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Inhibitory Effects of Selenium on Mutagenicity

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Antioxidants, such as 2(3)-*tert*-butyl-4-hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and glutathione, are effective inhibitors of a variety of chemical carcinogens and mutagens. Mutagencity tests employing the Ames *Salmonella*/microsome assay have shown that selenium as sodium selenite is effective in reducing the mutagenicity of both direct acting mutagens, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and mutagens requiring metabolic activation, such as 7,12-dimethylbenz[*a*]anthracene (DMBA). Sodium selenite, sodium selenate, selenium dioxide, and selenomethionine have been found to reduce the mutagenicity of DMBA when added to the surface of the Ames test plate. In addition, selenium has been shown to alter enzymes involved in the metabolic activation of mutagens. Incorporation of liver extracts derived from rats fed selenium-supplemented diets was found to result in lower reversion rates when compared to those of unsupplemented controls.

Selenium is an essential nutrient in the diet of numerous animals including man (van Rij et al., 1979). Although selenium can prevent various disease states in animals, it can be toxic if administered in sufficient quantitites (Harr et al., 1967). Animal studies have revealed that increased selenium intakes at nontoxic concentrations decreased the incidence and size of tumors induced in rats treated with a variety of chemicals which include: 3-methyl-4-(dimethylamino)azobenzene (Clayton and Baumann, 1949), 7,12-dimethylbenz[*a*]anthracene (Shamberger, 1970); *N*-(2-fluorenyl)acetamide (Harr et al., 1972), and 1,2-dimethylhydrazine (Jacobs et al., 1977a). Several reports have indicated that selenium also is effective in reducing mutagencity. Examples of compounds in which selenium reduced mutagenicity include 2-(acetylamino)fluorene, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, (acetoxyacetylamino)fluorene, acridine orange, and 7,12-dimethylbenz[*a*]anthracene (Jacobs et al., 1977b; Rosin and Stich, 1979; Martin et al., 1981).

Many chemical carcinogens and mutagens are frequently not carcinogenic or mutagenic per se but require metabolic activation to reactive intermediates, which can then combine with critical cellular components. A scheme of metabolic activation of a precarcinogen to an ultimate carcinogen is shown in Figure 1. The ultimate carcinogen may be formed directly or may be formed through one or more intermediates, termed proximate carcinogens, from the precarinogen. Upon formation, the electrophilic (relatively electron deficient) ultimate carcinogen may react with any available nucleophilic (electron rich) critical cellular macromolecule, such as DNA. Alternatively, it may be inactivated by binding to noncritical sites, by spontaneous breakdown, or by enzymatic detoxification. Many of the enzymes found to be involved in the metabolic activation of carcinogens and mutagens are located in the endoplasmic reticulum and cytosol of liver cells and in cells of other organs, such as the kidney, breast, and skin. Bacterial mutagenesis tests such as the Ames assay have, therefore, incorporated a mammalian metabolic activation system in order to obtain formation of the ultimate carcinogen.

In order to obtain maximum metabolic activity of the S-9 fraction, organ-donating animals must be exposed to an inducing agent prior to sacrifice. Polychlorinated biphenyls (PCB's), phenobarbital, and 3-methylcholanthrene are most frequently used as inducers. Studies have shown that both the inducing agent, as well as the amount of inducer utilized, can influence the types of metabolites formed (Sheikh et al., 1980; Yang et al., 1980; Schillaci et al., 1982).

Carcinogenic polycyclic aromatic hydrocarbons (PAH's) are an important class of chemical environmental pollutants. Of the PAH's thus far investigated, 7,12-dimethylbenz[*a*]anthracene (DMBA) is the most potent initiator of tumors in the conventional (mouse skin) initiation–promotion regimens (DiGiovanni and Juchau, 1980). In common with other PAH's, it is metabolized to reactive metabolites by the hepatic monoxygenase activation system (Chou et al., 1981). During activation, the metabolites formed in vitro by liver homogenate and microsomes are mostly *trans*-dihydrodiols, phenols, and mono- and dihydroxymethyl derivatives of DMBA (Chou et al., 1979; Chou and Yang, 1978, 1979). The three hydroxymethyl derivatives, 7-OHM-12-MBA, 7-M-12-OHMBA, and 7,12-diOHMBA, also can be further metabolized to form their corresponding diols, phenols, and other oxidation products of DMBA (Chou and Yang, 1979). Further metabolism of these hydroxyl derivatives leads to a complex mixture of metabolites (DiGiovanni and Juchau, 1980). The DMBA-*t*-3,4-dihydrodiol, 7-OHM-12-MBA-*t*-3,4-dihydrodiol, and the 7-OHM-12-MBA metabolites are among the most potent mutagens of DMBA metabolites tested by using Chinese hamster V79 cells (Slaga et al., 1979) and in the Ames *Salmonella*/microsome test (Wislocki et al., 1980). The bay-region 3,4-diol 1,2-epoxides of DMBA and 7-OHM-12-MBA have been implicated as being the ultimate carcinogenic forms of DMBA and 7-OHM-12-MBA (Figure 2; Chou and Yang, 1979; Dipple et al., 1979; Slaga et al., 1979). The 3,4-dihydrodiol derivatives also have been found to have greater DNA binding activity than DMBA itself (Chou et al., 1979).

