Biochemical and Molecular Action of Nutrients

Dietary Selenium and Arsenic Affect DNA Methylation In Vitro in Caco-2 Cells and In Vivo in Rat Liver and Colon[1,2]

Cindy D. Davis,[3] Eric O. Uthus and John W. Finley

U.S. Department of Agriculture, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota 58202-9034

ABSTRACT Selenium is an essential trace element for human health, and it has received considerable attention for its possible role as an anticarcinogenic agent. The purpose of the present study was to determine whether changes in the amount and the chemical form of selenium would affect DNA methylation and whether this effect would be modified by arsenic. Caco-2 cells, a human colon cancer cell line, were exposed to 0, 1 or 2 μmol supplemental selenite/L and 0, 1 or 2 μmol supplemental arsenite/L for 7 d. DNA isolated from Caco-2 cells not treated with selenite was significantly ($P < 0.0001$) hypomethylated compared with that from cells treated with 1 or 2 μmol selenite/L. DNA isolated from Caco-2 cells not treated with arsenite was significantly ($P < 0.0001$) hypomethylated compared with DNA isolated from cells treated with 1 or 2 μmol arsenite/L. In addition, methylation of the p53 promoter region of Caco-2 cells decreased when cells were cultured in the absence of selenite and in the absence of arsenite. Sixty weanling male Fischer 344 rats were fed a torula yeast–based diet supplemented with 0, 0.1 or 2 mg selenium/kg diet as either selenite or selenomethionine in the presence or absence of 5 mg arsenic/kg diet as arsenite for 6 wk. Similar to the results with Caco-2 cells, rats fed selenium-deficient diets had significantly ($P < 0.0001$) hypomethylated liver and colon DNA compared with rats fed 0.1 or 2.0 μg selenium/g diets as either selenite or selenomethionine. Thus, alterations in DNA methylation may be a potential mechanism, whereby deficient dietary selenium increases liver and colon tumorigenesis. J. Nutr. 130: 2903–2909, 2000.

KEY WORDS: • *selenium* • *arsenic* • *DNA methylation* • *rats* • *Caco-2 cells*

Selenium is an essential trace element for human health and has received considerable attention for its possible role as an effective, naturally occurring, anticarcinogenic agent. Epidemiologic studies reveal that selenium intakes correlate inversely with death from various types of cancer (Clark et al. 1996, Kneckt et al. 1990) and suggest an increased risk of colon cancer in humans in geographic areas where selenium is low in the soil (Clark et al. 1991). In a recent study by Clark et al. (1996), selenium supplementation reduced the morbidity and mortality rates from carcinomas at several sites in the body, including the colon. Diets high in selenium have been shown to suppress carcinogenesis in many animal tumor models (Chae et al. 1997, El-Bayoumy et al. 1996, Feng et al. 1999, Ip and Ganther 1990, Jao et al. 1996. Milner 1986, Reddy et al. 1996). In general, selenite is more effective than selenomethionine in inhibiting the development of chemically induced tumors (Feng et al. 1999, Ip and Hayes 1989, Thompson et al. 1984). It has been hypothesized that the production of partially methylated forms of selenium may be directly involved in the anticarcinogenic action of selenium (Ip and Ganther 1990).

Selenium is enzymatically methylated to monomethylated, dimethylated and trimethylated metabolites that use *S*-adenosylmethionine (SAM)[4] as the methyl donor. Inorganic arsenic also undergoes enzymatic methylation, yielding monomethylated, dimethylated and trimethylated species by using SAM as the methyl donor. Thus, the biomethylation of selenite may compete with that of arsenic for the methyl donor SAM. In support of this hypothesis, arsenite inhibits selenium methylation both in vivo and in vitro, and selenium is a potent inhibitor of arsenic methylation in vitro (Ganther and Baumann 1962, Hoffman and McConnell 1987, Tandon et al. 1986). It has been observed that diets that are deficient or excessive in selenium altered arsenate disposition and methylation (Ganther and Baumann 1962, Levander and Baumann 1966). In addition, when rats are injected with subacutely toxic doses of arsenic and selenium, exhalation of volatile selenium compounds is markedly reduced, retention of selenium in the liver is decreased and the amount of selenium arriving in the intestinal tract is increased (Kenyon et al. 1997). Kraus and Ganther (1989) suggested that arsenite may block detoxification of methylselenides by inhibiting further methylation to the trimethylselenonium ion. Furthermore, the

[1] The U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

[3] To whom correspondence should be addressed.
E-mail: cdavis@gfhnrc.ars.usda.gov

[4] Abbreviations used: PCR, polymerase chain reaction; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

0022-3166/00 $3.00 © 2000 American Society for Nutritional Sciences.
Manuscript received 15 May 2000. Initial review completed 27 June 2000. Revision accepted 31 August 2000.

Downloaded from https://academic.oup.com/jn/article/130/12/2903/4686287 by guest on 20 August 2022

cancer chemopreventive action of selenite in animals is almost completely abolished by the coadministration of arsenite (Ip and Ganther 1988). However, arsenic may form an insoluble complex with selenium, thereby removing it from the system independent of an effect on methylation per se (Berry and Galle 1994, Gailer et al. 2000)

In mammalian cells, the term "DNA methylation" refers to the postsynthetic methylation of deoxycytosine residues at the 5 position to form 5-methylcytosine (Richardson and Yung 1999). 5-Methylcytosine is synthesized by the enzyme cytosine DNA methyltransferase, which catalyzes the transfer of methyl groups from SAM to deoxycytosine residues in DNA. This produces 5-methylcytosine and *S*-adenosylhomocysteine (SAH). Abnormal patterns of DNA methylation have been recognized as a constant, but poorly understood, molecular change in human neoplasia (Richardson and Yung 1999). Vogelstein et al. (1983) established that genomic undermethylation of DNA was both an early event in human colorectal carcinogenesis and an event that was present in a very consistent fashion. Cravo et al. (1994) reported that genomic DNA hypomethylation is also present in the normal colonic mucosa of individuals who harbor colonic neoplasms; this indicates that the appearance of DNA hypomethylation may even precede histologic evidence of dysplasia. Transformed cells of virtually all types often have, simultaneously, widespread loss of methyl groups from normally methylated sites, increased total activity of DNA methyltransferase and more regional areas of hypermethylated DNA (Baylin et al. 1998).

