0026-895X/87/010112-05$02.00/0

MOLECULAR PHARMACOLOGY, 31:112-116

Inhibition of RNA and DNA Polymerases by the Product of the Reaction of Selenite with Sulfhydryl Compounds

GERALD D. FRENKEL, ADRIAN WALCOTT, and CHARLIEN MIDDLETON
Department of Biological Sciences, Rutgers University, Newark, New Jersey 07102
Received June 25, 1986; Accepted October 10, 1986

SUMMARY

Sodium selenite has previously been shown to inhibit DNA and RNA synthesis in both intact cells and isolated nuclei. Nevertheless, DNA and RNA polymerases, the enzymes responsible for this synthesis, are insensitive to inhibition by selenite. Several DNA and RNA polymerases have now been shown to be inhibited by selenite in the presence of sulfhydryl compounds. This inhibition is due to the reaction of selenite with the sulfhydryl compounds to form selenotrisulfide derivatives which inhibit the enzymes. The selenotrisulfides decrease the V_{max} of the polymerase reaction and increase the apparent K_m for the triphosphates, but do not alter the apparent K_m of the enzyme for the DNA template. There are differences in potency between selenotrisulfides formed from similar sulfhydryl compounds such as mercaptoethanol and mercaptoethylamine. There are also differences in the sensitivity of different polymerases to inhibition by the selenotrisulfides.

Selenium-containing compounds have been known for some time to have both beneficial and toxic effects at both the organismic and cellular levels (1, 2). Recent interest has been focused on the anticarcinogenic activity of selenium compounds, which has been demonstrated experimentally in animal systems (1–3) and epidemiologically in human populations (4–6). The mechanism of this anticarcinogenicity is not known, but it could be related to a "toxic" effect of selenium compounds, i.e., the inhibition of cellular proliferation (7–11).

Although the mechanism of this inhibition has not been fully elucidated, it is known that some selenium-containing compounds can inhibit cellular macromolecular synthesis. Thus, Gruenwedel and Cruikshank (7) showed that sodium selenite inhibits DNA, RNA, and protein synthesis in HeLa cells at concentrations several orders of magnitude below those which decrease cell viability (as measured by trypan blue exclusion). Inhibition of protein synthesis by selenite has also been demonstrated in rat hepatocytes (12). Abdullaev *et al.* (13) detected inhibition by selenite of RNA synthesis in intact loach embryos and also obtained evidence for inhibition of RNA polymerase activity in isolated nuclei. Medina and Oborn (14) also observed inhibition of DNA synthesis by selenite in mouse mammary cells in culture. We have previously reported (15) that, in addition to inhibiting synthesis in intact cells, selenite also inhibits DNA and RNA synthesis in isolated HeLa nuclei. This demonstrated that selenite does in fact inhibit the actual DNA and RNA synthetic reactions (as opposed to precursor nucleoside transport and/or phosphorylation). Nevertheless, selenite has been shown to have little or no effect on purified DNA and RNA polymerases (15, 16), the enzymes which catalyze cellular nucleic acid synthesis (17). Thus, the mechanism by which selenite inhibits nucleic acid synthesis remained unclear.

A number of biological effects of selenium compounds have previously been shown to be influenced by sulfhydryl compounds (18–26). In this report we describe our finding that, although selenite by itself has little effect on DNA and RNA polymerases, it does have significant inhibitory activity in the presence of sulfhydryl compounds. The inhibition results from the reaction of selenite with the sulfhydryl compounds to form selenotrisulfide derivatives (27).

