 μmoles; all in a final volume of 2.4 ml. The time of incubation at room temperature was 5 min.

thiocystine by converting it to thiocysteine which readily decomposes. However, in the presence of cystine, an equilibrium between thiocysteine and thiocystine is shifted toward the more stable compound.

Thiocystine as a substrate for rhodanese

It followed from the above observations that thiocystine should function as a substrate for rhodanese. The data of Table IV show that the enzyme can utilize thiocystine for transsulfuration. Compound from either of the methods of preparation served equally well. For transsulfuration to cyanide, thiocystine was equivalent to a

TABLE IV

THE FORMATION OF THIOCYANATE FROM THIOCYSTINE AND CYANIDE CATALYZED BY RHODANESE

The complete system contained: phosphate buffer, 0.1 M, pH 7.4, 1 mM in EDTA, 2.8 ml; rhodanese (thiosulfate-free), 1.6 units; KCN, 20 μmoles; all in a total volume of 3.6 ml. The time of incubation was 5 min at 25°. The data for thiosulfate are given for comparison purposes.

Modifications	*SCN^- formed (μmoles)*
Rhodanese omitted, thiocystine, 0.6 μmole	<0.03
Thiocystine, 0.2 μmole	0.09
Thiocystine, 0.4 μmole	0.18
Thiocystine, 0.6 μmole	0.29
Thiosulfate, 0.6 μmole	<0.03
Thiosulfate, 2.0 μmoles	0.14
Thiosulfate, 4.0 μmoles	0.25

7-fold greater concentration of thiosulfate. As noted by SÖRBO, in the rhodanese–cyanide system, the reaction rate depends not only upon donor substrate concentration but also upon the concentration of cyanide which is the acceptor substance. This was observed in the rhodanese–thiocystine–cyanide system also. Moreover, cyanide acts as an inhibitor of the enzyme[19].

Fig. 6 shows the optimum pH for the rhodanese–thiocystine–cyanide system to be 8.6. Spontaneous cyanolysis of thiocystine increases with pH and evidently displaces the optimum pH range upward. However, as noted above, thiocystine in the absence of cyanide is stable for at least 2 h at 37° in the pH range, 8.3–9.4.

DISCUSSION

The detection of thiocystine among the products of the action of cystathionase on cystine and also the utilization of thiocystine as a substrate by rhodanese provide evidence for the occurrence of the trisulfide as an intermediate in the coupled cystathionase–rhodanese transsulfuration system which converts cystine sulfur to thiocyanate, thiosulfate, sulfinates, and probably other products. The evidence is not unequivocal because of the complexities in the interrelationships between the sulfur compounds which occur in biological systems.

The investigations of CAVALLINI and co-workers[3,9] and of FLAVIN[7] demonstrated thiocysteine to be the initial product of the action of cystathionase on cystine.

$$CySSCy \rightarrow CySSH + CH_3COCOOH + NH_3 \quad (1)$$

Thiocystine apparently arises from thiocysteine and cystine according to Reaction 2.

$$CySSH + CySSCy \rightleftharpoons CySSSCy + CySH \quad (2)$$

The equilibrium is disturbed by the withdrawal of thiocystine through its decomposition to cysteine and free sulfur. These relationships are

$$CySSH \rightleftharpoons CySH + S \quad (3)$$

demonstrated in Fig. 5. Thiocysteine persists in weakly alkaline solution for some time, but when the compound was incubated with cysteine, prompt appearance of free sulfur resulted. Reaction 2 was shifted to the right by the addition of cystine, as indicated by a markedly decreased sulfur precipitation in the thiocystine–cysteine system.

The equilibrium of Reaction 3 is displaced to the right in neutral or acid solution by the insolubility of the sulfur. As the concentration of cysteine builds up, some of the sulfur is converted to hydrogen sulfide. As noted above, we confirmed the precipitation of free sulfur

$$2\,CySH + S \rightarrow CySSCy + H_2S \quad (4)$$

and the appearance of H_2S when the incubation of cystine with cystathionase was prolonged[3,7]. Thiocystine also decomposes in alkaline solution to provide free sulfur but the spontaneous reaction is too slow to account for the appearance of sulfur in the cysteine–cystathionase system. Furthermore, since cysteine is not a byproduct of the decomposition,

$$CySSSCy \rightarrow CySSCy + S \quad (5)$$

no H_2S would be produced if thiocystine were the only source of the sulfur precipitating in the incubation mixture. When the trisulfide was incubated with a 3-fold excess of cysteine for 17 h at pH 7, 0.8 mole of sulfide was produced per mole of thiocystine.

