Acceleration of Thiol-Induced Swelling of Rat Liver Mitochondria by Selenium†

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ABSTRACT: Liver mitochondria prepared from rats fed a diet deficient in selenium did not swell as rapidly in the presence of cysteine as mitochondria from rats fed the same diet supplemented with 0.5 ppm of Se as Na_2SeO_3, whether or not vitamin E was added to the diet. This effect of dietary Se in accelerating cysteine-induced mitochondrial swelling could be mimicked by adding 10^{-5} to 10^{-6} M Na_2SeO_3 *in vitro* to suspensions of liver mitochondria prepared from rats fed diets deficient in Se but adequate in vitamin E ("Se-deficient mitochondria"). The ability of selenite *in vitro* to stimulate thiol-induced swelling of Se-deficient mitochondria was also seen with mercaptoethylamine, but little or no stimulation by selenite was seen with thioglycolate, mercaptoethanol, dithiothreitol, or 2,3-dimercaptopropanol. The relative potency of various Se compounds in enhancing cysteine- or glutathione (GSH)-induced swelling of Se-deficient mitochondria was selenite > selenocystine > selenomethionine ~ selenate. Tellurite was the only oxyanion tested aside from Se compounds which could increase swelling of Se-deficient mitochondria in the presence of GSH, but its activity in this regard was much less than that of selenite. Arsenite, Hg^{2+}, and Cd^{2+} at 10^{-5} M all strongly inhibited the swelling of Se-deficient mitochondria caused by GSH plus selenite. GSH plus selenite-induced swelling was totally blocked by cyanide but only partially blocked by sodium amytal or antimycin A. These findings suggest that selenium may catalyze the transfer of electrons from GSH to cytochrome *c*.

The role of selenium in cellular metabolism is not definitely established at the present time. The beneficial nutritional effects of trace levels of selenium in the diet were first noted by Schwarz and Foltz (1957) who found that 0.1 ppm of dietary selenium could protect against liver necrosis in rats deficient in vitamin E. After this initial observation, several selenium-responsive nutritional diseases were discovered in other animals (Nesheim and Scott, 1961; Schubert *et al.*, 1961), and more recent reports have shown that selenium can exert beneficial effects even in animals adequately supplied with vitamin E, thereby demonstrating a specific nutritional need for Se independent of vitamin E (McCoy and Weswig, 1969; Thompson and Scott, 1969).

The close nutritional relationship between Se and vitamin E inspired the hypothesis that Se could function *in vivo* as an antioxidant (Tappel and Caldwell, 1967). Moreover, selenium has been shown to protect erythrocytes against oxidative damage, apparently by enhancing the activity of glutathione peroxidase (Rotruck *et al.*, 1972, 1973; Flohe *et al.*, 1973). However, the theory that vitamin E acts solely as a biological antioxidant has generated considerable controversy (Green, 1970), and reports have appeared which seem inconsistent with an antioxidant role for Se (Sprinker *et al.*, 1971; Hurt *et al.*, 1971a).

The so-called "respiratory decline" suffered by liver slices during prolonged incubations is one of the most striking manifestations of a deficiency of Se and vitamin E in rats (Chernick *et al.*, 1955). Additional evidence for a role for Se in respiration derives from the experiments of Bull and Oldfield (1967) who found an involvement of Se in the oxidation of pyruvate by rat liver preparations. Moreover, Lam and Olson (1964) observed that a Se metabolite with chromatographic properties similar to those of glutathione could stimulate the pyruvate oxidase activity of liver homogenates from Se-deficient rats.

The work described here demonstrates that Se can accelerate the respiration-dependent swelling of rat liver mitochondria caused by certain thiol compounds. Experiments with respiratory inhibitors indicate that the thiol-induced swelling catalyzed by Se in some cases may be mediated at the cytochrome *c* level.