Experimental Procedures

Materials. Sodium selenite was purchased from Gallard-Schlesinger Chemical Co. (Carle Place, NY). RNA polymerase II was extracted from wheat germ by the method of Jendrisak and Burgess (28) and purified by chromatography on DEAE-cellulose. Calf thymus DNA and DNA polymerase α were obtained from Cooper Biomedical Co., (Malverne, PA); *Escherichia coli* DNA polymerase I was obtained from New England Nuclear (Boston, MA). Radioactive nucleoside triphosphates were purchased from New England Nuclear, and unlabeled triphosphates were from PL Biochemicals (Milwaukee, WI). Mercaptoethanol was obtained from Eastman Kodak (Rochester, NY) and mercaptoethylamine from Sigma (St. Louis, MO). 2-Hydroxyethyldisulfide and cystamine were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Enzyme reactions. For RNA polymerase, the reaction mixture (0.3 ml) contained 50 mM Tris-HCl buffer (pH 7.9), 22 mM $MnCl_2$, 100 mM $(NH_4)_2SO_4$, 0.33 mM each of ATP, CTP, and GTP, 1.5 μM [5,6-^{3}H] UTP (final specific activity 22 Ci/mmol), and 18 μg/ml calf thymus DNA. For DNA polymerase the reaction mixture (0.3 ml) contained 50 mM Tris-HCl buffer (pH 7.9), 10 mM $MgCl_2$, 50 μM each of dATP, dCTP, and dGTP, 0.5 μM [*methyl*-^{3}H]dTTP (final specific activity 75

This research was supported in part by Research Grants ES-03413, ES-04087, and Minority Biomedical Research Grant RR-08223 from the National Institutes of Health. This is publication number 22 from the Department of Biological Sciences of Rutgers-Newark.

Ci/mmol), and 33 μg/ml calf thymus DNA. After incubation for 30 min at 37°, the reaction mixture was chilled, and 0.2 ml of salmon sperm DNA (0.25 mg/ml) and 0.5 ml of either 7% perchloric acid (for RNA polymerase) or 10% trichloroacetic acid (for DNA polymerase) were added. The precipitate was collected on a Whatman GF/C filter and washed with 6% trichloroacetic acid containing 0.1 M sodium pyrophosphate. The filter was dried and counted in a liquid scintillation counter.

The enzyme preparations which we have utilized normally contain sulfhydryl compounds at concentrations which would result in significant concentrations in the reaction. Since, in these experiments, control of the sulfhydryl concentration in the reaction was an obvious necessity, it was essential that the enzymes be dialyzed and/or diluted prior to use.

Reaction of selenite with sulfhydryl compounds. The reaction was carried out as described by Ganther (27). In the case of the mercaptoethanol derivative the initial concentrations were 15 mM selenite and 60 mM mercaptoethanol. In the case of mercaptoethylamine, in order to avoid decomposition of the selenotrisulfide it was necessary to carry out the reaction with more dilute solutions: 0.33 mM selenite and 1.33 mM mercaptoethylamine. The reactions were carried out for 30 min at 0°, during which time the reactions went to completion.

Results

In an earlier paper (15) we reported that DNA and RNA polymerases are resistant to inhibition by sodium selenite. We have now found that in the presence of a sulfhydryl compound such as mercaptoethanol, selenite is a highly potent inhibitor of the polymerases. Fig. 1 illustrates this for RNA polymerase II from wheat germ. In the absence of mercaptoethanol there was no significant inhibition of the enzyme by concentrations of selenite as high as 250 μM. However, in the presence of mercaptoethanol (at a 4:1 molar ratio), the enzyme was inhibited by the selenite (with 50% inhibition at approximately 20 μM). It should be noted that the concentrations of mercaptoethanol utilized in this experiment (as high as 1 mM) had no effect on the activity of the enzyme in the absence of selenite.

Ganther (27) has shown that selenite can react with sulfhydryl compounds to form a selenotrisulfide adduct, via the reaction first proposed by Painter (29):

$$4\ RSH + H_2SeO_3 \rightarrow RSSeSR + RSSR + 3H_2O \qquad (1)$$

In order to investigate whether this selenotrisulfide product is the inhibitory species of the polymerases, we have carried out the reaction of selenite with mercaptoethanol as described by Ganther (27). The spectrum of the product, shown in Fig. 2, is characteristic of the selenotrisulfide (27); based upon the molar absorptivity determined by Ganther (27), the results indicate essentially complete conversion of the selenite to the selenotrisulfide

$$OH—CH_2—CH_2—S—Se—S—CH_2—CH_2—OH$$

The effect of the reaction product on the activity of RNA polymerase II was examined. The results (Fig. 3) show that the enzyme was inhibited, with 50% inhibition at approximately 10 μM selenotrisulfide. We have also carried out reaction 1 with mercaptoethylamine as sulfhydryl compound, thereby forming the selenotrisulfide

