Laskowski, M., Jr., Widom, J. M., McFadden, M. L., and Scheraga, H. A. (1956), *Biochim. Biophys. Acta 19*, 581.
Leach, S. J., and Scheraga, H. A. (1960), *J. Biol. Chem. 235*, 2827.
Martin, C. J. (1961), *Biochim. Biophys. Acta 51*, 610.
Martin, C. J. (1962), *Federation Proc. 21*, 227.
Martin, C. J. (1964), *Biochim. Biophys. Acta 79*, 214.
Martin, C. J., and Frazier, A. R. (1963a), *J. Biol. Chem. 238*, 3268.
Martin, C. J., and Frazier, A. R. (1963b), *J. Biol. Chem. 238*, 3869.
Nelson, C. A., and Hummel, J. P. (1962), *J. Biol. Chem. 237*, 1567.
Neurath, H., Rupley, J. A., and Dreyer, W. J. (1956), *Arch. Biochem. Biophys. 65*, 243.
Oppenheimer, H. L., Mercouroff, J., and Hess, G. P. (1963), *Biochim. Biophys. Acta 71*, 78.
Rosenheck, K., and Doty, P. (1961), *Proc. Natl. Acad. Sci. U. S. 47*, 1775.
Schellman, J. A. (1958), *Comp. Rend. Trav. Lab. Carlsberg, Ser. Chim. 30*, 439.
Scheraga, H. A. (1957), *Biochim. Biophys. Acta 23*, 196.
Scheraga, H. A. (1961), Protein Structure, New York, Academic, p. 93.
Scott, R. A., and Scheraga, H. A. (1963), *J. Am. Chem. Soc. 85*, 3866.
Smillie, L. B. (1959), *Biochim. Biophys. Acta 34*, 548.
Steiner, R. F. (1964), *Biochim. Biophys. Acta 79*, 51.
Tanford, C. (1962), *J. Am. Chem. Soc. 84*, 1747.
Urnes, P., and Doty, P. (1961), *Advan. Protein Chem. 16*, 401.
Wetlaufer, D. B. (1962), *Advan. Protein Chem. 17*, 304.
Wetlaufer, D. B., Edsall, J. T., and Hollingworth, B. R. (1958), *J. Biol. Chem. 233*, 1421.
Yanari, S., and Bovey, F. A. (1960), *J. Biol. Chem. 235*, 2818.
Yang, J. T., and Doty, P. (1957), *J. Am. Chem. Soc. 79*, 761.

Reactions of Seleno- and Sulfoamino Acids with Hydroperoxides*

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This research was undertaken to explore the reactions of seleno- and sulfoamino acids with hydroperoxides because of their relevance to the mechanisms of selenium functions in animal biochemistry. Reactions of methionine, cystine, and their selenium analogs with hydrogen peroxide and several organic peroxides have been studied. Methionine reacts most rapidly with hydrogen peroxide, but selenocystine causes the most extensive decomposition. Both methionine and selenocystine react much more slowly with organic hydroperoxides. Chemical data on the intermediates produced suggest that under certain conditions the reactions may be catalytic. The chief conclusion is that selenocystine has the chemical capacity to act as an antioxidant by the reduction of peroxides.

Vitamin E and dietary selenium elicit a similar nutritional response in many animal species. Since vitamin E is primarily a lipid antioxidant, selenium may perform the same function. Several factors must be evaluated when considering an antioxygenic role for selenium: (1) It is metabolized in the same manner as sulfur; (2) only minute quantities of dietary selenium are required to elicit a biological response. In view of its dilution by corresponding sulfur compounds, the question arises how selenium compounds can produce such dramatic results. The answer probably lies in their relative reactivity, such that a small concentration of a selenium compound is equivalent to a higher concentration of its sulfur analog.