DNA methylation is an important epigenetic mechanism of transcriptional control (Baylin et al. 1998). Methylation of such CpG-rich regions in the 5′ flanking regions of certain genes, termed "CpG islands," is thought to inhibit transcription by directly impeding the binding of transcription factors to their *cis*-acting sites and/or by promoting the binding of methyl-DNA binding proteins, which restrict access of transcription factors to DNA (Richardson and Yung 1999). Hypomethylation correlates with gene expression, whereas methylation results in transcriptional suppression (Richardson and Yung 1999). Thus, alterations in DNA methylation could affect the expression of oncogenes and tumor-suppressing genes. Another mechanism whereby DNA hypomethylation may promote malignant transformation is by inducing regional alterations in DNA conformation and chromatin structure. Local conformational changes can promote genomic instability by increasing the accessibility of specific sequences to DNA-damaging agents (Keshet et al. 1986, Lewis and Bird 1991). The loss of methylated cytosines alter the conformation and stability of the chromatin structure, presumably by decreasing the number of binding sites for methyl-specific proteins. In the absence of methyl-directed protein binding, affected DNA sequences are rendered more accessible to oxidant- or enzyme-induced DNA strand breakage (Leteurtre et al. 1994, Smith 1991, Szyf et al. 1986). Thus, dysregulation of DNA methylation patterns and associated changes in DNA-protein binding may promote neoplasia not only by altering the transcription of cancer-related genes but also by altering local DNA structure and sequence accessibility to DNA-damaging agents.

Thus, cytosine DNA methyltransferase, as well as selenium and arsenic, compete for methyl donation from SAM. The purpose of the present study was to determine whether changes in the amount and the chemical form of selenium would affect DNA methylation and whether this effect is modified by arsenic. This was investigated in Caco-2 cells as well as in rats.

MATERIALS AND METHODS

Cell culture. Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD). Cells were maintained in Dulbecco's modified Eagle's medium (GIBCO BRL, Gaithersburg, MD) with 10% fetal bovine serum (Sigma Chemical Co., St. Louis, MO) in a humidified incubator at 37°C with an atmosphere of 5% CO_2. Sodium arsenite was purchased from Mallinckrodt (St. Louis, MO), and sodium selenite was purchased from Sigma Chemical Co. Exposure to arsenite, selenite or both occurred 24 h after cells were seeded onto culture flasks (675,000 cells were plated onto 25 cm^2 in 5-mL medium) to ensure that neither arsenite nor selenite affected attachment to the plastic substrate. Caco-2 cells were exposed to basal medium supplemented with 0, 1 or 2 μmol selenite/L and 0, 1 or 2 μmol arsenite/L; all determinations were performed in triplicate. The basal medium with 10% fetal bovine serum contained 0.53 μmol selenium/L and 0.32 μmol arsenic/L. Medium containing selenite and arsenite was changed every 2 d. After 7 d of growth in the presence of selenite and/or arsenite, flasks containing the cells were lysed with warmed (37°C) 0.5% sodium dodecyl sulfate in 10 mmol Tris and 10 mmol EDTA, pH 8.0, per L. DNA was isolated according to a procedure that involved enzymatic digestion of protein and RNA, followed by extraction with phenol and chloroform/isoamyl alcohol (24:1) (Gupta 1984). DNA concentration was determined spectrophotometrically at 260 nm by using a value of 50 A_{260} absorbance units/mg DNA to calculate its concentration.

Genomic DNA methylation. The methylation status of CpG sites in genomic DNA was determined by the in vitro methyl acceptance capacity of DNA by using [*methyl*-^{3}H]SAM as a methyl donor and a prokaryotic CpG DNA methyltransferase, as described previously (Choi et al. 1999, Cravo et al. 1994, Kim et al. 1995). The manner in which this assay is performed produces a reciprocal relationship between the endogenous DNA methylation status and the exogenous methyl-^{3}H incorporation. Briefly, 2 μg of DNA was incubated with 185 kBq of [*methyl*-^{3}H]SAM (Amersham Life Science Products, Piscataway, NJ), 4 U of *Sss*1 methyltransferase (New England Biolabs, Beverly, MA), 1× *Sss*1 buffer (50 mmol NaCl, 10 mmol Tris-HCl, 10 mmol EDTA and 1 mmol dithiothreitol, pH 8.0, per L) in a total volume of 50 μL for 3 h at 37°C. *Sss*1 methylase was denatured by heating at 65°C for 20 min. Reaction mixtures containing no enzyme were used as background controls for each DNA sample. The incubation mixtures were applied onto disks of Whatman DE-81 ion exchange filters (Fisher Scientific, Springfield, NJ) by using a vacuum filtration apparatus; the disks were then washed with 0.35 mol Na_2PO_4/L for 45 min. The disks were dried at 95°C for 30 min, and the resulting radioactivity of the DNA retained on the disks was measured by scintillation counting. All analyses were done in duplicate.

Quantitative HpaII-polymerase chain reaction assay for gene-specific methylation. The methylation status of the p53 gene was assessed by using polymerase chain reaction (PCR) with primers flanking the *Hpa*II cleavage sites (CCGG) within the gene, as previously described (Mass and Wang 1997). Genomic DNA (1.5 μg) from treated or control Caco-2 cells was digested with 6 U of *Hpa*II or *Msp*I (Stratagene, La Jolla, CA) in restriction enzyme buffer, incubated at 37°C overnight and then inactivated at 95°C for 10 min; a parallel restriction enzyme control contained no *Hpa*II or *Msp*I. The *Hpa*II restriction enzyme will cleave CCGG sequences that are not methylated at the internal or external cytosines, whereas the isoschizomer control, *Msp*I, will cleave both methylated and nonmethylated CCGG sequences. The p53 promoter region contains two CCGG sites. Primers were designed such that PCR amplification could not be performed if one of the CCGG sequences had been cleaved (Mass and Wang 1997). DNA resistant to *Hpa*II digestion is methylated on at least one of the two cytosines in the CCGG sequence and then can be amplified and analyzed quantitatively with PCR.

An aliquot (0.5 μg) of the digested DNA was added to a PCR mixture containing 200 μmol deoxynucleotide triphosphates/L, 50 pmol of primers (forward 5′-AGGGAATTCGGCACCAGGTCG-GCGAGAAT-3′, reverse 5′-AGGATCGATGGACTCATCAAG-TTCAGTCA-3′), 5 μL of 10× PCR buffer and 1 U of *Taq* polymerase (Promega Life Science, Madison, WI) in a total volume of 50 μL. After initial denaturation at 94°C for 5 min, the mixture was sub-

Downloaded from https://academic.oup.com/jn/article/130/12/2903/4686287 by guest on 20 August 2022

jected to 28 cycles of 94°C for 1 min, 58°C for 70 s, 72°C for 3 min and then a final extension step at 72°C for 5 min. A 10-μL aliquot of the reaction mixture was incubated 1:10,000 (v/v) with Vistra Green (Amersham Pharmacia Biotech, Piscataway, NJ) for 15 min before electrophoresis on 2% agarose gels. The fluorescence intensities of the PCR products after *Hpa*II digestion or without restriction enzyme treatment were quantified with a Storm 860 (Molecular Dynamics, Sunnyvale, CA). Values are expressed as the percentage of gene product resistant to *Hpa*II digestion. No PCR products were observed when DNA was incubated with *Msp*I.

