Effect of Dietary Selenium on the Interaction between 2-Acetylaminofluorene and Rat Liver DNA in Vivo

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ABSTRACT

Male weanling Charles River CD rats were fed a Torula yeast-based selenium-deficient diet or the same diet supplemented with selenium (0.5 ppm), as sodium selenite. Animals fed the basal diet for 3 weeks developed a functional selenium deficiency as evidenced by an almost total loss of hepatic, selenium-dependent glutathione peroxidase (glutathione:H2O2 oxidoreductase, EC 1.11.1.9) activity. In contrast, rats fed the selenium-supplemented diet exhibited an increase in glutathione peroxidase activity during the same interval.

There was no difference between selenium-deficient and selenium-supplemented rats with respect to the total amount of 2-acetylaminofluorene covalently bound to liver DNA in vivo at 1, 4, 16, 24, 96, or 168 hr following a single i.p. injection of [2-14C]acetylaminofluorene. However, alkaline sucrose gradient analysis revealed the production of DNA single-strand breaks in the livers of selenium-deficient rats at 4 hr after i.p. injection of 2-acetylaminofluorene (10 mg/kg). These lesions were apparently repaired at 24 hr after injection of the carcinogen. Under the same conditions, 2-acetylaminofluorene failed to produce evidence of DNA damage in the livers of selenium-supplemented rats. Administration of 2-acetylaminofluorene at the dose of 20 mg/kg resulted in extensive degradation of hepatic DNA in both groups of rats. Repair of this damage occurred at 48 to 72 hr after carcinogen administration and was unaffected by the selenium status of the animals.

The possible involvement of peroxidative processes in 2-acetylaminofluorene-induced DNA damage was investigated by measuring the hepatic malondialdehyde content of rats at 4 hr after a single i.p. injection of the carcinogen at a dose of 10 mg/kg. Neither the selenium-supplemented nor the selenium-deficient animals exhibited any significant alteration in lipid peroxidation as a result of carcinogen administration. In contrast, the administration of carbon tetrachloride (1.5 ml/kg) resulted in a statistically significant increase in the hepatic malondialdehyde concentration of selenium-deficient animals and a less marked increase in selenium-supplemented rats.

The results of these experiments indicate that the protective effect of dietary selenium against 2-acetylaminofluorene-induced hepatocarcinogenesis in rats is not mediated by an alteration in the binding of the carcinogen to liver DNA. Rather, the anticarcinogenic action of selenium appears to be related, at least in part, to its ability to protect hepatic DNA against indirect damage induced by the administration of 2-acetylaminofluorene. This protective effect of selenium is readily overcome by increasing the dose of the carcinogen. Moreover, under these conditions, dietary selenium has no apparent effect on the rate of DNA repair.

INTRODUCTION

The inhibitory effect of selenium on the process of chemical carcinogenesis has recently become a subject of considerable research interest. The ability of selenium to protect laboratory animals against the induction of cancer by different classes of carcinogenic chemicals has been demonstrated under a variety of experimental conditions (11, 14, 17, 36). Selenium has also been found to decrease the incidence of "spontaneous" tumors in C3H mice (34, 35). Furthermore, the epidemiological studies of Shamberger and Willis (39) suggest that selenium may act to protect the human population against certain forms of cancer. Taken together, these findings indicate that selenium is a naturally occurring inhibitor of carcinogenesis.

The biochemical basis for the anticarcinogenic effect of selenium is, as yet, unknown. In mammalian cells, selenium, as selenocysteine, is a component of the catalytic site of GSH-Px (32, 46). This selenium-dependent enzyme preferentially utilizes reduced glutathione as a hydrogen donor but catalyzes nonspecifically the reduction of H2O2 and a wide spectrum of organic peroxides (24). Selenium-dependent GSH-Px is widely distributed among various tissues, where its activity is highly dependent upon the dietary intake of selenium (4, 13).