Little comparison has been made of the reactivity of biologically important sulfur and selenium compounds. But several workers (Dennison and Condit, 1949; Woodbridge, 1959) report that alkyl selenides and diselenides are more efficient oil antioxidants and peroxide decomposers than their sulfur analogs. Since peroxides are the chief products of lipid peroxidation, a comparison of their reactions with some biological sulfides and selenides is appropriate. Accordingly, this paper reports a study of the action of sulfo- and seleno-amino acids on hydrogen peroxide and several organic peroxides.

Methods and Materials

Materials.—Selenocystine was prepared in good yield from sodium benzyl selenide and α-amino-β-chloropropionic acid methyl ester according to the directions of Painter (1947). Several workers (private communications) report difficulty in the synthesis of benzyl selenomercaptan. We were able to obtain this compound in 20–25% yield, if experimental conditions were carefully controlled. Air must be rigorously excluded. The reaction vessel was a 3-necked, 3-liter flask, equipped with a mechanical stirrer (center neck) and 3-way standard taper adapters (side necks). One adapter was fitted with (1) a condenser and $CaCl_2$—Drierite tube and (2) a small round-bottomed flask with rubber-tubing connection and a screw clamp. Selenium powder was stored in the flask until time for its addition. The other adapter was fitted with (1) a dropping funnel for admission of benzyl chloride and (2) an inlet for nitrogen gas. This apparatus made it possible to flush the system continuously with nitrogen and to exclude air until the latter stages of the preparation. Fractional distillation of the impure product gave a fraction, bp 115° (32 mm), which was used for subsequent reactions. Painter reported a bp of 102° (20 mm) and a best yield of 45%. All other reactions were carried out as he described with results comparable to his. Both selenocystine and selenomethionine are now commercially available from the Cyclo Chemical Co., Los Angeles, Calif.

Cystine disulfoxide was synthesized according to the method of Emiliozzi and Pichat (1959). Other reagent-grade chemicals were from commercial sources and satisfactory for use without further purification.

Methods.—The solubility and stability of selenocystine and cystine required the use of aqueous acid in all determinations. The following acids were used:

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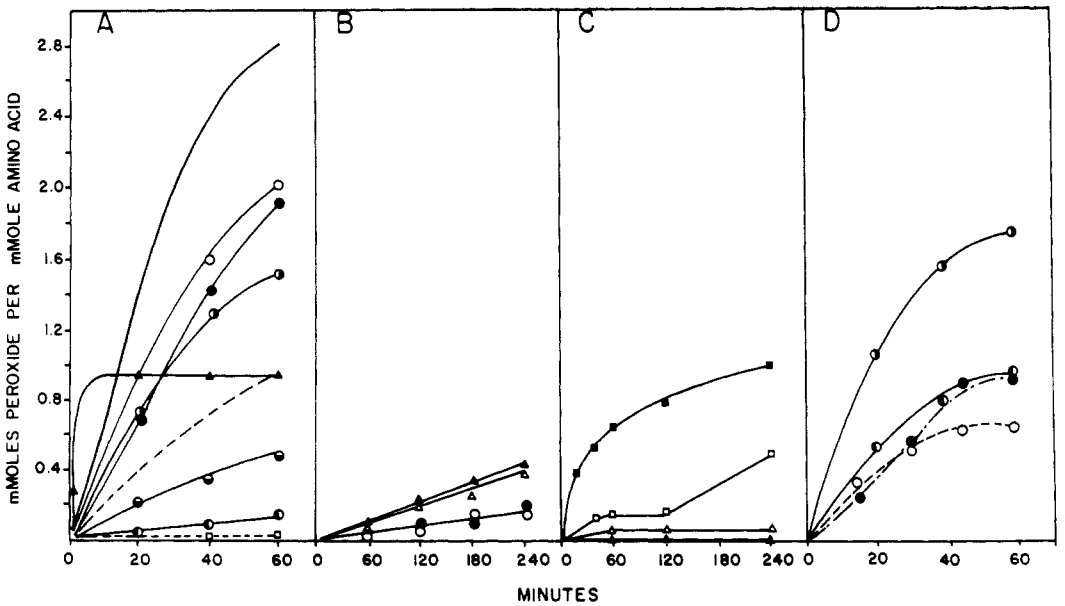


