Synergistic Effect of Vitamin E and Selenium in the Chemoprevention of Mammary Carcinogenesis in Rats

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ABSTRACT

The present study showed that vitamin E, although ineffective by itself, was able to potentiate the ability of selenium to inhibit the development of mammary tumors induced by dimethylbenz(a)anthracene (DMBA) in rats. Animals were maintained on a high-polyunsaturated fat (20% corn oil) diet in order to increase the degree of oxidant stress; additional selenium and/or vitamin E were present at a concentration of 2.5 and 1000 mg/kg of diet, respectively. It should be noted that rats tolerated these levels of supplementation very well with no obvious undesirable effect. Furthermore, our results indicated that vitamin E facilitated the anticarcinogenic action of selenium only when it was present during the proliferative phase. We then proceeded to examine whether DMBA administration would lead to any persistent damage in tissue peroxidation or changes in activities of enzymes associated with peroxide metabolism. It was found that DMBA resulted in an acute but modest increase in lipid peroxidation at 24 hr after carcinogen treatment. This perturbation was only of a transient nature. By comparing the response in a target tissue (mammary fat pad) and a non-target tissue (liver), it can be inferred that DMBA may have a differential effect on the degree of oxidant stress. The antagonistic effect of selenium and vitamin E in suppressing lipid peroxidation was then evaluated. Several conclusions can be drawn regarding the antioxidant potency of these agents in conjunction with their efficacies in cancer prevention. First, although vitamin E is a more effective antioxidant than selenium, it is apparent that systemic suppression of lipid peroxidation by vitamin E subsequent to a carcinogenic insult is not sufficient to inhibit tumor formation. Vitamin E supplementation increases significantly the microsomal hydroperoxidase activity. At the present time, it is unclear what role, if any, this enzyme plays in the synergistic effect of vitamin E and selenium in the inhibition of tumorigenesis. Secondly, the anticarcinogenic action of high levels of selenium is not related to its biochemical function in the regulation of the selenium-dependent glutathione peroxidase. The explanation for this is that the enzyme is already operating at near maximal capacity under normal physiological conditions. Additional selenium will not further increase its activity, since the enzyme protein becomes the limiting factor. Finally, vitamin E may be able to provide a more favorable climate against oxidant stress, thereby potentiating the action of selenium via some other mechanism.

INTRODUCTION

There is growing documentation that supranutritional levels of selenium, when supplemented in the diet or drinking water, can protect laboratory animals against the development of mammary neoplasia (10, 11, 14, 20, 21, 28, 32, 34–37). In general, the chemopreventive effect of selenium is manifested in the form of a lower tumor incidence, a reduction in tumor yield, and a longer latency period. Using the DMBA3-induced mammary tumor model, we have reported that the prophylactic response was affected by the dose of the carcinogen as well as by the fat intake of the animals (10). Furthermore, it was observed that the degree of inhibition was proportional to the level of dietary selenium up to 5 ppm, at which point toxicity, as indicated by a slight depression in weight gain, was evident.

One of the objectives of our research effort in selenium and cancer is to improve the anticarcinogenic efficacy of lower levels of selenium by combining it with other agents. The rationale for selecting vitamin E as a partner is 3-fold, (a) Both selenium and vitamin E share in common the role of endogenous antioxidants (31). (b) There is ample evidence in the literature that they have a sparing effect on each other in the prevention of several nutritional deficiency diseases (8). (c) Recent reports have suggested that vitamin E is capable of inhibiting tumorigenesis in various experimental systems (4, 5, 7, 29, 30).

In the present study, rats given DMBA 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 previously found that a continuous intake of selenium is necessary to achieve a maximal inhibitory response (11). 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. The effects of selenium and/or vitamin E on tissue lipid peroxidation were also evaluated in an attempt to determine if there was any correlation between the efficacies of these antioxidants in suppressing peroxidation and their ability to inhibit tumorigenesis.