In view of the above considerations, the possibility exists that the anticarcinogenic effect of this trace element may be a reflection of the ability of selenium-dependent GSH-Px to protect cells against environmentally induced peroxidative damage (2, 12, 28, 42). Alternatively, selenium may act like synthetic antioxidants to alter either the metabolism of carcinogenic chemicals or their interaction with important tissue macromolecules (44).

In an effort to obtain a better understanding of the molecular mechanisms underlying the anticarcinogenic effect of selenium, we have undertaken studies into the interaction between dietary selenium and the hepatocarcinogen, AAF. The latter was chosen as a model compound since its metabolism and interaction with tissue nucleophiles has been extensively studied (26). Moreover, Harr et al. (14) have shown that dietary selenium protects rats against the induction of liver tumor by AAF. The results of our initial studies, reported herein, indicate that this protective effect of selenium may be due, in part, to its ability to protect rat liver against AAF-induced DNA single-strand breaks.

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MATERIALS AND METHODS

Chemicals. AAF and 3,5-diaminobenzoic acid (Gold Label) were purchased from Aldrich Chemical Co., Milwaukee, Wis. [9-14C]AAF was obtained from ICN Chemical and Radioisotope Division, Irvine, Calif., and was diluted with unlabeled AAF to a final specific activity of 10 μCi/μmol. Cumene hydroperoxide and 1,1,3,3-tetraethoxypropane were purchased from Pfaltz and Bauer, Stamford, Conn. Bovine serum albumin, calf thymus DNA, NADPH (type X, tetrasodium salt), and glutathione reductase (type III) were from Sigma Chemical Co., St. Louis, Mo. T-4 viral DNA (M.W. 1.2 x 10^6) and λ viral DNA (M.W. 3.2 x 10^6) were obtained from Miles Laboratories Inc., Elkhard Ind. All other chemicals were of the highest purity available.

Animals and Diets. Male weanling CD rats were obtained from Charles River Breeding Laboratories (Wilmington, Mass.) and were immediately divided into 2 groups. One group was fed the powdered, Torula yeast-based selenium-deficient diet of Hafeman et al. (13). The second group was fed the same diet supplemented with selenium (0.5 ppm), as sodium selenite. Both diets were prepared by Teklad Test Diets (Madison, Wis.). Food and deionized water were available ad libitum. Total food consumption was identical in both groups of rats. All experiments involving AAF were begun at least 3 weeks after placing the rats on their respective diets. The selenium-supplemented animals increased their consumption of selenium from 2.5 to 10 μg/rat/day during this period. As shown below, the animals not given selenium developed a biochemically demonstrable selenium deficiency during this interval.

GSH-Px Assay. The GSH-Px activity of the cytosol fraction of rat liver homogenates was quantitated by the coupled enzyme assay of Paglia and Valentine (29) as modified by Little and O’Brien (24). In this procedure, the oxidation of NADPH is coupled to the oxidation of glutathione by means of excess glutathione reductase.

Rats were killed by decapitation, and their livers were perfused in situ with ice-cold 0.15 M KCl solution. The livers were then excised, weighed, and homogenized in 4 volumes of ice-cold PBS. The homogenates were centrifuged for 10 min at 750 g to sediment unbroken cells and nuclei. The resulting supernatants were then centrifuged for 60 min at 100,000 g to sediment mitochondria and microsomes. The resulting cytosol fraction was then diluted with 4 volumes of PBS for use in the GSH-Px assay. Protein concentration was determined by the method of Lowry et al. (25) with bovine serum albumin as a standard.

The reaction mixture contained the following components in a volume of 3 ml: 0.1 M Tris-HCl, pH 8.5; 0.25 mM reduced glutathione; 0.25 mM NADPH; 4 units of glutathione reductase; and 1 to 2 mg of cytosol protein. This mixture was allowed to stand at room temperature for 5 min before initiating the reaction with either cumene hydroperoxide (0.15 mM, final concentration) or H_2O_2 (final concentration, 0.25 mM). When H_2O_2 was used, 1 mM sodium azide was present in the incubation mixture to inhibit endogenous catalase activity.

