[CANCER RESEARCH 51, 4613–4617, September 1, 1991]

Inhibition of 7,12-Dimethylbenz(*a*)anthracene-induced Mammary Tumors and DNA Adducts by Dietary Selenite[1]

Jinzhou Liu, Karen Gilbert, Helen M. Parker, Wanda M. Haschek, and John A. Milner[2]

Division of Nutritional Sciences [J. L., J. A. M.], Department of Food Sciences [K. G., H. P., J. A. M.], and Department of Veterinary Pathobiology [W. H.], University of Illinois, Urbana, Illinois 61801

ABSTRACT

The present studies were designed to examine the influence of dietary selenite supplementation on the initiation phase of 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced mammary carcinogenesis and to correlate selenite-induced changes in the binding of DMBA metabolites to rat mammary cell DNA with the ultimate tumor incidence. Diets formulated to contain selenium, as sodium selenite at 0.1, 0.5, 1, 2, or 4 μg/g were fed for 2 weeks prior to and 2 weeks following treatment with DMBA (5 mg/kg body weight). Food intake and weight gain did not differ among treatments. Tumor incidence correlated inversely to the quantity of selenium consumed ($r = -0.99$). Final tumor incidences were 52, 32, 24, 14, and 10% for rats fed 0.1, 0.5, 1, 2, and 4 μg selenium/g, respectively. In a separate group of rats fed a diet containing 4 μg selenium/g during both the initiation and promotion stages the final tumor incidence was 4.8%. Selenite supplementation for 2 weeks markedly depressed the occurrence of individual and total DMBA-DNA adducts. The final mammary tumor incidence correlated positively with total DMBA-DNA adducts ($r = 0.99$). These studies clearly demonstrate that selenite can inhibit the initiation stage of mammary carcinogenesis. This reduction in tumor incidence is likely due to a reduction in carcinogen metabolism and ultimately adduct formation.

INTRODUCTION

Epidemiological studies reveal an increased incidence of breast, colon, rectum, and other cancers in individuals residing in geographic regions where the selenium content of the soil is low (1–3). Likewise, apparent selenium intake and plasma concentrations have been reported to correlate inversely with mortality from cancer (4, 5). Such data suggest that selenium may be a naturally occurring anticarcinogen. Laboratory investigations also reveal that selenium addition to the diet or drinking water can substantially reduce cancer risk in animals treated with various chemical carcinogens (6–14). Based upon published reports, the anticarcinogenic effects of selenium are not limited to a single form of this trace element, to a specific carcinogen, or to a specific tissue, suggesting a general metabolic phenomenon. Several studies also show that selenium, at intakes above that required to optimize glutathione peroxidase activity, can inhibit the incidence of virally induced tumors (14–17). Furthermore, selenium supplementation, both *in vivo* and *in vitro*, has been shown to suppress the growth of some neoplastic cells (18–20).

The greatest cancer protection offered by selenium generally occurs with chemical carcinogens requiring metabolic activation (6, 12, 21). Several studies indicate that dietary selenium can alter the ability of cells to metabolize carcinogenic compounds (22–24). In cultures of mouse embryo cells, selenite and selenodiglutathione markedly reduced DMBA-DNA[3] binding to a greater extent than either selenide or selenomethionine (25). Likewise, dietary selenium supplementation significantly reduced the ability of isolated rat mammary cells to metabolize DMBA, as indicated by a significant decrease in DMBA-DNA binding (26).

Previous investigations aimed at examining the influence of selenium supplementation on DMBA metabolism have been limited principally to *in vitro* studies. Whether selenium modifies the metabolic activation of DMBA *in vivo* as reflected by altered DNA adduct patterns remains unknown. In the present study, the major goals were to determine the ability of dietary selenium to alter the *in vivo* formation of DMBA-DNA adducts and correlate any changes in adducts with the occurrence of mammary tumors.

MATERIALS AND METHODS

Materials. Spleen phosphodiesterase, 7,12-dimethylbenz(*a*)anthracene, micrococcal nuclease, nuclease P_1, and apyrase were obtained from Sigma Chemical Co. (St. Louis, MO). T4 polynucleotide kinase was from Amersham Co. (Arlington Heights, IL). [γ-^{32}P]ATP was purchased from ICN Biochemicals, Inc. (Irvine, CA). Thin-layer chromatography was performed on precoated polyethyleneimine-cellulose plastic plates (Machery Nagel, Neumann, Germany). Kodak XAR-OMAT film (Sigma Chemical Co.) was used for autoradiography.

