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Evidence for the Involvement of Sulfhydryl Compounds in the Inhibition of Cellular DNA Synthesis by Selenite

GERALD D. FRENKEL and DARLENE FALVEY
Department of Biological Sciences, Rutgers University, Newark, New Jersey 07102
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SUMMARY

Previous studies have demonstrated that selenite inhibits cellular DNA synthesis. We have now found that endogenous cellular sulfhydryl compounds are involved in this effect of selenite. Treatment of cells with diethylmaleate, which produces a nonspecific depletion of cellular sulfhydryl compounds, resulted in a significant decrease in the sensitivity of DNA synthesis to inhibition by selenite. This decrease was eliminated by exogenous cysteine, but not by cystine. Similarly, DNA synthesis in nuclei isolated from diethylmaleate-treated cells was much less sensitive to inhibition by selenite than was synthesis in nuclei isolated from control cells. In contrast, treatment with buthionine sulfoximine, which specifically depletes the cells of glutathione, had no effect on the inhibition of DNA synthesis by selenite, indicating that cellular glutathione is not involved in the inhibition. Nevertheless, glutathione is able to compensate to some extent for the decreased level of sulfhydryl compounds in nuclei isolated from DEM-treated cells (although not as well as cysteine). Thus, although glutathione is able to potentiate the inhibition of cellular DNA synthesis by selenite, it apparently does not function in this capacity in the cell.

Selenium is an essential element at low levels and is toxic at higher levels (1). Some selenium compounds have also been shown to have anticarcinogenic activity in animal systems (2). It has been suggested that this property may result from inhibition of tumor cell proliferation (3, 4), and several laboratories have demonstrated that selenite does in fact possess antiproliferative activity (5). The molecular basis of this cytotoxic effect has not yet been established, although some investigators have proposed the inhibition of protein synthesis as a mechanism (6). However, the fact that selenite inhibits cellular DNA synthesis (7–9) has suggested this as an alternative possibility. Thus, as Medina has recently stated (10), "... one of the mechanisms of selenium modulation of carcinogenesis may be through inhibition of DNA synthesis. At this time, the molecular basis for this effect remains to be determined."

SH compounds have been implicated in a number of cellular effects of selenite (11–18). As part of our studies on the mechanism of the inhibition of cellular DNA synthesis by selenite we have investigated the possible involvement of SH compounds in this effect. The approach that we have taken is to treat cells with compounds that decrease the level of cellular SH compounds and examine the effect of this treatment on the inhibition of DNA synthesis by selenite. These experiments have provided direct evidence for the involvement of cellular SH compounds in this effect of selenite.

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Materials and Methods

Chemicals. Unlabeled deoxyribonucleoside triphosphates were purchased from P.L. Biochemical, [^{3}H]thymidine, and [^{3}H]dTTP from NEN (Boston, MA). DEM, BSO, and dithiobisnitrobenzene were purchased from Sigma Chemical Co. (St. Louis, MO), sodium selenite from Gallard-Schlesinger (Carle Place, NY).

DNA synthesis in intact cells. HeLa cells were grown in monolayer cultures (in 60-mm dishes), in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) containing 10% fetal calf serum (GIBCO). Sodium selenite was added to the cultures (at the concentrations indicated in the individual experiments) and incubation was continued for 1 hr at 37°. [^{3}H]Thymidine (84 Ci/mmol; final concentration, 6 μCi/ml) was then added and incubation continued for 15 min at 37°. The incorporation of radioactivity into acid-precipitable form was then measured as described previously (19). Where appropriate, DEM was added to the culture (at a final concentration of 1 mM) 2 hr before the addition of the selenite or BSO (final concentration, 1 mM) was added 24 hr before the selenite. All determinations were carried out in duplicate.

DNA synthesis in isolated nuclei. Nuclei were isolated and DNA synthesis was measured essentially as described previously (19). HeLa cells were grown in 100-mm dishes, removed from the dish with a rubber policeman into 50 mM sodium phosphate buffer (pH 7.6) containing 150 mM NaCl (7 ml/dish), and centrifuged for 10 min at 1000 rpm. The cell pellet was resuspended in 20 mM HEPES buffer (pH 7.4) containing 1 mM $MgCl_2$, 1 mM dithiothreitol, and 0.5 mM $CaCl_2$ (2 ml/dish). The cells were centrifuged again, and the pellet was resuspended in the same buffer (1 ml/dish). After 40 min at 0° the cells were broken with 12 strokes in a Dounce homogenizer and centrifuged for 10 min at 3000 rpm. The nuclei were resuspended in 50 mM HEPES buffer