From the above discussion, we conclude that in dilute solution, and in the presence of an excess of cystine, thiocysteine initially formed by the action of cystathionase is converted in appreciable amounts to the more stable thiocystine according to Reaction 2. CAVALLINI and co-workers observed thiocysteine in the cystathionase system to form an acid-stable, cyanolyzable compound[9]. The course of the cyanolysis of the enzymic reaction product (Fig. 4) resembles the cyanolysis of thiocystine in the presence of cysteine. The spectral changes with time in our cystathionase system (Fig. 3) point to rapid development of thiocystine. Finally, the isolation of thiocystine by isotopic dilution is strongly supportive although the isolation procedure involved a short exposure of the initial products to a low pH.

There is only presumptive evidence that thiocysteine in itself can act as a substrate for rhodanese since the compound has never been separated in pure form. However, it is reasonable to expect that it should serve as a substrate from its behavior on cold cyanolysis which is analogous to the action of cyanide on disulfide ion. Since Reaction 6

$$CySSH + CN^- \rightarrow CySH + SCN^- \quad (6)$$

$$CySSSCy + CN^- \rightarrow CySSCy + SCN^- \quad (7)$$

is the summation of Reaction 2 and Reaction 7 which is catalyzed by rhodanese, it is not obligatory for thiocysteine to serve as a substrate in order to obtain the results observed with the cystathionase–cystine–rhodanese–cyanide system.

The cystathionase system can produce disulfide sulfur resulting from the breakdown of thiocysteine (Reaction 2 *plus* 3). Disulfide sulfur has been postulated as a direct decomposition product of thiocysteine[3,10].

$$2\,CySSH + CySH \rightarrow CySSCy + H_2S_2 \quad (8)$$

Cystathionase acting alone on cystine functions as a desulfhydrase but production of hydrogen sulfide from free sulfur arising from Reactions 3 and 4 may occur only after an appreciable concentration of cysteine develops. In the presence of rhodanese and an acceptor such as cyanide or sulfite, sulfide does not appear but thiocystine and probably thiocysteine are rapidly converted to transsulfuration products. Furthermore, we observed that the reaction mixture of cystathionase and cystine could be acidified after a short incubation period and then neutralized before adding cyanide and rhodanese without decreasing the yield of thiocyanate (Table III). Under such conditions, disulfide ion and thiocysteine should be decomposed and sulfide ion lost.

Details for a metabolic pathway for transsulfuration from cystine to cyanide or sulfite have been elusive. Deamination of cystine to form mercaptopyruvate has been observed[20] and a mercaptopyruvate transsulfuration enzyme has been found in mammalian and bacterial systems[21,22] but the concerted action of the two enzymes to transfer cystine sulfur to an ultimate acceptor has not been demonstrated. The present observations raise interesting possibilities for a pathway by which there could

be a transfer of sulfur from cystine to a number of acceptors. If cystathionase and rhodanese should function *in vivo* as a coupled enzyme system, the intermediary compound, thiocystine, has sufficient stability to be transferred from the locus of one enzyme to that of another. The coupled system provides a mechanism for an efficient utilization of cystine sulfur which obviates the appearance of sulfide ion in tissues*.

It is also suggestive that KOJ, FRENDO AND GORNIAK[24,25] have made comparative estimations of the activities of rhodanese and cysteine desulfhydrase, *i.e.* cystathionase, in various tissues of a number of species and have found a strict correlation with regard to the presence and activities of the two enzymes. In the developing chick embryo, the appearance and increase in the two enzyme activities are parallel[26]. On the other hand, there is no correlation between the very large amounts of rhodanese and the content of thiocyanate in the tissues[27]. In this study we find also a large rhodanese requirement for optimal operation of the transsulfuration system. Such observations suggest the more important role for the system of transsulfuration may be for forming thiosulfate and other intermediates of sulfur metabolism rather than for the detoxication of cyanide.

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* Studies on the sulfur pool providing for thiocyanate production[23] have indicated that the sulfur of free cystine does not provide for the major part of cyanide detoxication. This probably results from the low concentration of cystine in tissue fluids. It is of interest to note that preliminary experiments have shown that a trisulfide corresponding to thiocystine but prepared from glutathione can also function as a substrate in the rhodanese system.