Animals and Diets. Female Sprague-Dawley rats were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). All rats were housed individually in screen-bottomed stainless steel cages in a room with controlled temperature and lighting (22°C; 12-h light-dark cycle). Distilled water and a basal semipurified diet were provided during a 5-day acclimation period. The quantity of selenium in the drinking water had been determined to be negligible. The basal diet contained (percentage by weight): casein, 17.2%; sucrose, 20.3%; cornstarch, 29.5%; cellulose fiber, 5%; corn oil, 20%; AIN-76 mineral mixture, 3.5% (Teklad Mills, Madison, WI); AIN-76 vitamin mixture, 1% (Teklad Mills); agar, 3%; choline dihydrogen citrate, 0.2%; and L-methionine, 0.3%. The quantity of selenium in the basal diet was adjusted to 0.1 μg/g by supplementation with selenite. Gel diets were prepared by mixing the dry ingredients in hot distilled water (50:50). Diets were stored at 4°C until fed. Fresh food was offered daily. The content of selenium in all experimental diets was verified by the method of McCarthy *et al.* (27), using a gas chromatograph equipped with an electron capture detector (5710 A w/ ECD; Hewlett-Packard, Avondale, PA).

Carcinogen. The carcinogen used in these studies was nonradioactive DMBA. DMBA was purified on silicic acid as described by Dipple *et al.* (28). DMBA was administered p.o. by intubation at 5 mg/kg body weight.

The P_1 nuclease-enriched ^{32}P-postlabeling method described by Reddy and Randerath (29) was used to quantitate individual DMBA-DNA adducts. Adducts were separated and identified as previously described (30, 31). Briefly, mammary DNA (5 μg) reconstituted in buffer (20 mM sodium succinate-10 mM $CaCl_2$, pH 6.0) was digested

Received 4/3/91; accepted 6/14/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by NIH Grant CA44567. Presented in part at the 14th International Congress of Nutrition, Seoul, Korea, 1989, and in part at the Annual Meeting of the Federation of the American Society of Experimental Biology, Washington, D.C., 1990 (FASEB J., *4*: A1042, 1990).

[2] To whom requests for reprints should be addressed, at Department of Nutrition, 126 Henderson Building South, The Pennsylvania State University, University Park, PA 16802.

[3] The abbreviations used are: DMBA, 7,12-dimethylbenz(*a*)anthracene; anti-G, *anti*-3,4-dihydrodiol-1,2-epoxide-deoxyguanosine; anti-A, *anti*-3,4-dihydrodiol-1,2-epoxide-deoxyadenosine; syn-G, *syn*-3,4-dihydrodiol-1,2-epoxide-deoxyguanosine; syn-A, *syn*-3,4-dihydrodiol-1,2-epoxide-deoxyadenosine.

with spleen phosphodiesterase (0.01 unit/sample) and micrococcal nuclease (0.06 unit/sample). Dephosphorylation of nucleotides was accomplished by adding nuclease P_1 (6 μg/sample) in a 0.25 M sodium acetate buffer (pH 5.0) containing 0.3 mM $ZnCl_2$. After neutralization of the sample with 0.5 M Tris base, the DMBA-deoxyribonucleoside monophosphates were labeled with ^{32}P by adding T4 kinase (4.3 units/sample) and [γ-^{32}P]ATP (110 μCi/sample) in buffer (0.2 M biocin-0.1 M $MgCl_2$-0.1 M dithiothreitol-10 mM spermidine, pH 9.5). The DMBA-DNA adducts were separated on the polyethyleneimine-cellulose thin-layer chromatographic plates and localized by autoradiography. For quantitation, individual adduct spots were removed and the radioactivity was determined. Quantities of individual adducts are expressed as nmol adduct/mol DNA assuming 1 μg of DNA equals 3240 pmol of deoxynucleoside 3'-monophosphates.

Experiment 1. This experiment was designed to determine whether there was a dose-dependent relationship between dietary selenium intake during the initiation phase of DMBA carcinogenesis and the occurrence of mammary tumors. Rats were weighed and randomly assigned to one of the following dietary selenium treatments: 0.1, 0.5, 1.0, 2.0, or 4.0 μg selenium/g (21–25 rats/treatment). After 2 weeks of experimental feeding each rat (55 days old) was intubated with DMBA. Rats continued to receive their assigned experimental diet for an additional 2 weeks following DMBA treatment (initiation phase). All rats were then offered the basal diet (0.1 μg selenium/g) for the remainder of the 43-week study. A separate group of rats was fed the 4.0 μg selenium/g diet throughout the entire study (initiation and promotion phase). Throughout the experiment food intake was monitored daily and body weights were measured weekly. Tumor number, location, and size were recorded weekly. At the end of the study, rats were sacrificed under CO_2, and their liver, lungs, and kidneys were removed for examination of tumor metastasis and/or selenium toxicity. Samples of mammary gland and all gross lesions were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for histopathological examination. The World Health Organization nomenclature and criteria were used for the classification of mammary tumors (31).