ABBREVIATIONS: SH, sulfhydryl; DEM, diethylmaleate; BSO, buthioninesulfoximine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

(pH 7.4) containing 100 mM NaCl and 1 mM $MgCl_2$ (2 ml/dish), centrifuged again, and resuspended in the same buffer (0.2 ml/dish). A 0.1-ml aliquot of the nuclei suspension was added to 0.1 ml of a 2-fold concentrated reaction mixture. The reaction mixture contained (final concentrations): 15 mM HEPES buffer (pH 7.4), 5 mM $MgCl_2$, 45 mM KCl, 2 mM ATP, 50 μM each of dGTP, dCTP, and dATP, and 5.4 μCi of [^{3}H]dTTP (79 Ci/mmol). After incubation for 10 min at 22°, acid-precipitable radioactivity was measured as described previously (19). Each reaction was carried out in duplicate.

Quantitation of cellular non-protein SH compounds. The level of cellular non-protein SH compounds was determined with Ellman's reagent (20), essentially as described by Sedlak and Lindsay (21). Cells were washed with 50 mM sodium phosphate buffer (pH 7.6) containing 150 mM NaCl and suspended in 0.2% sodium dodecyl sulfate containing 20 mM EDTA (final pH 7.4) (approximately 8×10^7 cells/ml). The suspension was mixed by vortexing and allowed to stand at room temperature for 10 min. The resulting cell lysate was chilled, 0.1 ml of 50% trichloroacetic acid was added, and the solution was centrifuged for 10 min at 10,000 × g. The supernatant was analyzed for SH content by adding 0.1 ml to a solution containing 30 mM Tris-HCl buffer (pH 8.3), 0.3 mM EDTA, 0.1 mM dithiobisnitrobenzene, and 80% (v/v) methanol. After 15 min at room temperature, the solution was centrifuged at 7000 × *g* for 15 min and the absorbance of the supernatant at 412 nm was determined. The SH content was determined based upon the absorbance obtained with a glutathione standard.

Results

In order to examine the role of SH compounds in the inhibitory effect of selenite on cellular DNA synthesis, we treated HeLa cells with DEM, an agent that has been shown to deplete these cells of non-protein SH compounds (22). Exposure of cells to 1 mM DEM for 2 hr resulted in a decrease in the level of non-protein SH to 14% of that of control cells (Table 1). The effect of DEM treatment on the inhibition of DNA synthesis by selenite is shown in Fig. 1. DNA synthesis was much less sensitive to inhibition by selenite in DEM-treated cells than in control cells. (In the experiment shown, 50% inhibition occurred in control cells with <10 μM selenite, in DEM-treated cells at approximately 150 μM selenite. In a second identical

TABLE 1

Effect of DEM and BSO on the level of cellular non-protein SH

Values are mean ± standard deviation.

Treatment	Non-protein SH
	nmol/10^6 cells
None	13.7 ± 1.4
DEM	2.5 ± 0.6
BSO	2.4 ± 0.8
DEM + BSO	0.5 ± 0.1

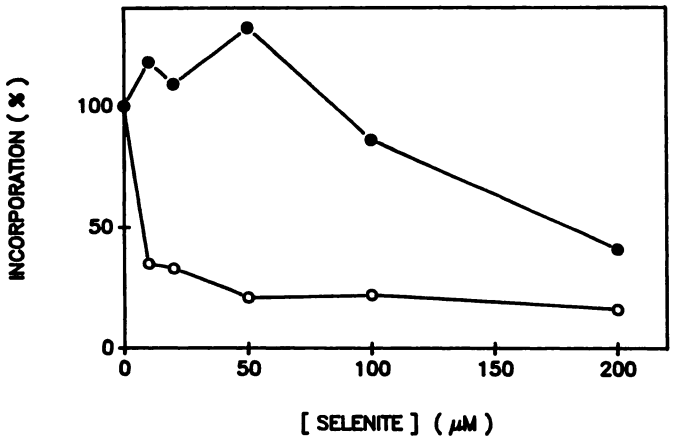

Fig. 1. Effect of selenite on DNA synthesis in intact cells. DNA synthesis in control (○) or DEM-treated (●) cells was measured in the presence of the indicated concentration of selenite as described in Materials and Methods. The results are presented as the percentage of incorporation in the absence of added selenite. The results are the means of duplicate determinations (which varied 10% or less). The results for control and DEM-treated cells at each concentration are significantly different (at $p < 0.005$ or less, *t* test).

