Symposium

Papers from the symposium on
Cancer — A molecular event

The papers appearing here as a unit publication were presented at a special symposium held late in 1983 at Lake Geneva, Wisconsin. Its purpose was to review and discuss lipid oxidation, antioxidants and selenium as factors modifying the process of carcinogenesis. National and international experts presented data dealing with these issues during the 2½ day meeting. The data presented indicated that intakes of lipid, antioxidants and selenium all are factors that modify the incidence of cancer in human beings and in experimental models. Possible mechanisms by which these factors modify susceptibility to cancer also were addressed. About 150 persons representing the fields of nutrition, chemistry, biochemistry, medicine, food technology and other, more specialized disciplines, attended the conference. We also would like to thank the American Oil Chemists' Society for its generous support. Furthermore the generous contributions from Hoffman LaRoche, Campbell Soup Co., Best Foods-CPC, the Eastman Chemical Products Health and Nutrition Division, and the Archer-Daniels Midland Company were of great assistance to the success of the conference.

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Anticarcinogenic Effect of Selenium in the Dimethylbenz(a)anthracene-Induced Mammary Tumor Model in Rats

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ABSTRACT

Using the dimethylbenz(a)anthracene-induced mammary tumor model in rats, our studies indicated that there was a dose-response relationship between dietary selenium supplementation and the inhibition of mammary carcinogenesis. The degree of inhibition was proportional to the level of dietary selenium up to 5 ppm, at which point toxicity in the form of a reduction in weight gain was evident. Moreover, it was observed that the chemopreventive efficacy of selenium was influenced by the dose of carcinogen as well as the fat intake of the animals. By supplementing selenium for defined periods of time, we concluded that selenium inhibited both the initiation and the promotion phases of chemical carcinogenesis, and that a continuous intake of selenium was necessary to achieve maximal suppression of tumor growth. In an attempt to improve the efficacy of lower levels of selenium, we conducted another series of experiments in which selenium and vitamin E were tested in combination. Results showed that although vitamin E alone had no prophylactic effect against tumorigenesis, it potentiated the ability of selenium to inhibit the development of mammary tumors. Further investigation suggested that the anticarcinogenic action of selenium could not be explained by its antioxidant function in lipid peroxidation. On the other hand, vitamin E might be able to provide a more favorable environment against oxidant stress to assist selenium in exerting its inhibitory effect through some other mechanisms.

INTRODUCTION

There is increasing evidence that selenium has a protective effect against tumorigenesis in laboratory animals. References to current experimental reports in the literature concerning selenium and cancer have been summarized by Dr. Shamberger in this conference. Most of these studies involve the use of inorganic selenium supplements either in the drinking water or in the diet at a concentration ranging from 0.5 to 6 ppm (mg/kg). These levels are considerably higher than the nutritional requirement of about 0.1 ppm established by the NRC for animals. A comparison of the results from several laboratories indicates that mice may be more sensitive to selenium inhibition of tumorigenesis than rats.

The breast cancer models that have been shown to be

responsive to selenium chemoprevention include both virus- and chemical carcinogen-induced mammary tumors (8,9,12, 18,21,22,26,27,32,33,35,36). Since selenium is effective in suppressing mammary neoplastic development induced by both methylnitrosourea and dimethylbenz(a)anthracene, it is unlikely that the primary action of selenium is exerted via changes in carcinogen metabolism. A report from Medina's laboratory (21) indicates that selenium markedly inhibits mammary tumorigenesis in BALB/cfC3H mice (MuMTVS positive), but has little effect on the incidence of neoplastic transformation in preneoplastic outgrowth lines or the growth rate of primary mammary tumors transplanted subcutaneously in BALB/c mice (MuMTVS negative). Thus there seems to be a decreasing sensitivity to selenium mediated inhibition as cells progress from normal to preneoplastic to neoplastic. This is in contrast to 2 other reports in which selenium was found to be effective in retarding the growth of a canine mammary tumor line in athymic nude mice (34) and the MT-W9B transplantable mammary tumor in W/F rats (10).

Our work has involved primarily the mammary tumor model induced by 7,12-dimethylbenz(a)anthracene (DMBA) in female Sprague-Dawley rats. The studies described in the present paper were designed to address the following questions. (a) What are the factors that influence the anticarcinogenic efficacy of selenium? (b) Are the different types of lesions found in the mammary gland subsequent to carcinogen administration equally sensitive to selenium inhibition? (c) How does the time and duration of selenium supplementation affect tumorigenesis? (d) Is selenium cytotoxic when present at high levels? (e) Can the chemopreventive effectiveness of selenium be improved by combining it with another agent? (f) Is the anticarcinogenic action of selenium related to its function in regulating the activity of selenium-dependent glutathione peroxidase? Details of the experimental protocol have been published previously (6,8,9,12). In all our studies, selenium in the form of sodium selenite was added to semi-purified synthetic diets.

