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Update on the Effects of Vitamins A, C, and E and Selenium on Carcinogenesis[1] (42424)

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Abstract. The effects of vitamins A, C, and E and of selenium on carcinogenesis are briefly summarized and updated. These vitamins and minerals were selected because they have been studied extensively in recent years with a variety of carcinogenesis models. The consumption of vitamin A and its precursors (carotenoids) has been negatively correlated with cancer at a number of sites, particularly the lung. Animal investigations on vitamin A involvement in carcinogenesis have generally been of three types: those assessing (i) the effect of vitamin A deficiency, (ii) the effect of excess vitamin A, or (iii) the effect of supplementation with synthetic analogs of vitamin A. Vitamin A deficiency had no effect on salivary gland carcinogenesis, enhanced urinary bladder, lung, and liver carcinogenesis, and inhibited colon carcinogenesis. Excess of various forms of vitamin A (i) enhanced or inhibited skin tumorigenesis, (ii) inhibited mammary carcinogenesis in rats (but not in mice), and carcinogenesis of the forestomach, liver, and urinary bladder (with one model, but not with another), or (iii) enhanced or did not influence lung carcinogenesis. Vitamin A analogs have (i) enhanced or inhibited skin tumorigenesis, (ii) inhibited salivary gland, mammary, and urinary bladder carcinogenesis, (iii) enhanced tracheal and liver carcinogenesis, and (iv) either enhanced or inhibited pancreas carcinogenesis, depending upon the model employed. Although retinoids have been shown to inhibit carcinogenesis at many sites, numerous negative studies have been reported and some reports have indicated enhanced carcinogenesis. The most convincing evidence for the involvement of vitamin C in cancer prevention is the ability of ascorbic acid to prevent formation of nitrosamine and of other N-nitroso compounds. In addition vitamin C supplementation was shown to inhibit skin, nose, tracheal, lung, and kidney carcinogenesis, to either not influence or enhance skin, mammary gland, and colon carcinogenesis, and to enhance urinary bladder carcinogenesis, when given as sodium ascorbate, but not when given as ascorbic acid. Like vitamin C, vitamin E can inhibit nitrosation. Vitamin E was shown to inhibit skin, cheek pouch, and forestomach carcinogenesis, to enhance or inhibit colon carcinogenesis, and to have no effect on or to inhibit mammary gland carcinogenesis, depending upon the method of vitamin E administration or the level of dietary selenium or dietary fat. Selenium effects on carcinogenesis have been recently reviewed and the present discussion only updates this area by indicating that enhancement of carcinogenesis by dietary selenium supplements has been observed in the liver, pancreas, and skin. In conclusion, interactions among these vitamins and minerals, other nutrients and other nonnutrient dietary components in cancer prevention should be vigorously pursued, since studies with vitamins A, C, and E and selenium have generally indicated enhancement, as well as inhibition, of carcinogenesis.

The effects of various vitamins and minerals on carcinogenesis have been under investigation since the importance of diet in cancer was first suspected. Unlike the general enhancement of carcinogenesis, which has been observed in animals fed high fat diets or diets in high calorie amounts, the observed effects of vitamins and minerals on carcinogenesis have differed, depending upon a wide range of experimental variables. This chapter will summarize and update data on the effects of vitamins A, C, and E and of selenium on carcinogenesis. These vitamins and minerals were included in this chapter because they have been studied extensively with a variety of carcinogenesis models and they have received considerable attention in recent years. Other vitamins and minerals may certainly be important in carcinogenesis; however, some boundary had to be imposed to limit this pre-

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sentation. Accordingly the effects of vitamins A, C, and E will be summarized. Selenium effects on carcinogenesis were the topic of a symposium at the FASEB meeting in 1984 (11) and will be only briefly updated here.

Effects of vitamin A and vitamin A analogs (retinoids) on carcinogenesis. The consumption of vitamin A and its precursors (carotenoids) was associated with a reduced rate of cancer at a number of sites (39, 53, 58, 75). Lung cancer incidence, in particular, was lower in people who consumed more carotene-rich foods or in those with higher serum β-carotene; this was the case in both smokers and nonsmokers (39, 58). Because of the inherent toxicity of the physiological forms of vitamin A (retinol, retinal, retinoic acid, and the various esters of retinol), synthetic analogs were developed to attempt to improve the therapeutic efficacy of the compounds, while avoiding the toxicity (45, 65). Some of these compounds have been extensively tested in animal studies and are currently under evaluation in clinical trials (51).

Animal investigations on the ability of vitamin A and related compounds to influence carcinogenesis have generally dealt with (i) the effect of vitamin A deficiency on cancer induction, (ii) the effect of vitamin A or carotenoid excesses in cancer inhibition, and (iii) supplementation with synthetic analogs of vitamin A. Table I summarizes the effects of retinoid studies of these three types on carcinogenesis at a number of sites in rodents.

Skin tumors in mice induced by 7,12-dimethylbenz(a)anthracene (DMBA) were inhibited by a variety of vitamin A analogs in one series of experiments (5); however, retinoic acid was shown to increase two-stage carcinogenesis with DMBA and 12-O-tetradecanoylphorbol-12-acetate (TPA) in studies from a separate laboratory (21). Skin tumorigenesis induced in mice by ultraviolet light was enhanced by retinoic acid feeding (16).