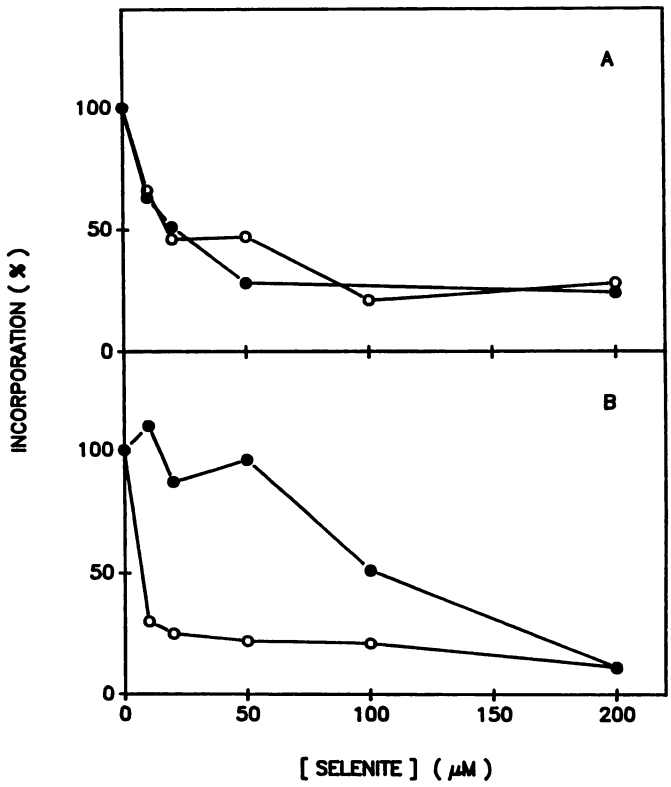

Fig. 2. Effect of selenite on DNA synthesis in intact cells in the presence of cysteine or cystine. The experiment was carried out as in Fig. 1, except that before addition to the cell cultures the selenite was mixed with either a 4:1 molar ratio of cysteine (A) or a 2:1 molar ratio of cystine (B). The results are presented as in Fig. 1.

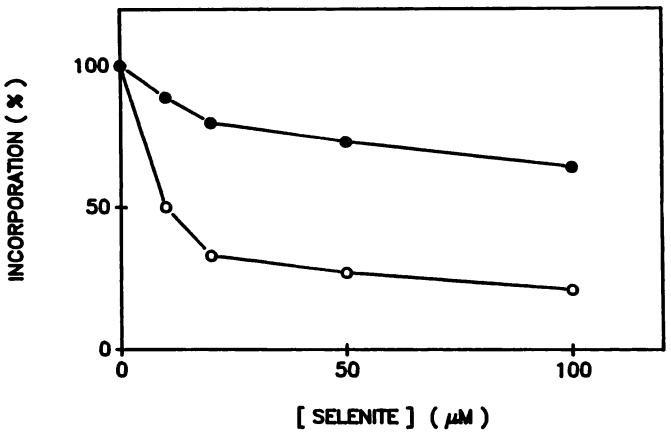

Fig. 3. Effect of selenite on DNA synthesis in isolated nuclei. Nuclei were isolated from control (○) or DEM-treated (●) cells and DNA synthesis was measured in the presence of the indicated concentration of selenite (see Materials and Methods). The results are presented as in Fig. 1. The results for nuclei from control and DEM-treated cells at each concentration are significantly different (at $p < 0.005$ or less, *t* test).

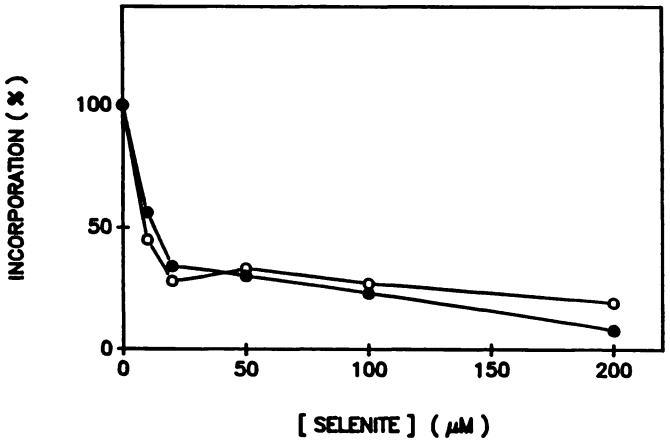

Fig. 4. Effect of selenite on DNA synthesis in BSO-treated cells. DNA synthesis in control (○) or BSO-treated (●) cells was measured in the presence of the indicated concentration of selenite as described in Materials and Methods. The results are presented as in Fig. 1.