Anticarcinogenic Efficacy of Selenium: Influence of Fat Intake and Carcinogen Dosage

The objective of the first experiment is to test the effect of graded levels of dietary selenium on mammary carcinogenesis, and to determine if the optimal level of supplementation depends on the dose of the carcinogen and the fat intake of the animals. Table I shows the effect of various levels of selenium supplementation on tumorigenesis in rats that were fed either a 5% or a 25% corn oil diet and given either 5 or 10 mg of DMBA at 50 days of age (7). Selenium supplementation of both diets was started from weaning and continued until the end of the experiment 22 weeks after DMBA administration. Mammary tumor pathology is defined according to the criteria of Young and Hallowes (38). In rats treated with DMBA at 50 days of age, over 90% of the tumors obtained are adenocarcinomas. Only adenocarcinomas are reported unless otherwise stated.

At 0.1 ppm of selenium, which is considered to meet the nutritional requirement of rats (control level), tumor incidence was higher in the 25% fat group than in the 5% fat group. We found that selenium had to be raised to 1.5 ppm before its chemopreventive effect became noticeable. The degree of inhibition was proportional to the level of dietary selenium up to 5 ppm, at which point a slight reduction in weight gain (about 10%) was evident. This decrease in growth was due to a lower food intake. Pair-feeding experiments, however, indicated that reduced food consumption alone was not sufficient to account for the striking suppression of tumorigenesis in those rats treated with 5 ppm of selenium (results not shown). In general, the selenium-mediated inhibitory responses included a lower tumor incidence, a reduction in tumor yield and a longer latency period. It should be noted that the anticarcinogenic efficacy of selenium was diminished by a larger dose of carcinogen. Moreover, selenium was unable to counteract completely the enhancing effect of fat in mammary carcinogenesis, since rats on a high-fat diet still developed more tumors than those on a low-fat diet at comparable levels of selenium supplementation.

TABLE I

Effect of Selenium Supplementation on DMBA-Induced Mammary Tumorigenesis in Rats Fed Either a 5% or a 25% Corn Oil Diet

Experiment	Dietary group	Selenium in diet (ppm)	Initial body wt.[a] (g)	Final body wt. (g)	Tumor incidence	Total no. of tumors	Average latency period[b] (days)
A	5% fat	0.1	152 ± 2[c]	290 ± 6[c]	12/30 (40.0%)	26	92 ± 7[c]
5 mg DMBA		0.5	150 ± 2	292 ± 6	11/30 (36.7%)	23	89 ± 6
		1.5	151 ± 3	289 ± 6	9/31 (29.0%)	19	97 ± 7
		2.5	150 ± 3	288 ± 7	7/29 (24.1%)	10	109 ± 8
	25% fat	0.1	153 ± 2	294 ± 6	21/30 (70.0%)	65	85 ± 6
		0.5	155 ± 3	290 ± 6	20/29 (68.9%)	66	86 ± 6
		1.5	151 ± 2	290 ± 7	16/29 (55.2%)	41	92 ± 7
		2.5	151 ± 3	288 ± 7	10/30 (33.3%)	21	106 ± 7
B	5% fat	0.1	154 ± 3	294 ± 7	21/30 (70.0%)	71	71 ± 6
10 mg DMBA		2.5	155 ± 3	290 ± 7	13/29 (44.8%)	32	81 ± 6
		5.0	139 ± 3	259 ± 6	7/30 (23.3%)	15	95 ± 7
	25% fat	0.1	156 ± 2	289 ± 6	30/30 (100%)	135	65 ± 5
		2.5	155 ± 3	290 ± 7	23/30 (76.7%)	85	73 ± 6
		5.0	142 ± 3	255 ± 6	16/29 (55.2%)	46	88 ± 7

[a]At the time of DMBA administration.

[b]Time between DMBA administration and the appearance of the first palpable tumor.

[c]Mean ± S.E.