MATERIALS AND METHODS

Animals and Diets. Female Sprague-Dawley rats were purchased from Charles River Breeding Laboratories, Wilmington, Mass. All animals used in this report were fed a synthetic diet containing 20% corn oil from weaning until termination of the experiment. Food and water were available ad libitum. The components of the basal diet are shown as follows: corn oil (Mazola), 20%; vitamin-free casein, 23.5%; dextrose, 44.7%; AIN-76 mineral mix, 4.1%; AIN-76 vitamin mix (with 1980 modification), 1.2%; Alphaceil, 5.9%; DL-methionine, 0.3%; and choline bitartrate, 0.2%. Stripped corn oil was not used because the amount of

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vitamin E supplemented in the diet (see below) far exceeded the quantity of vitamin E that is present in commercially available corn oil. According to the information given by the supplier, corn oil contains about 0.02% of \( \alpha \)-tocopherol. Thus, the corn oil in a 20% fat diet will contribute an additional 40 mg of \( \alpha \)-tocopherol per kg of diet. The cost of the stripped corn oil does not justify its use in studies concerning supplementation of vitamin E in large quantities.

The AIN-76 mineral and vitamin mixtures provide 0.1 mg of selenium and 50 mg of vitamin E per kg of diet, respectively. Additional selenium (2.5 mg/kg in the form of sodium selenite) and vitamin E (1000 mg/kg in the form of DL-\( \alpha \)-tocopheryl acetate) were tested singly and in combination. Selenium was supplemented starting 2 weeks before DMBA administration and continued until the end of the experiment. The schedule of vitamin E supplementation varied among experiments and will be indicated accordingly in each of the tables and charts in the text. Rats were weighed biweekly to monitor their growth.

**Mammary Tumor Induction and Statistical Analysis of Tumor Data.**

Mammary tumors were induced by i.g. administration of 10 mg of DMBA (Sigma Chemical Co., St. Louis, Mo.) at 50 days of age. The method of DMBA administration has been described in detail previously (9). Rats were palpated weekly to determine the appearance and the location of the tumors and were sacrificed 24 or 25 weeks after DMBA treatment. At autopsy, the rats were examined for nonpalpable tumors. All tumors were excised, fixed in Bouin’s reagent, and sectioned for histology. Mammary tumor pathology was confirmed according to the criteria of Young and Hallowes (38). Only adenocarcinomas are reported under “Results.”

In the first animal carcinogenicity study, the effect of selenium and/or vitamin E supplementation on the induction of mammary tumors by DMBA was examined. Both selenium and vitamin E were added to the diet starting 2 weeks before DMBA administration and continued until the animals were sacrificed 25 weeks later. Chart 1 illustrates the percentage of 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 versus Group 4; \( p < 0.05 \)).

Table 1 summarizes the total tumor yield (including nonpalpable tumors discovered at autopsy) 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 versus 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 (the 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. The inhibitory effect due to selenium-vitamin E was somewhat obscured when the data were expressed as number of tumors per tumor-bearing rat. Selenium supplementation also slightly prolonged the latency period; vitamin E failed to accentuate this delay as indicated in Group 4.

It should be noted that there was no difference in early mortality among the 4 groups; approximately 3 to 4 rats in each group had to be sacrificed at various times before termination of the experiment either when they became moribund or when the

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**RESULTS**

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Vitamin E, Selenium, and Mammary Carcinogenesis

Effect of selenium and/or vitamin E supplementation on DMBA-induced mammary carcinogenesis

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary supplement</th>
<th>Rats with tumors</th>
<th>Tumor incidence (%)</th>
<th>Total no. of tumors</th>
<th>Tumors/tumor-bearing rat</th>
<th>Latency period (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>28</td>
<td>93</td>
<td>132</td>
<td>4.7 ± 0.4</td>
<td>12.2 ± 1.0</td>
</tr>
<tr>
<td>2</td>
<td>Selenium</td>
<td>23</td>
<td>77</td>
<td>90</td>
<td>3.9 ± 0.4</td>
<td>15.0 ± 1.1</td>
</tr>
<tr>
<td>3</td>
<td>Vitamin E</td>
<td>27</td>
<td>90</td>
<td>122</td>
<td>4.5 ± 0.3</td>
<td>13.7 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>Selenium + vitamin E</td>
<td>18</td>
<td>60</td>
<td>60</td>
<td>3.3 ± 0.3</td>
<td>15.7 ± 0.9</td>
</tr>
</tbody>
</table>

Selenium and/or vitamin E were supplemented in the diet at a concentration of 2.5 and 1000 mg/kg, respectively.