FIG. 1.—Reaction of methionine, selenocystine, and cystine derivatives with various peroxides. (A) Methionine and selenocystine with hydrogen peroxide. Cystine (0.05 M) dissolved in 2 N H_2SO_4 + 0.10 M H_2O_2 (□). Selenocystine (0.05 M) dissolved in 2 N H_2SO_4 + H_2O_2; molarity of H_2O_2: 0.05 (◐), 0.10 (◒), 0.20 (◑), 0.30 (●), 0.40 (○). Reaction of 0.05 M selenocystine in 88% formic acid with H_2O_2; molarity of H_2O_2: 0.10 (– – –), 0.30 (——). Reaction of 0.05 M methionine in 2 N H_2SO_4 with 0.10 M H_2O_2 (▲). (B) Methionine and selenocystine with organic peroxides. Solvent, 50% ethanol–2 N H_2SO_4; molarity of amino acid and of hydroperoxide, 0.05. Selenocystine + *t*-butyl hydroperoxide (○), selenocystine + cumene hydroperoxide (●); methionine + *t*-butyl hydroperoxide (△), methionine + cumene hydroperoxide (▲). (C) Oxidized methionine or cystine derivatives with hydrogen peroxide. Amino acid (0.05 M) dissolved in 2 N H_2SO_4 + 0.10 M H_2O_2: either cysteic acid or methionine sulfone (▲), methionine sulfoxide (△), cystine disulfoxide (□), cysteine sulfinic acid (■). (D) Selenocystine with hydroperoxides in 2 N HCl. Molarity of selenocystine, 0.05. *t*-Butyl hydroperoxide, 0.05 M (○); cumene hydroperoxide (●); 0.09 M H_2O_2 (◐); 0.20 M H_2O_2 (◑).

2 N H_2SO_4, 88% formic, and 2 N HCl. In the case of organic peroxides, 50% ethanol–2 N sulfuric acid was the solvent. All reactions were carried out at room temperature. Peroxide decomposition was determined as follows: Twenty ml of acetic acid–2-propanol (3:2) was placed in a 300-ml Erlenmeyer flask and continuously flushed with nitrogen; 1 ml of saturated aqueous potassium iodide was added. An aliquot of the solution to be analyzed was pipetted into the acid-iodide solution and the nitrogen flow was continued for several minutes. The flask was tightly stoppered and stored in the dark for 20–60 minutes. Fifty ml of distilled water was then added and the solution was titrated with standard thiosulfate. Little air was admitted to the flask until after dilution with water. In no case was there appreciable decomposition in the absence of amino acid.

The absorbance spectra of cystine and selenocystine were determined in 2 N sulfuric acid. Whatman 3MM paper was used for all chromatographic analyses. Quantitative papers were run in 1-butanol–acetic acid–water (4:1:1; solvent A) and amino acid concentration was estimated by densitometric measurement at 575 mμ of its ninhydrin-positive reaction. Solvent A or ethanol–water–sulfuric acid (80:20:0.5; solvent B) was used for qualitative papers. Chromatograms were developed with ninhydrin or with the polychromatic copper-ninhydrin spray described by Moffat and Lytle (1959).

RESULTS

Reaction of Amino Acids with Hydroperoxides.—Cystine, selenocystine, methionine, and selenomethionine were tested for their ability to decompose hydrogen peroxide. Figure 1A shows that methionine reacts fastest but that selenocystine is far superior to both cystine and selenomethionine. Methionine reacts immediately upon addition and consumes 0.90 mole of peroxide per mole of amino acid within 10 minutes. Thus it appears to be a better peroxide decomposer than either selenoamino acid. However, under these experimental conditions the capacity of methionine is limited to 1 mole peroxide per mole amino acid. Even when the initial peroxide concentration was increased 4-fold, methionine showed no tendency to consume additional peroxide.