Experiment 2. This experiment examined the effect of dietary selenium on the *in vivo* formation of DMBA-DNA adducts. Rats were fed diets identical to those used in experiment 1 for 2 weeks prior to DMBA treatment. Mammary tissue from rats ($n = 4$) fed the basal diet but not intubated with DMBA was used to determine the frequency of nonspecific adducts. Twenty-four h after DMBA treatment, mammary tissue was removed and mammary cell DNA was isolated and purified as previously described (32).

Statistical Analysis. Food intake, body weight, and individual adducts were evaluated statistically using analysis of variance and applying the Fisher's least significant difference test for mean comparisons (StatView 512+; 1986 Abacus Concepts, Inc., Calabasas, CA). Correlation analysis was used to test the significance between the selenium quantities in diets and tumor incidence, and between selenium quantities and quantities of DMBA-DNA adducts (StatView 512+). The χ^2 test was applied to evaluate the significance of rat tumor incidence and total tumor numbers among dietary selenium treatments (33).

RESULTS

Food Intake and Weight Gain. Food intakes of rats did not differ among dietary selenium treatments ($P > 0.05$) in either experiment 1 or 2. Mean daily dry matter intake for rats across all treatments during the first 2 weeks of feeding was 11.7 g. During experiment 1 food intake reached a maximum at approximately 10 weeks post-DMBA treatment and remained relatively constant throughout the remainder of the 43-week study. Body weight gain of rats was also not significantly influenced by the dietary selenium intake in either study ($P > 0.05$). Weight gains for rats fed 4.0 μg selenium/g during both initiation and promotion were similar to those for rats fed 0.1 μg selenium/g (Fig. 1).

Tumor Incidence. In experiment 1, palpable mammary tumors first appeared on the 24th week after DMBA treatment (Fig. 2). Higher selenium intakes resulted in a delay in the time of first tumor appearance (Fig. 2). The first mammary tumors in rats fed either 2.0 or 4.0 μg selenium/g were not detected until week 34. The final tumor incidence correlated inversely with the log of the selenium content of the diet ($r = -0.99$; $P < 0.01$) (Fig. 3). Histopathological examination revealed that adenomas and fibroadenomas accounted for 80.5% of all tumors (Table 1). The remaining tumors consisted of mammary adenocarcinomas (7.3%), fibrosarcomas (7.3%), and undifferentiated sarcomas (4.9%). Malignant tumors appeared only in rats fed diets containing 0.1 or 0.5 μg selenium/g. There was no evidence of tumor metastasis in any of the experimental animals. An inverse relationship between the log of the dietary selenium content and the total tumor number ($r = -0.99$; $P < 0.01$) was observed.

DMBA-DNA Adducts. The predominant DMBA-DNA adduct formed *in vivo* was an *anti*-dihydrodiolepoxide bound to guanine (*anti*-G) (Fig. 4). The presence of *anti*-G adduct was dramatically reduced in rats fed supplemental selenite (Fig. 4). The greatest effect of dietary selenite occurred between 0.1 and 1.0 μg selenium/g. Overall, a highly significant inverse relation-

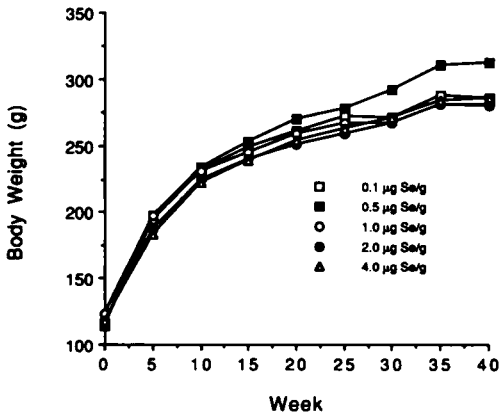

Fig. 1. Weight gain of rats fed diets with varying quantities of selenite (0.1, 0.5, 1.0, 2.0, 4.0, and 4.0 μg selenium/g). Diets were fed during the initiation phase of DMBA carcinogenesis. On the 4th week of feeding all rats were transferred to the basal diet (0.1 μg selenium/g).