Antioxidants are effective inhibitors of a wide variety of chemical carcinogens and mutagens. Ascorbate (ascorbic acid, vitamin C) has been found to have variable effects as an inhibitor of mutagenesis (Table I). Vitamin C was effective in reducing the mutagenicity of the direct acting *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, malonaldehyde, and β-propiolactone but did not reduce the mutagenicity of *N*-hydroxy-2-(acetylamino)fluorene. Vitamin E (α-tocopherol) and vitamin A (retinol) had similar variable results with direct-acting mutagens and premutagens.

The phenolic antioxidants 2(3)-*tert*-butyl-4-hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and

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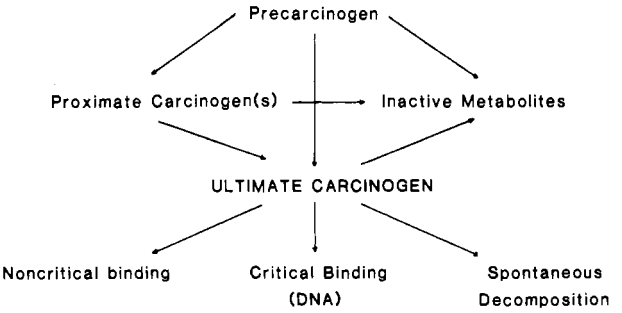

Figure 1. Schematic diagram of the metabolic activation of precarcinogen to an ultimate carcinogen.

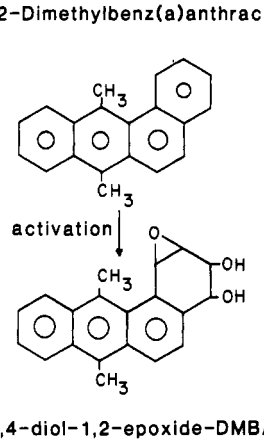

Figure 2. Chemical structure of 7,12-dimethylbenz[*a*]anthracene and its ultimate carcinogen.

propyl gallate also had varying effects when added to bacterial mutagenesis tests (Table I). The mutagenicity of benzo[*a*]pyrene was reduced, at least in some tests, by all three of these compounds. Variable results were also found with the antioxidants disulfiram, cysteine, and cysteamine (Table I). Glutathione, bisulfite, ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline), and a variety of other antioxidants caused a variable pattern in the reduction of mutagenicity of some mutagens (Table I). The mechanisms that have been proposed for the mode of action of antioxidants against chemically induced mutagenesis include (1) direct chemical reaction between inhibitor and the ultimate carcinogen, (2) induction of the detoxification processes, when the antioxidant is fed to the animal, or (3) inhibition of the activation processes (Calle and Sullivan, 1982).

Selenium as sodium selenite also has been shown to reduce the mutagenicity of 2-(acetylamino)fluorene, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and (acetoxyacetylamino)fluorene (Jacobs et al., 1977b; Rosin and Stich, 1979; Table II). Lofroth and Ames (1980) were unable to show that sodium selenite was mutagenic in the *Salmonella*/microsome test; however, sodium selenate gave rise to base-pair substitutions at a frequency of about 0.3 revertant/nmol. Shamberger et al. (1979) have reported that antioxidants, including selenium, markedly reduced mutagenesis in frame mutants of *Salmonella typhimurium*.

EXPERIMENTAL SECTION

Ames *Salmonella*/Microsome Test. The Ames test was performed as follows: 2 mL of molten top agar, tempered to 45 °C, was added to a sterile test tube (Martin et al., 1981). Immediately following were added 0.1 mL of the suspect mutagen, 0.1 mL of the tester strain from an early stationary phase culture (approximately 10^8 cells), and 0.5 mL of the S-9 mixture containing the metabolic activation system. Acridine orange was prepared in absolute ethanol and DMBA was dissolved in dimethyl sulfoxide. In some experiments, DMBA and its metabolites were dissolved in 81% Me_2SO and 19% methanol. Selenium-containing compounds were dissolved in water and filter-sterilized. The contents of the tube were mixed and poured over the surface of Petri plates containing solidified minimal glucose agar. The top agar was distributed evenly over the surface of the plate by gentle tilting and permitted to solidify, and the plates were incubated at 37 °C for 2 days. Top agar contained trace amounts of histidine and biotin, which permitted the growth of a light background lawn.

Dietary Selenium Experiments. Three- to four-week old female Sprague-Dawley rats were housed in stainless steel cages in a room with controlled temperature and lightening (12-h light and 12-h dark). Two groups of 20 rats each received drinking water supplemented with 0 or 5 ppm of selenium (as sodium selenite). All rats were fed a semipurified diet containing 0.15 ppm of selenium throughout the 21-day experimental period. The basal diet had the following composition (as a percentage): Torula yeast, 30.3; L-methionine, 0.3; sucrose, 32.0; corn starch, 17.9; vitamin mixture, 1.0; mineral mixture, 3.5; α cell, 5.0; corn oil, 10.0. The vitamin fortification mixture and the AIN mineral mixture were purchased from Teklad Mills, Madison, WI. Diet and water were supplied ad libitum in all studies. On day 19 of feeding, four groups of five rats from each water treatment received 0, 20, 100, or 500 mg of Aroclor 1254 (PCB)/kg of body weight. The PCB was suspended in corn oil. The injection volume was approximately 1 mL. Five days after PCB treatment and after an overnight fast, all rats were sacrificed by decapitation and the livers removed (Schillaci et al., 1982).