Reaction of Selenocystine with Hydrogen Peroxide.—To further clarify these reactions, additional studies were made on the selenocystine-H_2O_2 system. Figure 1A shows that the rate of reaction depends upon initial peroxide concentration. Oxygen consumption increases for approximately 1 hour, after which the reaction rate decreases. Figure 1A also indicates that the conversion is more efficient with peracid than with hydroperoxide. Similar observations have been made for sulfides. Both reactions are first order with respect to peroxide concentration (Fig. 2). Additionally, quantitative paper chromatography revealed that 50–80% of the selenocystine is consumed in less than 1 minute, after which its concentration remains relatively constant.

Reaction products determined by qualitative paper chromatography are shown in Table I. The mobility of a sulfur compound and its selenium analog was found to be essentially the same in each solvent system studied. Data from a typical chromatogram are given in Table II. The purity of amino acids was determined from both untreated standards and by comparison of differences immediately upon addition of H_2O_2 and at

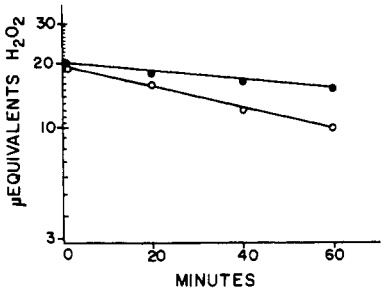


FIG. 2.—Decomposition of hydrogen peroxide by selenocystine. Selenocystine (0.05 M) + H_2O_2 (0.10 M) in 2 N H_2SO_4 (●); same reactants in 88% formic acid (○).

TABLE I
PRODUCTS FROM THE REACTION OF H_2O_2 WITH SULFO- OR SELENOAMINO ACIDS

Amino Acid	Products
Cystine	Cystine disulfoxide
	Cysteine sulfinic acid
	Cysteic acid
Selenocystine	Selenocystine seleninic acid
	Alanine
	Metallic selenium
Cystine disulfoxide	Cysteic acid
	Cysteine sulfinic acid
Cysteine sulfinic acid	Cysteic acid
Methionine	Methionine sulfoxide
Selenomethionine	Unidentified spot

TABLE II
IDENTIFICATION OF SULFO- OR SELENOAMINO ACIDS[a]

Amino Acid	R_a	Color
Cystine	0.50	Charcoal
Selenocystine	0.51	Charcoal
Cystine disulfoxide	0.32	Charcoal
Cysteine sulfinic acid	0.80	Pink
Cysteic acid	0.62	Pink
Alanine	1.00	Pink-purple
Methionine	1.22	Brown-yellow
Selenomethionine	1.23	Brown-yellow
Methionine sulfoxide	0.80	Pink-yellow
Methionine sulfone	0.87	Pink-yellow

[a] Results expressed as relative mobility with respect to alanine (R_a). Solvent, B; spray, copper-ninhydrin.

later time intervals. All amino acids were chromatographically pure with the exception of selenomethionine. The latter showed traces of its selenoxide and selenone, which were identified by comparison of their R_a values with those for the corresponding derivatives of methionine. Immediately upon addition of H_2O_2, selenomethionine and the oxidized contaminants were converted to a single unidentified compound, which shows an R_a different from either of the above. Since cystine causes no peroxide decomposition within the usual experimental period, its reaction products were determined on solutions which had been kept 6–8 hours.

The exact color development from the copper-ninhydrin spray depends upon amino acid concentration, but its use was most advantageous for identification of compounds with similar mobility, i.e., alanine and cysteine in solvent B. Small amounts of red selenium precipitate from dilute solutions of hydrogen peroxide (e.g., 0.10 M) within 5 minutes and can be seen visually. In more concentrated solutions (e.g., 0.40 M) its precipitation may require several hours or even days.