Methylation-specific PCR. Methylation-specific PCR was carried out essentially according to the method of Wong et al. (1999) and was based on the principle that the treatment of DNA with bisulfite results in the deamination of unmethylated cytosine residues into uracil. Methylated cytosine residues, on the other hand, would remain unchanged. Thus, the DNA sequences of methylated and unmethylated genomic regions after bisulfite conversion would differ and would be distinguishable by sequence-specific PCR primers.

Bisulfite conversion was carried out by using the reagents provided in a CpGenome DNA modification kit (Intergen, Purchase, NY). Caco-2 DNA (1 μg) was treated with sodium bisulfite according to the manufacturer's recommendations. After conversion, the bisulfite-converted DNA was suspended in a total volume of 25 μL.

Each chemically modified DNA sample was amplified with each of three oligonucleotide primer sets, including "U, M and W," supplied in the CpG WIZ p16 amplification kit (Intergen Company, Purchase, NY). The "U primer" set will anneal to unmethylated DNA that has undergone a chemical modification. The "M primer" set will anneal to methylated DNA that has undergone a chemical modification. The "W primer" set serves as a control for the efficiency of chemical modification; this primer will anneal to any DNA (unmethylated or methylated) that has not undergone chemical modification. After initial denaturation at 95°C for 5 min, the mixture was subjected to 35 cycles of 95°C for 45 s, 60°C for 45 s, 72°C for 1 min and then a final extension step at 72°C for 5 min. A 10-μL aliquot of the reaction mixture was incubated 1:10,000 (v/v) with Vistra Green (Amersham Pharmacia Biotech) for 15 min before electrophoresis on 2% agarose gels. The fluorescence intensity of the PCR products with each of the different primer sets was quantified with a Storm 860 (Molecular Dynamics).

Animals and diets. Sixty male weanling Fischer 344 rats were purchased from Sasco (Omaha, NE). All rats were housed individually in stainless steel wire-bottomed cages in a room with controlled temperature and light. Animals were provided free access to demineralized water and purified diet. The basal diet was a selenium-deficient, torula yeast–based diet. The basal diet contained 30% torula yeast, 0.3% DL-methionine, 59% sucrose, 5% corn oil, 3.5% selenium-deficient AIN-76A mineral mix (American Institute of Nutrition 1977), 1.2% calcium carbonate, 1% AIN-76A vitamin mix, 0.1% choline bitartrate and 0.001% menadione sodium bisulfite complex. The basal diet contained <1.6 μg selenium/kg diet and <44.1 μg arsenic/kg diet by analysis. The basal diet was supplemented with a 0, 0.1 or 2 mg selenium/kg diet as either selenite or selenomethionine in the presence or absence of 5 mg arsenite/kg diet. Six rats were allowed free access to each of these 10 diets for 6 wk before killing. Rats were exsanguinated by cardiac puncture following ketamine/xylazine anesthesia.

This study was approved by the Animal Care Committee of the Grand Forks Human Nutrition Research Center, and the animals were maintained in accordance with the NIH guidelines for the care and use of laboratory animals.

DNA isolation. Portions of the liver and colon were promptly removed, rinsed and immediately frozen in liquid nitrogen. Livers and colons were stored at −70°C until DNA isolation. DNA was isolated from 0.5-g portions of liver and colon by using a standard procedure that involved enzymatic digestion of protein and RNA, followed by extraction with phenol and chloroform/isoamyl alcohol (24:1) (Gupta 1984).

Liver SAM and SAH. Portions of fresh liver were weighed and homogenized at 11,500 × *g* in 0.4 mol $HClO_4$/L with a Mark II Tissuemizer (Tekamr, Cincinnati, OH). Samples were centrifuged at 2000 × *g* at 4°C for 30 min. The supernatant was stored at −70°C until analysis. SAM and SAH were measured with a Dionex 40000i (Dionex Corp., Sunnyvale, CA) according to the procedure of Bottiglieri (1990).

Plasma homocysteine. Total homocysteine was determined in heparinized plasma by using HPLC according to the procedure of Durand et al. (1996).

Selenium status. Selenium concentrations in the plasma and liver were determined by hydride-generation atomic absorption spectrometry according to a previously published procedure (Finley et al. 1996). Samples were prepared for analysis by predigestion in nitric acid and hydrogen peroxide, followed by high-temperature ashing while in the presence of $MgNO_3$ as an aid to prevent Se volatilization.

Glutathione peroxidase enzyme activity was determined according to the coupled enzymatic method of Paglia and Valentine (1967), which uses hydrogen peroxide as the substrate.

Blood arsenic. Whole blood was digested with magnesium nitrate-saturated nitric acid. Digestion tubes were placed in an aluminum block heater until the samples appeared dried. The samples were then ashed in a muffle oven at 635°C for 12 h. Ashed samples were diluted in hydrochloric acid. The samples were analyzed by hydride generation with a Perkin–Elmer FIAS-100 and a Perkin–Elmer 5100PC atomic absorption spectrophotometer (Perkin–Elmer Cetus, Norwalk, CT).

Statistical analyses. The data were analyzed by a two-way ANOVA (diet selenium and arsenic) using the SAS General Linear Models program (SAS Version 6.12; SAS Institute, Cary, NC). Tukey's contrasts were used to differentiate among means for variables that had been significantly ($P < 0.05$) affected by selenium or by a selenium × arsenic interaction. Values are reported as means ± SEM in the text. Data with unequal variances (plasma selenium and blood arsenic concentrations) were not transformed before statistical analysis because the unequal variances did not affect the results.

RESULTS

Cell culture studies. By using *Sss*I methylase, the relative content of 5-methyl cytosine was assessed in samples of DNA from Caco-2 cells grown in the presence of 0, 1 or 2 μmol added selenite, arsenite or both/L for 2 wk. In this assay, the amount of methyl groups incorporated into DNA in the presence of ^{3}H-SAM and bacterial *Sss*I methylase is proportional to the original number of CpG sites available for methylation. Thus, it is inversely proportional to the methylation status of DNA. The DNA isolated from Caco-2 cells not treated with selenite was significantly ($P < 0.0001$) hypomethylated compared with that isolated from cells treated with 1 or 2 μmol selenite/L (**Fig. 1**). Similarly, the DNA isolated from Caco-2 cells not treated with arsenite was significantly ($P < 0.0001$) hypomethylated compared with Caco-2 cells treated with 1 or 2 μmol arsenite/L. However, in contrast to the results observed with selenite, in which there were no differences in DNA methylation between cells treated with 1 or 2 μmol selenite/L, cells treated with 2 μmol arsenite/L were significantly hypomethylated compared with cells treated with 1 μmol arsenite (77,292 dpm/ng DNA versus 60,500 dpm/ng DNA for 2 and 1 μmol arsenite/L, respectively).