Three groups of 20 rats each were assigned to one of three diets containing varying quantities of selenium. Selenium as sodium selenite was added to the semipurified diet at 0.1 (control diet), 2.5, or 5 μg/g. Weight gain and feed intake were determined periodically in all rats in the second study. All rats were fed their respective diet for 3 weeks. Five days before sacrifice, while still on the test diet, the rats were injected in groups of five with PCB at levels of 0, 20, 50, or 100 mg/kg. At the termination of the study, the rats were weighed and sacrificed and the livers quickly removed (Schillaci et al., 1982).

RESULTS AND DISCUSSION

In our laboratories we have examined the influence of selenium on the mutagenicity of two compounds: DMBA and acridine orange (*N,N,N',N'*-tetramethyl-3,6-acridinediamine). The Ames *Salmonella*/microsome mutagenicity test was utilized, and in initial experiments, selenium was added to the molten top agar prior to its addition to the surface of the minimal agar, an in vitro addition. In one of the first experiments, selenium as sodium selenite was added in increasing amounts to assays that contained 20 μg of DMBA (Figure 3). Increasing the amount of sodium selenite up to 692 μg (16 ppm of Se) was increasingly effective in reducing the mutagenicity of DMBA. Studies also have been performed in which the amount of sodium selenite added per plate was kept constant and the level of DMBA varied (Martin et al., 1981; Arciszewska et al., 1982; Table III).

Other forms of selenium were also examined to determine their efficacy in reducing the mutagenicity of DMBA (Table IV). Results obtained indicate that selenium as sodium selenite, selenium dioxide, and selenomethionine caused a significant decrease in the number of DMBA-induced revertants at concentrations of 1, 2, or 4 ppm of Se. Selenomethionine caused a 72% decrease in the

Table I

mutagen	test system (A = Ames)	mammalian metabolizing capability	inhibition (+ = >30%; − = <30%)	reference	comments
			Ascorbate		
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	A 1535	−	+	Rosin and Stich (1979)	
2-(acetoxyacetylamino)fluorene	A 98	−	−	Rosin and Stich (1979)	
2-(hydroxyacetylamino)fluorene	A 1538	+ (nuclei S-9)	−	Sakai et al. (1978)	ascorbate increases mutagenicity
benzo[*a*]pyrene	A 98	+	−	Calle and Sullivan (1982)	
N-nitrodimethylamine	A 100, 1530	+	+	Khudoley et al. (1981)	
			α-Tocopherol		
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	A 1535	−	−	Rosin and Stich (1979)	
2-(acetoxyacetylamino)fluorene	A 98	−	−	Rosin and Stich (1979)	
benzo[*a*]pyrene	A 98	+	−	Calle and Sullivan (1982)	
			Retinol		
2-fluorenamine	A 98	+	+	Baird and Birnbaum (1979)	
adriamycin	A 98	−	−	Baird and Birnbaum (1979)	
benzo[*a*]pyrene	A 98	+	+	Calle and Sullivan (1982)	
			2(3)-*tert*-Butyl-4-hydroxyanisole (BHA)		
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	A 1535	−	−	Rosin and Stich (1979)	
2-(acetoxyacetylamino)fluorene	A 98	−	−	Rosin and Stich (1979)	
benzo[*a*]pyrene	A 98, 1538	+	+	McKee and Tometsko (1979)	
benzo[*a*]pyrene	A 98	+	−	Calle and Sullivan (1982)	
			Butylated Hydroxytoluene (BHT)		
methyl methanesulfonate	A 100	−	−	McKee and Tometsko (1979)	
2,4-dinitro-1-fluorobenzene	A 98	−	−	McKee and Tometsko (1979)	
benzo[*a*]pyrene	A 98, 1538	+	+	McKee and Tometsko (1979)	
benzo[*a*]pyrene	A 98	+	−	Calle and Sullivan (1982)	
			Propyl Gallate		
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	A 1535	−	+	Rosin and Stich (1979, 1980)	
2-(acetoxyacetylamino)fluorene	A 98	−	+	Rosin and Stich (1979, 1980)	
2-(hydroxyacetylamino)fluorene	A 98	−	−	Rosin and Stich (1980)	PG > mutagenicity
4-nitroquinoline 1-oxide	A 98, 100	−	−	Rosin and Stich (1980)	PG > mutagenicity
			Glutathione		
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	A 1535	−	+	Rosin and Stich (1979)	
quinoline	A 100	+	+	Hollstein et al. (1978)	
benzo[*a*]pyrene	A 98	+	−	Calle and Sullivan (1982)	
benzo[*a*]pyrene	A 98	+	+	Benson et al. (1978)	inhibition required added glutathione *S*-transferase
			Bisulfite		
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	A 1535	−	+	Rosin and Stich (1979)	
2-(acetoxyacetylamino)fluorene	A 98	−	−	Rosin and Stich (1979)	
benzo[*a*]pyrene	A 98	+	±	Calle and Sullivan (1982)	
			Ethoxyquin		
benzo[*a*]pyrene	A 98	+	−	Calle and Sullivan (1982)	
benzo[*a*]pyrene	A 98, 100	+	+	Batzinger et al. (1978)	host-mediated assay hamster feeder cell layer

