[CANCER RESEARCH 50, 1206–1211, February 15, 1990]

Activity of Methylated Forms of Selenium in Cancer Prevention[1]

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ABSTRACT

The anticarcinogenic activity of selenium in animal models is well established. The active forms of selenium involved have not been identified to date, but conversion of selenium via hydrogen selenide (H_2Se) to methylated forms such as dimethylselenide and trimethylselenonium ion is an important metabolic fate. By controlling the entry of selenium into various points within this pathway through selection of appropriate starting compounds, it is possible to pinpoint more closely the form(s) of selenium responsible for its anticarcinogenic activity. Selenobetaine in the chloride form [$(CH_3)_2Se^+CH_2COOH$] and its methyl ester are extensively metabolized in the rat to mono-, di-, and trimethylated selenides, largely bypassing the inorganic H_2Se intermediary pool. The chemopreventive efficacy of these selenobetaines was determined at 1 and 2 ppm selenium supplemented in the diet throughout the duration of the experiment using the dimethylbenz(a)anthracene induced mammary tumor model in rats. There was a dose-dependent inhibitory response to both compounds, and they appeared to be slightly more active than selenite. These doses were without any adverse effects on the animals. Coadministration of selenobetaine with arsenite (5 ppm arsenic) enhanced the tumor-suppressive effect of selenobetaine, although arsenic by itself was totally inactive. Arsenite is known to inhibit certain steps in selenium methylation. The substantial prophylactic efficacy of methylated selenides and the enhancement by arsenite suggest that partially methylated forms of selenium may be directly involved in the anticarcinogenic action of selenium.

INTRODUCTION

With few exceptions, the selenium compounds that have been examined in previous animal cancer chemoprevention experiments were those readily available from commercial sources. Over 90% of such studies reported in the literature have used either selenite or selenomethionine as the test reagent (1). In general, selenite is more effective than selenomethionine in inhibiting the development of chemically induced tumors (2, 3) as well as the growth of implanted neoplastic cells (4, 5). In addition, two synthetic selenium compounds, *p*-methoxybenzeneselenol and benzylselenocyanate, have also been found to have cancer-inhibitory activity (6–10). Recently, we have been exploring a postulate that both selenomethionine and selenite must be further metabolized in order to exert their anticarcinogenic activities. Two lines of indirect evidence are cited below in support of this hypothesis: (*a*) the prophylactic efficacy of selenomethionine is greatly compromised under a situation in which selenomethionine is preferentially incorporated into tissue proteins in place of methionine (11); and (*b*) the chemopreventive action of selenite is almost completely abolished by coadministration of arsenite which is known to interfere with the formation of methylated selenium metabolites (12). These two pieces of information, together with an earlier observation that a continuous intake of supplemental selenium is necessary to achieve maximal protection against cancer (13), suggest that some active species of selenium with antitumorigenic potential and with a relatively short half-life is generated only when the supply of selenium is maintained at a certain level.

Prior to developing the rationale of the design of novel selenium compounds that will provide clues towards identification of the active form(s) involved in cancer prevention, it is essential to appreciate how selenium is metabolized by the animals (14). As shown in Fig. 1, selenite (SeO_3^{2-}) is reduced to hydrogen selenide (H_2Se) via selenodiglutathione (GS-Se-SG) and glutathione selenopersulfide (GS-SeH). Hydrogen selenide is an important intermediate because the selenium in this pool can be channeled either to the assimilatory pathway of selenium utilization in the synthesis of selenoproteins such as glutathione peroxidase (15, 16) or to the detoxification pathway of sequential methylation by *S*-adenosylmethionine to methylselenol [CH_3SeH], dimethylselenide (CH_3SeCH_3), and trimethylselenonium ion [$(CH_3)_3Se^+$]. Dimethylselenide is exhaled in the breath when large amounts of selenite are administered, while trimethylselenonium is one of the metabolites identified in the urine associated with either normal or high selenium intake (14).