Disparity between equivalence of peroxide and

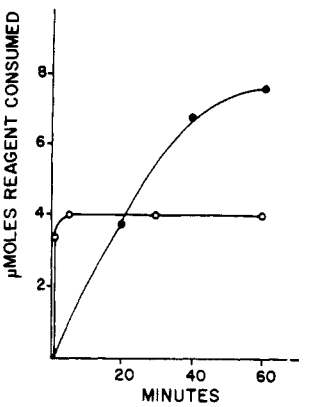


FIG. 3.—Rate of consumption of selenocystine and hydrogen peroxide. Reaction system: 0.05 M selenocystine in 2 N H_2SO_4 and 0.20 M H_2O_2: selenocystine (○), H_2O_2 (●).

selenocystine consumption (Fig. 3) and also the number of products formed indicate the complexity of the reaction. This apparent disparity will be more fully considered under Discussion.

Reaction of Methionine and Selenocystine with Organic Hydroperoxides.—By contrast to their rapid oxidation by H_2O_2, both methionine and selenocystine react slowly with organic peroxides. Methionine consumed only 0.4 mole peroxide per 4 hours and selenocystine consumed only 0.2 mole in the same time (Fig. 1B).

Oxidations in the Presence of HCl.—It should be pointed out that studies of this type have often been carried out in hydrochloric acid. When 2 N HCl was used in our experiments, we observed a significant increase in reaction rate for H_2O_2 and a profound increase for organic peroxides (Fig. 1D). This effect is probably owing to the participation of chlorine produced *in situ*. Both Maclaren (1961) and Njaa (1962) indicate similar effects.

Reactions of Cystine and Methionine Oxides with H_2O_2.—The rapid and extensive reaction of selenocystine suggests that its oxidation products are active peroxide decomposers. Oxygenated derivatives of selenocystine are not available; however, the reactions of cystine disulfoxide, cysteine sulfinic acid, and cysteic acid were studied. Methionine sulfoxide and methionine sulfone were also tested. Control samples, containing no peroxides, were run in all cases. Data given in Figure 1C show that cysteine sulfinic acid reacts at an appreciable rate but that the disulfoxide first undergoes a lag period of 2 hours; cysteic acid caused no decomposition. Additionally, the reaction of methionine sulfoxide is very slight and methionine sulfone is completely unreactive.

Attempts to determine hydrogen peroxide by its absorbance in the ultraviolet revealed that selenocystine also absorbs strongly in this region. It exhibits a maximum at 290 mμ (Fig. 4) and obeys Beer's law. An extinction coefficient of 330 was calculated for a 0.002 M solution of the amino acid in 2 N H_2SO_4. Cystine shows no similar peak absorbance.

DISCUSSION

Peroxide Determination.—Disparity between the equivalence of peroxide and selenocystine consumption has been mentioned. This is probably owing to the participation of solvent and perhaps to the presence of appreciable quantities of oxidized intermediates of selenocystine. In a study of the hydrogen peroxide oxidation of radio-labeled thiolsulfinates to thiolsulfonates, Barnard and Percy (1960) also observed that the thiol-

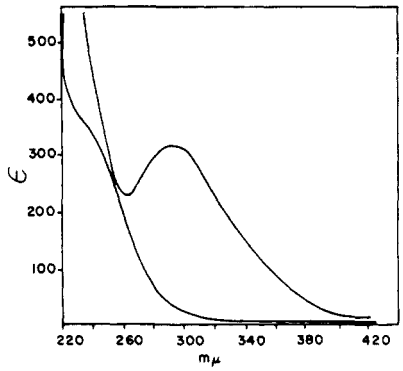


FIG. 4.—Absorption spectra of selenocystine and cystine. Amino acid (0.002 M) in 2 N H_2SO_4.

sulfinate was more rapidly consumed than oxidant. Their study revealed the following: (1) Transient but significant amounts of disulfide and trace amounts of sulfonic acid were formed. (2) One equivalent of oxidant caused the disappearance of several equivalents of thiolsulfinate to yield disulfide and thiolsulfonate in equal amounts. (3) The reaction was catalyzed by sulfinyl radicals.