benzo[*a*]pyrene	A 98	+	±	Calle and Sullivan (1982)	also + for 6- and 10-MeBaP	
			Disulfiram			
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	A 1535	−	±	Rosin and Stich (1979)		
2-(acetoxyacetylamino)fluorene	A 98	−	−	Rosin and Stich (1979)		
benzo[*a*]pyrene	A 98	+	+	Calle and Sullivan (1982)		
			Cysteine			
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	A 1535	−	+	Rosin and Stich (1978a)		
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	A 1535	−	+	Rosin and Stich (1978b)		
2-(acetoxyacetylamino)fluorene	A 98	−	+	Rosin and Stich (1978b)		
2-(hydroxyacetylamino)fluorene	A 98	−	+	Rosin and Stich (1978b)		
benzo[*a*]pyrene	A 98	+	+	Calle and Sullivan (1982)		
			Cysteamine			
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	A 1535	−	+	Rosin and Stich (1979)		
2-(acetoxyacetylamino)fluorene	A 98	−	+	Rosin and Stich (1979)		
2-(hydroxyacetylamino)fluorene	A 1538	+	+	Sakai et al. (1978)		
benzo[*a*]pyrene	A 98	+	−	Calle and Sullivan (1982)		

number of spontaneous revertants at 1 ppm. Selenium as sodium selenate (Na_2SeO_4) appeared to be weakly mutagenic for strain TA100, as reported by Ames (Lofroth and Ames, 1980). Increasing concentrations of selenium as sodium selenite, sodium selenate, or selenium dioxide, from 4 to 40 ppm, resulted in an increasing reduction in the number of DMBA-induced revertants. All of the inorganic forms of selenium tested were not toxic to the *Salmonella* tester strains at the concentrations employed (Table V).

The influence of sodium selenite on the mutagenicity of some selected metabolites of DMBA has also been examined (Table VI). DMBA and the metabolites 7-OHM-12MBA, 12-OHM-7MBA, 7,12-diOHMBA, 3-OH-DMBA, and 5,6-diol-DMBA were tested at 20 μg/plate. Although 7-OHM-12MBA, 12-OHM-7MBA, and 3-OH-DMBA were mutagenic, their mutagenic activities were less than that of DMBA (25.3%, 27.2%, and 40.6%, respectively). Selenium (20 ppm) as Na_2SeO_3 reduced the mutagenicity of 7-OHM-12MBA and 12-OHM-7MBA to background levels. The metabolites 7,12-diOHMBA and 5,6-diol-DMBA were not found to be mutagenic in these studies (data not presented). In addition, DMBA and the metabolites 7-OHM-12MBA, 12-OHM-7MBA, and 3-OH-DMBA (20 μg/plate) were not mutagenic in the absence of S-9.

Acridine orange previously has been shown to be mutagenic in the Ames *Salmonella*/microsome assay (McCann et al., 1975) and to increase the mutation rate to phage resistance in excision-deficient and recombination-deficient strains of *Escherichia coli* (Hass and Webb, 1978). Acridines and a large number of acridine derivatives have been shown to cause both addition and frameshift mutations (Nasim and Brychcy, 1979). Figure 4 shows the results obtained when acridine orange was tested in the Ames *Salmonella*/microsome test in the presence and absence of selenium as sodium selenite. Selenium was added in vitro to the molten top agar and poured onto the plates. In this test, strain TA100 was utilized. Results shown in panel A indicate that acridine orange concentrations above 30 μg/plate were bactericidal to the tester strain. Addition of 8 ppm of selenium resulted in a significant reduction in the number of revertants at all concentrations of acridine orange tested. Increasing selenium concentrations reduced the number of revertants caused by acridine orange (20 μg/plate; panel B).

Rasco et al. (1977) have shown that selenium inhibited the activity of human lymphocyte aryl hydrocarbon hydroxylase by 50% when added to the incubation mixture. In studies with isolated microsomes from selenium-supplemented or nonsupplemented rats it was observed that selenium administration led to an increase in ring hydroxylation and a decrease in N-hydroxylation of 2-(acetylamino)fluorene, thereby shifting metabolism toward detoxification pathways (Marshall et al., 1979). Although, Heidelberger (1975) has suggested that covalent interactions, particularly with DNA, may be an essential initial step in the process of mutagenesis or carcinogenesis, Wortzman et al. (1980) were unable to detect any influence of selenium on 2-(acetylamino)fluorene binding to rat liver DNA, yet tumor incidence was reduced. Furthermore, their results indicate DNA repair was not responsible for the anticarcinogenic property of selenium against 2-(acetylamino)fluorene. Harbach and Swenberg (1981) showed that selenium supplementation was capable of decreasing the metabolism of dimethylhydrazine in liver but increased extrahepatic metabolism and alkylation. The reason for the discrepancies between metabolism and binding of the metabolites to DNA are not known, unless the binding