Methods and Materials

Animals and Diets. Weanling male Fischer 344 albino rats[1] (Microbiological Associates, Walkersville, Md.) were housed individually in hanging wire cages and given the appropriate diet and distilled water *ad libitum* for at least 5 weeks. The composition of the Se- and vitamin E-deficient diet has been given (Levander *et al.*, 1973b). Where indicated, the deficient diet was supplemented with either 100 ppm of vitamin E as *dl*-α-tocopheryl acetate (powder, General Biochemicals, Chagrin Falls, Ohio), 0.5 ppm of Se as Na_2SeO_3, or both.

Reagents. All chemicals were laboratory reagent grade. Selenomethionine, selenocystine, and all thiol compounds except cysteine were purchased from Sigma Chemical Co., St. Louis, Mo. Cysteine hydrochloride monohydrate (Reagent Chemical) was from Fisher Scientific Co., Fair Lawn, N. J.

Mitochondrial Swelling. Swelling of rat liver mitochondria was carried out essentially by the method of Neubert and Lehninger (1962) except that the mitochondria were washed only once. Data are presented either graphically in the form of mitochondrial swelling curves or tabularly in terms of the decline in absorbance $\times 10^3$ produced by the swelling agent above the spontaneous rate at the time intervals specified.

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[1] Mention of a proprietary product does not imply endorsement by the U. S. Department of Agriculture.

TABLE I: Effect of Dietary Se or Vitamin E on Cysteine-Induced Swelling of Rat Liver Mitochondria.

Supplement to Diet	$-10^3\Delta A_{520}$ (45 min)[a] [Cysteine] (mM) 1.0	5.0	10.0
Vitamin E	50 ± 30	168 ± 40	114 ± 9
Vitamin E + Se	287 ± 36**	325 ± 44*	246 ± 58
Se	487 ± 12***	526 ± 9**	521 ± 12***

[a] Each value represents swelling of mitochondria after 45 min at room temperature in 0.125 M KCl–0.02 M Tris-HCl (pH 7.4), corrected for spontaneous swelling as described in text. Values are means of three observations ± standard error. Where indicated, statistical significance refers to comparisons between vitamin E *vs.* vitamin E + Se groups and between vitamin E *vs.* Se groups. Levels of significance of $P < 0.05$, $P < 0.01$, and $P < 0.001$ are indicated by one, two, and three asterisks, respectively.

The data in the curves or tables represent means obtained from three to five different mitochondrial preparations for each dietary treatment. Where statistical tests were performed, comparisons were made with the Student's *t* test for unpaired values (Steel and Torrie, 1960). Levels of significance of $P < 0.05$, $P < 0.01$, and $P < 0.001$ are indicated in the tables by one, two, and three asterisks, respectively. Lipid peroxides in mitochondria were estimated by the method of Corwin (1962), which depends upon the color produced at 532 nm by the reaction of malondialdehyde with thiobarbituric acid.

Results

Stimulation of Cysteine-Induced Swelling by Dietary Selenium. The ability of dietary selenium to increase the swelling of rat liver mitochondria caused by 1–10 mM cysteine is shown in Table I. Mitochondria prepared from rats fed the diet supplemented with both Se and vitamin E underwent two to five times as much swelling in the presence of cysteine as mitochondria prepared from animals fed the same diet supplemented with vitamin E alone ("Se-deficient mitochondria"). This increase in cysteine-induced mitochondrial swelling due to dietary Se was not accompanied by any increase in mitochondrial lipid peroxide formation (Table II). When vitamin E was omitted from the Se-fortified diet, the mitochondrial swelling caused by cysteine was more extensive than that observed in either of the vitamin E supplemented groups. Production of lipoperoxides during cysteine-induced swelling was also significantly higher in the vitamin E deficient group when compared to that seen in either of the two groups receiving vitamin E in the diet.