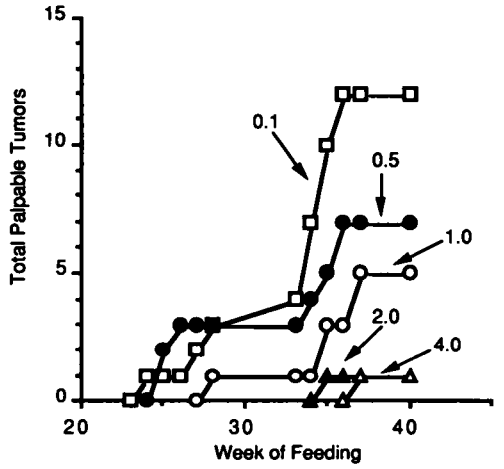

Fig. 2. Influence of dietary selenite intake on the onset of DMBA-induced mammary tumors. Rats were fed increasing quantities of selenite during the initiation phase of carcinogenesis. All rats were intubated with DMBA (5 mg/kg body weight) at 55 days of age.

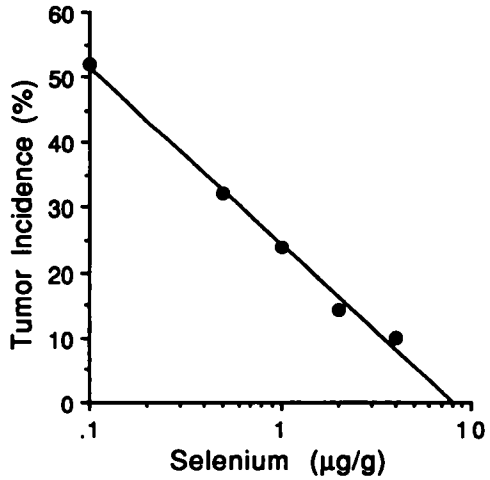

Fig. 3. Relationship of dietary selenite intake to the incidence of DMBA-induced mammary tumors. The correlation coefficient between the log of dietary selenium and the tumor incidence was $r = -0.996$, $P < 0.01$.

Table 1 *Influence of dietary selenite on DMBA-induced mammary tumors*

Dietary selenium (μg/g)		Total no. of rats	Total no. of tumors*	No. of malignant tumors
Initiation	Promotion			
0.1	0.1	23	15 (a)	3
0.5	0.1	25	10 (a, b)	3
1.0	0.1	25	6 (b, c)	1
2.0	0.1	21	4 (b, c)	0
4.0	0.1	21	2 (b, c)	0
4.0	4.0	21	1 (c)	0

* Values not sharing a common letter differ ($P < 0.05$).

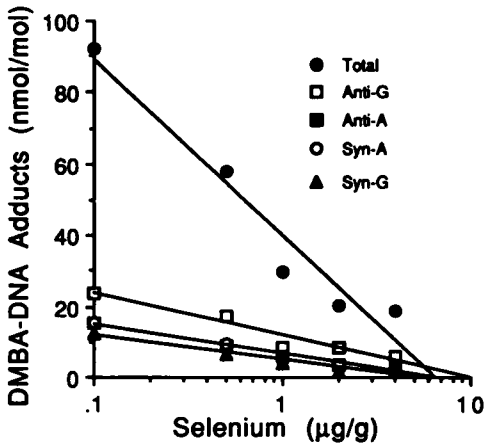

Fig. 4. Relationship of dietary selenium content to the occurrence of DMBA-DNA adducts.

ship between the log of the selenium content of the diet and the presence of anti-G adduct was found. Likewise selenite supplementation resulted in a marked depression in all identified adducts. Binding due to anti-A, syn-A, and syn-G adducts was maximally reduced when rats were fed diets containing 2 μg selenium/g or more. The occurrence of anti-A, syn-G, and syn-A adducts also correlated inversely with the log of the dietary selenium intake ($P < 0.05$) (Fig. 4).

Correlation of DMBA-DNA Adducts and Tumor Incidence. A highly positive relationship of the quantity of anti-G and total DNA-DMBA adducts with final tumor number was observed ($r = 0.99$; $P < 0.01$). Rat mammary tumor incidence was positively correlated with the amounts of anti-G ($r = 0.98$), anti-A ($r = 0.98$), syn-A ($r = 0.99$), syn-G ($r = 0.95$), and total DMBA-DNA ($r = 0.99$) adducts (Fig. 5).