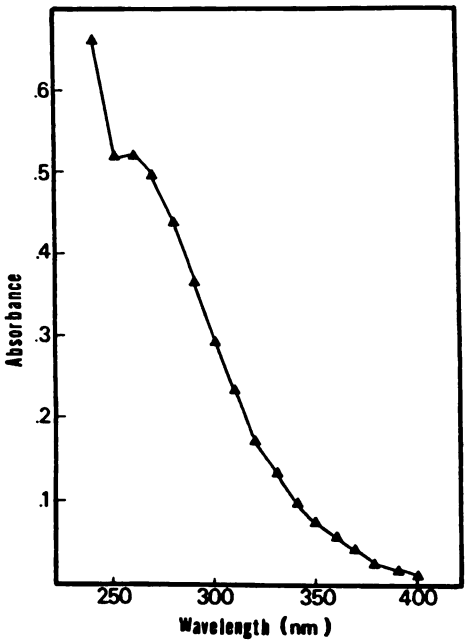


Fig. 2. Absorbance spectrum of the product of the reaction of sodium selenite with 2-mercaptoethanol. The reaction was carried out as described in Experimental Procedures, and the absorbance spectrum was determined in a Gilford spectrophotometer.

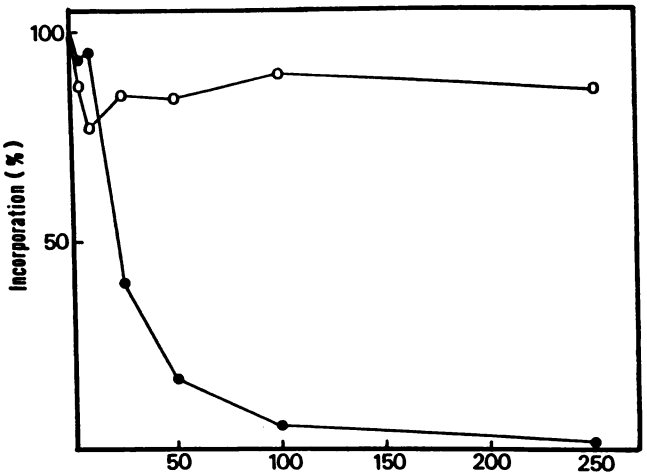


Fig. 1. Effect of sodium selenite on the activity of RNA polymerase II. Reactions were carried out as described in Experimental Procedures in the presence of the indicated concentration of selenite, either alone (○) or with a 4:1 molar ratio of 2-mercaptoethanol (●). The results are expressed as a percentage of the synthesis in the absence of selenite.

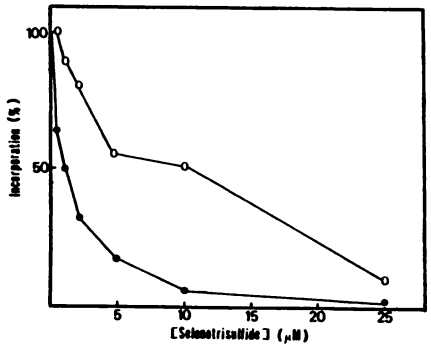


Fig. 3. Inhibition of RNA polymerase II by selenotrisulfides. Reactions were carried out in the presence of the indicated concentration of the selenotrisulfide product of the reaction of selenite with mercaptoethanol (○) or mercaptoethylamine (●). The results are expressed as a percentage of the synthesis in the absence of any added compound.

$NH_2—CH_2—CH_2—S—Se—S—CH_2—CH_2—NH_2$

(A spectrum similar to that shown in Fig. 2 was obtained.) This reaction product was even more potent in inhibiting the polymerase, with 50% inhibition at approximately 1 μM (Fig. 3).