Stimulation of Cysteine-Induced Swelling by Selenium Added in Vitro. Figure 1 shows that the addition of 10^{-6} M selenium as Na_2SeO_3 *in vitro* markedly stimulated the cysteine-induced swelling of Se-deficient liver mitochondria. Addition of selenite in the absence of cysteine had only a negligible effect on swelling. A direct comparison revealed that cysteine-induced swelling could be similarly stimulated either by the *in vitro* addition or by the dietary supplementation of Se (Figure 2).

Effect of Selenite and Various Thiols on Swelling. The ability of selenite added *in vitro* at a level of 10^{-5} M to increase the mitochondrial swelling caused by various thiol compounds is shown in Table III. Once again, the addition of selenite *in vitro* caused a significant increase in the cysteine-induced swelling of Se-deficient liver mitochondria. A smaller but still significant stimulatory effect of *in vitro* selenite was seen when mercaptoethylamine was used as the thiol instead of cysteine. No significant effects of *in vitro* selenite were noted with thioglycolate, mercaptoethanol, dithiothreitol, or 2,3-dimercaptopropanol.

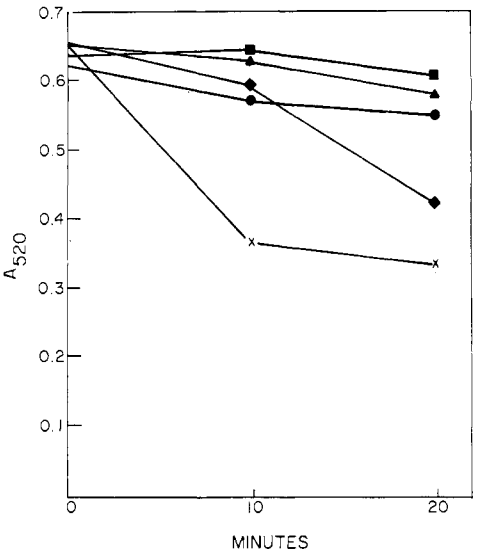

FIGURE 1: Effect of *in vitro* selenite on swelling of Se-deficient rat liver mitochondria induced by cysteine. Mitochondria prepared from animals fed a diet supplemented with vitamin E only. Incubation conditions as in Table I. Additions to KCl–Tris buffer were: cysteine, 10^{-2} M (■); Na_2SeO_3, 10^{-6} M (▲); Na_2SeO_3, 5×10^{-6} M (●); cysteine, 10^{-2} M, plus Na_2SeO_3, 10^{-6} M (◆); cysteine, 10^{-2} M, plus Na_2SeO_3, 5×10^{-6} M (×).

Stimulation of Cysteine- or Glutathione-Induced Swelling by Different Selenium Compounds. A comparison of the ability of various chemical forms of Se added *in vitro* to increase the cysteine-induced swelling of Se-deficient liver mitochondria is shown in Figure 3. Selenite was by far the most active form of Se in this regard followed by selenocystine and then selenate or selenomethionine. The relative ability of the different Se compounds to stimulate thiol-induced swelling was the same

TABLE II: Effect of Dietary Se or Vitamin E on Mitochondrial Lipoperoxidation in Cysteine-Induced Mitochondrial Swelling.

Supplement to Diet	$10^3\Delta A_{532}$ (45 min)[a] [Cysteine] (mM) 1.0	5.0	10.0
Vitamin E	8 ± 8	41 ± 11	−10 ± 12
Vitamin E + Se	12 ± 4	−3 ± 3*	3 ± 3
Se	98 ± 10***	198 ± 9***	169 ± 17***

[a] Each value represents the level of mitochondrial lipoperoxides as estimated by the thiobarbituric acid reaction according to Corwin (1962). Incubation conditions and statistical comparisons are as in Table I. Values are corrected for zero time lipoperoxide levels.