We have focused our attention on synthetic selenium compounds that can enter the metabolic pathway beyond H_2Se. The two prototypes of the second generation selenium compounds that were tested in the present study for their anticarcinogenic activities are selenobetaine [$(CH_3)_2Se^+CH_2COOH$] and its methyl ester. Using ^{14}C and ^{75}Se doubly-labeled substrates, Foster *et al.* (17) have provided evidence that selenobetaine tends to lose a methyl group before scission of the CH_3Se—CH_2COOH bond to form methylselenol (Fig. 1, *Box A*); whereas selenobetaine methyl ester tends to undergo facile breakage of the $(CH_3)_2Se$—$CH_2CO_2CH_3$ bond to form dimethylselenide directly (Fig. 1, *Box B*). By feeding these relatively stable, nonvolatile compounds, it is possible to generate *in vivo* a higher proportion of methylated selenides compared to selenite, and to vary the proportion of doubly-methylated *versus* monomethylated selenides entering the pathway. The present paper therefore reports the effect of chronic selenobetaine and selenobetaine methyl ester administration at 2 different doses on the DMBA[2]-induced mammary tumor model in female rats. Comparable levels of selenite were included as positive control groups since there is a substantial body of data on the inhibitory responses to selenite.

A useful extension of this approach is to test synthetic organoselenium compounds that do not release selenium to the inorganic pool. Synthesis of selenoproteins such as glutathione peroxidase would be precluded, and, more generally, the question of whether selenium must flow through the inorganic H_2Se pool in order for its anticarcinogenic activity to be manifested could be addressed. Ebselen [2-phenyl-1,2-benzisoselenazol-3(2*H*)-one] is a synthetic selenium compound with intrinsic antioxidant and antiinflammatory properties (18). In contrast to selenite and selenobetaine, ebselen apparently does not release selenium to the inorganic H_2Se or methylselenol pools. Several ebselen metabolites have been identified in the liver

Received 5/11/89; revised 10/30/89; accepted 11/9/89.

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[1] This project was supported by Grant CA45164 from the National Cancer Institute, NIH, and by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, WI. Preliminary reports were presented at the 80th annual meeting of the American Association for Cancer Research, San Francisco, May 1989, and at the Joint AACR/Japanese Cancer Association Meeting in Honolulu, May 1989.

[2] The abbreviation used is: DMBA, dimethylbenz(a)anthracene.

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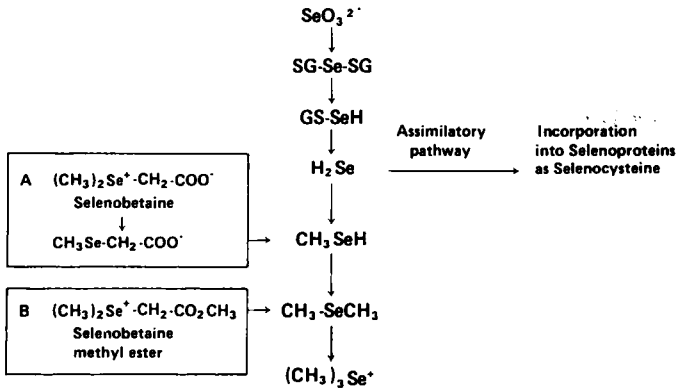

Fig. 1. This schematic flow chart shows the metabolism of selenite ($SeO_3^{=}$) via reduction and methylation reactions, as illustrated in the center portion of the diagram. It also shows that hydrogen selenide (H_2Se) is the precursor for selenium incorporation into selenoproteins. *Box A* and *Box B* indicate the main sites where selenobetaine and its methyl ester enter the metabolic pathway. Refer to the "Introduction" for further detail.

perfusion system (19). In all of these metabolites, selenium remains attached to the phenyl moiety. *In vivo* metabolism studies in plasma and urine also showed that all metabolites of ebselen have in common that the isoselenazolone ring is opened and that selenium glucuronide is the major metabolite (20). Thus for the purpose of our study, ebselen represents an organic selenium-containing reagent in which the selenium is not bioavailable (21).