Inhibition of the polymerase by the products of reaction 1 is most likely due to the selenotrisulfides. However, in order to be certain that the disulfides, $R—S—S—R$ [which in these cases are the compounds 2-hydroxyethyldisulfide ($OH—CH_2—CH_2—S—S—CH_2—CH_2—OH$) and cystamine ($NH_2—CH_2—CH_2—S—S—CH_2—CH_2—NH_2$), respectively], are not causing the inhibition, the effect of these compounds on the polymerase was examined. As shown in Fig. 4, neither of these compounds had any effect on the activity of the enzyme. Thus, the most likely inhibitory species are the selenotrisulfides.

Preliminary information as to the mechanism of the inhibition of the polymerase by the selenotrisulfides has been obtained from a Michaelis-Menten kinetic study. When the reaction rate was measured as a function of ribonucleoside triphosphate concentration, the selenotrisulfide was found to increase the apparent K_m of the enzyme for this substrate and also to decrease the V_{max} of the reaction (Fig. 5). In contrast, the selenotrisulfides had no significant effect on the apparent K_m of the enzyme for the DNA template (Fig. 6).

We have examined several other polymerases and have found that inhibition by selenotrisulfides is not limited to RNA polymerase II. As an example, the effect of the selenotrisulfides on the activity of *E. coli* DNA polymerase I and calf thymus DNA polymerase α is shown in Fig. 7. The inhibitory effect of the two selenotrisulfides on the three enzymes is summarized in Table 1. These results show that different polymerases exhibit different degrees of sensitivity to these compounds. Thus, with mercaptoethanol-selenotrisulfide, 50% inhibition with DNA polymerase α, RNA polymerase II, and polymerase I occurs at 1, 10, and 78 μM, respectively. Similarly, with mercaptoethylamine-selenotrisulfide, 50% inhibition of these enzymes occurs at 1, 1, and 38 μM, respectively. It is also apparent that there is a significant difference in potency between the two compounds, but only with some enzymes and not with others.

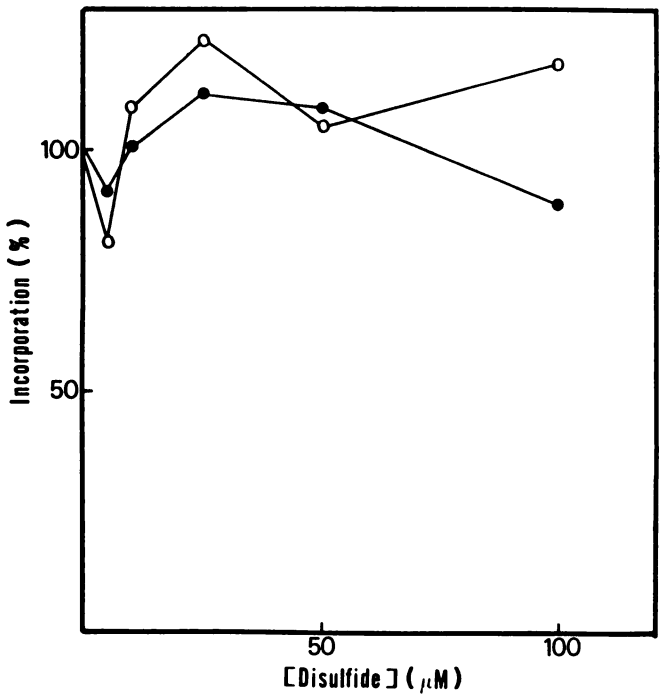


Fig. 4. Effect of disulfides on RNA polymerase II. Reactions were carried out in the presence of the indicated concentration of 2-hydroxyethyldisulfide (○) or cystamine (●) (the disulfides of mercaptoethanol and mercaptoethylamine, respectively). The results are expressed as a percentage of the synthesis in the absence of any added compound.