Tumors became very big and necrotic and the rats started to lose weight. Otherwise, the animals seemed to tolerate both selenium (2.5 mg/kg) and vitamin E (1000 mg/kg) supplementation very well with no apparent ill effect on the final body weight at autopsy. The final body weights and organ weights are presented in Table 2. No significant difference was detected in any of the supplemented groups when compared to the controls.

In view of the well-known antioxidant property of both selenium and vitamin E, we proceeded to perform a series of experiments to examine (a) whether DMBA administration resulted in any change in lipid peroxidation and (b) whether selenium and vitamin E might have an antagonistic effect. Both the mammary fat pad (target organ) and the liver (non-target organ) were selected for this study. In the first experiment designed to evaluate the acute effect of the carcinogen, groups of rats were given a p.o. dose of 10 mg DMBA dissolved in 1 ml of corn oil (same as in the carcinogenicity experiment) and were sacrificed after 6, 24, or 48 hr. Control rats were given the vehicle only. As shown in Chart 2, DMBA caused a modest but insignificant increase of lipid peroxidation in the mammary fat pad at 24 hr. In contrast, the enhancement in the liver at the same time point was statistically significant. By 48 hr, the peroxidation values in both tissues had returned to control levels, suggesting that the perturbation due to DMBA was probably a transient phenomenon.

The effects of selenium and/or vitamin E on lipid peroxidation are illustrated in Chart 3. All animals in this experiment were sacrificed 24 hr after the administration of 10 mg DMBA (Subgroup B) or the vehicle (Subgroup A). Selenium was found to be ineffective in inhibiting lipid peroxidation in either the control or DMBA-treated rats. A high intake of vitamin E suppressed in vitro peroxidation by about 50% in both Subgroup A and Subgroup B, whereas a combination of selenium and vitamin E did not result in further inhibition compared to vitamin E alone.

It is possible that DMBA administration may lead to a delayed but persistent damage to the defense mechanism of the cells against peroxide accumulation. A third experiment was therefore initiated in which the animals were sacrificed 2 months after carcinogen treatment. The effects of selenium and/or vitamin E supplementation on tissue lipid peroxidation and the activities of 3 enzymes associated with peroxide metabolism were investigated. The 3 enzymes that we focused on were the selenium-dependent glutathione peroxidase, the selenium-dependent glutathione peroxidase, and the microsomal hydroperoxidase. The reason for assaying the last enzyme is that it has been shown that cytochrome P-450 can accept oxygen from a variety of donors, including cumene hydroperoxide and hydrogen peroxide (24). Since no attempt was made to separate the selenium-independent and selenium-dependent glutathione peroxidases...
animals were sacrificed 24 hr after the administration of 10 mg DMBA (Subgroup B) or the vehicle (Subgroup A). NS, not significant.

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before analysis, the former was assayed as part of the total glutathione peroxidase activity using cumene hydroperoxide as the substrate.

It is important to point out that we found that DMBA had no effect on lipid peroxidation or on the activities of the 3 enzymes in both the mammary fat pad and the liver when these biochemical functions were determined 2 months after carcinogen administration. In order to avoid redundancy, control values (from rats treated with vehicle only) were not presented. Table 3 shows the effect of dietary selenium and/or vitamin E supplementation on lipid peroxidation in rats that were given 10 mg DMBA at 50 days of age and were sacrificed 2 months later. Results of this experiment were very similar to that described in Chart 3 in that vitamin E significantly suppressed peroxidation whereas selenium had no effect. A comparison of the peroxidative potential in rats killed 3 days or 2 months after DMBA treatment revealed no significant alteration (results not shown), suggesting that the degree of oxidant stress was unaffected as a function of time after carcinogenic insult.

Table 4 describes the enzyme activities in the mammary fat pad and the liver. There was no change in the total glutathione peroxidase activity in any of the supplemented groups. With respect to the selenium-dependent glutathione peroxidase activity, selenium supplementation produced a slight but insignificant increase in the mammary fat pad and only a 25% elevation in the liver. Vitamin E, on the other hand, resulted in a 50% increase ($p < 0.01$) in the microsomal hydroperoxidase activity. It remained to be determined whether this was sufficient to account for the suppression of lipid peroxidation by vitamin E as reported in Table 3.