Table II. Selenite

mutagen	test system (A = Ames)	mammalian metabolizing capability	inhibition (+ = >30%; − = <30%)	reference	comments
N-methyl-*N*′-nitro-*N*-nitrosoguanidine	A 1535	−	+	Rosin and Stich (1979)	
2-(acetoxyacetylamino)fluorene	A 98	−	±	Rosin and Stich (1979)	
2-(hydroxyacetylamino)fluorene	A 1538	+ (intestinal S-9)	−	Schut and Thorgeirsson (1979)	
2-(hydroxyacetylamino)fluorene	A 1538	+ + +	+ + +	Jacobs (1977)	
2-(acetylamino)fluorene	A 1538			Jacobs (1977)	
malonaldehyde	A	−	+ + +	Shamberger et al. (1979)	
β-propiolactone	A	−	+ + +	Shamberger et al. (1979)	
benzo[*a*]pyrene	A 98	+ + +	+ + +	Calle and Sullivan (1982)	
7,12-dimethylbenz[*a*]anthracene	A 98			Martin et al. (1981)	
acridine orange	A 100	+ + +	+ + +	Martin et al. (1981)	
1,2-dimethylhydrazine	A G46	+ +	−	Moriya et al. (1979)	host-mediated assay
azoymethane	A G46	+ +	−	Moriya et al. (1979)	host-mediated assay

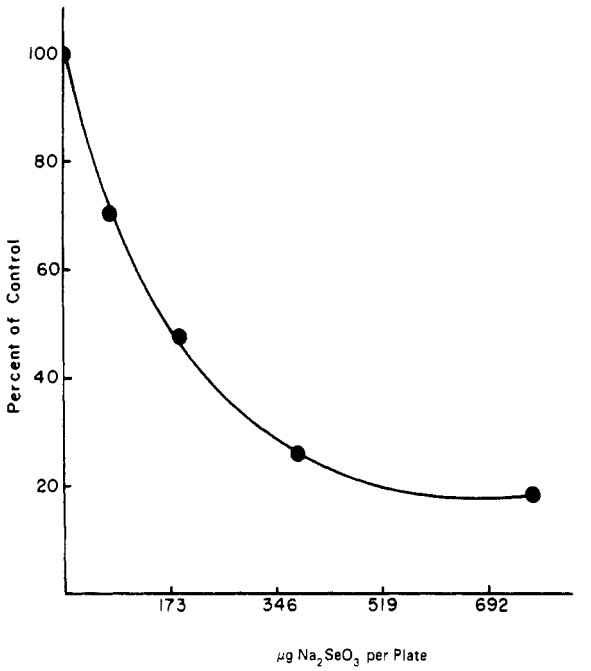

Figure 3. Inhibition of 7,12-dimethylbenz[*a*]anthracene mutagenesis by increasing amounts of selenium. Selenium, 20 μg of DMBA/plate, and S-9 mixture were incorporated into the top agar with *S. typhimurium* test strain TA98. Histidine revertants were scored after 72 h of incubation. Percent of control was calculated by assuming that the number of revertants on plates containing no selenium and 20 μg of DMBA equaled 100. All results are the average of at least three experiments, using three or six plates per variation per experiment. Background spontaneous revertants (34 ± 4) have been subtracted. The average standard deviation was 12.8%.

Table III. Increasing DMBA Levels in the Presence of 20 ppm of Se

μg of DMBA/plate	Na_2SeO_3 revertants[a] −Se	Na_2SeO_3 revertants[a] +20 ppm of Se	% reduction
10	180	113	37
50	259	136	47
100	186	73	61

[a] Strain TA100 served as the test organism.

measured is not indicative of the potential of the metabolites to elicit a cellular transformation.

In a further attempt to understand the antimutagenic effects of selenium, we supplemented the rats' diet or drinking water with sodium selenite (Schillaci et al., 1982). Animals were maintained on their appropriate selenium-supplemented diets for 3 weeks. In these studies, livers were isolated from rats receiving various supplemental selenium levels. These livers were used to prepare the S-9 activation system. DMBA served as the mutagen. Rats were induced with Aroclor 1254 at four levels: 20, 50, 100, or 500 mg of Aroclor 1254/kg of rat body weight. Increasing the quantity of Aroclor administered from 20 to 100 mg/kg resulted in an approximate 280% increase in the number of revertants (Figure 5). S-9 preparations made from livers isolated from rats induced with 20 or 100 mg/kg, and receiving 5 ppm of Se in their drinking water, had an apparent altered metabolic activation activity for DMBA, as measured by mutagenicity. At 20 and 100 mg/kg Aroclor treatment, a 71% and 31% reduction in the number of revertants was observed by using S-9 preparations from selenium-treated animals compared to controls. Treatment of control rats with 500 mg/kg Ar-