NADPH oxidation was quantitated by recording the decrease in absorption at 340 nm in a Gilford model spectrophotometer. Under these conditions, the oxidation of NADPH was linear for 1 to 2 min and was proportional to the protein concentration of the cytosol fraction. Nonenzymatic oxidation of NADPH was similarly quantitated through the use of blank reaction mixtures in which the cytosol was replaced by an equal volume of PBS. After subtracting the blank values, results were recorded as nmol NADPH oxidized per mg protein per min.

In Vivo Binding of AAF to Hepatic DNA. Rats were given i.p. injections of [9-14C]AAF (100 μCi/10 μmol/kg) and were killed at specified intervals thereafter. Hepatic DNA was isolated by a modification of the method of Irving and Vaezy (16). Livers were homogenized in 15 volumes of 6% sodium p-amino salicylate and then extracted 3 times (1 hr, 15 min, and 15 min) with phenol:cresol (500 g phenol, 70 ml m-cresol, 55 ml H_2O and 0.5 g 8-hydroxyquinoline). After each extraction, the phases were separated by centrifugation for 20 min at 10,000 x g in the type GSA rotor of a Sorvall Model RC-2 centrifuge. Nucleic acids were precipitated from the final aqueous phases by the addition of 2 volumes of cold (—20°) ethanol:m-cresol (9:1, v/v) and wound onto glass rods. The precipitate was then dissolved in 10 ml of 1 x SSC. rRNA was precipitated by the addition of an equal volume of ice-cold 6 M potassium acetate (pH 6.0). After allowing the mixture to stand at 0-4° for 1 hr, the rRNA was sedimented by centrifugation at 12,000 x g. DNA was precipitated from the resulting supernatant by the addition of 2 volumes of absolute ethanol (—20°). The precipitate was wound onto a glass rod, dissolved in 10 ml of 1 x SSC, and centrifuged for 1 hr at 100,000 x g to remove glycogen. The resulting clear supernatant was incubated at 37° for 30 min with 3 to 4 mg of heat-treated (100° for 10 min) pancreatic RNase. The mixture was then extracted 3 times with phenol (saturated with 1 x SSC) and centrifuged for 22 min at 10,000 x g to separate the phases. The combined aqueous phases were extracted with ether to remove dissolved phenol and then bubbled with N_2 to remove the ether. Solid sodium acetate was added to a final concentration of 4% (w/v), and the DNA was precipitated by the addition of 2 volumes of 2-ethoxyethanol. The DNA was wound onto a glass rod, washed with absolute ethanol, and dried under a stream of nitrogen. The final preparation was free of RNA as measured by the orcinol procedure (33) and contained an average of 3% protein, as determined by the procedure of Lowry et al. (25). No radioactivity was associated with the contaminating protein.

The purified DNA was hydrolyzed at 100° for 10 min in 1.7 M perchloric acid. After cooling, an aliquot was removed for determination of DNA content by the method of Burton (3), using calf thymus DNA as a standard. A second aliquot was dissolved in 10 ml of Scintisol (Isolab Inc., Akron, Ohio) and counted on a Beckman Model LS-335 liquid scintillation spectrometer. Counting efficiency was estimated following the addition of a [14C]toluene internal standard. Results are reported as dpm/mg DNA.

DNA Damage and Repair. The induction of single-strand breaks by AAF and their subsequent repair were determined by the method of Zubroff and Sarma (47). Rats were given a single i.p. injection of AAF (10 or 20 mg/kg) dissolved in corn oil. Control rats received an equal volume of corn oil alone. At specified intervals thereafter, the animals were killed by decapitation, and a 1- to 2-g piece of liver was excised and placed in a chilled watch glass containing ice-cold squashing solution (0.024 M EDTA:0.75 M NaCl, pH 7.4). The liver was gently disrupted with a spatula and then centrifuged for 2 min at 200 x g to sediment large tissue fragments. The resulting supernatant primarily consisted of a suspension of hepatocytes and...
M. S. Wortzman et al.

hepatocyte nuclei which were lysed and subjected to alkaline sucrose sedimentation, as described below.