On the basis of our observation concerning the effect of vitamin E on lipid peroxidation, we decided to ascertain if vitamin E facilitated the anticarcinogenic action of selenium by exerting its effect on the initiation or promotion phase of DMBA-induced mammary carcinogenesis. In this 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. The time of DMBA administration (50 days of age, 10 mg i.g.) was taken as 0; minus and plus signs represent the time before and after DMBA treatment, respectively. The different dietary groups involved in this study and the results of the carcinogenicity experiment are shown in Table 5.

Tumor incidence was reduced by selenium supplementation, although the difference between Group 1 and Group 2 was not statistically significant. As in the previous carcinogenicity experiment, vitamin E enhanced the prophylactic effect of selenium, but only when it was present in the postinitiation 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). The data on total tumor yield further confirmed the conclusion that the potentiating effect of vitamin E was exerted primarily on the proliferation stage of chemical carcinogenesis, since the total number of tumors in Groups 3 and 5 was significantly different from Group 2.

**DISCUSSION**

The present study shows that although vitamin E supplementation alone has no prophylactic effect against tumorigenesis, it potentiates the ability of selenium to inhibit the development of mammary neoplasia induced by DMBA. Furthermore, our results indicate that vitamin E facilitates the anticarcinogenic action of selenium only when it is present during the promotion or proliferative phase. The concept of using more than one nutritional supplement in an attempt to produce either a synergistic or an additive response in cancer prevention has been explored by several investigators (23, 27, 33), including our own laboratory (13). In terms of effectiveness, the most noteworthy example is the simultaneous supplementation of selenium and retinoid (vitamin A analogue). One drawback with this regimen is that levels of retinoid that normally inhibit tumorigenesis have also been observed to result in depressed growth. Our present approach seems rather promising, since rats are able to tolerate high levels of vitamin E very well with no obvious undesirable effect. Experiments are now under way to determine if lower levels of vitamin E can be used without compromising its efficacy in enhancing the anticarcinogenic action of selenium.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Effect of selenium and/or vitamin E supplementation on lipid peroxidation in mammary fat pad and liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group⁴</td>
<td>Dietary supplement⁵</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Selenium</td>
</tr>
<tr>
<td>3</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>4</td>
<td>Selenium + vitamin E</td>
</tr>
</tbody>
</table>

⁴ There were 8 rats/group. All rats received 10 mg DMBA i.g. at 50 days of age and were killed 2 months later.

⁵ Selenium and/or vitamin E were supplemented in the diet at a concentration of 2.5 and 1000 mg/kg, respectively. Both selenium and vitamin E supplementations were started 2 weeks before DMBA administration and continued until the animals were sacrificed.

⁶ Mean ± S.E.

⁷ Group 3 is statistically different from Group 1 and Group 2 ($p < 0.001$).

⁸ Group 4 is statistically different from Group 1 and Group 2 ($p < 0.001$).
Table 4
Effects of dietary selenium and/or vitamin E supplementation on the activities of glutathione peroxidase and microsomal hydroperoxidase in mammary fat pad and liver

<table>
<thead>
<tr>
<th>Group</th>
<th>Supplement</th>
<th>Total glutathione peroxidase</th>
<th>Selenium-dependent glutathione peroxidase</th>
<th>Microsomal hydroperoxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammary fat pad</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>99 ± 6</td>
<td>44 ± 3</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>Selenium</td>
<td>98 ± 6</td>
<td>52 ± 4</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>3</td>
<td>Vitamin E</td>
<td>105 ± 8</td>
<td>41 ± 3</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>4</td>
<td>Selenium + vitamin E</td>
<td>112 ± 9</td>
<td>54 ± 4</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>364 ± 28</td>
<td>151 ± 9</td>
<td>92 ± 6</td>
</tr>
<tr>
<td>2</td>
<td>Selenium</td>
<td>381 ± 29</td>
<td>186 ± 10*</td>
<td>85 ± 6</td>
</tr>
<tr>
<td>3</td>
<td>Vitamin E</td>
<td>352 ± 25</td>
<td>160 ± 9*</td>
<td>138 ± 9*</td>
</tr>
<tr>
<td>4</td>
<td>Selenium + vitamin E</td>
<td>370 ± 30</td>
<td>194 ± 11*</td>
<td>131 ± 8*</td>
</tr>
</tbody>
</table>

* There were 8 rats/group. All rats received 10 mg DMBA i.g. at 50 days of age and were killed 2 months later.