Table IV. Inhibition of 7,12-Dimethylbenz[a]anthracene Mutagenesis by Selenium as Sodium Selenite, Sodium Selenate, Selenium Dioxide, and Selenomethionine

concentration, ppm	revertants[a,b]							
	Na_2SeO_3		Na_2SeO_4		SeO_2		Se-methionine	
	-DMBA	+DMBA[c]	-DMBA	+DMBA	-DMBA	+DMBA	-DMBA	+DMBA
no addition	131[a]	388[a]	131[a]	388[a]	131[a]	388[a]	166[a]	420[a]
1	129[a]	435[a]	165[a]	527[b]	148[a]	300[b]	57[b]	127[b]
2	126[a]	312[b]	232[b]	480[b]	138[a]		42[b]	81[c]
4	91[a]	304[b]	231[b]	423[a]	161[a]	278[b]	23[b]	9[d]
no addition	132[a]	395[a]	130[a]	239[a]	153[a]	281[a]		
1	127[a]	243[b]	128[a]	151[b]	133[a]	252[a]		
20	110[a]	259[b]	15[b]	52[c]	122[a]	204[b]		
40	74[b]	189[c]	7[b]	11[c]	103[b]	159[b]		

[a] Results are the average of five plates. The average standard error of the mean was 8.03%. [b] Vertical means with unlike superscripts vary, $p < 0.05$. Strain TA100 served as the test organism. [c] DMBA was added at 50 μg/plate.

Table V. Influence of Form of Selenium and the Presence of DMBA on the Viability of *S. typhimurium* TA100

	colony forming units[a] × 10^8
no addition[b]	7.8[a]
Na_2SeO_3, 5 ppm of Se	6.8[a]
Na_2SeO_3, 20 ppm of Se	5.9[a]
Na_2SeO_4, 5 ppm of Se	7.4[a]
Na_2SeO_4, 20 ppm of Se	8.9[a]
SeO_2, 5 ppm of Se	7.7[a]
SeO_2, 20 ppm of Se	4.0[a]
selenomethionine, 1 ppm of Se	1.5[b]
0.02 mg of DMBA	7.6[a]

[a] Average of three plates. [b] Vertical means with unlike superscripts vary, $p < 0.05$.

Table VI. Influence of Selenium on the Mutagenicity of DMBA and Selected Metabolites

test compound	revertants[a]	
	-Se	+Se (20 ppm)
DMBA[b]	367 ± 47[a]	212 ± 38[b]
7-OHM-12MBA	100 ± 46[a]	0 ± 23[b]
12-OHM-7MBA	93 ± 23[a]	0 ± 42[b]
3-OH-DMBA	149 ± 51[a]	38 ± 37[b]

[a] Horizontal means with unlike superscripts vary, $p < 0.05$. [b] Revertants of strain TA100 are the average of five plates. The average standard error of the mean was 16.1%. Selenium as sodium selenite was added at 20 ppm.

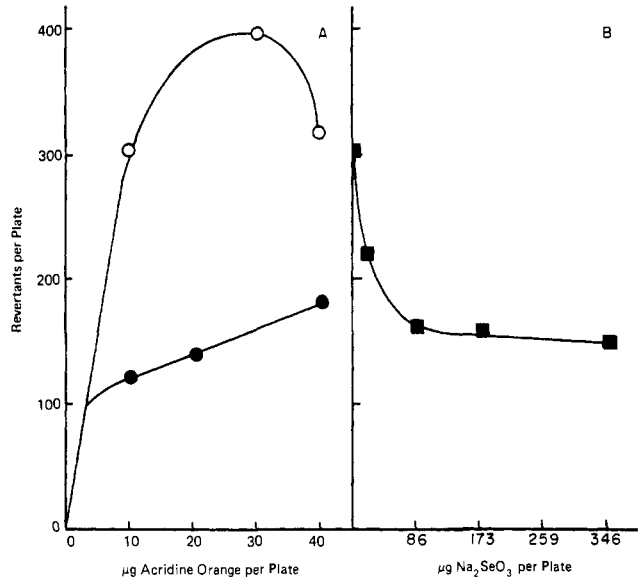

Figure 4. Inhibition of acridine orange mutagenesis by selenium. Acridine orange and selenium were incorporated into the top agar with *S. typhimurium* test strain TA100 and S-9 mixture. Histidine revertants were scored after 72 h of incubation. (A) Open circles, increasing amounts of acridine orange per plate; closed circles, increasing concentration of acridine orange in the presence of 346 μg of sodium selenite. (B) Closed squares, increasing amounts of sodium selenite per plate, in the presence of 20 μg of acridine orange per plate. Results are the average of three experiments using three plates per variation per experiment. Background spontaneous revertants (151 ± 14) have been subtracted. The average standard error of the mean was 7.8% of the reported number of revertants.