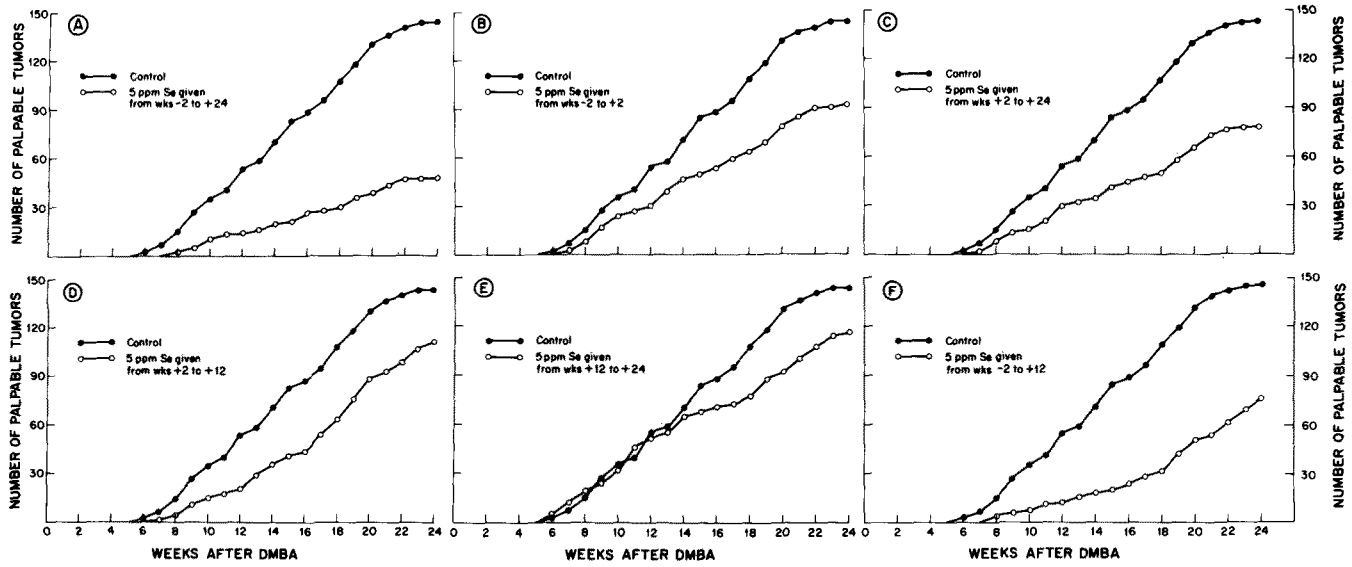

FIG. 1. Effect of selenium supplementation (5 ppm) for various periods of time on the development of palpable mammary tumors. The time of DMBA administration (50 days of age) was taken as 0; minus and plus signs represent the time in weeks before and after DMBA administration. The schedule of selenium supplementation is indicated in panels A to F. The control group (0.1 ppm selenium) is reproduced in each panel for comparison. There were 35 rats/group.

Effect of Selenium Supplementation on the Development of Hyperplastic Alveolar Nodules (HANs) and the Formation of Adenocarcinomas and Fibroadenomas in Older Rats

HANs are a form of dysplasia in the mammary gland produced subsequent to carcinogen treatment. Normally they can be detected before the appearance of palpable tumors. There is, however, some controversy as to the precancerous nature of these lesions in the rat model. In the present experiment, these nodules were identified and counted in stained whole mount preparation of the mammary gland (2) obtained from rats that were killed 8 weeks after DMBA (5 mg dose given at 50 days of age). The average number of HANs found per rat was 18.0 ± 3.6 in the 0.1 ppm selenium control group and 7.1 ± 1.6 in the 2.5 ppm selenium supplemented group ($P < 0.05$). Further investigation is necessary to evaluate the usefulness of this system in assessing the inhibitory effect of selenium in the early stages of neoplastic transformation.

Age is an important factor in the induction of mammary cancer in rats (1,4,23,28). The animals are most susceptible to carcinogenesis between 50-60 days of age and become more and more resistant as they get older. Consequently, the incidence is very low in rats treated with DMBA when they are over 100 days old. Moreover, there is a proportionate increase in fibroadenoma formation in this experimental model. By feeding animals a high fat diet and using a pulse-dose protocol, we were able partially to overcome the resistance of these older rats to mammary tumorigenesis induced by DMBA (7).

We were interested to find out whether selenium was equally effective in inhibiting the development of adenocarcinomas and fibroadenomas in rats that were maintained on a 20% corn oil diet and were given DMBA when they were 120 days of age. A multiple dose schedule was adopted with the administration of 5 mg of DMBA per week for 4 consecutive weeks. Selenium supplementation (2.5 ppm in the diet) was initiated immediately after the first dose of DMBA. Animals were killed 24 weeks after the last dose. Results in Table II show that the number of adenocarcinomas was reduced by 50% in the selenium-treated group. Interestingly, this was not accompanied by a comparable

TABLE II

Effect of Selenium Supplementation on Induction of Mammary Adenocarcinomas and Fibroadenoma in Adult Female Rats

Dietary selenium (ppm)	No. of rats	No. of adenocarcinomas	No. of fibroadenomas
0.1	30	29	18
2.5	30	14	16

Rats were given 5 mg of DMBA per week for 4 consecutive weeks; the first dose was given when the rats were 120 days of age.

suppression of fibroadenoma formation. These lesions generally appeared later in the course of the experiment. It is unclear at this time whether the immunogenicity or pathogenesis of the different tumor types have an effect on their responsiveness to selenium inhibition.

Prophylaxis of Mammary Neoplasia by Selenium Supplementation

In the first experiment described above, selenium was given for the entire duration of the study, and it was not possible to ascertain at which time point selenium was most effective in cancer chemoprevention. In order to answer this question, we conducted a new series of experiments in which the effect of selenium supplementation during the initiation and promotion (or proliferation) phases of DMBA-induced mammary carcinogenesis was examined.