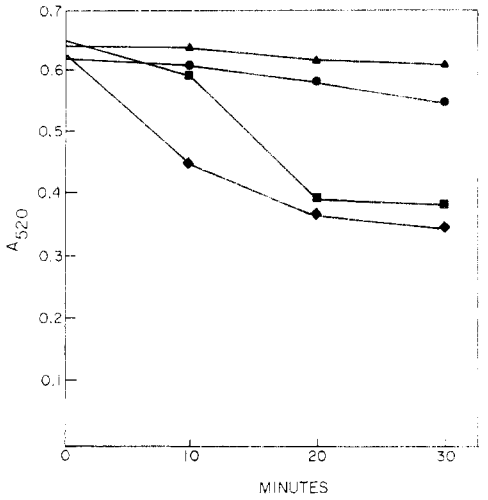

FIGURE 2: Effect of dietary *vs. in vitro* selenite on swelling of rat liver mitochondria induced by cysteine. Incubation conditions as in Table I. Mitochondria prepared from animals fed a diet supplemented either with vitamin E only (+E mitochondria) or with vitamin E plus Se (+E + Se mitochondria): swelling of +E mitochondria in KCl–Tris buffer (▲) or in KCl–Tris buffer plus 10^{-2} M cysteine and 10^{-5} M selenite (■); swelling of +E + Se mitochondria in KCl–Tris buffer (●) or in KCl–Tris buffer plus 10^{-2} M cysteine (◆).

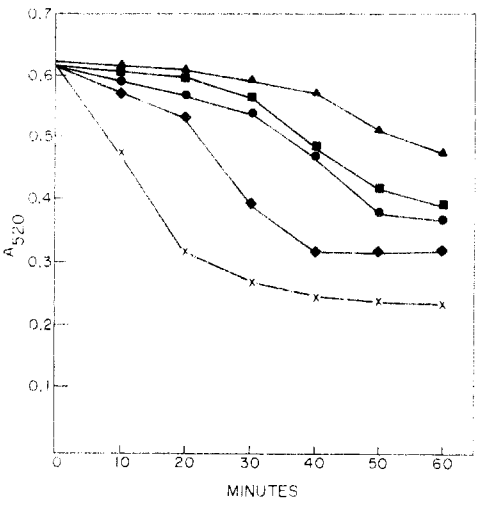

FIGURE 3: Effect of various Se compounds *in vitro* on cysteine-induced swelling of Se-deficient rat liver mitochondria. Incubation conditions as in Table I. Mitochondria prepared from animals fed a diet supplemented with vitamin E only. In addition to 10^{-2} M cysteine, all tubes contained the following Se compounds at 10^{-5} M: (▲) none; (■) Na_2SeO_4; (●) selenomethionine; (◆) selenocystine; or (×) Na_2SeO_3.

TABLE III: Effect of *in Vitro* Selenite on Thiol- or Dithiol-Induced Swelling of Se-Deficient Rat Liver Mitochondria.

Compound, 10 mM	$-10^3\Delta A_{520}$ (60 min)[a]	
	−Selenite	+Selenite[b]
Cysteine	135 ± 14	280 ± 20***
Mercaptoethylamine	−27 ± 13	48 ± 10**
Thioglycolate	−6 ± 16	24 ± 11
Mercaptoethanol	−13 ± 15	39 ± 21
Dithiothreitol	−58 ± 8	−2 ± 30
2,3-Dimercaptopropanol	−47 ± 15	−56 ± 16

[a] Rat liver mitochondria were incubated for 60 min under conditions as in Table I. Animals were fed a diet supplemented with vitamin E only. Values are means of five observations ± standard error. Where indicated, statistical significance refers to comparisons between −selenite *vs.* +selenite treatments. [b] 10^{-5} M.

when glutathione (GSH)[2] was used as the thiol instead of cysteine (data not shown).

Effect of Various Oxyanions plus Glutathione on Swelling. Neither arsenite nor arsenate was able to replace selenite in stimulating GSH-induced swelling of Se-deficient mitochondria (Table IV). Tellurite had some activity in enhancing swelling in medium containing GSH but tellurate was essentially without effect. Sulfate, sulfite, thiosulfate, molybdate, metavanadate, Cu^{2+}, and Zn^{2+} at concentrations of 10^{-4}–10^{-5} M also had little or no effect on swelling of Se-deficient mitochondria in the presence of GSH (data not shown).