Linear 5 to 20% sucrose gradients (16 ml) prepared in 0.9 m NaCl:0.3 m NaOH were layered on top of a 2.3-ml shelf of 2.3 m sucrose:0.9 m NaCl:0.3 m NaOH. The gradients were then layered with 0.5 ml of lysing solution (0.3 m NaCl:0.3 EDTA:0.5% sodium lauryl sulfate:0.1 m Tris, pH 12.5). A 0.5-ml aliquot of the cell suspension was then gently layered onto the lysing solution and, in turn, was covered with an additional 0.2 ml of lysing solution. The cells were allowed to lyse for 30 min before filling the tubes with light mineral oil. The filled tubes were then placed on a Beckman SW27 swinging bucket rotor and centrifuged at 20° for 30 min, at 25,000 rpm in a Beckman model L5 ultracentrifuge. The rotor was allowed to decelerate without braking. After piercing the bottom of the tubes, the gradients were pumped through an ISCO Model 328 fraction collector. Twenty fractions of 1 ml each were collected from each gradient.

The relative DNA content of the fractions was determined fluorimetrically after reaction with 3,5-diaminobenzoic acid hydrochloride. To each tube, 50 μl bovine serum albumin (2 mg/ml), 50 μl 4 M HCl, and 0.2 ml ice-cold 50% TCA were added, and the fractions were allowed to remain at 4° overnight to precipitate the DNA. The tubes were then centrifuged at 1000 x g for 15 min, and the resulting precipitates were allowed to dry at room temperature overnight.

The DNA-containing precipitates were reacted for 30 min at 70° with 20 μl of 3,5-diaminobenzoic acid hydrochloride. After cooling, 1 ml of 0.6 M perchloric acid was added and the fluorescence of the samples at 510 nm was measured in an Aminco Bowman spectrophotofluorometer following excitation at 420 nm. Results are reported as the percentage of the total fluorescence contained in each fraction. T-4 viral DNA (M.W. 1.2 x 10^6) and λ viral DNA (M.W. 3.2 x 10^5) were sedimented in parallel gradients under the same conditions in order to obtain an estimate of the molecular weight of hepatic DNA fragments released as a result of AAF treatment.

**Lipid Peroxidation.** Lipid peroxidation was quantitated in terms of hepatic MDA content according to the procedure of Slater (40). Rats were treated with corn oil, AAF (10 mg/kg), or carbon tetrachloride (1.5 ml/kg) and were killed 4 hr thereafter. The livers were excised, weighed, and homogenized in an equal volume of ice-cold 10% TCA. The homogenates were then centrifuged for 5 min at 1000 x g in a Sorvall RC-2 refrigerated centrifuge, and the resulting supernatant was brought to 5 ml with 5% TCA. An equal volume of 0.67% (w/v) 2-thiobarbituric acid was added, and the mixture was heated in a boiling water bath for 10 min. After cooling, the colored product was extracted into 4 ml of n-butyl alcohol, and the absorbance at 532 nm was recorded. MDA standards, containing 0.2 to 1.6 nmol/ml, were prepared by hydrolyzing 1,1,3,3-tetraethoxypropane overnight in 5% TCA. Results are reported as nmol/g liver.

**RESULTS**

**Effect of Dietary Selenium on Hepatic GSH-Px Activity.** Hepatic GSH-Px activity was measured at various intervals after placing the experimental animals on the test diets in order to document their functional selenium status. Since rat liver is now known to contain both selenium-dependent and selenium-independent GSH-Px activity (21-23), dietary effects on the 2 enzyme activities were evaluated through the use of appropriate substrates. Thus, cumene hydroperoxide was used to evaluate both enzymes, whereas H_2O_2 was used to study dietary effects on the selenium-dependent enzyme alone. This distinction was made possible by the fact that selenium-independent GSH-Px has very little activity toward H_2O_2 as a substrate (21, 22).