In this experiment, 245 rats were divided randomly into 7 groups of 35 each. All were fed a high fat ration (25% corn oil) since diets rich in fat are known to promote the development of mammary neoplasia. Control rats in Group 1 received 0.1 ppm of selenium, while Groups 2 to 7 were supplemented with 5 ppm of selenium in the diet for various periods of time as indicated below. The time of DMBA administration (50 days of age) was taken as 0; minus and plus signs represent the time in weeks before and after DMBA administration (10 mg), respectively. The schedule of selenium treatment in Groups 2 to 7 was as follows: Group 2, −2 to +24; Group 3, −2 to +2; Group 4,

+2 to +24; Group 5, +2 to +12; Group 6, +12 to +24; and Group 7, −2 to +12. All rats were killed 24 weeks after DMBA administration. The reason for using 5 ppm of selenium was that we were afraid the inhibitory response might not be detected with a lower level of selenium, especially in those groups that received selenium supplementation for only a short period of time.

Figure 1, panels A to F, shows the time course of palpable mammary tumor development in the different groups. The control group (Group 1) is reproduced in each panel for comparison. Table III summarizes the final tumor incidence and the total tumor yield in Groups 1 to 7. The following conclusions can be drawn after careful analysis of the data. (a) A continuous intake of selenium is necessary to achieve maximal inhibition of tumorigenesis, such as in rats that were supplemented with selenium for the longest period of time (−2 to +24 weeks, Fig. 1A; Group 2 in Table III). (b) Selenium can inhibit both the initiation and promotion phases of carcinogenesis. This is suggested by the observation that a decrease in tumorigenesis was evident when selenium was supplemented either around the time of DMBA administration (−2 to +2 weeks, Fig. 1B; Group 3 in Table III) or during the proliferation phase of tumor development (+2 to +24 weeks, Fig. 1C; Group 4 in Table III). (c) The inhibitory effect of selenium in the early promotion phase probably is reversible, since we found that the chemopreventive response was severely diminished when selenium supplementation was limited from +2 to +12 weeks (Fig. 1D; Group 5 in Table III). (d) In rats that were supplemented with selenium from +12 to +24 weeks, there was only an insignificant reduction in the number of tumors found (Fig. 1E; Group 6 in Table III), suggesting that the efficacy of selenium is much attenuated when it is given long after carcinogenic injury. It should be pointed out that the schedule of dividing the promotion phase into 2 parts was an arbitrary one and should not be construed as the distinction of 2 separate events with identifiable phenotypic manifestation, but rather as a temporal relationship in terms of tumor development (early versus late).

TABLE III

Effect of Selenium Supplementation (5 ppm in the Diet) for Various Periods of Time on DMBA-Induced Mammary Tumorigenesis

Group[a]	Period of selenium supplement[b] (week)	Tumor incidence	Total no. of tumors	% of inhibition
1	none	97.1%	152	–
2	−2 to +24	45.7%	52	65.8%
3	−2 to +2	71.6%	101	33.5%
4	+2 to +24	68.5%	89	41.4%
5	+2 to +12	85.7%	126	17.1%
6	+12 to +24	91.4%	124	18.4%
7	−2 to +12	57.1%	80	47.4%

[a]Rats were given 10 mg of DMBA intragastrically. There were 35 rats per group.

[b]The time of DMBA administration was taken as 0; minus and plus signs represent the time in weeks before and after DMBA administration, respectively.

TABLE IV

Effect of Selenium Treatment In Vitro on Labeling Index of Mammary Explants Cultured with DMBA and on Subsequent Tumorigenesis in W/F Rats Following Transplantation

Selenium treatment[a]	Labeling index in explants[b]	Rats with tumors[c]
None	15.3 ± 3.1%	12/25
10^{-6} M	13.5 ± 2.4%	10/25
5×10^{-6} M	12.8 ± 2.0%	8/25
10^{-5} M	8.7 ± 1.4%	4/25
5×10^{-5} M	3.2 ± 0.5%	2/25

[a]Selenium in the form of sodium selenite was used.

[b]Mammary explants were incubated with DMBA (1 μg/ml) in the presence of insulin, estradiol, progesterone and prolactin for the first 3 days. On the fourth day, the culture was replenished with fresh hormone-supplemented medium but without DMBA. ^{3}H-Thymidine was added to the culture on day 6. Labeling was allowed to continue for 24 hr before the explants were fixed for autoradiography.

[c]Mammary explants were transplanted in the subscapular fat pad of isologous hosts using day 7 culture.