Effect of Sulfhydryl Inhibitors on Glutathione-Induced Swelling Catalyzed by Selenium. The swelling of Se-deficient mitochondria caused by the addition of 10^{-2} M GSH plus 10^{-5} M selenite to the incubation medium was strongly inhibited by 10^{-5} M Cd^{2+} or Hg^{2+} (Table V, trial A). The organic mercurials, *p*-hydroxymercuribenzoate (HgOHBzO) and *p*-chloromercuriphenylsulfonate, were less effective than mercuric ion in inhibiting such mitochondrial swelling (Table V, trial B). Arsenite at 10^{-5} M strongly inhibited GSH swelling of Se-deficient mitochondria catalyzed by 10^{-5} M selenite, although arsenate had no effect at 10^{-4} M and only a partial inhibitory effect at 10^{-3} M (Table V, trial C). Neither tellurite nor tellurate at 10^{-5} M had any inhibitory effect on GSH plus selenite swelling, but tellurate had some inhibitory action at 10^{-4} M (data not shown). Iodoacetate at 10^{-3} M markedly inhibited swelling of Se-deficient mitochondria induced by GSH plus selenite, but 10^{-2} M *N*-ethylmaleimide was necessary to obtain similar inhibition (data not shown).

Effect of Respiratory Inhibitors on Glutathione- or Cysteine-Induced Swelling Catalyzed by Selenium. The GSH plus selenite-induced swelling of Se-deficient mitochondria was completely blocked by cyanide but only partially blocked by amytal or antimycin A (Figure 4). On the other hand, the cysteine plus selenite-induced swelling of Se-deficient mitochondria was totally blocked by all three inhibitors (data not shown).

Discussion

The results reported here demonstrate that selenium can increase the thiol-induced swelling of rat liver mitochondria under certain experimental conditions. Selenium was especially effective in promoting mitochondrial swelling due to cysteine or glutathione. The fact that selenite added directly *in vitro* to mitochondria prepared from animals fed Se-deficient but vitamin E adequate diets was about as effective as dietary selenite in stimulating cysteine-induced swelling indicated that any necessary conversion of selenite to a form active in accelerating swelling could occur *in vitro*. The amounts of Se needed to enhance thiol-induced swelling of mitochondria from Se-deficient rats by direct addition *in vitro* (10^{-5}–10^{-6} M) are similar to the concentrations found in the livers of animals fed diets nutritionally adequate in selenium (Hurt *et al.*, 1971b). Also, in the studies with glutathione, the ratio of the

[2] Abbreviations used are: GSH, glutathione; HgOHBzO, *p*-hydroxymercuribenzoate.

TABLE IV: Effect of Arsenic and Tellurium Oxyanions on GSH-Induced Swelling of Se-Deficient Rat Liver Mitochondria.

Compound, 10^{-5} M	$-10^3\Delta A_{520}$ (60 min)[a]
None	−5 ± 22
Na_2SeO_3	466 ± 24
$NaAsO_2$	−14 ± 19
Na_2HAsO_4	70 ± 27
K_2TeO_3	187 ± 56
K_2TeO_4	15 ± 20

[a] Incubation conditions and diet as in Table III except that the thiol used was 10^{-2} M GSH. Values are means of three observations ± standard error.

concentrations of glutathione to selenite used in the incubation medium (10^3) is similar to that found in tissues under normal physiological conditions.

The relative inability of Se added *in vitro* to increase the swelling of Se-deficient mitochondria due to thiols other than cysteine or GSH suggests that some molecular specificity is involved in this phenomenon. Although the basis for this specificity is not known at present, studies with a chemically defined model system based upon the results of the work reported here suggest that the lack of effect of Se seen with thioglycolate or mercaptoethanol may be related to a lack of reactivity of these substances, whereas the inactivity of Se observed with dithiothreitol or 2,3-dimercaptopropanol may be due to an inhibitory effect of these dithiols on respiration (Levander *et al.*, 1973a).