The results in Chart 1 show that hepatic GSH-Px activity increased significantly over a period of 3 to 4 weeks when weaning rats were fed the selenium-supplemented diet. This effect was observed when either cumene hydroperoxide (Chart 1a) or H_2O_2 (Chart 1b) was used as the substrate for the enzymatic reaction. In contrast, GSH-Px activity measured with cumene hydroperoxide remained at initial levels in rats fed the selenium-deficient diet. More importantly, the activity measured with H_2O_2 decreased to almost undetectable levels within 3 weeks after feeding the deficient diet to the experimental animals (Chart 1b). These results are indicative of an almost total absence of hepatic selenium-dependent GSH-Px in the rats fed the selenium-deficient diet.

On the basis of these results, all experiments involving AAF were commenced at least 3 weeks after placing the animals on their respective diets. Moreover, hepatic GSH-Px activity was routinely measured 1 to 2 days prior to each experiment in order to verify the selenium status of the animals.

**Effect of Selenium on the Binding of AAF to Hepatic DNA.** The covalent binding of carcinogens to DNA in target tissues is generally considered to be a crucial event in the induction of cancer (15). Furthermore, studies conducted over the past several years have demonstrated a positive association between the ability of various agents to protect animals against the induction of cancer and their ability to inhibit the binding of carcinogens to target tissue DNA (44). For these reasons, it
was deemed important to investigate the effect of dietary selenium on the binding of AAF to hepatic DNA. Accordingly, rats from each dietary group were given a single i.p. injection of [9-\(^{14}\)C]AAF and sacrificed at 1, 4, 16, 24, 96, or 168 hr thereafter. Hepatic DNA was extracted and purified by the method of Irving and Veazey (16), and the extent of binding of the labeled carcinogen as quantitated by liquid scintillation spectrometry. As shown in Chart 2, selenium did not significantly alter either the initial binding of AAF to hepatic DNA (1 to 24 hr) or the retention of bound carcinogen at later intervals (96 and 168 hr).

**DNA Damage and Repair.** In addition to binding covalently to DNA, AAF and N-acetoxy 2-acetylaminofluorene have been shown to induce the formation of DNA single-strand breaks *in vivo* and *in vitro* (19, 30, 31, 41, 43). Since damage to DNA and its subsequent misrepair may be important factors in the induction of cancer, it was of interest to ascertain the effect of selenium on these processes. Accordingly, pilot experiments were carried out in selenium-deficient animals to study the effect of varying amounts of AAF (5 to 20 mg/kg) on the alkaline sucrose sedimentation profile of hepatic DNA. These studies revealed a dose-dependent decrease in the DNA sedimentation rate at 4 hr after carcinogen administration. Dose levels of 10 and 20 mg/kg were chosen for further study. The former was the lowest dose which produced a clear separation between the DNA peaks of control and carcinogen-treated animals. The larger dose produced marked damage, such that the bulk of the DNA remained at the top of the gradient. This permitted a more detailed analysis of the influence of selenium on the rate of DNA repair.

As shown in Chart 3a, hepatic DNA from selenium-deficient or selenium-supplemented control rats given corn oil injections sedimented into the 2.3 M sucrose shelf underlying the alkaline sucrose gradients (Fractions 3 to 5). A similar sedimentation pattern was observed at 4 hr after the injection of AAF (10 mg/kg) into selenium-supplemented rats (Chart 3b). In contrast, a significant decrease in the sedimentation rate of hepatic DNA from selenium-deficient rats was observed at 4 hr after treatment with the carcinogen. Although the average molecular weight of the DNA from these animals was still in excess of 10^8, the observed shift in the sedimentation profile is indicative of AAF-induced DNA single-strand breaks.