The amount of *Hpa*II-resistant DNA that was amplified by the PCR specific for the p53 promoter region in selenite- and/or arsenite-exposed cells significantly ($P < 0.0001$) increased with the concentration of selenite in which the cells were cultured (2.48 versus. 6.76 versus 13.34% for 0, 1 and 2 μmol selenite/L averaged over all three arsenite concentrations, respectively) and increased significantly ($P < 0.0001$) when the cells were cultured with 2 μmol arsenite/L regardless of selenite concentration (10.31%) rather than 0 or 1 μmol arsenite/L (5.64 and 6.63%, regardless of selenite concentration, respectively) (**Fig. 2**). In all samples, incubations with the isoschizomer *Msp*I resulted

Downloaded from https://academic.oup.com/jn/article/130/12/2903/4686287 by guest on 20 August 2022

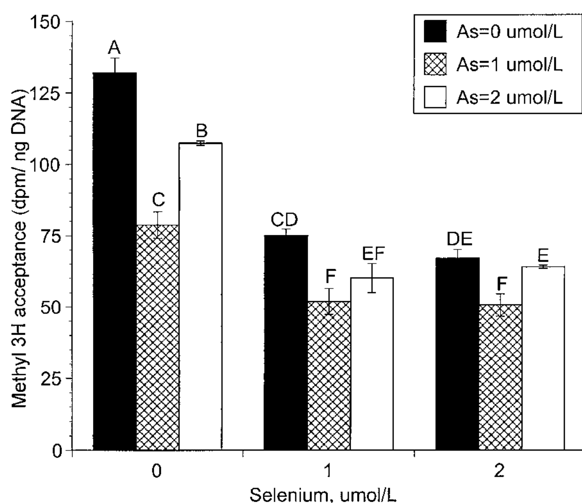

FIGURE 1 Analysis of global DNA methylation status in Caco-2 cells cultured in the presence of 0, 1 or 2 μmol selenite/L and 0, 1 or 2 μmol arsenite/L for 1 wk. The extent of global DNA methylation is inversely proportional to the incorporation of methyl groups by bacterial *Sss*I methyltransferase in the presence of [*methyl*-^{3}H]-*S*-adenosylmethionine. Values are means ± SEM, $n = 3$. Bar graphs without common superscript letters are significantly ($P < 0.05$) different as determined by Tukey's contrasts.

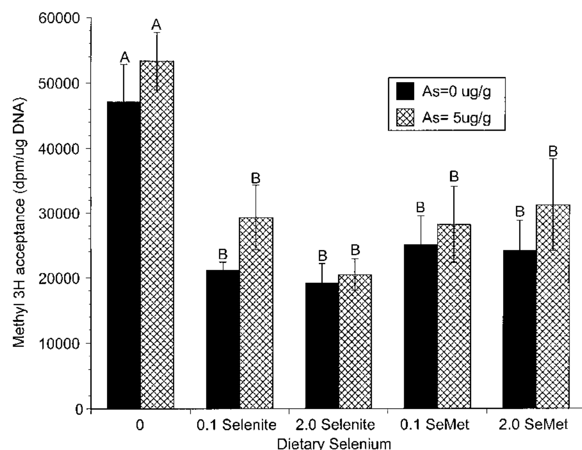

FIGURE 3 Analysis of global DNA methylation status in liver DNA of rats fed torula yeast–based diets supplemented with 0, 0.1 or 2 mg selenium/kg diet as either selenite or selenomethionine in the presence or absence of 5 mg arsenite/kg diet for 6 wk. The extent of global DNA methylation is inversely proportional to the incorporation of methyl groups by bacterial *Sss*I methyltransferase in the presence of [*methyl*-^{3}H]-*S*-adenosylmethionine. Values are mean ± SEM, $n = 6$. Bar graphs without common superscript letters are significantly ($P < 0.05$) different as determined by Tukey's contrasts.

in complete cleavage of the CCGG sites, and no PCR products were produced.

In contrast to the results observed when investigating methylation of the p53 promoter region of Caco-2 cells, all of the samples showed complete methylation of the promoter region for the p16 tumor suppressor gene. No PCR products from sample DNA were visualized when the primers for unmethylated DNA were used; however, products were visualized when a control DNA sample supplied with the kit and the primers for unmethylated DNA were used (data not shown).

Animal studies. The concentration and chemical form of selenium and arsenic in the diet did not significantly influence food intake or body weight (data not shown). The weight of the rats at the end of the study was 224 ± 2 g.

Similar to the results with Caco-2 cells, rats fed selenium-deficient diets had significantly ($P < 0.0001$) lower amounts of methylated DNA in their livers and colons compared with rats fed 0.1 or 2.0 μg selenium/g diet as either selenite or selenomethionine (**Figs. 3, 4**). The chemical form of selenium did not influence liver DNA methylation. However, rats fed 2 μg selenomethionine/g diet had significantly ($P < 0.0001$) hypermethylated colonic DNA compared with rats fed 0.1 or 2.0 μg selenium/g diet as

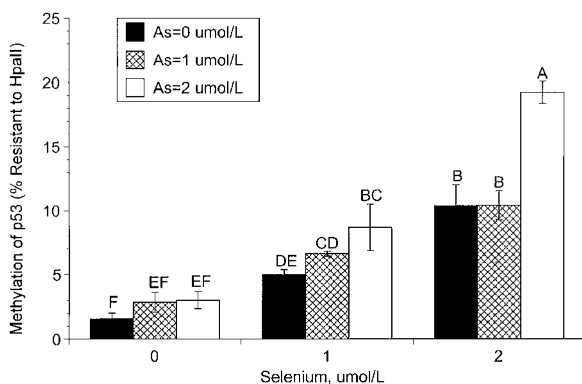

FIGURE 2 Quantitative polymerase chain reaction (PCR) assay for resistance to *Hpa*II digestion in the p53 promoter gene of Caco-2 cells cultured in the presence of 0, 1 or 2 μmol selenite/L and 0, 1 or 2 μmol arsenite/L for 1 wk. An aliquot of DNA (1.5 μg) was digested with 6 U of *Hpa*II; a parallel restriction enzyme control reaction contained no *Hpa*II enzyme. An aliquot (0.5 μg) of the digested DNA was amplified in a PCR that spanned bases 638–978 of the p53 promoter. A 10-μL aliquot of the PCR was incubated 1:10,000 with Vistra Green for 15 min before electrophoresis on 2% agarose gels. The fluorescence intensity of the PCR products after *Hpa*II digestion or with no restriction enzyme treatment was quantified with a Storm 860. Data are expressed as the percentage of gene product resistant to *Hpa*II digestion. Values are mean ± SEM, $n = 3$. Bar graphs without common superscript letters are significantly ($P < 0.05$) different as determined by Tukey's contrasts.