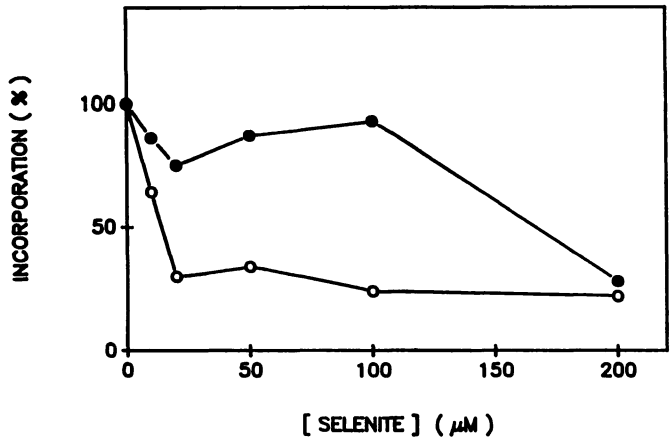

Fig. 5. Effect of selenite on DNA synthesis in cells treated with BSO and DEM. DNA synthesis in control cells (○) or cells treated with BSO and DEM (●) was measured in the presence of the indicated concentration of selenite as described in Materials and Methods. The results are presented as in Fig. 1.

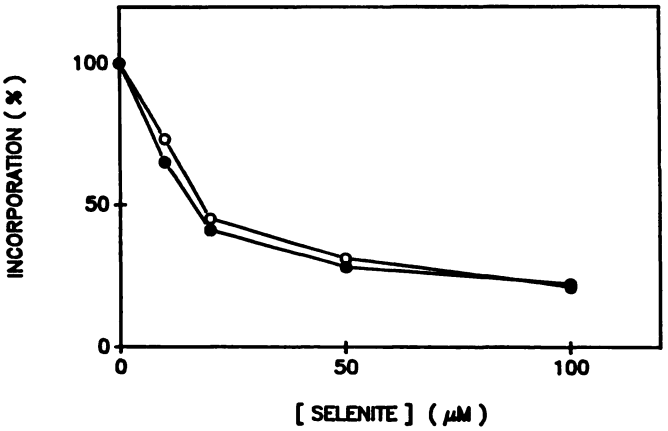

Fig. 6. Effect of selenite on DNA synthesis in nuclei isolated from BSO-treated cells. Nuclei were isolated from control (○) or BSO-treated (●) cells and DNA synthesis was measured in the presence of the indicated concentration of selenite (see Materials and Methods). The results are presented as in Fig. 1.

experiment the values obtained were <10 μM and 125 μM, respectively.)

In order to confirm that in this experiment the difference between control and DEM-treated cells is in fact due to the difference in the level of SH compounds, the effect of selenite was determined in the presence of added cysteine. The results (Fig. 2A) show that, in the presence of cysteine, DNA synthesis in DEM-treated cells was as sensitive to inhibition by selenite as synthesis in control cells; thus, the difference between control and DEM-treated cells was eliminated by the SH compound. In contrast, cystine (the disulfide of cysteine) had relatively little effect on the inhibition of DNA synthesis by selenite (Fig. 2B).

To further examine the involvement of endogenous SH compounds in the inhibition of cellular DNA synthesis by selenite, we took advantage of our earlier observation (9) that DNA synthesis in isolated nuclei is also inhibited by selenite. Nuclei were isolated from the DEM-treated cells and the effect of selenite on DNA synthesis was examined. The results (Fig. 3) show that DNA synthesis in nuclei isolated from DEM-treated cells was significantly less sensitive to selenite than was synthesis in nuclei isolated from control cells. There was a reproducible 10-fold difference in the concentration of selenite that results in 50% inhibition of synthesis in nuclei isolated from control or DEM-treated cells. [In contrast, the sensitivity of synthesis to *N*-ethylmaleimide, a reagent that inhibits DNA polymerase α but not DNA polymerase β (23), was the same in the two types of nuclei (data not shown).] This result with isolated nuclei demonstrates that the difference between DEM-treated and control cells (Fig. 1) is not the result of differences in uptake or phosphorylation of the [^{3}H]thymidine precursor but rather reflects a real difference in the effect of selenite on DNA synthesis.