When the study was carried out at 24 hr after AAF administration, it was found that the sedimentation of hepatic DNA from selenium-deficient animals had returned to control values (Chart 3c). As before, AAF (10 mg/kg) was without effect in selenium-supplemented rats.

The effect of selenium on AAF-induced DNA damage was also studied after administration of the carcinogen at a dose of 20 mg/kg. The data presented in Chart 4a show that the higher dose of AAF produced a marked degradation of hepatic DNA in both dietary groups at 4 hr after injection. In each case, the majority of the single-strand DNA sedimented at a molecular weight of less than 3 x 10^8. The progressive accumulation of higher molecular weight species of DNA at 24 and 48 hr (Chart 4b and c) is evidence of ongoing repair of the damage. However, a complete return to the pretreatment sedimentation pattern did not occur until 48 to 72 hr after injection of the carcinogen (Chart 4d). More importantly, the data revealed no significant difference between the 2 dietary groups with respect to the rate of DNA repair.

Taken together, the results of these studies indicate that
selenium deficiency enhances the susceptibility of rat liver DNA to damage induced by AAF. Thus, only selenium-deficient rats evidence the production of DNA single-strand breaks following the administration of AAF at the relatively low dose of 10 mg/kg. The protective effect of selenium, however, is overcome by increasing the dose of the carcinogen. Under these conditions, both dietary groups evidence a comparable and marked degradation of hepatic DNA at 4 hr after AAF injection. Moreover, the parallel changes in the DNA sedimentation patterns of both groups at later intervals indicate that selenium does not affect the overall rate of DNA repair.

**Effect of AAF on Hepatic Lipid Peroxidation.** Since dietary selenium protected hepatic DNA from damage induced by AAF without altering the binding of the carcinogen to DNA, it appeared that the damage was produced by an indirect effect of AAF. In view of the function of selenium-dependent GSH-Px in protecting cells against peroxidative damage (2, 12, 28, 42), we conducted studies to ascertain if the DNA damage induced by AAF was associated with an overall increase in peroxidation when administered to a dose level sufficient to induce DNA single-strand breaks in selenium-deficient rats. As expected, the administration of CCl₄ was followed by a significant increase in lipid peroxidation in selenium-deficient rats and a less marked effect in selenium-supplemented animals. This protective effect of dietary selenium against CCl₄-induced hepatic lipid peroxidation is in accord with the results obtained by other investigators (2, 12, 42).

**DISCUSSION**

The studies described in this report were undertaken in order to elucidate the molecular mechanisms underlying the anticarcinogenic effect of selenium. The investigations focused on the effect of dietary selenium on the interaction between AAF and hepatic DNA in vivo. The rationale for studying this aspect of the overall problem was based on 2 considerations. First, dietary selenium has been shown to delay markedly the appearance of AAF-induced tumors in rat liver (14). Second, AAF has been extensively studied and is known to form covalent adducts at both the C-8 and N-2 positions of guanine in rat liver DNA in vivo (20, 45).

As shown in Table 1, dietary selenium had no effect on the concentration of MDA in control animals given injections of corn oil. These results indicate that the basal level of hepatic lipid peroxidation is not dependent on their functional selenium status. A slight increase in the hepatic concentration of MDA was observed in both groups following AAF administration. This change was not statistically significant and indicates that AAF does not produce an overall increase in peroxidation when administered at a dose level sufficient to induce DNA single-strand breaks in selenium-deficient rats. Without altering the binding of the carcinogen to DNA, it appeared that the damage was produced by an indirect effect of AAF. In view of the function of selenium-dependent GSH-Px in protecting cells against peroxidative damage (2, 12, 28, 42), we conducted studies to ascertain if the DNA damage induced by AAF was associated with an overall increase in peroxidation when administered to a dose level sufficient to induce DNA single-strand breaks in selenium-deficient rats. As expected, the administration of CCl₄ was followed by a significant increase in lipid peroxidation in selenium-deficient rats and a less marked effect in selenium-supplemented animals. This protective effect of dietary selenium against CCl₄-induced hepatic lipid peroxidation is in accord with the results obtained by other investigators (2, 12, 42).