DISCUSSION

DMBA is recognized as an initiator of both mammary and skin cancer (7, 34–37). The covalent binding of DMBA metabolites to DNA has been implicated as a critical step in the initiation phase of these cancers (38–43). Binding to DNA is known to involve primarily the bay region dihydrodiol epoxide route, with both *syn-* and *anti*-dihydrodiol epoxides contributing to binding. Our previous studies have demonstrated that *in vitro* selenium supplementation is effective in inhibiting the formation of DMBA-DNA adducts (25, 26) with a principal reduction in the occurrence of *anti*-dihydrodiol epoxide adducts. The present study demonstrated that dietary selenium is likewise effective in reducing the *in vivo* formation of total and individual DMBA-DNA adducts. As the quantity of sodium selenite in the diet increased, the quantity of individual and total DMBA-DNA adducts decreased markedly ($P < 0.05$). Unlike the *in vitro* studies, the present *in vivo* studies reveal that selenite supplementation is equally effective in reducing the occurrence of both *syn-* and *anti*-dihydrodiol epoxide adducts from DMBA. Quantitative differences in the *in vivo* and *in vitro* formation of individual adducts may reflect the quantity of DMBA that ultimately comes in contact with the mammary cell. Nevertheless, selenite supplementation is clearly effective in inhibiting the occurrence of DMBA-DNA adducts in mammary tissue both *in vivo* and *in vitro*.

In the present study supplemental dietary selenite provided during the initiation phase of mammary carcinogenesis significantly inhibited the incidence and total number of tumors resulting from DMBA treatment. This observation is consistent with other investigations (9, 10) and shows that diets containing 1.0 μg selenium/g or more are effective in inhibiting this phase of mammary carcinogenesis. Ip (9) suggested that approximately one-half of the anticarcinogenic effect of selenite could be explained by a suppression of initiation. In the present study mammary carcinogenesis was inhibited by 50, 75, or 83% in rats fed diets containing 1.0, 2.0, or 4.0 μg selenium/g, respectively, during initiation phase compared to rats fed a diet containing 0.1 μg selenium/g. While the degree of inhibition of

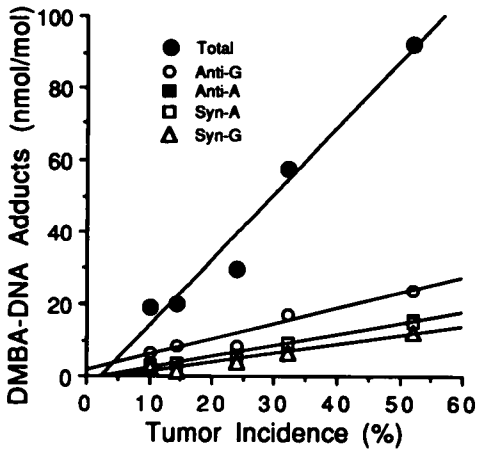

Fig. 5. Relationship between total and individual DMBA-DNA adducts and the incidence of DMBA-induced mammary tumors. The correlation coefficients between tumor incidence and DMBA-DNA adducts were: total binding, $r = 0.971$; anti-G, $r = 0.961$; anti-A, $r = 0.982$; syn-A, $r = 0.973$; and syn-G, $r = 0.965$.

the initiation phase of mammary carcinogenesis is dependent upon the quantity of selenite consumed, the greatest percentage depression occurred when the diet was increased from 0.1 to 1.0 μg selenium/g. While it is difficult to extrapolate these observations to recommendations for humans, a moderate increase in dietary selenium for human beings may have the greatest impact on reducing cancer risk while minimizing selenosis. Consistent with other reports (8, 9, 11), selenite supplementation resulted in a lengthening of time before the first appearance of tumors. The present study revealed that this delay progressively increased as the quantity of dietary selenite was increased.

In this study, benign tumors accounted for more than 80% of all tumors. The ability of DMBA to induce benign or malignant tumor appears to depend on the dosage administered. Dao (42) reported that the ratio of malignant tumors to hyperplastic alveolar nodules varied from 1:16 to 8:17 over a range of 5 to 15 mg of DMBA administered intragastrically to the rat. The lower dosage of DMBA administered in this experiment (5 mg/kg) likely accounts for the high percentage of benign tumors. Selenite supplementation was effective in reducing the incidence of both benign and malignant tumors. Selenite supplementation at 1.0 μg selenium/g was effective in eliminating the occurrence of malignant mammary tumors.