In view of our previous finding that arsenite reduces the effectiveness of selenite in chemoprevention but enhances that of trimethylselenonium ion (12), the selenobetaine and selenobetaine methyl ester experiments were carried out in the absence and presence of arsenite in order to evaluate how arsenite would affect the activity of these two novel selenium compounds.

MATERIALS AND METHODS

Diet and selenium Supplementation. Female Sprague-Dawley rats 40 days of age were purchased from Charles River Breeding Laboratories, Wilmington, MA. They were maintained on the AIN-76A diet (substituting dextrose for sucrose) as described previously (22) for the entire duration of the experiment. The AIN-76 mineral mix used in the diet provided 0.1 ppm selenium as sodium selenite. For the mammary cancer chemoprevention studies, additional selenite, selenobetaine, selenobetaine methyl ester, or ebselen was added to the basal diet starting 1 week before DMBA administration and continued until the animals were sacrificed. Selenite was supplemented at 3 different dose levels: 1, 2, or 3 ppm selenium. Selenobetaine and its methyl ester were added to the diet at 1 or 2 ppm selenium, with or without 5 ppm arsenic in the form of sodium arsenite. Ebselen was present in the diet at a concentration of 10 ppm selenium. All diets were prepared in batches every week and stored in the cold room. Fresh food was offered to the animals every 2 days (every 3 days on weekends); any diet left uneaten in the food cup was discarded. The selenium content of the various diets was regularly checked for quality control.

Mammary Tumor Induction.. Mammary tumors were induced by intragastric administration of 10 mg DMBA (Sigma) between 7 and 8 weeks of age (23). Rats were palpated weekly to determine the appearance and location of tumors and were killed between 24 and 25 weeks after DMBA treatment. At autopsy, the mammary gland was exposed for the detection of nonpalpable tumors. Only confirmed adenocarcinomas were reported in the results. Tumor incidences at the final time point were compared by χ^2 analysis and the total tumor yield compared by frequency distribution analysis as described previously (24).

selenium Compounds. Ebselen was a gift from Ciba-Geigy Pharmaceuticals Division, Suffern, NY. Selenobetaine was synthesized by reaction of dimethylselenide with 2-bromoacetic acid in nitromethane:H_2O (1:1) overnight at 25°C (25). Selenobetaine methyl ester was synthesized similarly using methyl bromoacetate at 0°C. The water phase from the reaction mixture was applied to a SP-Sephadex (H^+) column and eluted with 0.01 N HCl at room temperature. Under these conditions selenobetaine was retarded and came off after other reaction products; selenobetaine methyl ester was retained on the column and was eluted with 0.25 M ammonium formate (pH 4). Purity of the compounds was assessed using thin layer electrophoresis on cellulose plates at 10°C in pyridine:acetic acid:water (20:5:2000), pH 5.3. Selenonium compounds were located by spraying with Dragendorff's reagent (25).

Biochemical Analysis. selenium concentrations in blood, liver, and mammary gland from rats in the DMBA carcinogenesis experiments were determined by the fluorometric procedure of Olson *et al.* (26). The ability of ebselen to maintain liver selenium-dependent glutathione peroxidase activity was evaluated in a selenium depletion/repletion protocol. Weanling rats were fed the AIN-76A diet without selenium in the mineral mix for 3 weeks. Our analysis indicated that this selenium-deficient diet contained approximately 0.03–0.04 ppm Se. The animals were then divided into the following groups (6/group) and maintained for an additional 3 weeks on these dietary treatment: selenium-deficient diet; selenite supplementation (0.1 ppm selenium); or ebselen (10 ppm selenium). Liver glutathione peroxidase activity in the 105,000 × *g* cytosol fraction was measured by the coupled assay procedure of Paglia and Valentine (27) using hydrogen peroxide as the substrate.