Since several forms of Se added *in vitro* had at least some activity in enhancing the swelling of Se-deficient mitochondria in the presence of GSH or cysteine, one could conclude that there was a rather nonspecific effect of Se in increasing certain types of thiol-mediated mitochondrial swelling. An alternate possibility could be that all the compounds of Se tested were metabolized to the same form and that this metabolite was then the catalytically active species. Experiments with GSH carried out in a chemically defined system in which no metabolism would occur revealed pronounced differences in the reactivity of various Se derivatives toward thiols (Levander *et al.*, 1973a). Of course, variations in mitochondrial permeability to the different Se compounds used could also account for the differences in catalytic activity observed here.

The inability of any oxyanions other than selenite or selenate to increase appreciably the swelling of Se-deficient mitochondria in the presence of GSH is of interest since Lawrence (1969) found that tellurate and metavanadate in addition to selenite and selenate were able to catalyze disulfide interchange. The only oxyanion not containing Se which stimulated mitochondrial swelling in the presence of GSH was tellurite and this species had less than half the catalytic activity of selenite. These results indicate that Se probably does not enhance the mitochondrial swelling due to GSH by catalyzing sulfhydryl–disulfide exchange, a mechanism advanced to explain the Se-catalyzed activation of thiol enzymes by sulfhydryl compounds (Dickson and Tappel, 1969).

The studies which showed that several sulfhydryl inhibitors could block the swelling of Se-deficient mitochondria caused by the combination of GSH plus Se are of possible biological significance since the phenomenon of respiratory decline characteristic of mitochondria from rats deficient in vitamin E

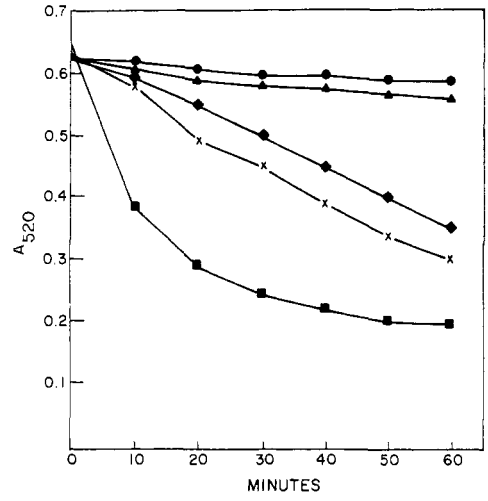

FIGURE 4: Effect of respiratory inhibitors on GSH plus selenite-induced swelling of Se-deficient rat liver mitochondria. Incubation conditions as in Table I except that all tubes contained 10^{-3} M DL-β-hydroxybutyrate. Mitochondria prepared from animals fed a diet supplemented with vitamin E only. In addition to 10^{-2} M GSH, all tubes contained the following additions: (▲) none; (■) Na_2SeO_3, 10^{-5} M; (●) Na_2SeO_3, 10^{-5} M, plus KCN, 10^{-3} M; (◆) Na_2SeO_3, 10^{-5} M, plus antimycin A, 0.12 μg/ml; or (×) Na_2SeO_3, 10^{-5} M, plus sodium amytal, 1.8×10^{-3} M.

and Se can be induced by the addition of arsenite or Cd^{2+} *in vitro* (Corwin and Schwarz, 1963). Since the arsenite and heavy metals were effective in blocking GSH plus selenite-induced mitochondrial swelling at levels as low as 10^{-5} M, this would suggest that the inhibitors were not acting by complexing the glutathione which was present in the medium in a

TABLE V: Effect of Various Sulfhydryl Inhibitors on GSH Plus Selenite-Induced Swelling of Se-Deficient Rat Liver Mitochondria.