In the present studies, individual DNA adducts (*anti*-G, *anti*-A, *syn*-A, *syn*-G) and the total DMBA-DNA adducts correlated positively with the incidence of rat mammary tumors. DiGiovanni *et al.* (43) reported the occurrence of anti-A adducts correlated with the skin tumor-initiating activity of DMBA in mice. A similar relationship between the occurrence of anti-A adducts and mammary tumors was observed in the present study using the rat model. Unlike the studies of DiGiovanni (43) a significant relationship between each of the major *syn*- and *anti*-dihydrodiol epoxide adducts and ultimate tumor formation was observed. From the present data it is impossible to determine which adduct is most important in tumor induction since all were equally influenced by dietary selenite. The amount of DMBA exposure is known to influence the quantity of individual adducts (25). It is unknown whether dosage of DMBA or species differences account for our inability to relate a single alteration in DMBA-DNA adducts to tumor incidence.

The mechanism by which selenium alters the *in vivo* formation of adducts is unknown. Support for altered metabolism within the mammary cell *per se* comes from the ability of selenite to dramatically inhibit binding in cells in culture. It is generally believed that glutathione serves an important role in the protection against chemically induced cancer (44, 45). LeBoeuf *et al.* (46) reported that high selenium intake significantly increased the concentrations of hepatic nonprotein sulfhydryls. Chung and Maines (47) demonstrated that selenium increased the activities of γ-glutamylcysteine synthetase and glutathione disulfide reductase, hence increasing the concentration of glutathione. While glutathione was not determined in the present studies it is conceivable that increased liver or nonhepatic tissue glutathione concentrations in rats fed supplemental selenite reduced the effective concentration of DMBA within the mammary cell. The greater reduction in binding as the diet was increased from 0.1 to 1.0 μg selenium/g may reflect the development of a new steady-state concentration in tissue selenium concentrations. Additional studies are needed to clarify the effect that alterations in DMBA detoxification of nonmammary tissue have on mammary tissue DMBA-DNA binding.

In conclusion, dietary selenium is effective in inhibiting the *in vivo* metabolism of DMBA to metabolites capable of binding to DNA. This reduction in binding was found to correlate significantly with ultimate tumor formation.