RESULTS

In an initial 40-day toxicological study, we had already ascertained that the growth rate of rats fed up to 2 ppm selenium as either selenobetaine or selenobetaine methyl ester, with or without 5 ppm arsenic in the diet, was identical to that of controls given the basal regimen containing 0.1 ppm selenium as selenite.[3] Thus we were confident that changes in weight gain would not be a confounding factor in the interpretation of the DMBA carcinogenesis experiment involving these compounds administered chronically at 1 or 2 ppm selenium. Fig. 2 illustrates the cumulative appearance of palpable mammary tumors as a function of time after DMBA intubation in a total of 13 treatment groups which were all set up in a single design. There were 30 rats in each group. Fig. 2*A* shows the results from the 2 control groups given either the basal diet containing 0.1 ppm selenium or the basal diet plus 5 ppm arsenic. The rate of tumor appearance was quite similar between these two groups, suggesting that arsenic by itself had no effect on mammary carcinogenesis. The selenite data from 3 different doses (1, 2, and 3 ppm selenium) are shown in Fig. 2*B*. The dose-response relationship and the magnitude of inhibition of tumorigenesis at these selenium levels were within our expectation based on previous experiences. The coadministration of selenite and arsenite was omitted from the current design because of the already enormous scope of the study (close to 400 rats used) and also because we have recently reported (12) that arsenite diminished significantly the inhibitory response to 3 ppm selenite selenium. Fig. 2*C* summarizes the selenobetaine results at 1 or 2 ppm selenium, with or without arsenic. It appeared that selenobetaine by itself was slightly more active than selenite in chemoprevention, as evidenced by the dose-related biopotency data showing that selenobetaine at 1 and 2 ppm selenium was approximately equivalent to 2 and 3 ppm selenium from selenite. Interestingly, arsenite was found to enhance the protective efficacy of selenobetaine, especially at the higher level of supplementation of 2 ppm selenium. The selenobetaine methyl

[3] Unpublished data.

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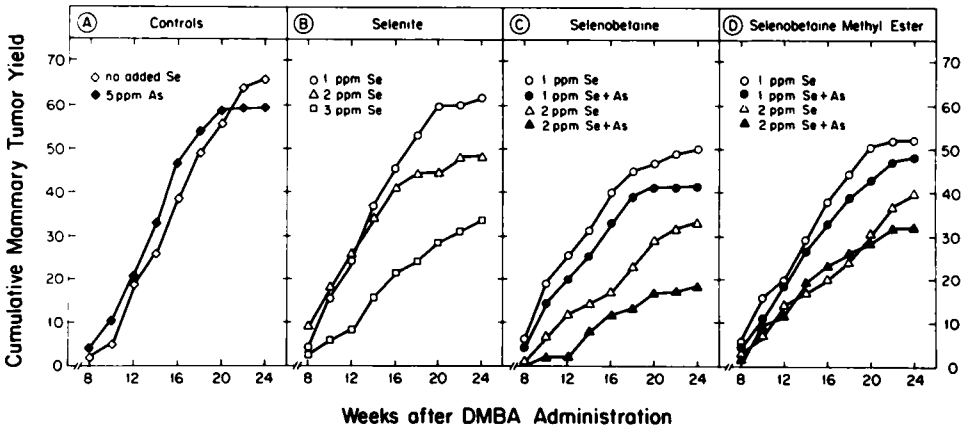

Fig. 2. Cumulative appearance of palpable mammary tumors as a function of time after DMBA administration. Three selenium compounds were investigated in these chemoprevention experiments: selenite (*B*), selenobetaine (*C*), and selenobetaine methyl ester (*D*). Two control groups were also included (*A*): no added selenium (with only 0.1 ppm selenium in the basal diet) and arsenic supplementation alone. There were 30 rats/group.

ester data, as depicted in Fig. 2*D*, are quite similar qualitatively to the selenobetaine experiment, although the arsenite effect was dampened considerably. Thus on a comparable selenium weight basis (1 or 2 ppm), the methyl ester was equal to the parent compound in its effectiveness in protection against mammary carcinogenesis, but there was minimal potentiation of its activity by arsenite.