10^{-2} M GSH	10^{-5} M Selenite	Compd (Concn, M)	$-10^3\Delta A_{520}$(60 min)[a] Trial A	Trial B	Trial C
+	−	None	11	15	32
−	+	None	184	114	89
+	+	None	484	518	484
+	+	$CdCl_2$ (10^{-6})	487		
+	+	$CdCl_2$ (10^{-5})	81***		
+	+	$HgCl_2$ (10^{-6})	492	515	
+	+	$HgCl_2$ (10^{-5})	−7***	−12***	
+	+	HgOHBzO (10^{-4})		466	
+	+	HgOHBzO (10^{-3})		349**	
+	+	Sulfonate[b] (10^{-5})		493	
+	+	Sulfonate[b] (10^{-4})		164***	
+	+	$NaAsO_2$ (10^{-6})			486
+	+	$NaAsO_2$ (10^{-5})			106***
+	+	Na_2HAsO_4 (10^{-4})			484
+	+	Na_2HAsO_4 (10^{-3})			345***

[a] Incubation conditions and diet as in Table III. Values are means of three observations. Where indicated, statistical significance refers to comparisons between GSH plus selenite group (positive control) *vs.* GSH plus selenite plus inhibitor group. [b] *p*-Chloromercuriphenylsulfonate.

1000-fold excess. Two other points of attack of the inhibitors seem possible. The inhibitors could either react with the Se catalyst itself or with the mitochondrial site at which the Se catalyst presumably functions. Although the results presented here do not allow a distinction to be made between these two possibilities, experiments with a chemically defined system suggest that the latter alternative might be the preferred one (Levander *et al.*, 1973a). The high inhibitory potency of arsenite, Cd^{2+}, and Hg^{2+} on GSH plus selenite swelling implicates a dithiol group in this type of swelling. The specific role of a dithiol in GSH plus selenite swelling is also suggested by the relatively low inhibitory activity of monothiol inhibitors such as organic mercurials, iodoacetate, or *N*-ethylmaleimide. In fact, such high levels of the latter two inhibitors were needed to see their effects that these compounds may have acted directly on the glutathione in the incubation medium. The failure to inhibit GSH plus selenite-induced swelling by tellurite would apparently rule out any direct role for the Se-catalyzed oxidation of GSH *per se* in the swelling process described here since Tsen and Tappel (1958) found that tellurite was a potent inhibitor of this type of catalysis.

The respiratory inhibitor experiments show that thiol-selenite swelling is dependent to some degree upon a functional respiratory chain. The experiments with GSH plus selenite in which total blockade of swelling was observed with cyanide but only partial blockade was seen with amytal or antimycin A suggested that the swelling caused by the GSH plus selenite combination might be mediated partly at the cytochrome *c* level. This concept led to the development of a chemically defined system based upon the Se-catalyzed reduction of cytochrome *c* by sulfhydryl compounds which has been used as a model to study the biological function of Se (Levander *et al.*, 1973a). The fact that amytal or antimycin A allowed some GSH plus selenite-induced swelling of Se-deficient mitochondria but completely blocked the cysteine plus selenite-induced swelling indicates some chemical specificity in the swelling phenomenon but the molecular basis for this difference is not clear at this time.

The results reported here may help to explain the complex and puzzling nutritional relationship between Se and vitamin E. It should be emphasized that all of the effects of *in vitro* Se discussed above were obtained with animals that had been fed diets which lacked Se but which contained adequate levels of vitamin E. Therefore, these effects due to Se must be regarded as specific effects of Se acting in its own right and not as a quasi substitute for vitamin E. In the one experiment in which Se was tried in a diet lacking vitamin E (Tables I and II), Se was not able to replace vitamin E as an antioxidant since lipoperoxidation associated with cysteine-induced swelling occurred to a significant extent in the group not receiving vitamin E. This result is in agreement with other work from our laboratory which showed that unlike vitamin E, Se was not able to protect against the mitochondrial lipoperoxidation caused by swelling agents such as ascorbate, iron, or a combination of reduced plus oxidized glutathione (Higgs and Morris, 1973). Since Se apparently has little antioxidant activity under the conditions of our mitochondrial swelling studies, the question of the role of Se in mitochondrial function remains open. The ability of *in vitro* Se to catalyze mitochondrial swelling in the presence of GSH or cysteine which is sensitive to respiratory inhibitors suggests that Se may play a role in respiratory processes. This hypothesis would be consistent with earlier work which showed an involvement of Se in the respiratory decline of rat liver preparations (Schwarz, 1962; Bull and Oldfield, 1967).