Cytotoxic Effect of High Levels of Selenium

In order to better evaluate if high levels of selenium have any cytotoxic effect, we proceeded to use the DMBA-treated mammary transplant technique in which organ cultures were incubated with different concentrations of selenium (as sodium selenite) before grafting to hosts for observation of tumorigenesis. Details of this procedure have been described previously (11). Mammary explants from female W/F rats were exposed to DMBA (1μg/ml) in the presence of insulin, estradiol, progesterone and prolactin for the first 3 days. On the fourth day, fresh hormone-supplemented medium without DMBA was replenished and the culture was continued for 3 more days. Selenium was present during the entire period of the culture. Explants were then transplanted in the subscapular fat pad of isologous hosts. Results are shown in Table IV.

When selenium in the culture was increased from 10^{-6} to 5×10^{-5} M there was a gradual inhibition of tumorigenesis following transplantation of the DMBA-treated mammary explants. Only 2 out of 25 rats developed tumors upon receiving the transplants that had been exposed to 0.05 mM of selenium in the medium. In contrast, 12 out of 25 rats in the control group developed tumors (no added selenium in the medium). The proliferative activity of the culture also was determined immediately before transplantation. Tritiated thymidine was added to the medium on day 6. Labeling was allowed to continue for 24 hr before the explants were fixed for autoradiography (13). It can be seen from Table IV that high levels of selenium markedly suppressed DNA synthesis in the culture. Those explants that had a low proliferative rate also had a low potential to develop into tumors when grafted to the recipients. The present finding thus provides a model to study the cytotoxic effect of selenium and its chemopreventive action in the initiation phase of neoplastic transformation.

Improvement of Selenium Chemoprevention by Combination with Vitamin E

Since high levels of selenium (e.g. 5 ppm) lead to a slight depression in growth of the animals, we have been trying to improve the anticarcinogenic efficacy of lower levels of selenium by combining it with other agents. Our experience with vitamin E proved to be most promising. The rationale for selecting vitamin E is two-fold. First, selenium and vitamin E share in common the role of endogenous antioxidants. Second, there is ample evidence in the literature which shows that they have a sparing effect on each other in the prevention of several nutritional deficiency diseases.

In this experiment, rats were fed a 20% corn oil diet containing 0.1 ppm selenium and 50 mg vitamin E per kg of diet (NRC recommended requirement). Additional selenium (2.5 ppm) and vitamin E (DL-α-tocopheryl

acetate, 1,000 mg/kg of diet) were tested singly and in combination. Rats were maintained on a high polyunsaturated fat diet, thus enabling us to evaluate the efficacy of the vitamin E and selenium combination treatment under a more vigorous condition of oxidant stress. Selenium was supplemented in the diet for the entire duration of the experiment, while additional vitamin E was present for various lengths of time, depending on the experimental design. The reason for adopting this protocol is that we have found previously that a continuous intake of selenium is necessary to achieve a maximal inhibitory response. By supplementing vitamin E for a defined period either around the time of or after DMBA administration, we can examine the effect of vitamin E during the initiation and promotion phases of mammary carcinogenesis.

In the first animal carcinogenicity study, both selenium and vitamin E were added to the diet starting 2 weeks before DMBA administration (10 mg at 50 days of age) and continued until the animals were sacrificed 25 weeks later. Figure 2 illustrates the per cent incidence of rats with palpable tumors as a function of time in the 4 experimental groups. Selenium supplementation (Group 2) led to a modest reduction compared to the controls (Group 1); the difference, however, was not statistically significant. Vitamin E by itself had no effect (Group 3), but a combination of selenium and vitamin E resulted in the only significant inhibitory response (Group 1 vs Group 4, $P < 0.05$).

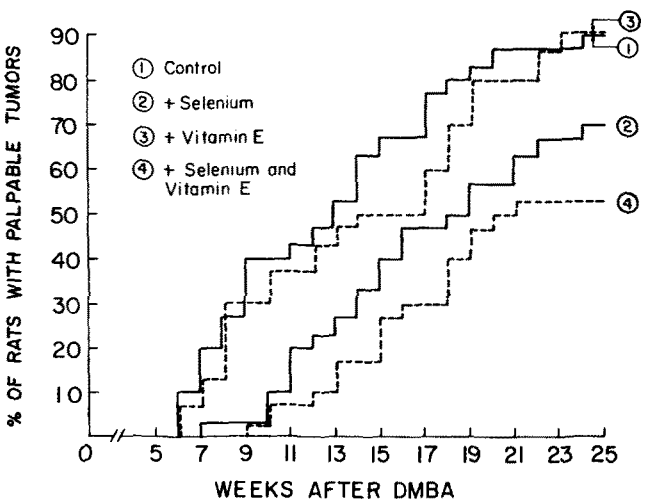

FIG. 2. Effect of selenium and/or vitamin E supplementation on the cumulative palpable mammary tumor incidence in rats fed a 20% corn oil diet. The control diet contained 0.1 ppm of selenium and 50 mg of vitamin E per kg of diet. Additional selenium and vitamin E were present at 2.5 ppm and 1,000 mg/kg of diet, respectively.