DEM is a nonspecific reagent in that it reduces the cellular level of all non-protein SH compounds (24). The cells can be specifically depleted of glutathione, the most abundant of the SH compounds (25), by treatment with BSO (26), which inhibits its biosynthesis. This reagent has been used to demonstrate, among other things, that glutathione is involved in protection against radiation-induced damage (27). We have examined the effect of BSO treatment on the sensitivity of DNA synthesis to selenite. In spite of the fact that BSO treatment reduced the level of non-protein SH about as much as DEM treatment (Table 1), it did not result in any detectable difference in the inhibition of DNA synthesis by selenite (Fig. 4). Furthermore, treatment of cells with BSO in addition to DEM (Fig. 5) did not result in a decrease in the sensitivity of DNA synthesis to selenite below that of DEM treatment alone (cf. Fig. 1), in spite of the fact that the additional BSO treatment did result in a further reduction in the level of cellular SH compounds (Table 1). Thus, nonspecific depletion of the cells of SH compounds (by DEM) resulted in a decrease in the inhibition of DNA synthesis by selenite but specific depletion of glutathione (by BSO) did not. Similar results were obtained when DNA synthesis in isolated nuclei was examined. As shown in Fig. 6, selenite inhibited synthesis equally in nuclei isolated from control or BSO-treated cells. Similarly, inhibition of synthesis in nuclei isolated from cells treated with both BSO and DEM (Fig. 7) was the same as in nuclei isolated from cells treated with DEM alone (cf. Fig. 3).

These results demonstrate that a cellular SH compound is

involved in the inhibition of DNA synthesis by selenite but that glutathione, which is the most abundant cellular SH compound, is not. A possible explanation for this is that glutathione is unable to potentiate the inhibitory effect of selenite on DNA synthesis. In order to investigate this possibility, we examined the effect of selenite on DNA synthesis in isolated nuclei in the presence of glutathione. The results show that glutathione was able to partially compensate for the deficiency in the nuclei that results from SH depletion of the cells (Fig. 8B), although it was less effective in this regard than cysteine (Fig. 8A). (These results should be compared with those in Fig. 3, which show the effect of selenite in the absence of added SH compound.) Thus, the lack of effect of BSO in cells and isolated nuclei cannot be ascribed simply to the inability of glutathione to function in the inhibition of DNA synthesis by selenite.

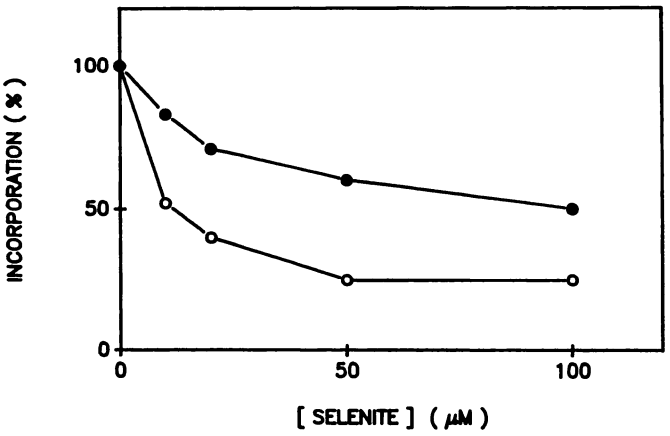

Fig. 7. Effect of selenite on DNA synthesis in nuclei isolated from cells treated with BSO and DEM. Nuclei were isolated from control cells (○) or cells treated with BSO and DEM (●) and DNA synthesis was measured in the presence of the indicated concentration of selenite (see Materials and Methods). The results are presented as in Fig. 1.