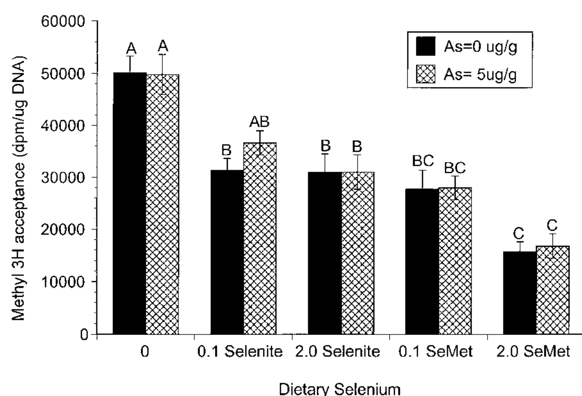

FIGURE 4 Analysis of global DNA methylation status in colon DNA of rats fed torula yeast–based diets supplemented with 0, 0.1 or 2 mg selenium/kg diet as either selenite or selenomethionine in the presence or absence of 5 mg arsenite/kg diet for 6 wk. The extent of global DNA methylation is inversely proportional to the incorporation of methyl groups by bacterial *Sss*I methyltransferase in the presence of [*methyl*-^{3}H]-*S*-adenosylmethionine. Values are mean ± SEM, $n = 6$. Bar graphs without common superscript letters are significantly ($P < 0.05$) different as determined by Tukey's contrasts.

Downloaded from https://academic.oup.com/jn/article/130/12/2903/4686287 by guest on 20 August 2022

TABLE 1

Effect of dietary selenium and arsenic on plasma homocysteine, liver S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and the ratio of SAM/SAH in rats[1]

Dietary selenium	Dietary arsenic	Plasma homocysteine	Liver SAM	Liver SAH	Liver SAM/SAH
	mg/kg diet	*μmol/L*	*nmol/g*		
Deficient	0	3.07 ± 0.14[c]	95.7 ± 4.3	11.5 ± 0.4	8.3 ± 0.3
	5	3.17 ± 0.09[c]	98.3 ± 3.9	12.5 ± 0.9	8.1 ± 0.5
0.1 Selenite	0	7.27 ± 0.16[ab]	96.0 ± 4.4	12.9 ± 0.4	7.4 ± 0.2
	5	6.82 ± 0.55[ab]	99.5 ± 3.7	12.6 ± 0.4	7.9 ± 0.4
2.0 Selenite	0	6.91 ± 0.36[ab]	102.5 ± 3.4	12.7 ± 0.7	8.1 ± 0.3
	5	6.30 ± 0.16[ab]	96.2 ± 4.5	13.0 ± 0.4	7.5 ± 0.4
0.1 Selenomethionine	0	6.87 ± 0.43[ab]	99.1 ± 5.0	12.4 ± 0.9	8.1 ± 0.3
	5	6.31 ± 0.35[ab]	103.1 ± 4.1	13.0 ± 0.6	8.0 ± 0.4
2.0 Selenomethionine	0	6.19 ± 0.26[b]	85.6 ± 5.0	11.4 ± 0.1	7.5 ± 0.5
	5	7.77 ± 0.29[a]	93.9 ± 2.8	12.6 ± 0.4	7.5 ± 0.2
Selenium[2]		0.0001	NS	NS	NS
Arsenic[2]		NS	NS	NS	NS
Selenium × arsenic[2]		0.007	NS	NS	NS

[1] Values are means ± SEM ($n = 4–6$). Means in a column without common superscript letters are significantly different ($P < 0.05$) as determined by Tukey's contrasts. NS, not significant, $P \geq 0.05$.
[2] Significant effects as determined by two-way ANOVA.

selenite and rats fed 0.1 μg selenium/g diet as selenomethionine. Dietary arsenic did not significantly affect liver or colon DNA methylation.

Rats fed the selenium-deficient diets had significantly ($P < 0.0001$) lower plasma homocysteine concentrations compared with rats fed 0.1 or 2.0 μg selenium/g diet as either selenite or selenomethionine (**Table 1**). The chemical form of selenium did not influence plasma homocysteine concentrations. An interaction between selenium and arsenic showed that dietary arsenic significantly increased plasma homocysteine concentrations only when rats were fed a diet containing 2 μg Se/g diet as selenomethionine. Neither dietary selenium nor dietary arsenic significantly affected liver SAM, liver SAH or the SAM/SAH ratio.

Liver glutathione peroxidase activity was significantly ($P < 0.0001$) lower in rats fed deficient dietary selenium than in rats fed 0.1 or 2.0 μg selenium/g diet as either selenite or selenomethionine (**Table 2**). Dietary arsenic increased liver glutathione peroxidase activity only when rats were fed 2.0 μg Se/g diet as selenite. Liver cytosolic and plasma selenium concentrations were significantly ($P < 0.0001$) less in the selenium-deficient rats than in the those supplemented with selenium, regardless of the chemical form (Table 2). The selenium concentrations increased with dietary selenium. However, both liver cytosol and plasma selenium concentrations were significantly greater in rats fed 2.0 mg selenomethionine/kg diet than in those fed 2.0 mg selenite/kg diet. Dietary arsenic did not influence liver cytosolic or plasma selenium concentrations. Blood arsenic concentrations were significantly ($P < 0.0001$) elevated when rats were fed high

Downloaded from https://academic.oup.com/jn/article/130/12/2903/4686287 by guest on 20 August 2022

TABLE 2

Effect of dietary selenium and arsenic on indicators of selenium and arsenic status in rats[1]