The complete mammary tumor data at autopsy are summarized in Table 1. Nonpalpable tumors found at the time of killing the animals were included in all the calculations. The outcome of statistical comparisons between the control and experimental groups is indicated in Table 1, Footnote *g*. Overall, the tumor incidence data paralleled closely the tumor yield data, although the latter probably represented a more sensitive marker of inhibitory responses. In general, it can be seen that statistical significance of tumorigenesis suppression is achieved only at higher levels of selenium supplementation, and often when arsenite is also present in the diet. Changes induced by the selenium compounds in the other 3 parameters listed in Table 1 (number of tumors per tumor-bearing rat, latency period of tumor appearance, and mean tumor weight) were only minimal, although the trend towards a lower tumor multiplicity in the selenium-treated rats certainly confirmed the reduced tumor incidence and yield as mentioned above. It is interesting to point out that the lack of a striking effect on the number of tumors per tumor-bearing rat has been observed previously with selenite and selenomethionine (11–13). In other words, those rats which develop at least one tumor will have, on the average, close to the same number of tumors independent of treatment. Thus the major effect of selenium is to reduce the number of tumor-bearing rats. This implies that there may be differences in sensitivity to selenium-mediated inhibition of tumorigenesis among individual animals.

The body weights, organ weights, and tissue selenium levels of the DMBA-treated rats are presented in Table 2. The mean body weights (shown at 6, 14, and 24 weeks after DMBA) of all 13 groups of rats were very close to each other, suggesting that chronic feeding of selenite, selenobetaine, and its methyl ester at these doses did not affect the growth of the animals and that the suppression of tumorigenesis by these selenium compounds was independent of selenium toxicity. As expected, there was no change in the weight of liver, kidney, and spleen in any of the selenium-treated rats compared to the control group.

Tissue selenium levels in these DMBA-treated rats are also shown in Table 2. Ingestion of selenite, selenobetaine, and selenobetaine methyl ester resulted in an increase in selenium concentrations in blood, liver, and mammary gland; the mag-

Table 1 *Mammary tumor data at autopsy of DMBA-treated rats given different selenium compounds with or without arsenite*[a]

Treatment group	Final tumor incidence	Total tumor yield[b]	Tumors/TBR[c]	Latency period[d] (wk)	Mean tumor wt[e] (g)
Control	25/30 (83%)	71	2.8	15	1.9 ± 0.3
Arsenite[f]	27/30 (90%)	65	2.4	13	2.3 ± 0.4
Selenite					
1 ppm selenium	24/30 (80%)	66	2.8	13	1.4 ± 0.3
2 ppm selenium	21/30 (70%)	52	2.5	12	1.9 ± 0.4
3 ppm selenium	17/30 (57%)[g]	38[g]	2.2	15	1.7 ± 0.3
Selenobetaine					
1 ppm selenium	19/30 (63%)	52	2.7	12	2.0 ± 0.3
1 ppm selenium + arsenic	19/30 (63%)	46[g]	2.4	12	1.8 ± 0.3
2 ppm selenium	14/30 (47%)[g]	35[g]	2.5	16	1.5 ± 0.3
2 ppm selenium + arsenic	10/30 (33%)[g]	20[g]	2.0	14	1.7 ± 0.3
Selenobetaine methyl ester					
1 ppm selenium	24/30 (80%)	55	2.3	14	1.8 ± 0.2
1 ppm selenium + arsenic	22/30 (73%)	51	2.3	13	2.1 ± 0.3
2 ppm selenium	18/30 (60%)	44[g]	2.4	16	1.8 ± 0.4
2 ppm selenium + arsenic	17/30 (57%)[g]	38[g]	2.2	13	1.7 ± 0.4

[a] Rats were killed 24–25 weeks after DMBA administration.
[b] Includes both palpable and nonpalpable tumors.
[c] TBR, tumors/tumor-bearing rat.
[d] Median time to appearance of all tumors.
[e] Mean ± SE.
[f] Arsenite was present in the diet as 5 ppm arsenite arsenic.
[g] $P < 0.05$ compared to the corresponding control value.