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The experimental approach involved a comparison between the effects of AAF in rats fed a selenium-deficient diet with those in rats fed the same diet supplemented with selenium (0.5 ppm). Consequently, it was necessary to know the functional selenium status of the animals prior to their being exposed to the carcinogen. This was achieved by measuring hepatic selenium-dependent GSH-Px activity at various intervals after placing the rats on the test diets. As shown in Chart 1, selenium-dependent enzyme activity decreased to nearly undetectable levels within 3 weeks after placing weanling rats on the selenium-deficient diet. In contrast, rats fed the diet supplemented with selenium displayed increased enzyme activity during this same interval. Accordingly, all experiments involving AAF were commenced at least 3 weeks after placing the rats on their respective diets.

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Selenium supplemented</th>
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<tbody>
<tr>
<td>Corn oil</td>
<td>1.96 ± 0.09 (9)</td>
<td>2.00 ± 0.15 (5)</td>
</tr>
<tr>
<td>AAF</td>
<td>2.14 ± 0.17 (5)</td>
<td>2.10 ± 0.24 (5)</td>
</tr>
<tr>
<td>CCl₄</td>
<td>2.34 ± 0.18 (10)</td>
<td>2.53 ± 0.07 (5)</td>
</tr>
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*Mean ± S.E.*

Numbers in parentheses, number of rats.

*Student's t test.*
The effect of dietary selenium on the total binding of AAF to hepatic DNA in vivo is shown in Chart 2. The data show that the selenium had no effect on either the initial (1 to 24 hr) binding of the carcinogen to DNA or on the retention of covalently bound AAF at later intervals (96 and 168 hr). Moreover, the data suggest that selenium does not affect the type of adduct formed, since it is known that adducts formed at the C-8 and N-2 positions of guanine turn over at greatly different rates (20). Daoud and Griffin (5) similarly found that the addition of selenium to the drinking water of rats maintained on a commercial diet did not affect the binding of AAF to liver DNA in vivo. Taken together, these results indicate that the protective effect of selenium is not mediated by an alteration in the binding of AAF to target tissue DNA. In this respect, the mechanisms underlying the anticarcinogenic action of selenium differ from those of the synthetic antioxidant butylated hydroxytoluene, which has been shown to decrease the binding of AAF to rat liver DNA (9, 10).

In contrast to its lack of effect on the binding of AAF to liver DNA, selenium exerted a significant protective effect against AAF-induced DNA single-strand breaks (Chart 3). Thus, a readily discernible decrease in the sedimentation rate of hepatic DNA in alkaline sucrose was observed at 4 hr after the injection of AAF (10 mg/kg) into selenium-deficient rats. Under the same conditions, the sedimentation of DNA from selenium-supplemented rats was not different from that of control animals given injections of corn oil. However, as is also shown in Chart 3, the DNA sedimentation profile from selenium-deficient rats returned to control values at 24 hr after the injection of AAF. These results indicate that selenium deficiency enhances the susceptibility of rat liver DNA to AAF-induced single-strand breaks. Selenium-deficient rats, however, apparently retain the capacity to repair rapidly DNA damage resulting from a single exposure to a relatively low dose of the carcinogen. The protective effect of selenium was abolished, however, when the dose of AAF was increased to 20 mg/kg (Chart 4a). Under these conditions, both groups of rats exhibited comparable damage to hepatic DNA and comparable rates of repair of that damage (Charts 4b, c, and d). Thus, selenium does not appear to exert a significant effect on DNA repair processes.