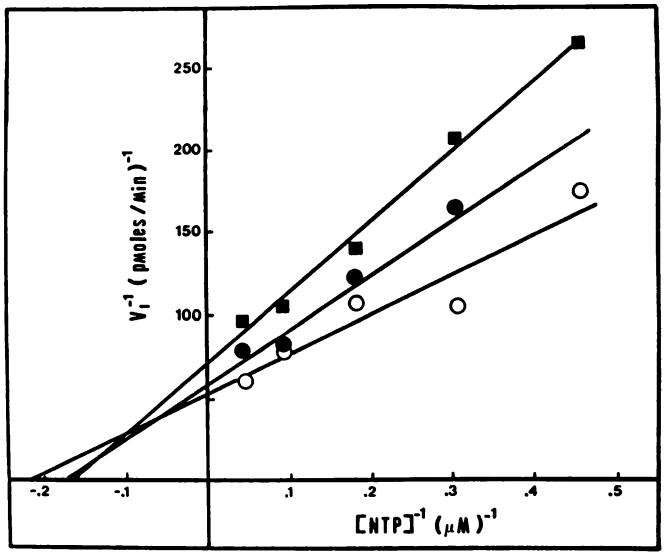


Fig. 5. Effect of selenotrisulfide on the reaction kinetics of RNA polymerase II. Reactions were carried out in the presence of the indicated concentration of ATP, GTP, and CTP (with the concentration of UTP kept constant at 1.5 μM), either with no addition (○), or in the presence of the product of the reaction of selenite with mercaptoethanol at a concentration of 2 μM (●) or 5 μM (■). The results are presented in the Lineweaver-Burke form.

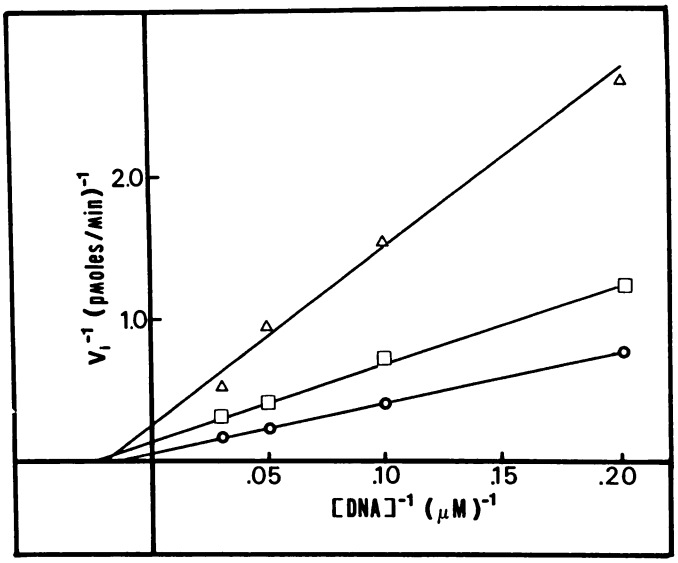


Fig. 6. Effect of selenotrisulfides on the reaction kinetics of RNA polymerase II. Reactions were carried out in the presence of the indicated concentration of DNA, either with no addition (○), or in the presence of the selenotrisulfide product of the reaction of selenite with mercaptoethanol (□) or mercaptoethylamine (△) (each at a concentration of 2 μM). The results are presented in the Lineweaver-Burke form.

Discussion

Sulfhydryl compounds are known to be involved in the metabolism of selenite (18, 19), as well as in its antagonistic effect

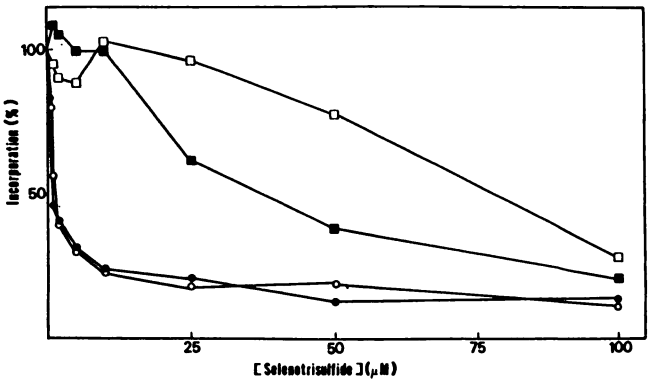


Fig. 7. Effect of selenotrisulfides on the activity of DNA polymerases. Reactions with *E. coli* DNA polymerase I (□, ■) or with calf thymus DNA polymerase α (○, ●) were carried out as described in Experimental Procedures in the presence of the indicated concentration of the selenotrisulfide product of the reaction of selenite with mercaptoethanol (□, ○) or mercaptoethylamine (■, ●). The results are expressed as a percentage of the synthesis in the absence of any added compound.