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Table 2 *Body weights, organ weights, and tissue selenium levels at autopsy of DMBA-treated rats given different selenium compounds with or without arsenite*[a]

	Body wt at times after DMBA (g)			Organ wt (g/100 g body wt)			Tissue selenium (μg/ml or g wet wt)		
	6 wk	14 wk	24 wk	Liver	Kidney	Spleen	Blood	Liver	Mammary gland
Control	231 ± 3	278 ± 4	297 ± 4	3.4 ± 0.1	0.71 ± 0.01	0.18 ± 0.01	0.40 ± 0.02	0.55 ± 0.04	0.08 ± 0.02
Arsenite	229 ± 3	281 ± 5	295 ± 5	3.2 ± 0.1	0.69 ± 0.01	0.16 ± 0.01	0.42 ± 0.02	0.59 ± 0.05	0.08 ± 0.02
Selenite									
1 ppm selenium	234 ± 4	282 ± 5	302 ± 6	3.3 ± 0.1	0.65 ± 0.01	0.18 ± 0.01	ND[b]	ND	ND
2 ppm selenium	231 ± 4	280 ± 5	298 ± 7	3.2 ± 0.1	0.72 ± 0.02	0.19 ± 0.01	0.62 ± 0.04[c]	1.1 ± 0.1[c]	0.19 ± 0.02[c]
3 ppm selenium	227 ± 4	275 ± 6	290 ± 7	3.3 ± 0.2	0.68 ± 0.01	0.19 ± 0.01	0.83 ± 0.05[c]	1.4 ± 0.1[c]	0.26 ± 0.03[c]
Selenobetaine									
1 ppm selenium	232 ± 4	277 ± 5	294 ± 6	3.6 ± 0.2	0.73 ± 0.02	0.20 ± 0.01	ND	ND	ND
1 ppm selenium + arsenic	233 ± 5	273 ± 5	291 ± 6	3.3 ± 0.1	0.71 ± 0.01	0.18 ± 0.01	ND	ND	ND
2 ppm selenium	230 ± 4	271 ± 4	290 ± 5	3.6 ± 0.1	0.69 ± 0.02	0.17 ± 0.01	0.51 ± 0.05	1.0 ± 0.1[c]	0.14 ± 0.02[c]
2 ppm selenium + arsenic	233 ± 4	275 ± 5	292 ± 7	3.4 ± 0.1	0.70 ± 0.01	0.16 ± 0.01	0.64 ± 0.05[c]	1.4 ± 0.1[c, e]	0.19 ± 0.02[c]
Selenobetaine methyl ester									
1 ppm selenium	234 ± 3	279 ± 4	300 ± 5	3.4 ± 0.1	0.69 ± 0.01	0.18 ± 0.01	ND	ND	ND
1 ppm selenium + arsenic	230 ± 4	277 ± 4	297 ± 6	3.2 ± 0.2	0.72 ± 0.01	0.16 ± 0.01	ND	ND	ND
2 ppm selenium	228 ± 4	276 ± 5	294 ± 6	3.6 ± 0.1	0.68 ± 0.01	0.17 ± 0.01	0.47 ± 0.05	0.74 ± 0.08[c, d]	0.12 ± 0.02[d]
2 ppm selenium + arsenic	227 ± 4	274 ± 6	299 ± 7	3.5 ± 0.1	0.71 ± 0.02	0.16 ± 0.01	0.54 ± 0.06[c]	0.98 ± 0.07[c, e]	0.14 ± 0.02[c]