REFERENCES

1. Shamberger, R. J., Tytko, S. A., and Willis, C. E. Antioxidants and cancer. Part VI. Selenium and age-adjusted human cancer mortality. Arch. Environ. Health, *31:* 231–235, 1976.
2. Cowgill, U. M. The distribution of selenium and cancer mortality in the continental United States. Biol. Trace Elem. Res., *5:* 345–361, 1983.
3. Yu, S. Y., Chu, Y. J., Gong, X. L., and Hou, C. Regional variation of cancer mortality and its relation to selenium levels in China. Biol. Trace Elem. Res., *7:* 21–29, 1985.
4. Schrauzer, G. N., White, D. A., and Schneider, C. J. Cancer mortality correlation studies. III. Statistical associations with dietary selenium intakes. Bioinorg. Chem., *7:* 23–34, 1977.
5. Schrauzer, G. N., White, D. A., and Schneider, C. J. Cancer mortality correlation studies. IV. Association with dietary intakes and blood levels of certain trace elements. Notably Se antagonists. Bioinorg. Chem., *7:* 35–56, 1977.
6. Milner, J. A. Inhibition of chemical carcinogenesis and tumorigenesis by selenium. *In:* L. A. Poirier, P. M. Newberne, and M. W. Pariza (eds.), Essential Nutrients in Carcinogenesis, pp. 449–463. New York: Plenum Press, 1986.
7. Shamberger, R. J. Relationship of selenium to cancer: I. Inhibitory effect of selenium on carcinogenesis. J. Natl. Cancer Inst., *44:* 931–936, 1970.
8. Thompson, H. J., Meeker, L. D., and Kokoska, S. Effect of inorganic and organic form of dietary selenium on the promotional stage of mammary carcinogenesis in the rat. Cancer Res., *44:* 2803–2806, 1984.
9. Ip, C., and Daniel, F. B. Effects of selenium on 7,12-dimethylbenz(*a*)anthracene-induced mammary carcinogenesis and DNA adduct formation. Cancer Res., *45:* 61–65, 1985.
10. Welsch, C. W., Goodrich-Smith, M., Brown, C. K., Greene, H. D., and Hamel, E. J. Selenium and the genesis of murine mammary tumors. Carcinogenesis (Lond.), *2:* 519–522, 1981.
11. Ip, C. Prophylaxis of mammary neoplasia by selenium supplementation in the initiation and promotion phases of chemical carcinogenesis. Cancer Res., *41:* 4386–4390, 1981.
12. Jacobs, M. M., Jansson, B., and Griffin, A. C. Inhibitory effects of selenium on 1,2-dimethylhydrazine and methylazoxymethanol acetate induction of colon tumors. Cancer Lett., *2:* 133–138, 1977.
13. Nayini, J., Bayoumy, K., Sugie, S., Cohen, L. A., and Reddy, B. S. Chemoprevention of experimental mammary carcinogenesis by the synthetic organoselenium compound, benzylselenocyanate, in rats. Carcinogenesis (Lond.), *10:* 541–546, 1989.
14. Medina, D. Mechanism of selenium inhibition of tumorigenesis. *In:* L. A. Poirier, P. M. Newberne, and M. W. Pariza (eds.), Essential Nutrients in Carcinogenesis, pp. 465–472. New York: Plenum Press, 1986.
15. Whanger, P., Schmitz, J. A., and Exon, J. H. Influence of diet on the effects of selenium in the genesis of mammary tumors. Nutr. Cancer, *3:* 240–248, 1982.
16. Schrauzer, G. N., and Ishmael, D. Effects of selenium and of arsenic on the genesis of spontaneous mammary tumors in inbred C_3H mice. Ann. Clin. Lab. Sci., *4:* 441–447, 1974.
17. Milner, J. A. Effect of selenium on virally induced and transplantable tumor models. Fed. Proc., *44:* 2568–2572, 1985.
18. Milner, J. A., and Hsu, C. Y. Inhibitory effect of selenium on the growth of L1210 leukemic cells. Cancer Res., *41:* 1652–1656, 1981.
19. Fico, M. E., Poirier, K. A., Watrach, A. M., Watrach, M. A., and Milner, J. A. Differential effects of selenium on normal and neoplastic canine mammary cells. Cancer Res., *46:* 3384–3388, 1986.
20. Ip, C., Ip, M. M., and Kim, U. Dietary selenium intake and growth of the MT-W9B transplantable rat mammary tumor. Cancer Lett., *14:* 101–107, 1981.
21. Thompson, J. J., and Becci, P. J. Effect of graded dietary levels of selenium on tracheal carcinomas induced by 1-methyl-1-nitrosourea. Cancer Lett., *7:* 215–219, 1979.
22. Rasco, M. A., Jacobs, M. M., and Griffin, A. C. Effects of selenium on aryl hydrocarbon hydroxylase activity in cultured human lymphocytes. Cancer Lett., *3:* 295–301, 1977.
23. Marshall, M. V., Arnott, M. S., Jacobs, M. M., *et al.* Selenium effects on the carcinogenicity and metabolism of 2-acetylaminofluorene. Cancer Lett., *7:* 331–338, 1979.
24. Arciszewska, L. K., Martin, S. E., and Milner, J. A. The antimutagenic effect of selenium on 7,12-dimethylbenz(*a*)anthracene and metabolites in the Ames *Salmonella*/microsome system. Biol. Trace Elem. Res., *4:* 259–267, 1982.
25. Milner, J. A., Pigott, M. A., and Dipple, A. Selective effects of selenium on 7,12-dimethylbenz(*a*)anthracene-DNA binding in fetal mouse cell cultures. Cancer Res., *45:* 6347–6354, 1985.
26. Ejadi, S., Bhattacharya, I. D., Voss, K., Singletary, K., and Milner, J. A. *In vitro* and *in vivo* effects of sodium selenite on 7,12-dimethylbenz(*a*)anthracene-DNA adduct formation in isolated rat mammary epithe-