Dietary selenium	Dietary arsenic	Liver glutathione peroxidase	Liver cytosol selenium	Plasma selenium	Blood arsenic
	mg/kg diet	*U/mg protein*	*mmol/L*		*μmol/L*
Deficient	0	21.4 ± 3.7[c]	0.20 ± 0.06	0.31 ± 0.03	8.8 ± 0.4
	5	21.8 ± 4.9[c]	0.13 ± 0.04	0.37 ± 0.08	1598 ± 102
0.1 Selenite	0	1033.3 ± 37.6[b]	1.51 ± 0.04	6.95 ± 0.18	8.5 ± 0.5
	5	826.1 ± 94.6[b]	1.46 ± 0.14	6.88 ± 0.32	1644 ± 159
2.0 Selenite	0	1160.7 ± 92.1[b]	2.62 ± 0.22	8.17 ± 0.24	8.6 ± 0.6
	5	1578.7 ± 133.9[a]	2.73 ± 0.19	8.09 ± 0.22	1613 ± 118
0.1 Selenomethione	0	1045.8 ± 146.0[b]	1.66 ± 0.12	7.25 ± 0.22	10.7 ± 0.5
	5	960.7 ± 78.0[b]	1.68 ± 0.14	6.81 ± 0.12	1585 ± 324
2.0 Selenomethione	0	1018.7 ± 89.4[b]	3.59 ± 0.28	10.96 ± 0.29	7.2 ± 1.0
	5	1159.4 ± 107.9[ab]	4.11 ± 0.11	10.55 ± 0.59	1759 ± 179
Selenium[2]		0.0001	0.0001	0.0001	NS
Arsenic[2]		NS	NS	NS	0.0001
Selenium × arsenic[2]		0.02	NS	NS	NS

[1] Values are means ± SEM ($n = 5$ or 6). Means in a column without common superscript letters are significantly different ($P < 0.05$) as determined by Tukey's contrasts. NS, not significant, $P \geq 0.05$.
[2] Significant effects as determined by two-way ANOVA.

dietary arsenic. Dietary selenium did not influence blood arsenic concentrations.

DISCUSSION

These results demonstrate that a dietary deficiency of selenium causes global hypomethylation of liver and colon DNA in experimental animals. A possible direct role for DNA hypomethylation in the neoplastic process has been proposed based on experimental animal findings. In rodents, the depletion of methyl groups by dietary restriction leads to a decrease in SAM that results in liver carcinogenesis and in liver DNA hypomethylation, which precedes tumor development (Christman et al. 1993, Phgribny et al. 1995, Wainfin and Poirier 1992). In humans, selenium supplementation has been shown to significantly decrease the incidence of liver cancer in selenium-deficient persons living in Qidong, China (Yu et al. 1991 and 1997). Global hypomethylation has also been associated with colon carcinogenesis in both experimental animals and humans (Cravo et al. 1994, Feinberg and Vogelstein 1983). We have observed previously that animals fed selenium-deficient diets have significantly increased formation of aberrant crypt foci, a preneoplastic lesion for colon cancer, compared with rats fed nutritional or supranutritional amounts of dietary selenium in the form of the inorganic salts, selenite and selenate (Feng et al. 1999). In addition, rats fed selenium-deficient diets tended to have increased formation of aberrant crypt foci compared with those fed organic selenium as selenomethionine (Feng et al. 1999). Furthermore, selenium-deficient rats also have been shown to have a significantly greater karyorhectic index (a measure of acute carcinogen-induced nuclear toxicity) in the colonic mucosa when they were treated with azoxymethane. These data suggest that selenium deficiency increases the risk of colon cancer (Nelson et al. 1996). Thus, alterations in DNA methylation may be a potential mechanism whereby deficient dietary selenium increases colon and liver tumorigenesis.

Experimental antitumorigenic effects of selenium have been consistently associated with supranutritional concentrations of exposure to the element, that is, concentrations for animals of >1 μg/g diet (Coombs and Gray 1998). These concentrations are at least 10 times those required to prevent clinical signs of selenium deficiency and to support near-maximal tissue activities of selenoenzymes in animals. It should be noted that the majority of animal tumor model studies have used selenite as the source of selenium (Coombs and Gray 1998) The lack of a dose response in either colon or liver DNA methylation to changes in dietary selenite from 0.1 to 2.0 μg selenium/g diet suggests that alterations in DNA methylation do not appear to be the mechanism for the chemopreventive effect of supranutritional concentrations of selenium.

Caco-2 cells, a human-derived colon adenocarcinoma cell line, were used to determine whether selenite also would affect DNA methylation in a cell culture model. Plasma selenium concentrations ranged from 0.31 to 8.2 μmol/L in rats fed 0–2 mg selenite/kg diet. Because the main differences in DNA methylation were observed when animals were fed deficient dietary selenium, we exposed Caco-2 cells to 0–2 μmol selenite/L. However, it should be noted that in experimental animals, most of the plasma selenium is bound to protein rather than free. Similar to our results in experimental animals, we observed that deficient selenium caused global hypomethylation in Caco-2 cells. In addition, methylation of the p53 promoter region of Caco-2 cells decreased when cells were cultured in the absence of selenite. In contrast to the results with the tumor suppressor gene p53, the tumor suppressor gene p16 was completely methylated regardless of dietary treatments. Caco-2 cells are a colon cancer–derived cell line. Many cancer cells, despite widespread genomic hypomethylation, demonstrate hypermethylation of tumor suppressor genes. Aberrant hypermethylation in cancer cells often occurs in the CpG-rich promoter regions (CpG islands) of many tumor suppressor genes and is associated with gene inactivation (Baylin et al. 1998, Richardson and Yung 1999). Hypermethylation-induced inactivation of the p16 gene is an early event in oncogenesis (Nuovo et al. 1999). However, p53 may be responding to the global state of methylation.

The results of dietary arsenic on methylation of DNA in experimental animals and in Caco-2 cells were less consistent than the results with dietary selenium. Dietary arsenic did not significantly affect liver or colon DNA methylation in rats. However, Caco-2 cells treated with 0 or 2 μmol arsenite/L were significantly hypomethylated compared with cells treated with 1 μmol arsenite/L. Furthermore, despite higher global methylation, Caco-2 cells treated with 1 μmol arsenite/L had significantly less methylation of the p53 tumor suppressor gene than Caco-2 cells treated with 2 μmol arsenite/L. These results suggest that moderate concentrations of arsenic affect DNA differently than low or high concentrations of arsenic. These results also suggest that arsenic and selenium are not competing with cytosine DNA methyltransferase for methyl donation from SAM as originally hypothesized. One possible explanation is that the concentrations of selenium and arsenic in cells are at least 2 orders of magnitude lower than the concentration of SAM. Thus, it is unlikely that the metabolism of selenium and arsenic will reduce the supply of SAM to an extent that there will not be sufficient SAM available for cytosine DNA methyltransferase. Furthermore, in the present study, despite changes in DNA methylation, neither dietary selenium nor dietary arsenic significantly affected liver SAM, liver SAH or the SAM/SAH ratio. Thus, a competitive mechanism whereby the availability of SAM affects the methylation of DNA is not supported as an explanation for the hypomethylation of DNA during selenium deficiency.