TABLE 1

Inhibition of polymerases by selenotrisulfides

The data are taken from Figs. 3 and 7. IC_{50} is the approximate concentration of the compound which produces 50% inhibition of the enzyme.

Enzyme	IC_{50}	
	Mercaptoethanol-selenotrisulfide	Mercaptoethylamine-selenotrisulfide
	μM	
RNA polymerase II	10	1
E. coli DNA polymerase I	78	38
Calf thymus DNA polymerase α	1	1

TABLE 2

Inhibition of polymerases by mercury compounds

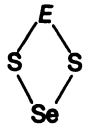
Reactions were carried out as described in Experimental Procedures. IC_{50} is the approximate concentration of the compound which produces 50% inhibition of the enzyme.

Enzyme	IC_{50}	
	Methyl mercury	$HgCl_2$
	μM	
RNA polymerase II	1.5	0.15
E. coli DNA polymerase I	100	10
Calf thymus DNA polymerase α	0.7	0.5

on mercury toxicity (20–24). In addition, certain toxic effects of selenium compounds, such as inhibition of amino acid transport (12), genotoxicity (25), and inhibition of protein synthesis (26) have been shown to be potentiated by SH compounds. We have found that the inhibition by selenite of a variety of DNA and RNA polymerases is also potentiated by sulfhydryl compounds, via formation of selenotrisulfide adducts. Thus, the inhibition of cellular DNA and RNA synthesis by selenite (7, 13–15) could result from the formation of selenotrisulfide derivative(s) via reaction with endogenous sulfhydryl compound(s). This possibility is currently under investigation.

Although the mechanism of the inhibition of the polymerases by the selenotrisulfides is not yet clear, some preliminary information has been obtained. First, binding of the enzyme to the DNA template does not appear to be significantly altered by the compounds, as evidenced by a lack of effect on the apparent K_m of the enzyme for the DNA. There does appear, however, to be an effect on the apparent K_m of the enzyme for the triphosphates, as well as on the V_{max} at saturating DNA or triphosphates. Second, there is clearly both chemical and enzymatic specificity in the effect, as evidenced by the differences in potency of selenotrisulfides formed from two similar sulfhydryl compounds and by the large difference in sensitivity of different enzymes to the compounds (see Table 1).

One possible mechanism for the interaction of the selenotrisulfide with the enzymes is suggested by the finding of Ganther and Corcoran (30), that addition of selenite to ribonuclease inactivates the enzyme as a result of the formation of selenotrisulfides in which the Se bridges two enzyme sulfhydryls, i.e.,



It is conceivable that, in the case of the polymerases, sulfhydryl groups of the protein are unable to react with selenite itself, but are able to substitute for those of the added selenotrisulfide, thereby forming the above type of adduct. This possibility is currently under investigation.

If this mechanism of action is in fact correct, the relative sensitivity of different enzymes to the selenotrisulfides might be expected to parallel their relative sensitivity to SH reagents such as mercurials. A major difficulty in assessing this stems from the fact that the relative sensitivity of enzymes to SH reagents can differ for different reagents (31). As an illustration of this, Table 2 shows the sensitivity of the three polymerases to two different mercury compounds. Because of the variation in the effects of the two mercurials on each of the enzymes, it is difficult to draw firm conclusions from a comparison of these data with the sensitivity of the enzymes to selenotrisulfides, as shown in Table 1. Nevertheless, the data are compatible with the involvement of enzyme sulfhydryl(s) in the inhibition by the selenotrisulfides.