Since dietary selenium did not affect the binding of AAF to hepatic DNA at any of the time intervals studied (Chart 2), the induction of DNA single-strand breaks appeared to result from an indirect action of the carcinogen. The possible involvement of free radicals in this process was suggested by the studies of Bartsch and Hecker (1) and Floyd et al. (6–8). These investigators have shown that in the presence of either H2O2 or lipid hydroperoxides the proximate carcinogenic metabolic of AAF, i.e., N-hydroxy-2-acetylaminofluorene undergoes a 1-electron oxidation in vitro to yield nitroso free radicals. The reaction is catalyzed by heme or heme-containing peroxidases and results in the formation of N-acetoxy-2-acetylaminofluorene and 2-nitrosofluorene via the dismutation of 2 nitroso free radicals.

Although reactions analogous to those described above have not as yet been demonstrated under in vivo conditions, it was believed that the protective effect of selenium against AAF-induced DNA strand breaks might result from its ability to interfere with free radical processes. For example, through its role in GSH-Px selenium might serve to lower the peroxide content of the liver and thus reduce the amount of substrate available for the free radical activation of AAF. Alternatively, selenium-dependent GSH-Px activity could function to lower the level of potentially damaging peroxide radical species that might be generated from the reaction between nitroso free radicals and cell constituents in vivo.

In an initial effort to discern between these possibilities, we measured the hepatic MDA content of rats in both dietary groups prior to and following exposure to AAF. MDA is a product of lipid peroxidation (27) which reportedly possesses carcinogenic activity (38). Moreover, Shamberger (37) found an increase in the MDA content of mouse skin following treatment with the carcinogen DMBA. Accordingly, it seemed reasonable to ascertain if rat liver was similarly affected by exposure to AAF at a dose level and time interval at which AAF-induced DNA damage had been observed.

The data in Table 1 show that the basal hepatic MDA content was the same in both dietary groups. These results suggest that selenium deficiency is not associated with increased levels of lipid peroxides which might potentially serve as substrates for the free radical activation of AAF. Moreover, the data also show that AAF treatment did not produce a significant alteration of the hepatic content of rats from either dietary group. In contrast, a single injection of the hepatotoxin, carbon tetrachloride, resulted in a significant increase in the hepatic MDA concentration of selenium-deficient rats. A less marked increase was also observed in selenium-supplemented animals. This protective effect of selenium against CCl4-induced lipid peroxidation is in accord with the findings of other investigators (2, 14, 42).

In summary, the protective effect of dietary selenium against the hepatocarcinogenic action of AAF does not appear to be mediated by an alteration in the binding of the carcinogen to liver DNA. Rather, the results of our initial studies indicate that the anticarcinogenic effect of selenium may result, at least in part, from its ability to protect hepatic DNA from indirect damage induced by the administration of AAF. This protective effect, however, is easily overridden by increasing the dose of the carcinogen. Therefore, the reported anticarcinogenic effect of selenium in humans most probably results from the fact that the human population, in general, is exposed to low levels of environmental carcinogens over prolonged periods. Similarly, the protective effect of dietary selenium against AAF-induced hepatocarcinogenesis reported by Harr et al. (14) is most probably a reflection of the relatively low level of carcinogen fed to their animals, i.e., 8 to 11 mg/kg/day. Our results indicate that dietary selenium would protect rat liver DNA from damage under those conditions.

Although selenium-dependent GSH-Px is the most probable candidate for mediating this protective effect, our results indicate that AAF does not produce an overall increase in peroxidation in the liver. It is still possible, however, that peroxides generated near the cell nucleus might be responsible for the observed induction of DNA single-strand breaks. Alternatively, other reactive species such as hydroxyl radicals might be involved in this process. These potential indirect effects of AAF are currently being studied in our laboratory.

Finally, the observation that inorganic selenium salts inhibit tumor induction (35) and the mutagenic effect of carcinogens such as AAF (18) suggests that non-enzyme-bound selenium may play a role in protecting DNA from the indirect damage produced by the administration of AAF.
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