Acknowledgments

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References

Bull, R. C., and Oldfield, J. E. (1967), *J. Nutr. 91*, 237.
Chernick, S. S., Moe, J. G., Rodnan, G. P., and Schwarz, K. (1955), *J. Biol. Chem. 217*, 829.
Corwin, L. M. (1962), *Arch. Biochem. Biophys. 97*, 51.
Corwin, L. M., and Schwarz, K. (1963), *Arch. Biochem. Biophys. 100*, 385.
Dickson, R. C., and Tappel, A. L. (1969), *Arch. Biochem. Biophys. 131*, 100.
Flohe, L., Gunzler, W. A., and Schock, H. H. (1973), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 32*, 132.
Green, J. (1970), *in* The Fat-Soluble Vitamins, DeLuca, H. F., and Suttie, J. W., Ed., Madison, Wis., University of Wisconsin Press, p 293.
Higgs, D. J., and Morris, V. C. (1973), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 32*, 885 Abstr.
Hurt, H. D., Cary, E. E., Allaway, W. H., and Visek, W. J. (1971a), *J. Nutr. 101*, 363.
Hurt, H. D., Cary, E. E., and Visek, W. J. (1971b), *J. Nutr. 101*, 761.
Lam, K. W., and Olson, R. E. (1964), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 23*, 183 Abstr.
Lawrence, P. J. (1969), *Biochemistry 8*, 1271.
Levander, O. A., Morris, V. C., and Higgs, D. J. (1973a), *Biochemistry 12*,
Levander, O. A., Morris, V. C., Higgs, D. J., and Varma, R. N. (1973b), *J. Nutr. 103*, 536.
McCoy, K. E. M., and Weswig, P. H. (1969), *J. Nutr. 98*, 383.
Nesheim, M. C., and Scott, M. L. (1961), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 20*, 674.
Neubert, D., and Lehninger, A. L. (1962), *J. Biol. Chem. 237*, 952.
Rotruck, J. T., Pope, A. L., Ganther, H. E., and Hoekstra, W. G. (1972), *J. Nutr. 102*, 689.
Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., and Hoekstra, W. G. (1973), *Science 179*, 588.
Schubert, J. R., Muth, O. H., Oldfield, J. E., and Remmert, L. F. (1961), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 20*, 689.
Schwarz, K. (1962), *Vitam. Horm. (New York) 20*, 463.
Schwarz, K., and Foltz, C. M. (1957), *J. Amer. Chem. Soc. 79*, 3292.
Sprinker, L. H., Harr, J. R., Newberne, P. M., Whanger, P. D., and Weswig, P. H. (1971), *Nutr. Rep. Int. 6*, 335.
Steel, R. G. D., and Torrie, J. H. (1960), Principles and Procedures of Statistics, New York, N. Y., McGraw-Hill, p 73.
Tappel, A. L., and Caldwell, K. A. (1967), *in* Selenium in Biomedicine, Muth, O. H., Oldfield, J. E., and Weswig, P. H., Ed., Westport, Conn., AVI Publishing Co., p 345.
Thompson, J. N., and Scott, M. L. (1969), *J. Nutr. 97*, 335.
Tsen, C. C., and Tappel, A. L. (1958), *J. Biol. Chem. 233*, 1230.