Selenium and/or vitamin E were supplemented in the diet at a concentration of 2.5 and 1000 mg/kg, respectively. Both selenium and vitamin E supplementations were started 2 weeks before DMBA administration and continued until the animals were sacrificed.

Cumene hydroperoxide was used as the substrate to assay for the total glutathione peroxidase activity.

Values are expressed as nmol NADPH oxidized per min per mg protein.

Hydrogen peroxide was used as the substrate to assay for the selenium-dependent glutathione peroxidase activity. Values are expressed as nmol NADPH oxidized per min per mg protein.

Values are expressed as nmol tetramethylphenyldiamine oxidized per min per mg protein.

Mean ± S.E.

Significantly different from Group 1 (p < 0.01).

Significantly different from Group 1 (p < 0.05).

Table 5
Effect of selenium and/or vitamin E supplementation on DMBA-induced mammary carcinogenesis

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary supplement</th>
<th>Duration of vitamin E supplementation (wk)</th>
<th>Rats with tumors</th>
<th>Tumor incidence (%)</th>
<th>Total no. of tumors</th>
<th>Tumors per tumor-bearing rat</th>
<th>Latency period (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td></td>
<td>23</td>
<td>92</td>
<td>113</td>
<td>4.9 ± 0.4</td>
<td>10.1 ± 1.0</td>
</tr>
<tr>
<td>2</td>
<td>Selenium</td>
<td></td>
<td>18</td>
<td>72</td>
<td>73</td>
<td>4.1 ± 0.4</td>
<td>12.3 ± 1.1</td>
</tr>
<tr>
<td>3</td>
<td>Selenium + vitamin E</td>
<td>-2 to +24</td>
<td>12</td>
<td>48</td>
<td>36</td>
<td>3.0 ± 0.3</td>
<td>13.7 ± 1.1</td>
</tr>
<tr>
<td>4</td>
<td>Selenium + vitamin E</td>
<td>-2 to +2</td>
<td>19</td>
<td>76</td>
<td>66</td>
<td>3.5 ± 0.4</td>
<td>12.8 ± 1.0</td>
</tr>
<tr>
<td>5</td>
<td>Selenium + vitamin E</td>
<td>+2 to +24</td>
<td>14</td>
<td>56</td>
<td>40</td>
<td>2.9 ± 0.3</td>
<td>12.5 ± 1.1</td>
</tr>
</tbody>
</table>

* There were 25 rats/group. All rats received 10 mg DMBA i.g. at 50 days of age and were killed 24 weeks later.

Selenium and/or vitamin E were supplemented in the diet at a concentration of 2.5 and 1000 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.

The time of DMBA administration was taken as Time 0; - and +, time in weeks before and after DMBA administration, respectively.

Includes rats with nonpalpable tumors discovered at autopsy.

Significantly different from Group 1 (p < 0.01).

Significantly different from Group 1 (p < 0.05).

Significantly different from Group 1 (p < 0.05).

Latency period is denoted as the time between DMBA administration and the appearance of the first palpable tumor. Only Group 3 is different from Group 1 (p < 0.05).

Number of rats which survived until the end of the experiment for Groups 1 to 5 was as follows: 22, 22, 23, 23, 23, respectively.

As indicated in Table 1, supplementation of vitamin E alone offered no protection against mammary neoplasia. We have reported recently that vitamin E excess also failed to overcome the enhancement of mammary carcino genesis caused by selenium depletion (12). These observations are in contrast to those in the literature which showed that vitamin E reduced experimental tumorigenesis in several organ sites including the breast (5, 7), skin (30), colon (4), and oral mucosa (29). In most of these studies, the controls were either vitamin E or fed laboratory chow. Our results, however, did agree with the conclusion of King and Otto (15) who found that 0.2% tocopherol in the diet was totally ineffective in inhibiting DMBA-induced mammary tumorigenesis in rats. The reason for the discrepancy among all these studies remains unclear. Before any consensus can be established regarding the efficacy of vitamin E, consideration should be given to the influence of exogenous factors such as dietary history and characteristics of the tumor model.