[a] Results are expressed as mean ± SE.
[b] ND, not determined.
[c] $P < 0.05$ compared to corresponding control value.
[d] $P < 0.05$ compared to corresponding 2 ppm selenite selenium value.
[e] $P < 0.05$ compared to corresponding selenobetaine or selenobetaine methyl ester value without arsenic.

nitude of the increase was more pronounced in the latter two organs compared to the increase in the blood. There was a trend towards lower selenium accumulation with selenobetaine and the methyl ester (in the absence of arsenic) compared to selenite, but only the selenobetaine methyl ester values quality for statistical significance (Table 2, Footnote *d*). In contrast, the coadministration of arsenic seemed to result in higher selenium retention in rats given selenobetaine and its methyl ester compared to those given the selenium compounds alone; however, the difference is significant only with selenobetaine and only in the liver (Table 2, Footnote *e*). Thus, even though tissue selenium level is clearly dependent on intake, it is not a particularly reliable and consistent marker for host protection against tumorigenesis.

Results of the DMBA-induced carcinogenesis experiment with ebselen showed that ebselen has no cancer-chemopreventive activity, at least at the dose of 10 ppm selenium tested here. The final tumor incidences of the 2 groups were: control, 76%; ebselen, 68%. The total mammary tumor yield (25 rats/group) was 42 for the control group and 38 for the ebselen-treated group. Ebselen, at a level of 10 ppm selenium in the diet, was well tolerated by the animals with no adverse effect on growth. The ability of ebselen to restore hepatic glutathione peroxidase activity following selenium deprivation was evaluated in a selenium depletion/repletion protocol as described in "Materials and Methods." Results presented below are expressed as a percentage of the control activity from rats that were maintained throughout on the basal diet containing 0.1 ppm selenium: continuous 6-week selenium depletion, 34%; 3-week selenium depletion/3-week repletion by 0.1 ppm selenite selenium, 96%; 3-week selenium depletion/3-week repletion by 10 ppm ebselen selenium, 31%. Thus it can be concluded that unlike selenite, the selenium in ebselen is not released into the H_2Se pool for incorporation into selenoproteins such as glutathione peroxidase. This experiment further reinforces the notion that the selenium moiety must be converted to some active form for prevention of tumorigenesis.

DISCUSSION

The most significant implication of the selenobetaine and selenobetaine methyl ester chemoprevention experiments is that the partially methylated selenides may be directly involved in the anticarcinogenic action of selenium. Our understanding of how selenobetaine and its methyl ester enter the selenium metabolic pathway (refer to Fig. 1), as detailed in the previous work by Foster *et al.* (17), gave us the opportunity to select for two starting selenium compounds that can generate large amounts of methylated selenium metabolites independent of the intermediary pool of inorganic H_2Se. The data in this paper indicate that the two selenobetaines are at least as effective compared to inorganic selenite in cancer protection. The fact that coadministration of arsenite had diametrically opposed effects on the activity of selenite and selenobetaine supports a mode of action of the methylated selenides independent of the metabolic pool entered by selenite. It is possible that the anticarcinogenic effects of selenobetaine might be exerted without the involvement of selenoproteins as a class, as exemplified by glutathione peroxidase; some role involving selenium-binding proteins (28) cannot be ruled out.