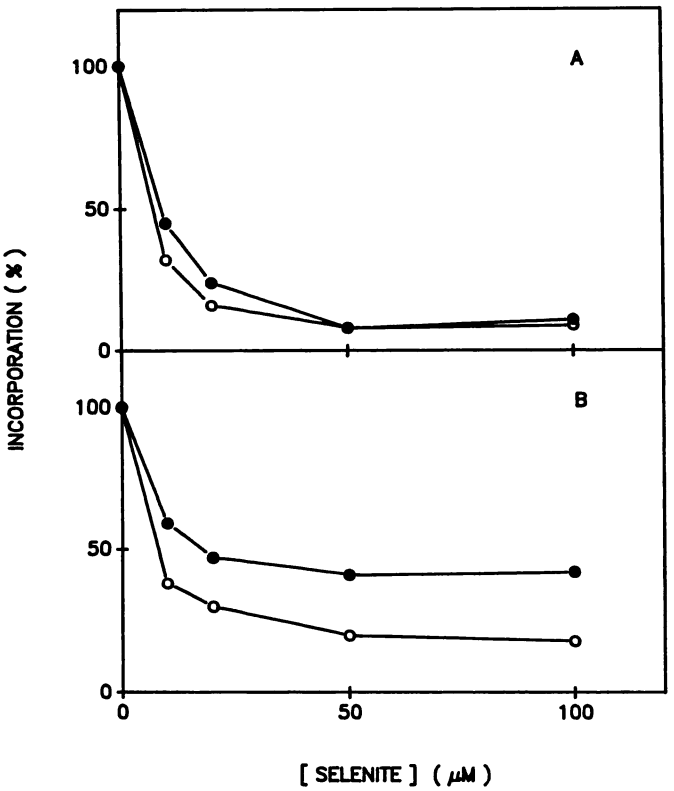

Fig. 8. Effect of selenite on DNA synthesis in isolated nuclei in the presence of SH compounds. Nuclei were isolated from control (○) or DEM-treated (●) cells and DNA synthesis was measured (as described in Materials and Methods) in the presence of the indicated concentration of selenite and either cysteine (A) or glutathione (B) (at a 4:1 molar ratio). The results are presented as the percentage of synthesis in the absence of added selenite. (The SH compounds alone had no effect on synthesis.)

Discussion

Although a number of studies have suggested the possible involvement of SH compounds in the cellular effects of selenium compounds (11–18), our results provide the first direct evidence for this in living cells. Nonspecific depletion of SH compounds from cells, by treatment with DEM, results in a significant decrease in the inhibitory effect of selenite on DNA synthesis; this demonstrates the involvement of at least one such SH compound in this effect of selenite. Cellular SH compounds probably react with exogenously added selenite to form selenotrisulfides (28), which are most likely the inhibitory agents of DNA synthesis. However, specific depletion of glutathione from the cell, by treatment with BSO, has no detectable effect on the inhibition of DNA synthesis by selenite. Thus, under the conditions of our experiments, glutathione is not involved in the inhibition of DNA synthesis by selenite. It may be that in the cell (or in isolated nuclei) selenodiglutathione is not readily formed or is unstable compared with selenotrisulfides of other cellular SH compounds (28, 29). Because of this, selenodiglutathione does not accumulate to any significant extent in the cell, and depletion of the cells of glutathione has no impact on the inhibition of DNA synthesis by selenite. We are currently investigating this possibility, as well as the identity of the cellular SH compounds that are involved in the inhibition of DNA synthesis by selenite.

There have been many reported examples of the depletion of cellular SH compounds resulting in an increase in the sensitivity of the cells to toxic agents (30). This has generally been interpreted as demonstrating that they play a role in cellular protective and/or repair processes. Selenite is the first example, to our knowledge, of depletion of SH compounds resulting in a decrease in sensitivity to a toxic agent. Thus, at least in the case of selenite, SH compounds can also increase the toxicity of an exogenous agent.

We recently reported (31) that inhibition by selenite of DNA synthesis by purified DNA polymerase, the principal enzymatic activity in DNA synthesis (23), also requires an SH compound. Thus, the present results, which demonstrate the involvement of SH compounds in the inhibition by selenite of DNA synthesis in the cell, suggest that the latter effect may result from a direct inhibitory effect of selenite on the polymerase. This possibility is under investigation.

The levels of selenite that inhibit DNA synthesis in cells in culture are comparable to the levels in blood that have been found to be toxic in human beings. For example, the typical Se level in human blood in the United States has been reported to be approximately 2.5 μM (32). At this concentration there is no significant inhibition of DNA synthesis in tissue culture. In areas of China where individuals exhibit toxic effects due to excess Se, the typical level in blood has been reported to be approximately 40 μM (33); significant inhibition of DNA synthesis in cultured cells occurs at this concentration (see Fig. 1). This suggests that our findings may be relevant to the effects

of selenite in human beings. It may prove to be possible, for example, to enhance the anticarcinogenicity and/or decrease the toxic side effects of selenium compounds by manipulating cellular SH content. This in turn may facilitate the future development of selenium compounds as chemopreventive agents.

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Send reprint requests to: Dr. Gerald Frenkel, Department of Biological Sciences, Rutgers University, 101 Warren Street, Newark, NJ 07102