Two other biological effects have been demonstrated for selenotrisulfides. They have been shown to inhibit protein synthesis both *in vivo* and *in vitro* (26, 32–35). They have also been shown to inhibit the growth of tumors *in vivo* as well as the proliferation of tumor cells in culture (36–38) (the latter at concentrations as low as 0.2 μM). Our results raise the possibility that this inhibitory effect on cell proliferation and, thus, perhaps also the anticarcinogenicity of selenium, could be the direct result of the inhibition of the DNA polymerase. If this turns out to be the case, then the design of selenotrisulfide compounds with selectivity for inhibition of tumor cell DNA polymerases could prove to be a valuable approach in the search for specific inhibitors of tumor cell proliferation. Our finding that there is considerable variation in the sensitivity of DNA polymerases to selenotrisulfides (see, for example, Fig. 7) suggests that this may indeed be feasible.

References

1. Shamberger, R. J. *Biochemistry of Selenium.* Academic Press, New York (1983).
2. Vernie, L. N. Selenium in carcinogenesis. *Biochim. Biophys. Acta* **738**:203–217 (1984).
3. Shamberger, R. J. The genotoxicity of selenium. *Mutat. Res.* **154**:29–48 (1985).
4. Shamberger, R. J., and C. Willis. Selenium distribution and human cancer mortality. *CRC Crit. Rev. Clin. Lab. Sci.* **2**:211–221 (1971).
5. Schrauzer, G. N., D. A. White, and C. J. Schneider. Cancer mortality correlation studies. III. Statistical associations with dietary selenium intakes. *Bioinorg. Chem.* **7**:23–34 (1977).
6. Salonen, J. T., G. Alfthan, J. K. Huttunen, and P. Puska. Association between