From a biochemical standpoint, both vitamin E and selenium are recognized as endogenous antioxidants (31). Vitamin E restricts the formation of peroxides by neutralizing free radicals, while selenium discharges its duty in an indirect way by being an integral component of glutathione peroxidase, an enzyme responsible for the removal of hydrogen peroxide and organic hydroperoxides. Thus, they work in tandem in controlling excessive accumulation of peroxides in the cells. Although the activity of the selenium-dependent glutathione peroxidase may respond...
slightly to high levels of selenium supplementation (6, 18), deple-
tion of this trace element invariably leads to much lower enzyme
levels (8). As shown in Table 4, the activity of the selenoenzyme
(assayed in the presence of hydrogen peroxide) in the liver was
only slightly elevated with 2.5 mg/kg of selenium in the diet,
confirming our previous observation in the mammary tissue
under similar nutritional condition (14). This relationship sug-
uggests that, in control animals receiving selenium (0.1 mg/kg) in the diet,
the enzyme is already operating at near maximal capacity. Ad-
ditional selenium will not further increase its activity significantly,
since the enzyme protein becomes the limiting factor. Medina et al.
(19) have also observed that DMBA administration (6 weekly
doses of 1 mg each) led to a persistent reduction in the selenium-
dependent glutathione peroxidase activity in the mammary gland
of mice 13 weeks after the last carcinogen treatment. This was
in contrast to our finding which showed no deleterious effect due to
DMBA. Differences in susceptibility between species and
carcinogen administration protocol may account for this discrep-
ancy even though DMBA is equally effective in inducing mam-
mary tumors in both rats and mice.

A second glutathione peroxidase activity, which is not depend-
ent on selenium, has been described in rat liver by Lawrence
and Burk (16). Unlike the selenium-dependent enzyme, it has
little activity toward hydrogen peroxide, but shows a greater affin-
ity for organic hydroperoxides. The method of using cumene
hydroperoxide as the substrate measures both the selenium-
dependent and selenium-independent glutathione peroxidases.
Our data showed that neither excess vitamin E nor selenium had
any effect on the total activity. It is therefore unlikely that the
anticarcinogenic action of vitamin E and selenium is mediated by
this reaction involving peroxide metabolism. Interestingly, vitamin
E supplementation was found to increase significantly the micro-
somal hydroperoxidase activity. At the present time, it is unclear
what role, if any, this enzyme plays in the synergistic effect of
vitamin E and selenium in the inhibition of tumorogenesis.

Several conclusions can be drawn from the effects of vitamin
E and selenium on lipid peroxidation in conjunction with their
efficacies on cancer prevention. Although vitamin E is a more
potent antioxidant than selenium, it is apparent that systemic
suppression of lipid peroxidation by vitamin E alone is not suffi-
cient to inhibit tumor formation. Likewise, the anticarcinogenic
action of high levels of selenium is not related to its biochemical
function in the regulation of glutathione peroxidase. Since the
anticarcinogenic effect of vitamin E is nonspecific in relation to target
tissue, it may provide a more favorable climate against oxidant
stress, thereby potentiating the action of selenium via some other
mechanism. The first-line defense against neoplastic dis-
ease is the host immune system. Not only is immunity depressed
under antioxidant-deficient conditions but, more importantly,
without supplementation with vitamin E and selenium in quantities
in excess of established dietary requirements is found to produce
immunostimulatory effects (1). An “adequate” intake of antioxi-
dants would obviously be advantageous, particularly in those
animals consuming high levels of dietary fat, since diets rich in
polyunsaturated lipid (and therefore of high peroxidative poten-
tial) are known to suppress certain immune functions (1). Further
research is needed to determine whether immunological rein-
forcement as a result of vitamin E and selenium supplementation
is the mechanism by which they work synergistically to produce
an inhibitory response in tumorogenesis.

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