Table V summarizes the total tumor yield and the data on the number of tumors per tumor-bearing rat and the time of first tumor appearance. Rats supplemented with selenium produced fewer tumors (90 in Group 2 vs 132 in Group 1, $P < 0.01$), whereas those given vitamin E did not manifest any meaningful reduction (Group 3). In contrast, rats supplemented with both selenium and vitamin E developed the least number of tumors (Group 4), with a tally even lower than that of the selenium-supplemented group (difference between Group 2 and Group 4 was statistically significant, $P < 0.05$). These observations suggested that vitamin E, although ineffective by itself, was able to potentiate the anticarcinogenic action of selenium.

We decided to ascertain if vitamin E exerted its effect on the initiation or promotion phase of DMBA-induced mammary carcinogenesis. In the second experiment, vitamin E was tested only in combination with selenium. Selenium was supplemented in the diet from −2 to +24 weeks, while vitamin E was supplemented for different periods of time: −2 to +24 weeks, −2 to +2 weeks, and +2 to +24 weeks. Results in Table VI show that vitamin E enhanced the prophylactic effect of selenium only when it was present in the post-initiation or promotion phase (Groups 3 and 5). Supplementation with vitamin E around the time of DMBA administration (−2 to +2 weeks) produced no beneficial effect (Group 4).

Effect of Selenium and/or Vitamin E on Lipid Peroxidation and Glutathione Peroxidase Activity

In view of the well known antioxidant property of both selenium and vitamin E, we proceeded to investigate their effects on the peroxidative potential of the mammary tissue. Lipid peroxidation was measured by the thiobarbituric acid method (24). The principal reactant is considered to be malondialdehyde (MDA), which is produced by lipid

TABLE V

Effect of Selenium and/or Vitamin E Supplementation on DMBA-Induced Mammary Carcinogenesis

Group[a]	Dietary supplement[b]	Rats with tumors[c]	Tumor incidence[d] (%)	Total no. of tumors[e]	Tumors per tumor-bearing rat[f]	Latency period[g] (wk)
1	None	28	93	132	4.7 ± 0.4	12.2 ± 1.0
2	Selenium (Se)	23	77	90	3.9 ± 0.4	15.0 ± 1.1
3	Vitamin E	27	90	122	4.5 ± 0.3	13.7 ± 1.0
4	Se + Vit E	18	60	60	3.3 ± 0.3	15.7 ± 0.9

In this experiment, both selenium and vitamin E were supplemented starting 2 weeks before DMBA administration and continued until the animals were sacrificed.

[a]There were 30 rats per group. All rats received 10 mg DMBA i.g. at 50 days of age and were killed 25 weeks later.

[b]Selenium (Se) and/or vitamin E (Vit E) were supplemented in the diet at a concentration of 2.5 mg/kg and 1,000 mg/kg, respectively.

[c]Include rats with nonpalpable tumors discovered at autopsy.

[d]Only Group 4 is statistically different from Group 1 ($P < 0.05$).

[e]Groups 2 and 4 are different from Group 1 ($P < 0.01$). Group 4 is different from Group 2 ($P < 0.05$).

[f]Values are expressed as mean ±S.E. Only Group 4 is different from Group 1 ($P < 0.05$).

[g]Latency period is denoted as the time between DMBA administration and the appearance of the first palpable tumor. Values are expressed as mean ±S.E. Groups 2 and 4 are different from Group 1 ($P < 0.05$).

TABLE VI

Effect of Selenium and/or Vitamin E Supplementation on DMBA-Induced Mammary Carcinogenesis

Group[a]	Dietary supplement[b]	Duration of vitamin E supplementation[c] (wk)	Rats with tumors[d]	Tumor incidence[e] (%)	Total no. of tumors[f]	Tumors per tumor-bearing rat[g]	Latency period[h] (wk)
1	None	–	23	92	113	4.9 ± 0.4	10.1 ± 1.0
2	Selenium (Se)	–	18	72	73	4.1 ± 0.3	12.3 ± 1.1
3	Se + Vit E	−2 to +24	12	48	36	3.0 ± 0.3	13.7 ± 1.1
4	Se + Vit E	−2 to +2	19	76	66	3.5 ± 0.4	12.8 ± 1.0
5	Se + Vit E	+2 to +24	14	56	40	2.9 ± 0.3	12.5 ± 1.1

In this experiment, additional selenium was present in the diet for the entire duration of the study in Groups 2 to 5, while vitamin E was present for different periods of time.

[a]There were 25 rats per group. All rats received 10 mg DMBA i.g. at 50 days of age and were killed 24 weeks later.

[b]Selenium (Se) and/or vitamin E (Vit E) were supplemented in the diet at a concentration of 2.5 mg/kg and 1,000 mg/kg, respectively. Additional selenium was supplemented starting 2 weeks before DMBA administration and continued until the end of the experiment. Vitamin E was present for different periods of time as indicated in column 3.