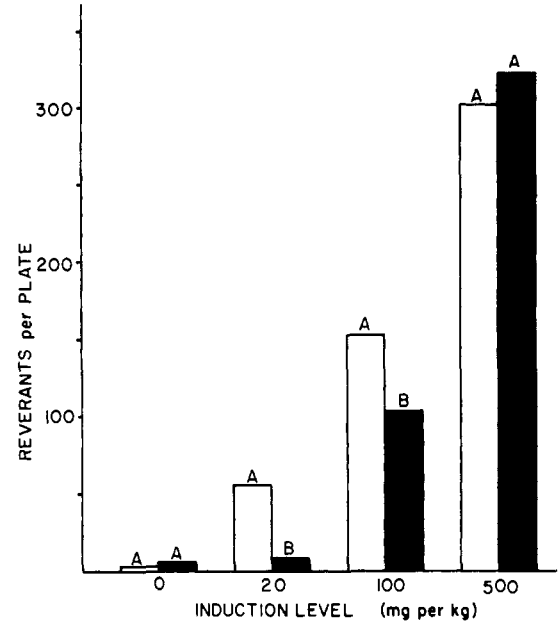

Figure 5. Effects of supplementation of selenium to drinking water on the activation activity of rat liver S-9 preparations. Selenium (5 ppm) as sodium selenite was added to the drinking water for 3 weeks prior to induction with Aroclor. Animals were induced with 0, 20, 100, or 500 mg of Aroclor 1254/kg of body weight 5 days prior to sacrifice. 20 μg of DMBA was added per plate, with *S. typhimurium* TA98 serving as the tester strain. The reported colony forming units (CFU) have had spontaneous revertants (37) subtracted. The open bars represent S-9 derived from rats receiving no selenium supplementation, while the solid bars represent S-9 derived from rats receiving selenium-supplemented drinking water. Values not sharing a common superscript letter differ significantly, $P < 0.05$.

oclor approximately doubled the total number of revertants mediated by their liver S-9 fractions as compared to rats receiving 100 mg/kg. At 500 mg/kg Aroclor no significant difference in the number of revertants mediated by S-9 preparations between control and selenium-supplemented rats was observed.

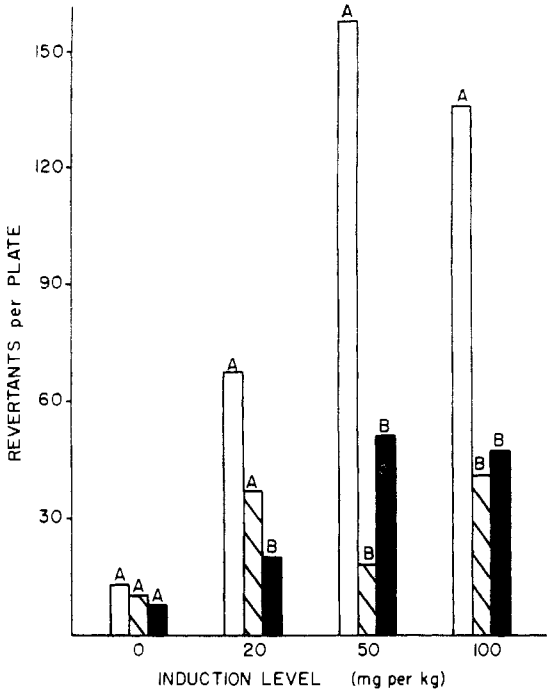

Figure 6. Effects of dietary selenium supplementation on the activation activity of rat liver S-9 preparations. Selenium (0.1, 2.5, and 5 ppm) as sodium selenite was added to the diet for 3 weeks prior to induction. Aminals were induced with either 20, 50, or 100 mg/kg of body weight Aroclor 1254 5 days prior to sacrifice. 20 μg of DMBA was added per plate with *S. typhimurium* TA98 serving as the tester strain. The CFU have had the spontaneous revertants (67) subtracted. The open bars represent S-9 derived from rats fed a diet containing 0.1 ppm of selenium, the striped bars 2.5 ppm of selenium, and the solid bars 5 ppm of selenium. Values not sharing a common superscript letter differ significantly, $p < 0.05$.

Selenium supplementation in the diet also significantly reduced the capability of the rat liver fractions to transform DMBA as indicated by the decrease in mutagenicity (Figure 6). When 2.5 ppm of Se was added to the diet, the number of revertants per plate was reduced 46% when compared to the control 0.15-ppm diet, at the 20 mg/kg level of Aroclor induction. When two other levels of induction were examined, 50 and 100 mg/kg, decreases of 89% and 70%, respectively, were observed. Selenium supplementation at 5 ppm reduced the revertants per plate from 68 to 20 (71% reduction), 154 to 51 (68%), and 136 to 47 (65%) at the 20, 50, and 100 mg/kg induction levels, respectively. The effects of dietary selenium supplementation on the final body weight, daily food intake, plasma selenium level, and S-9 selenium level are shown in Table VII. Supplemental selenium resulted in increases in both the plasma and S-9 selenium levels.

When selenium was added in vitro, a 52% reduction in Ames test activity resulted with a Se/DMBA molar ratio of $10^{1.1}$ (Martin et al., 1981). When rats were given supplemental selenium, the molar ratio of Se/DMBA in the S-9 was $10^{-2.29}$. This represents a more than 3 log difference in the relative amount of selenium. It is, therefore, doubtful that selenium per se directly affected the Ames test but rather altered the rats' microsomal enzymatic activities.