serum selenium and the risk of cancer. *Am. J. Epidemiol.* **120**:342–349 (1984).
7. Gruenwedel, D. W., and M. K. Cruikshank. The influence of sodium selenite on the viability and intracellular synthetic activity (DNA, RNA and protein synthesis) of HeLa S-3 cells. *Toxicol. Appl. Pharmacol.* **50**:1–7 (1979).
8. Poirier, K. A., and J. A. Milner. The effects of various seleno-compounds on Ehrlich ascites tumor cells. *Biol. Trace Elem. Res.* **1**:25–34 (1979).
9. Medina, D., and C. J. Oborn. Differential effects of selenium on the growth of mouse mammary cells *in vitro*. *Cancer Lett.* **13**:333–344 (1981).
10. Greeder, G. A., and J. A. Milner. Factors influencing the inhibitory effect of selenium on mice inoculated with Ehrlich ascites tumor cells. *Science* (Wash. D. C.) **209**:825–827 (1980).
11. Milner, J. A., and C. Y. Hsu. Inhibitory effects of selenium on the growth of L1210 leukemic cells. *Cancer Res.* **41**:1652–1656 (1981).
12. Hogberg, J., and A. Kristoferson. Inhibition of amino acid uptake in isolated hepatocytes by selenite. *FEBS Lett.* **107**:77–80 (1979).
13. Abdullaev, G. B., N. Kh. Mekhtiev, F. I. Abdullaev, and K. A. Kafiani. Effects of sodium selenite on RNA synthesis of loach embryos. *Biokhimiya* (translation) **45**:77–81 (1980).
14. Medina, D., and C. J. Oborn. Selenium inhibition of DNA synthesis in mouse mammary epithelial cell line YN-4. *Cancer Res.* **44**:4361–4365 (1984).
15. Frenkel, G. D. Effects of sodium selenite and selenate on DNA and RNA synthesis *in vitro*. *Toxicol. Lett.* **25**:219–223 (1985).
16. Abdullaev, G. B. Different degrees of inhibition of DNA-dependent RNA polymerases from wheat germ by sodium selenite. *Biokhimiya* (translation) **49**:1674–1678 (1984).
17. Kornberg, A. *DNA Replication*. Freeman & Co., San Francisco (1980).
18. Nickerson, W. J. and G. Falcone. Enzymatic reduction of selenite. *J. Bacteriol.* **85**:763–771 (1963).
19. Ganther, H. E. Enzymic synthesis of dimethyl selenide from sodium selenite in mouse liver extracts. *Biochemistry* **5**:1089–1098 (1966).
20. Stillings, B. R., H. Lagally, P. Bauersfeld, and J. Soares. Effect of cysteine, selenium and fish protein on the toxicity and metabolism of methylmercury in rats. *Toxicol. Appl. Pharmacol.* **30**:243–254 (1974).
21. Naganuma, A., and N. Imura. Bis(methylmercuric) selenide as a reaction product from methylmercury and selenite in rabbit blood. *Res. Commun. Chem. Pathol. Pharmacol.* **27**:163–173 (1980).
22. Iwata, H., T. Masukawa, H. Kito, and M. Hayashi. Involvement of tissue sulfhydryls in the formation of a complex of methylmercury with selenium. *Biochem. Pharmacol.* **30**:3159–3163 (1981).
23. Mochizuki, T., T. Kobayashi, and R. Doi. *In vitro* effects of mercury-selenium compounds on enzymes. *Toxicol. Lett.* **14**:201–206 (1982).
24. Mochizuki, T., T. Kobayashi, and R. Doi. Comparison of cytotoxicity of mercury-selenium and mercury compounds on cultured cells. *Toxicol. Lett.* **22**:47–51 (1984).
25. Whiting, R. F., L. Wei, and H. F. Stich. Unscheduled DNA synthesis and chromosome aberrations induced by organic selenium compounds in the presence of glutathione. *Mutat. Res.* **78**:159–169 (1980).
26. Vernie, L. N., W. S. Bont, and P. Emmelot. Inhibition of *in vitro* amino acid incorporation by sodium selenite. *Biochemistry* **13**:337–341 (1974).
27. Ganther, H. E. Selenotrisulfides. Formation by the reaction of thiols with selenious acid. *Biochemistry* **7**:2898–2905 (1968).
28. Jendrisak, J. A., and R. R. Burgess. A new method for the large-scale purification of wheat germ DNA-dependent RNA polymerase II. *Biochemistry* **14**:4639–4645 (1975).
29. Painter, E. P. The chemistry and toxicity of selenium compounds with special reference to the selenium problem. *Chem. Rev.* **28**:179–213 (1941).
30. Ganther, H. E. and C. Corcoran. Selenotrisulfides. II. Cross-linking of reduced pancreatic ribonuclease with selenium. *Biochemistry* **8**:2557–2563 (1969).
31. Webb, J. L. *Enzyme and Metabolic Inhibitors*, Vol. II. Academic Press, New York, 860–861 (1966).
32. Vernie, L. N., J. G. Collard, A. P. M. Eker, A. De Wildt, and I. T. Wilders. Studies on the inhibition of protein synthesis by selenodiglutathione. *Biochem. J.* **180**:213–218 (1979).
33. Vernie, L. N., W. S. Bont, G. B. Ginjaar, and P. Emmelot. Elongation factor 2 as the target of the reaction product between sodium selenite and glutathione (GSSeSG) in the inhibiting of amino acid incorporation *in vitro*. *Biochim. Biophys. Acta* **414**:283–292 (1975).
34. Vernie, L. N., H. B. Ginjaar, I. T. Wilders, and W. S. Bont. Amino acid incorporation in a cell-free system derived from rat liver studied with the aid of selenodiglutathione. *Biochim. Biophys. Acta* **518**:507–517 (1978).
35. Vernie, L. N., M. De Vries, L. Karreman, R. J. Topp, and W. S. Bont. Inhibition of amino acid incorporation in a cell-free system and inhibition of protein synthesis in cultured cells by reaction products of selenite and thiols. *Biochim. Biophys. Acta* **739**:1–7 (1983).
36. Vernie, L. N., C. J. Homburg, and W. S. Bont. Inhibition of the growth of malignant mouse lymphoid cells by selenodiglutathione and selenodicysteine. *Cancer Lett.* **14**:303–308 (1981).
37. Poirier, K. A., and J. A. Milner. Factors influencing the antitumorigenic properties of selenium in mice. *J. Nutr.* **113**:2147–2154 (1983).
38. Fico-Santoro, M., A. Lebowitz, and J. A. Milner. Effect of selenodiglutathione on the metabolism of canine mammary tumor cells. *Fed. Proc.* **45**:1077 (1986).

Send reprint requests to: Dr. Gerald D. Frenkel, Department of Biological Sciences, Rutgers University, Newark, NJ 07102.