Rats fed the selenium-deficient diets had significantly lower plasma homocysteine concentrations than rats fed 0.1 or 2.0 μg selenium/g diet as either selenite or selenomethionine. Similarly, Bunk and Combs (1981) observed a qualitative decrease of free homocysteine concentrations in plasma from selenium-deprived chicks. Homocysteine is formed from the hydrolysis of SAH to form homocysteine and adenosine. Possibly, a decrease in plasma homocysteine concentrations is a reflection of changes in liver homocysteine concentrations. Studies are under way to determine whether liver homocysteine concentrations are affected by selenium deficiency. Another explanation for the decreased plasma homocysteine concentrations in the selenium-deficient animals is that more homocysteine is directed toward the transulfuration pathway to increase glutathione production. The effect of selenium deficiency on glutathione production is currently being investigated.

In conclusion, these results demonstrate that deficient dietary selenium causes global hypomethylation of liver and colon DNA in experimental animals and in Caco-2 cells. Thus, alterations in DNA methylation may be a potential mechanism whereby deficient dietary selenium increases tumorigenesis but is not the mechanism for the chemoprotective effect of supranutritional intakes of selenium.

Downloaded from https://academic.oup.com/jn/article/130/12/2903/4686287 by guest on 20 August 2022

ACKNOWLEDGMENTS

The authors would like to thank Mary Briske-Anderson and Brenda Skinner for culture and treatment of the Caco-2 cells, Denice Schafer and her staff for care of the animals, Sheila Bichler for performing the statistical analysis and Laura Idso, Tiffany Carbaugh, Brian Gregoire, Rhonda Poellet, Kim Baurichter and Thomas Zimmerman for technical assistance.

LITERATURE CITED

American Institute of Nutrition (1977) Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J. Nutr. 107: 1340–1348.

Baylin, S. B., Herman, J. G., Graff, J. R., Vertino, P. M. & Issa, J.-P. (1998) Alterations in DNA methylation: A fundamental aspect of neoplasia. Adv. Cancer Res. 141–196.

Berry, J. P. & Galle, P. (1994) Selenium-arsenic interaction in renal cells: Role of lysosomes: Electron microprobe study. J. Submicrosc. Cytol. Pathol. 26: 203–210.

Bottliglieri, T. (1990) Isocratic high performance liquid chromatographic analysis of S-adenosylmethionine in animal tissues: The effect of exposure to nitrous oxide. Biomed. Chromatogr. 4: 239–244.

Bunk, M. J. & Combs, G. F. (1981) Evidence for an impairment in the conversion of methionine to cysteine in the selenium-deficient chick. Proc. Soc. Exp. Biol Med. 167: 87–93.

Chae, Y.-H., Upadhyaya, P. & El-Bayoumy, K. (1997) Structure-activity relationships among the ortho-, meta- and para-isomers of phenylenebis(methylene)selenocyanate (XSC) as inhibitors of 7,12-dimethylbenz(*a*)anthracene-DNA binding in mammary glands of female CD rats. Oncol. Rep. 4: 1067–1071.

Choi, S.-W., Stickel, F., Baik, H. W., Kim, Y.-I., Seitz, H. K. & Mason, J. B. (1999) Chronic alcohol consumption induces genomic but not p53-specific DNA hypomethylation in rat colon. J. Nutr. 129: 1945–1950.

Christman, J. K., Sheikhnejad, G., Dizik, M., Abileah, S. & Wainfan, E. (1993) Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary methyl deficiency. Carcinogenesis 14: 551–557.

Clark, L. C., Combs, G. F., Turnbull, B. W., Slate, E. H., Chalker, D. K., Chow, J., Davis, L. S., Glovar, R. A., Graham, G.F., Gross, E. G., Krongrad, A., Lesher, J. L., Park, K., Sanders, B. S., Smith, C. L. & Taylor, J. R. (1996) Effect of selenium supplementation for cancer prevention in patients with carcinoma of the skin: A randomized controlled trial. J. Am. Med. Assoc. 276: 1957–1963.

Clark, L. C., Cantor, K. P. & Allaway, W. H. (1991) Selenium in forage crops and cancer mortality in U.S. counties. Arch. Environ. Health. 46: 37–42.

Coombs, G. F. & Gray, W. P. (1998) Chemopreventive agents: Selenium. Pharmacol. Ther. 79: 179–192.

Cravo, M., Fidalgo, P., Pereira, A. D., Gouveia-Oliveira, A., Chaves, P., Selhub, J., Mason, J. B., Mira, F. C. & Leitao, C. N. (1994) DNA methylation as intermediate biomarker in colorectal cancer: Modulation by folic acid supplementation. Eur. J. Cancer Prev. 3: 473–479.

Durand, P., Fortin, L. J., Sussier-Cacan, S., Davignon, J. & Blache, D. (1996) Hyperhomocysteinemia induced by folic acid deficiency and methionine load-applications of a modified HPLC method. Clin. Chim. Acta 252: 83–93.

El-Bayoumy, Chae, Y.-H., Upadhyaya, P. & Ip, C. (1996) Chemoprevention of mammary cancer by diallyl selenide, a novel organoselenium compound. Anticancer Res. 16: 2911–2916.

Feinberg, A. P. & Vogelstein, B. (1983) Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301: 89–92.

Feng, Y., Finley, J. W., Davis, C. D., Becker, W. K., Fretland, A. J. & Hein, D. W. (1999) Dietary selenium reduces the formation of aberrant crypts in rats administered 3,2′-dimethyl-4-aminobiphenyl. Toxicol. Appl. Pharmacol. 157: 36–42.

Finley, J., Matthys, L., Schuler, T. & Korynta, E. (1996) Selenium content of foods purchased in North Dakota. Nutr. Res. 16: 723–728.

Gailer, J., George, G. M., Pickering, I. J., Prince, R. C., Ringwald, S. C., Pemberton, J. E., Glass, R. S., Younis, H. S., DeYoung, J. E. & Aposhian, H. V. (2000) A metabolic link between arsenite and selenite: The seleno-bis(S-glutathionyl)arsinium ion. J. Am. Chem. Soc. 122: 4637–4639.

Ganther, H. E. & Baumann, C. A. (1962) Selenium metabolism. I. Effects of diet, arsenic and cadmium. J. Nutr. 77: 210–216.

Gupta, R. C. (1984) Nonrandom binding of the carcinogen N-hydroxy-2-acetylaminoflourene to repetitive sequences of rat liver DNA *in vivo*. Proc. Natl. Acad. Sci. U.S.A. 81: 6943–6947.