[c]The time of DMBA administration was taken as time 0; minus and plus signs represent the time in weeks before and after DMBA administration, respectively.

[d]Includes rats with nonpalpable tumors discovered at autopsy.

[e]Only Groups 3 and 5 are statistically different from Group 1 ($P < 0.01$).

[f]Groups 2, 3, 4 and 5 are all different from Group 1 ($P < 0.05$). Groups 3 and 5 are different from Group 2 ($P < 0.02$).

[g]Values are expressed as mean ±S.E. Groups 3, 4 and 5 are different from Group 1 ($P < 0.02$). Groups 3 and 5 are different from Group 2 ($P < 0.05$).

[h]Latency period is denoted as the time between DMBA administration and the appearance of the first palpable tumor. Values are expressed as mean ±S.E. Only Group 3 is different from Group 1 ($P < 0.05$).

TABLE VII

Effect of Selenium and/or Vitamin E Supplementation on Lipid Peroxidation and Selenium-Dependent Glutathione Peroxidase (GSH-Px) Activity in the Mammary Fat Pad

Group[a]	Dietary supplement[b]	Lipid peroxidation[c]	Se-dependent GSH-Px[d]
1	None	355 ± 30	44 ± 3
2	Selenium (Se)	320 ± 28	52 ± 4
3	Vitamin E	142 ± 12[e]	41 ± 3
4	Se + Vit E	121 ± 10[e]	54 ± 4

[a]There were 8 rats per group. All rats received 10 mg DMBA i.g. at 50 days of age and were killed 2 mo later.

[b]Selenium and/or vitamin E (Vit E) were supplemented in the diet at a concentration of 2.5 mg/kg and 1,000 mg/kg, respectively. Both selenium and vitamin E supplementations were started 2 weeks before DMBA administration and continued until the animals were sacrificed.

[c]Values are expressed as nmol MDA formed/g tissue, mean ±S.E.

[d]Hydrogen peroxide was used as the substrate to assay for the Se-dependent GSH-Px activity. Values are expressed as nmol NADPH oxidized/min/mg protein, mean ±S.E.

[e]Statistically different from Groups 1 and 2 ($P < 0.001$).

peroxidation and released upon heating the sample in an acid medium. Results in Table VII show that vitamin E significantly suppressed peroxidation, whereas selenium had no effect. A combination of selenium and vitamin E did not result in further inhibition compared to vitamin E alone. With respect to the selenium-dependent glutathione peroxidase activity as measured by the coupled assay of Paglia and Valentine (25), selenium supplementation produced only a slight but insignificant increase. This suggests that in control rats receiving 0.1 ppm of selenium, the enzyme already is operating at near maximal capacity. Additional selenium will not further increase its activity, since the enzyme protein becomes the limiting factor.

DISCUSSION

The present study confirms previous findings by other investigators that selenium supplementation above dietary requirement inhibits tumorigenesis. In addition, our observations lead to the conclusion that the optimal level of selenium for manifestation of this protective effect depends on the dose of the carcinogen and the nutritional status of the animal, specifically in relation to fat intake. Higher selenium supplementation is necessary to neutralize the insult produced by a larger dose of DMBA. Moreover, we found that selenium was unable to counteract completely the enhancing effect of dietary fat in mammary carcinogenesis, since rats fed a high fat diet still developed more tumors than those fed a low fat diet at comparable selenium intake level. By supplementing selenium for defined lengths of time, we showed that selenium can inhibit both the initiation and promotion phases of carcinogenesis and that a continuous intake of selenium is necessary to achieve maximal inhibition of tumorigenesis.

In the present study, we found that additional selenium supplement failed to increase glutathione peroxidase activity in the mammary tissue. Selenium is an integral component of this enzyme which is involved in the destruction of hydroperoxides (5). Our observation is in agreement with the report by Lane and Medina (14). These results suggest that the anticarcinogenic action of selenium may not be mediated by its antioxidant function via glutathione peroxidase. Vitamin E is a much more potent antioxidant than selenium. Our experiment using a combination of vitamin E and selenium indicates that although the suppression of lipid peroxidation by vitamin E alone is not sufficient to inhibit tumor formation, vitamin E may provide a favorable environment against oxidant stress to facilitate selenium in exerting its anticarcinogenic action through some other mechanisms.

Little information is available on the mode of action of selenium. Reports from Griffin's laboratory showed that selenium impedes activation and accelerates detoxification of 2-acetylaminofluorene (3,16). Other evidence that supports a role for selenium in the initiation phase includes protection of liver DNA against single-strand breakage induced by 2-acetylaminofluorene (37) and facilitation of the repair process (15). Pharmacological levels of selenium

have been reported to potentiate the immune response of the host (29-31). Exposure to high concentrations of selenium is known to inhibit DNA synthesis (20) such that cells are blocked in the S-G2 phase of the cell cycle (17). A modulation of mitochondrial function by selenium also has been suggested as one of the early effects of growth inhibition (19). It is likely that selenium may be acting through several mechanisms. Any working hypothesis concerning the mode of action of selenium should accommodate the observation that selenium inhibits both virus- and chemical carcinogen-induced tumors and that it is effective during the proliferative or promotion phase of tumorigenesis.