The mechanism of action by which arsenite enhances the anticarcinogenic activity of selenobetaine is unknown. Arsenite is known to interfere with the formation of dimethylselenide by inhibiting the microsomal thiol-*S*-methyltransferase that uses *S*-adenosylmethionine to methylate H_2Se (29). The same enzyme can methylate methylselenol to form dimethylselenide and possibly could methylate the latter to form trimethylselenonium. However, a recent report from Hoffman's laboratory suggests that there is a thioether-*S*-methyltransferase enzyme present in the lung which is specific for the final methylation reaction and which is not sensitive to arsenic (30). This newly characterized enzyme may account for part, but not necessarily all, of the conversion of dimethylselenide to trimethylselenonium. Through arsenic-mediated inhibition of the methyltransferase reaction, the partially methylated selenium metabolites, such as methylselenol or possibly dimethylselenide, could be expected to accumulate. The fact that arsenic could potentiate the anticarcinogenic activity of selenobetaine is a further indication that the methylated selenides are important metabolites for cancer prevention. Our data in Fig. 2 also indicate that the arsenic effect with selenobetaine methyl ester is much attenuated compared to that with selenobetaine. This could be ex-

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plained by reasoning that the further along the methylation pathway at which selenium is introduced, the less inhibition by arsenic will become apparent, and more of the selenium metabolites will be fully methylated to trimethylselenonium and excreted in the urine. Furthermore, there is good justification to expect that the arsenic effect on selenobetaine methyl ester would be minimal if a significant share of dimethylselenide conversion to trimethylselenonium is catalyzed by the new arsenic-insensitive thioether-*S*-methyltransferase enzyme as reported by Hoffman's group (30).

If the methylated selenides are indeed active species in cancer prevention, what could be their mechanism of action? Dimethylselenide, as a small hydrophobic molecule, might have activity by occupying hydrophobic sites in critical macromolecules. Monomethylated derivatives of selenium might form mixed selenenyl sulfide derivatives of proteins (PS-$SeCH_3$), analogous to inactivation of proteins through mixed disulfide formation with methylmercaptan, a toxic product of methionine metabolism. By the same token, formation of methylselenylated bases in nucleic acids might also occur (31). Even though reduction is a characteristic feature of selenium metabolism, there is the possibility that mono- and dimethylated selenide intermediates might undergo oxidation, as an alternative to further methylation, forming methylseleninic acid (CH_3SeO_2H) or dimethylselenoxide (CH—SeO—CH_3). Although evidence for their formation is almost nonexistent, such metabolites might be significant with regard to the biological effects of selenium at high levels of administration. Of interest is the study by Palmer *et al.* (32) in which various forms of selenium were injected into chick embryo, a closed system where there is no excretion of selenium and where the detoxifying enzymes might be poorly developed. They found that methylseleninic acid was much more toxic than selenate, selenite, selenoamino acids, dimethylselenoxide, or trimethylselenonium. Thus, monomethylated forms of selenium may be more cytotoxic than the nonmethylated or the fully methylated forms. On the other hand, dimethylselenoxide, as a more reactive analogue of dimethyl sulfoxide, might mimic the free radical-scavanging properties of dimethylsulfoxide (33) and thereby alter critical stages in carcinogenesis.

In our carcinogenesis experiments reported here, selenobetaine and the methyl ester were given to the animals beginning 1 week before DMBA administration and continued until sacrifice. Thus the action of these selenium compounds could be exerted at either the initiation or promotion stage of carcinogenesis, or both. This design is intentional, because when the chemopreventive effect of selenite was first characterized by one of the authors a decade ago (34), the supplementation of selenite was maintained throughout the initiation and promotion phases. Subsequently it was found that the protective effect of selenite, at least in the DMBA model, was primarily expressed during the tumor progression period (35). We had no *a priori* knowledge of whether selenobetaine would be effective in cancer prevention, and if so, how it would affect the carcinogenic process. On this basis, we decided to expose the animals to these second generation selenium compounds before, during, after DMBA treatment to cover all eventualities. Future experiments will be refined to delineate their role in initiation *versus* neoplastic progression. In closing, as we have pointed out previously (3, 12), selenium metabolism is a key area of future research in developing agents and strategies for chemoprevention.

ACKNOWLEDGMENTS

The authors are grateful to Cassandra Hayes, Todd Parsons, Rita Pawlak, Janet Treichel, and Robert Burrow for their technical assistance with the experiments and to Cathy Russin for her help in preparation of the manuscript.

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