On the basis of their observations, they suggest that (1) the major pathway for the conversion of thiolsulfinate to thiolsulfonate is not a direct oxidation but an induced disproportion with -S-S- bond cleavage; (2) sulfinyl radicals are derived from the spontaneous disproportionation of disulfoxide; (3) the latter compound is formed by direct oxidation of thiolsulfinate; and (4) disulfide produced in the reaction undergoes direct oxidation to thiolsulfinate. Additionally, they report similar reactions with diselenides, although the final reaction products may differ.

It seems likely that the same series of reactions occurs with selenocystine. This would account for differences in the rates of consumption of peroxide and selenocystine and for failure to observe complete disappearance of the amino acid from reaction solutions. Cysteic acid appears to be quite stable under these experimental conditions and is the final product from cystine, whereas selenocystine yields alanine and metallic selenium. This, too, seems to parallel the results reported by Barnard and Percy (1960).

Lavine (1936) has shown that both cystine disulfoxide and cysteine sulfinic acid release iodine from acid iodide solutions; cysteic acid does not undergo this reaction. Since solutions were analyzed by iodometric titration, the full extent of peroxide decomposition would be masked in the presence of these compounds or their selenium analogs. Such error in the determination of peroxide content would give a conservative estimate of the decomposing activity of selenocystine. Therefore even if oxidized selenocystine compounds are present, comparisons of cystine-selenocystine reactivity reported here are valid.

Methionine-Selenomethionine.—Since selenomethionine appears to be unstable under the experimental conditions, it is impossible to compare its activity with that of methionine. Under other experimental conditions, Olcott *et al.* (1961) showed that selenomethionine was more efficient than methionine in decomposing menthane hydroperoxide and in inhibiting the autoxidation of lipids.

Methionine-Selenocystine.—The ease with which methionine is converted to its sulfoxide indicates that it has significant ability to decompose peroxides. In addition to its other metabolic reactions it probably augments the animal body's antioxidant pool (Tappel, 1962b). Compared with selenocystine, however, its antioxidant capacity is limited. Since the two compounds have structural differences aside from the sulfur and selenium atoms, precise comparisons cannot be made.

Cystine-Selenocystine.—GENERAL CONSIDERATIONS OF SULFUR AND SELENIUM CHEMISTRY.—On the other hand, the facile and extensive reduction of hydroperoxides by selenocystine, as compared to cystine, may be predicted on the basis of the known chemistry of the two types of compounds. The oxidation and antioxidant properties of sulfur compounds have been studied extensively, although relatively little similar information is available for selenium compounds. Data from several sources are consistent with the findings of this report.

First, Denison and Condit (1949) found various dialkyl selenides to be more effective inhibitors of oil oxidation than their sulfur analogs. Woodbridge (1959) made a comprehensive review of the antioxidant activity of a number of sulfur and selenium compounds and emphasized the influence of reaction parameters. In a study of the autoxidation of squalene, he found that neither dialkyl or diaryl monosulfides or monoselenides inhibited the reaction. By contrast, dialkyl diselenides were powerful inhibitors and much more active than dialkyl disulfides.

Second, while no comparable data is available on selenium compounds, a large volume of work has been reported on the reaction of sulfides with hydroperoxides and peracids. A number of mechanisms have been proposed, but the currently accepted view is that an S_{N^2} nucleophilic substitution occurs at oxygen (Davies, 1961; Bateman and Hargrove, 1954; Swern, 1949). This assumes that the peroxidic oxygen is electrophilic and is readily released in the presence of a nucleophilic group, e.g.:

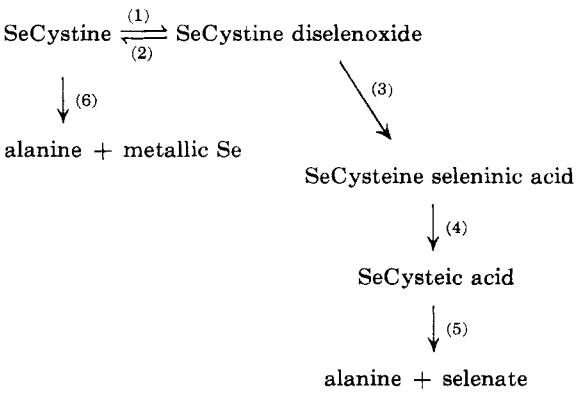
$$\ddot{Y} + \underset{\displaystyle R}{\overset{\displaystyle |}{O}}\!\!-\!OR' \longrightarrow Y\!-\!OR + {}^{-}OR'$$