lial cells. Carcinogenesis (Lond.), *10:* 823–826, 1989.
27. McCarthy, T. P., Brodie, B., Milner, J. A., and Beville, R. F. Improved method for selenium determination in biological samples by gas chromatography. J. Chromatogr., *225:* 9–16, 1981.
28. Dipple, A., Tomaszewski, J. E., Moschel, R. C., Bigger, C. A. H., Nebzydoski, J. A., and Egan, M. Comparison of metabolism-mediated binding to DNA of 7-hydroxymethel-12-methyl-benz(*a*)anthracene and 7,12-dimethylbenz(*a*)anthracene. Cancer Res., *39:* 1154–1158, 1979.
29. Reddy, M. V., and Randerath, K. Nuclease P_1-mediated enhancement of sensitivity of ^{32}P-postlabeling test for structurally diverse DNA adducts. Carcinogenesis (Lond.), *7:* 1543–1551, 1986.
30. Singletary, K. W., and Milner, J. A. DNA binding and adduct formation of 7,12-dimethylbenz(*a*)anthracene by rat mammary epithelial cell aggregates *in vitro*. Carcinogenesis (Lond.), *7:* 95–98, 1986.
31. Young, S., and Hallowes, R. C. Tumors of the mammary gland. *In:* V. S. Turusov (ed.), Pathology of Tumors in Laboratory Animals, Vol. 1, IARC Scientific Publication 5, pp. 31–73. Lyon, France: International Agency for Research on Cancer, 1973.
32. Singletary, K. W., Parker, H. M., and Milner, J. A. Identification and *in vivo* formation of ^{32}P-postlabeled rat mammary DMBA-DNA adducts. Carcinogenesis (Eynsham), *11:* 1959–1963, 1990.
33. Cochran, W. G. Some methods for strengthening the common c^2 test. Biometrics, *10:* 417–451, 1954.
34. Huggins, C., Grand, L. C., and Brillantes, F. P. Mammary cancer induced by a single feeding of polynuclear hydrocarbons, and its suppression. Nature (Lond.), *189:* 204–207, 1961.
35. Gruenstein, M., Meranze, D. R., Thatcher, D., and Shimkin, M. B. Carcinogenic effects of intragastric 3-methylcholanthrene and 7,12-dimethylbenz(*a*)anthracene in Wistar and Sprague-Dawley rats. J. Natl. Cancer Inst., *36:* 483–495, 1966.
36. Ip, C., and Hayes, C. Tissue selenium levels in selenium-supplemented rats and their relevance in mammary cancer protection. Carcinogenesis (Lond.), *10:* 921–925, 1989.
37. L'Abbe, M. R., Fischer, P. W. F., Campbell, J. S., and Chavez, E. R. Effects of dietary selenium on DMBA-induced carcinogenesis in rats fed a diet high in mixed fats. J. Nutr., *119:* 757–765, 1989.
38. Dipple, A., and Nebzydoski, J. A. Evidence for the involvement of a diol-epoxide in the binding of 7,12-dimethylbenz(*a*)anthracene to DNA in cells in culture. Chem.-Biol. Interact., *20:* 17–26, 1978.
39. Moschel, R. C., Baird, W. M., and Dipple, A. Metabolic activation of carcinogen 7,12-dimethylbenz(*a*)anthracene for DNA binding. Biochem. Biophys. Res. Commun., *76:* 1092–1098, 1977.
40. Dipple, A., Pigott, M., Moschel, R. C., and Costantino, N. Evidence that binding of 7,12-dimethylbenz(*a*)anthracene to DNA in mouse embryo cell cultures results in extensive substitution of both adenine and guanine residues. Cancer Res., *43:* 4132–4135, 1983.
41. DiGiovanni, J., Sawyer, T. W., and Fisher, E. P. Correlation between formation of a specific hydrocarbon-deoxyribonucleoside adduct and tumor-initiating activity of 7,12-dimethylbenz(*a*)anthracene and its 9- and 10-monofluoroderivatives in mice. Cancer Res., *46:* 4336–4341, 1986.
42. Dao, T. L. Carcinogenesis of mammary gland in rat. Prog. Exp. Tumor Res., *5:* 157–216, 1964.
43. DiGiovanni, J., Sawyer, T. W., and Fisher, E. P. Correlation between formation of a specific hydrocarbon-deoxyribonucleoside adduct and tumor-initiating activity of 7,12-dimethylbenz(*a*)anthracene and its 9- and 10-monofluoroderivatives in mice. Cancer Res., *46:* 4336–4341, 1986.
44. Chasseaud, L. F. The role of glutathione and glutathione *S*-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv. Cancer Res., *29:* 175–274, 1979.
45. Booth, J., and Sims, P. Metabolism of benz(*a*)anthracene epoxides by rat liver. Biochem. Pharmacol., *23:* 2547–2555, 1974.
46. LeBoeuf, R. A., Zentner, K. L., and Hoekstra, W. G. Effect of dietary selenium concentration and duration of selenium feeding on hepatic glutathione concentrations in rats. Proc. Soc. Exp. Biol. Med., *180:* 348–352, 1985.
47. Chung, A. S., and Maines, M. A. Effect of selenium on glutathione metabolism induction of γ-glutamylcysteine synthetase and glutathione reductase in the rat liver. Biochem. Pharmacology. *30:* 3217–3223, 1981.