Hoffman, J. L. & McConnell, K. P. (1987) Periodate-oxidized adenosine inhibits the formation of dimethylsenide and trimethylselenonium ion in mice treated with selenite. Arch. Biochem. Biophys. 254: 534–540.

Ip, C. & Ganther, H. (1988) Efficacy of trimethylselenonium versus selenite in cancer chemoprevention and its modulation by arsenite. Carcinogenesis 9: 1481–1484.

Ip, C. & Ganther, H. E. (1990) Activity of methylated forms of selenium in cancer prevention. Cancer Res. 50: 1206–1211.

Ip, C. & Hayes, C. (1989) Tissue selenium levels in selenium-supplemented rats and their relevance in mammary cancer protection. Carcinogenesis 10: 921–925.

Jao, S.-W., Shen, K.-L., Lee, W. & Ho, Y.-S. (1996) Effect of selenium on 1,2-dimethylhydrazine-induced intestinal cancer in rats. Dis. Colon Rectum. 39: 628–631.

Kenyon, E. M., Hughes, M. F. & Levander, O. A. (1997) Influence of dietary selenium on the disposition of arsenate in the female B6C3F$_1$ mouse. J. Toxicol. Environ. Health 51: 279–299.

Keshet, L., Lieman-Hurwitz, J. & Cedar, H. (1986) DNA methylation affects for formation of active chromatin. Cell 44: 535–543.

Kim, Y. I., Christmas, J. K., Fleet, J. C., Cravo, M. L., Salomon, R. N., Smith, D., Ordovas, J., Selhub, J. & Mason, J. B. (1995) Moderate folate deficiency does not cause global hypomethylation of hepatic and colonic DNA or c-myc specific hypomethylation of colonic DNA in rats. Am. J. Clin. Nutr. 61: 1083–1090.

Kneckt, P., Aromaa, A., Maatela, J., Alfthan, G., Aaran, R. K., Hakama, M., Hakulinen, T., Peto, R. & Teppo, L. (1990) Serum selenium and subsequent risk of cancer among Finnish men and women. J. Natl. Cancer Inst. 82: 864–868.

Kraus, R. J. & Ganther, H. E. (1989) Synergistic toxicity between arsenic and methylated selenium compounds. Biol. Trace Elem. Res. 20: 105–113.

Leteurtre, F., Kohlhagen, G., Fesen, M. R., Tanizawa, A., Kohn, K. W. & Pommier, Y. (1994) Effects of DNA methylation of topoisomerase I and II cleavage activities. J. Biol. Chem. 269: 7893–7900.

Levander, O. A. & Baumann, C. A. (1969) Selenium metabolism studies on the distribution of selenium in rats given arsenic. Toxicol. Appl. Pharmacol. 9: 98–105.

Lewis, J. & Bird, A. (1991) DNA methylation and chromatin structure. FEBS Lett. 285: 155–159.

Mass, M. J. & Wang, L. (1997) Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: A model for the mechanism of carcinogenesis. Mutat. Res. 386: 263–277.

Milner, J. A. (1986) Inhibition of chemical carcinogenesis and tumorigenesis by selenium. In: Essential Nutrients in Carcinogenesis (Poirier, L. A., Newberne, P. M. & Pariza, M. W., eds.), pp. 449–463, Plenum Press, New York.

Nelson, R. L., Abcarian, H., Nelson, T. M., Misumi, A., Kako, H., Rizk, S. & Sky-Peck, H. (1996) The effect of dietary selenium deficiency on acute colorectal mucosal nucleotoxicity induced by several carcinogens in the rodent. Am. J. Surg. 172: 85–88.

Nuovo, G. J., Plaia, T. W., Belinsky, S. A., Baylin, S. B. & Herman, J. G. (1999) In situ detection of the hypermethylation-induced inactivation of the p16 gene as an early event in oncogenesis. Proc. Natl. Acad. Sci. U.S.A. 96: 12754–12759.

Paglia, D. & Valentine, W. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70: 158–169.

Pogribny, I. P., Basnakian, A. G., Miller, B. J., Lopatina, N. G., Poirier, L. A. & James, S. J. (1995) Breaks in genomic DNA and within the p53 gene are associated with hypomethylation in livers of folate/methyl-deficient rats. Cancer Res. 55: 1894–1901.

Reddy, B. S., Wynn, T. T., El-Bayoumy, K., Upadhyaya, P., Fiala, E. & Rao, C. V. (1996) Evaluation of organoselenium compounds for potential chemopreventive properties in colon cancer. Anticancer Res. 16: 1123–1128.

Richardson, B. & Yung, R. (1999) Role of DNA methylation in the regulation of cell function. J. Lab. Clin. Med. 134: 333–340.

Smith, S. S. (1991) DNA methylation in eukaryotic chromosome stability. Mol. Carcinog. 4: 91–92.

Szyf, M., Meisels, E. & Razin, A. (1986) Biological role of DNA methylation: Sequence specific single-strand breaks associated with hypomethylation of GATC sites in DNA. J. Bacteriol. 168: 1487–1490.

Tandon, S. K., Magos, L. & Webb, M. (1986) The stimulation and inhibition of the exhalation of volatile selenium. Biochem. Pharmacol. 35: 2763–2766.

Thompson, R. H., Meeker, L. D. & Kokoska, S. (1984) Effect of an inorganic and organic form of dietary selenium on the promotional stage of mammary carcinogenesis in the rat. Cancer Res. 44: 2803–2806.

Wainfan, E. & Poirier, L. A. (1992) Methyl groups in carcinogenesis: Effects on DNA methylation and gene expression. Cancer Res. 52: 2071–2077.

Wong, I.H.N., Lo, Y.M.D., Zhang, J., Leiw, C.-T., Ng., M.H.L., Wong, N., Lai, P.B.S., Lau, W. Y., Hjelm, M. & Johnson, P. J. (1999) Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients. Cancer Res. 59: 71–73.

Yu, S.-Y., Zhu, Y. J., Li, W.-G., Huang, Q.-S., Huang, C. Z., Zhang, Q. N. & Hou, C. (1991) A preliminary report on the intervention trials of primary liver cancer in high-risk populations with nutritional supplementation of selenium in China. Biol. Trace Elem. Res. 29: 289–294.

Yu, S. Y., Zhu, Y. J. & Li, W. G. (1997) Protective role of selenium against hepatitis B virus and primary liver in Qidong. Biol. Trace Elem. Res. 56: 117–124.

Downloaded from https://academic.oup.com/jn/article/130/12/2903/4686287 by guest on 20 August 2022