The presence of electron-attracting groups in the peroxide increases the rate of reaction by increasing the electrophilic character of oxygen (Cerniani and Modena, 1959; Overberger and Cummins, 1953; Swern, 1947). Similarly, electron-releasing groups attached to or in the vicinity of sulfur increase its electron density, i.e., its nucleophilic properties, with a corresponding increase in rate. Selenium is known to have more nucleophilic character than sulfur; therefore, under the same experimental conditions, its compounds may be predicted to react faster with peroxides than their sulfur analogs.

Third, studies on the ultraviolet-absorption spectra of saturated disulfides and diselenides (Bergson *et al.*, 1962) demonstrate a characteristic absorption in both cases. More interestingly, although their spectra are similar, diselenides show peak absorbance at longer wavelengths than do disulfides. Several of the diselenides studied have structures very similar to selenocystine and show peak absorbance at approximately 300 mμ. This may be compared with our data for selenocystine (Fig. 4), which exhibits a maximum at 290 mμ. This smaller excitation energy for diselenides also favors the higher reactivity shown by selenocystine in these reactions.

ROLE OF OXIDIZED INTERMEDIATES OF CYSTINE AND SELENOCYSTINE.—Most available chemical evidence indicates only a quantitative rather than a qualitative difference in the reactions of sulfur and selenium compounds. On the basis of the chromatographic data (Table I) the following oxidation pathway is indicated

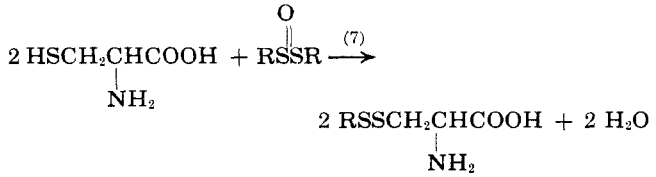
(only selenocystine shown):



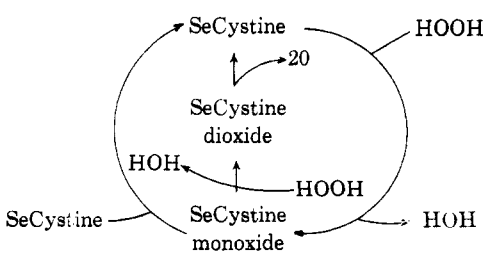
Since cystine oxidation proceeds by way of the monoxide (thiolsulfinate) which is unstable (Toennies, 1934; Toennies and Lavine, 1936), selenocystine should follow the same pathway. Failure to observe the diselenoxide is not surprising and is probably owing to its instability and high reactivity.

The decomposer activity of oxidized derivatives of cystine (Fig. 1C) points to reaction (1) as rate limiting. Therefore the higher activity of selenocystine may be owing in large measure to the ease of its oxidation to the dioxide. On the other hand, and using the same arguments presented above, oxidized derivatives of selenocystine can reasonably be expected to reduce peroxides more readily than their sulfur analogs. This is in accord with the finding that selenocystine is oxidized immediately and essentially completely through several oxidation levels.

Lavine (1936) has demonstrated the ease of the dismutative decomposition of cystine disulfoxide to cystine; Small *et al.* (1947) also report the rapid and quantitative reaction of cysteine with thiolsulfinates, i.e.,



Under conditions where cysteine is readily available, both reactions (2) and (7) are competitive with the further oxidation of the compound. That is to say, under many biochemical conditions the oxidation of selenocystine may well be catalytic. The slow rate of reaction of cystine makes its participation in such oxidations negligible. For selenocystine, the following rapid reactions may be visualized:



BIOCHEMICAL SIGNIFICANCE AND CONCLUSION.—Data presented in this paper are compatible with an antioxidant function for selenium compounds. It has been shown that these compounds, particularly selenocystine, react rapidly and probably catalytically to reduce hydroperoxides. The superior action of selenocystine is owing in part to the ease of its conversion to the dioxide. The chemical capacity of selenocystine to undergo these reactions suggests a possible biological function.