In summary, these studies strongly suggest that selenium alters the ability of liver activation enzymes to transform DMBA into its ultimate carcinogenic forms. In support of this theory, Marshall et al. (1979) reported a shift in the production of 2-(acetylamino)fluorene metabolites by supplementation of selenium (4 ppm) to the drinking water of rats. Liver microsomes from rats receiving selenium produced more ring hydroxylation and less N-hydroxylation products than control rats, thereby shifting metabolism toward detoxification. In our first experiments, in which selenium was added in vitro, it may have been effective by one or more of the following modes of action. (i) Selenium may react directly with the ultimate carcinogen, preventing it from interacting with DNA. (ii) Selenium may stimulate or activate enzymes involved in the detoxification of the ultimate carcinogen. Or (iii) selenium may inhibit the activation enzymes. When selenium was administered in the diet, it may have been effective due to (i) direct chemical interaction with the ultimate carcinogen, although this is unlikely as the in vivo levels were not greatly increased, (ii) selenium supplementation may have caused the induction of detoxification enzymes, or (iii) selenium may have caused the induction of activation enzymes that hydroxylate at sites other than those that form the ultimate carcinogen. Research in our laboratories is currently under way in order to determine the mechanisms by which selenium reduces mutagenicity.

Table VII. Effects of Supplemental Selenium on Food Intake, Weight Gain, and in Vivo Concentration

dietary selenium, ppm[a]	final body weight, g	daily food intake, g	plasma selenium, μg/g	S-9 selenium, μg/g
	Experiment 1: Water Supplementation			
0	185		0.50	0.42
5	141		0.85	0.59
	Experiment 2: Diet Supplementation			
0.1	153	12.6	0.56	0.42
2.5	123	12.4	0.70	0.59
5.0	124	11.0	0.91	0.65

Registry No. 7,12-DMBA, 57-97-6; 7-OHM-12-MBA, 568-75-2; 12-OHM-7-MBA, 568-70-7; 7,12-diOHMBA, 2564-65-0; 3-OH-DMBA, 57266-83-8; 5,6-diOH-DMBA, 16033-60-6; Se, 7782-49-2; Na_2SeO_3, 10102-18-8; SeO_2, 7446-08-4; Na_2SeO_4, 13410-01-0; acridine orange, 65-61-2; selenomethionine, 1464-42-2; Aroclor 1254, 11097-69-1.

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Antioxidants and Cancer

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As a corollary of the free radical theory of carcinogenesis, antioxidants have been postulated to exert an anticarcinogenic effect by inhibiting the activation of carcinogens via oxidative processes in vivo, stabilizing potential precursors of carcinogens or cocarcinogens such as polyunsaturated fatty acids, and acting as scavengers of genotoxic free radicals. High concentrations of the food antioxidants BHA and BHT protect against chemically induced neoplasia in animals by a mechanism that appears to involve their capacity to accelerate the catabolism and excretion of carcinogens via the mixed function oxidase complex rather than their antioxidant properties. Also, the protective effect of diets high in selenium against chemically induced carcinogenesis appears to be unrelated to the normal physological role of this element as a constituent of the enzyme glutathione peroxidase. The natural antioxidants vitamin E and vitamin C are less active as inhibitors of chemical carcinogenesis, but they inhibit the formation of potentially carcinogenic products of oxidative rancidity in foods and their action in quenching genotoxic free radicals is suggestive of an anticarcinogenic effect in vivo.

Interest in the possible anticarcinogenic effect of antioxidants stems from the free radical theory of carcinogenesis, according to which tumor formation is a result of the genotoxic action of oxy and peroxy radicals formed by one-electron oxidation of carcinogens, or of reduced oxygen species such as hydroxyl and superoxide radicals, hydrogen peroxide, and organic peroxides formed as secondary products (Ts'o et al., 1977). Evidence in support of this hypothesis includes the demonstration that ionizing radiation produces reduced oxygen compounds in biological materials (Bielski and Gebicki, 1977), that some organic peroxides have mutagenic and tumorigenic activity (Kotin and Falk, 1963), that diets high in polyunsaturated fatty acids predispose to chemically induced carcinogenesis (King et al., 1979; Hopkins et al., 1981; Ip, 1982), and that malonaldehyde, a decomposition product of fatty acid peroxides formed in vivo, is carcinogenic (Shamberger et al., 1974; Bird et al., 1982). The demonstration that under certain conditions vitamin E and certain synthetic antioxidants as well as selenium, a constituent of a peroxide-decomposing enzyme, have anticarcinogenic activity in animals has provided further support for this hypothesis.

As a corollary of this theory, antioxidants have been postulated to exert their anticarcinogenic effect by inhibiting the activation of carcinogens via oxidative processes in vivo, by stabilizing potential precursors of carcinogens or cocarcinogens such as polyunsaturated fatty acids, and by acting as scavengers of genotoxic free radical intermediates. However, there is no single mechanism that explains the anticarcinogenic action of all antioxidants, and recent research indicates that the inhibitory effect of some such compounds is due in part to their ability to alter the action of enzymes involved in the metabolism of carcinogens rather than to their antioxidant properties per se.

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