ACKNOWLEDGMENT

This work was supported by grant CA 27706 from the National Cancer Institute, NIH.

REFERENCES

1. Dao, T.L., Science 165:810 (1969).
2. Dao, T.L.; D. Sinha, S. Christakos and R. Varela, Cancer Res. 35:1128 (1975).
3. Daoud, A.H., and A.C. Griffin, Cancer Lett. 5:231 (1978).
4. Haslam, S.Z., Int. J. Cancer 23:374 (1979).
5. Hoekstra, W.G., Fed. Proc. 34:2083 (1975).
6. Horvath, P.M., and C. Ip, Cancer Res. 43, In press.
7. Ip, C., Ibid. 40:2785 (1980).
8. Ip, C., Ibid. 41:2683 (1981).
9. Ip, C., Ibid. 41:4386 (1981).
10. Ip, C.; M.M. Ip and U. Kim, Cancer Lett. 14:101 (1981).
11. Ip, C., and D. Sinha, Ibid. 11:277 (1981).
12. Ip, C., and D. Sinha, Carcinogenesis 2:435 (1981).
13. Koyama, H.; D. Sinha and T.L. Dao, J. Natl. Cancer Inst. 48:1671 (1972).
14. Lane, H.W., and D. Medina, Cancer Res. 43:1558 (1983).
15. Lawson, T., and D. Birt, Proc. Am. Assoc. Cancer Res. 22:93 (1981).
16. Marshall, M.V.; M.S. Arnott, M.M. Jacobs and A.C. Griffin, Cancer Lett. 7:331 (1979).
17. Medina, D.; H.W. Lane and C.M. Tracey, Cancer Res. 43:2460 (1983).
18. Medina, D.; H. Lane and F. Shepherd, Anticancer Res. 1:377 (1981).
19. Medina, D.; F. Miller, C.J. Oborn and B.B. Asch, Cancer Res. 43:2100 (1983).
20. Medina, D., and C.J. Oborn, Cancer Lett. 13:333 (1981).
21. Medina, D., and F. Shepherd, Ibid. 8:241 (1980).
22. Medina, D., and F. Shepherd, Carcinogenesis 2:451 (1981).
23. Meranze, D.R.; M. Gruenstein and M.B. Shimkin, Int. J. Cancer 4:480 (1969).
24. Ohkawa, H.; N. Ohishi and K. Yagi, Anal. Biochem. 95:351 (1979).
25. Paglia, D.E., and W.N. Valentine, J. Lab. Clin. Med. 70:158 (1967).
26. Schrauzer, G.N.; J.E. McGinness and K. Kuehn, Carcinogenesis 1:199 (1980).
27. Schrauzer, G.N.; D.A. White and C.J. Schneider, Bioinorg. Chem. 8:387 (1978).
28. Sinha, D., and T.L. Dao, J. Natl. Cancer Inst. 64:519 (1980).
29. Spallholz, J.E.; J.L. Martin, M.L. Gerlach and R.H. Heinzerling, Proc. Soc. Exp. Biol. Med. 143:685 (1973).
30. Spallholz, J.E.; J.L. Martin, M.L. Gerlach and R.H. Heinzerling, Infect. Immun. 8:841 (1973).
31. Spallholz, J.E.; J.L. Martin, M.L. Gerlach and R.H. Heinzerling, Proc. Soc. Exp. Biol. Med. 148:37 (1975).
32. Thompson, H.J., and P.J. Becci, J. Natl. Cancer Inst. 65:1299 (1980).
33. Thompson, H.J.; L.D. Meeker, P.J. Becci and S. Kokoska, Cancer Res. 42:4954 (1982).
34. Watrach, A.M.; J.A. Milner and M.A. Watrach, Cancer Lett. 15:137 (1982).
35. Welsch, C.W.; M. Goodrich-Smith, C.K. Brown, H.D. Greene and E.J. Hamel, Carcinogenesis 2:519 (1981).
36. Whanger, P.D.; J.A. Schmitz and J.H. Exon, Nutrition & Cancer 3:240 (1982).
37. Wortzman, M.S.; H.J. Besbris and A.M. Cohen, Cancer Res. 40:2670 (1980).
38. Young, S., and R.C. Hallowes, in Pathology of Tumors in Laboratory Animals, ed., V.S. Turusov, Vol. 1, Tumors of the Rat, Part 1. Lyon:IARC (1973), p. 31.

[Received June 20, 1984]