In studies on petroleum stabilization, Kennerly and Patterson (1956) report that peroxide inhibitors function best at low peroxide concentrations, whereas decomposers function best in the presence of high peroxide concentrations; both function as antioxidants but by different mechanisms. Synergism has been shown between such inhibitor-decomposer combinations. The best explanation is that the inhibitor depresses autoxidation by rapidly removing free-radical initiator chains. If some peroxides are formed despite inhibitor action, the peroxide decomposer reduces them; this decreases initiator chains and consequently the amount of inhibitor consumed.

Although sulfur compounds have been found to inhibit peroxidation, the inhibitor action of selenocystine has not been evaluated. But the close nutritional relationship of selenium to vitamin E has been the subject of many studies (Wolf *et al.*, 1963; Tappel, 1962a; Schultze, 1960). Vitamin E is known to be a peroxide inhibitor. Thus it is possible that vitamin E–selenocystine (or other selenium compounds) forms a synergistic combination. Such synergism would account for the biochemical effects of these compounds generally and the dramatic results observed under conditions of high peroxidative stress particularly. Although other modes of action have not been eliminated, the results of these experiments suggest that selenocystine may act as an important biological antioxidant.

REFERENCES

Barnard, D., and Percy, E. J. (1960), *Chem. Ind. (London)*, 1332.

Bateman, L., and Hargrove, K. R. (1954), *Proc. Roy. Soc. (London), Ser. A: 224*, 389.

Bergson, G., Cleason, G., and Schotte, L. (1962), *Acta Chem. Scand. 16*, 1159.

Cerniani, A., and Modena, G. (1959), *Gazz. Chim. Ital. 89*, 843.

Davies, A. G. (1961), Organic Peroxides, London, Butterworths.

Dennison, G. H., and Condit, P. C. (1949), *Ind. Eng. Chem. 41*, 944.

Emiliozzi, R., and Pichat, L. (1959), *Bull. Soc. Chim. France*, 1887.

Kennerly, G. W., and Patterson, W. L., Jr. (1956), *Ind. Eng. Chem. 48*, 1917.

Lavine, T. F. (1936), *J. Biol. Chem. 113*, 583.

Maclaren, J. A. (1961), *Experientia 17*, 346.

Moffat, E. D., and Lytle, R. I. (1959), *Anal. Chem. 31*, 926.

Njaa, L. R. (1962), *Acta Chem. Scand. 16*, 1359.

Olcott, H. S., Brown, W. D., and Van Der Vean, J. (1961), *Nature 191*, 1201.

Overberger, C. G., and Cummings, R. A. (1953), *J. Am. Chem. Soc. 75*, 4250.

Painter, E. P. (1947), *J. Am. Chem. Soc. 69*, 229.

Schultze, M. O. (1960), *Ann. Rev. Biochem. 29*, 391.

Small, L. D., Bailey, J. H., and Cavallito, C. J. (1947), *J. Am. Chem. Soc. 69*, 1710.

Swern, D. (1947), *J. Am. Chem. Soc. 69*, 1692.

Swern, D. (1949), *Chem. Rev. 45*, 1.

Tappel, A. L. (1962a), *Symp. Foods. Lipids Oxidation, 2nd Corvallis, Ore. 1961*, 367.

Tappel, A. L. (1962b), *Vitamins Hormones 20*, 493.

Toennies, G. (1934), *J. Am. Chem. Soc. 56*, 2198.

Toennies, G., and Lavine, F. (1936), *J. Biol. Chem. 113*, 571.

Wolf, E., Kollonitsch, V., and Kline, C. H. (1963), *J. Agr. Food Chem. 11*, 355.

Woodbridge, D. T. (1959), M.Sc. thesis, University of London.