Vitamin A deficiency did not influence salivary gland carcinogenesis induced in hamsters with locally applied DMBA (6); however, supplementation with 13-cis-retinoic acid inhibited the genesis of this tumor (61). Supplementation with several vitamin A analogs or with retinyl acetate inhibited mammary carcinogenesis induced by *N*-methyl-nitrosourea (MNU) or by DMBA in rats (42–44). In contrast, spontaneous (35) and DMBA-induced (78) mammary tumors in mice were not inhibited by retinoid feeding.

Forestomach tumors in hamsters induced by benzo(a)pyrene (BP) or DMBA were inhibited by feeding retinyl palmitate (7). Urinary bladder carcinogenesis induced by butyl-(4-hydroxy-butyl)nitrosamine (BBN) in mice or rats has been consistently inhibited by a wide variety of vitamin A analogs (41, 45, 65). Similarly, MNU-induced urinary bladder carcinogenesis in rats was inhibited by 13-cis-retinoic acid supplementation (66). However, urinary bladder cancer induced in rats by *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) was not influenced by retinyl palmitate supplementation, although vitamin A deficiency enhanced urinary-bladder carcinogenesis by this compound (10).

Effects of retinoids on respiratory tract carcinogenesis have been extensively studied because of the strong inverse correlation between human lung cancer rates and vitamin A intake (39, 58). Vitamin A deficiency was shown to elevate carcinogenesis in rats by 3-methylcholanthrene (3MC) (47). Supplementation with retinyl acetate, however, did not influence lung cancer in the 3MC model (47). In addition, lung carcinogenesis induced in hamsters by BP on ferric oxide was enhanced by retinyl acetate (63). Tracheal carcinogenesis in hamsters was also enhanced by retinoid supplementation (67).

Mixed effects of retinoid supplementation were observed on liver tumorigenesis. Aflatoxin B1 carcinogenesis was enhanced in vitamin A-deficient rats in studies by Newberne *et al.* (48) and retinoic acid supplementation inhibited 3′-methyl-4-dimethylaminoazobenzene (DMAB) hepatocarcinogenesis in rats (13). The feeding of various analogues of vitamin A enhanced hepatic tumorigenesis induced by *N*-nitrosobis(2-oxopropyl)amine (BOP) in hamsters (3) or by azaserine (AZA) in rats (34) in studies designed to test the ability of retinoids to inhibit pancreatic carcinogenesis.

Colon carcinogenesis was induced by AFB_1 only in rats deficient in vitamin A (49). This lesion was suppressed, however, in vitamin A-deficient rats treated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (46). Consumption of various vitamin A analogs had

no effect on colon carcinogenesis by MNU (62), but supplementation with 13-cis-retinoic acid inhibited 1,2-dimethylhydrazine (DMH)-induced colon cancer (49).

BOP-induced pancreatic carcinogenesis of ductular morphology was elevated in hamsters fed high doses of several analogs of vitamin A following a high dose of carcinogen, as shown in Fig. 1 (3), and inhibition was not observed in hamsters treated with lower doses of either retinoids or carcinogens (3). In contrast, pancreatic carcinogenesis of acinar morphology was inhibited by some of the same analogs as were used in the hamster studies (34).

In summary, although retinoids have been shown to inhibit carcinogenesis at many sites in a number of models, a considerable number of negative studies have been reported and some studies have shown enhancement of carcinogenesis. In addition, the influence of carotenoids on carcinogenesis has not been adequately studied.

Effects of vitamin C on carcinogenesis. The most convincing evidence for the involvement of vitamin C in cancer prevention is the ability of ascorbic acid to prevent *N*-nitroso compound formation (37, 38, 40, 69). The potential for this blockage of nitrosamine formation

TABLE I. SUMMARY OF THE INFLUENCE OF RETINOIDS ON CHEMICAL CARCINOGENESIS

Site	Species	Agent	Retinoid	Effect	Reference(s)
Skin	Mice	DMBA	Vit. A. analogues	↓	(5)
	Mice	DMBA + TPA	Retinoic acid	↑	(21)
	Mice	UV light	Retinoic Ac	↑	(16)
Salivary glands	Hamsters	DMBA	Vit. A def.[a]	NE[a]	(6)
		DMBA	13 cis ret. Ac[a]	↓	(61)
Mammary gland	Rats	DMBA	Ret. acetate	↓	(42)
	Rats	MNU	Ret. acetate + analogues	↓	(43, 44)
	Mice	DMBA	analogues	NE	(78)
	Mice	None	Retinyl acetate	NE	(35)
Forestomach	Hamsters	DMBA	Vit. A palmitate	↓	(7)
	Hamsters	BP	Vit. A palmitate	↓	(7)
Urinary bladder	Rats	BBN	Vit. A analogues	↓	(41, 65)
	Mice	BBN	Vit. A + analogues	↓	(45)
	Rats	MNU	13 cis ret. Ac.	↓	(66)
	Rats	FANFT	Vit. A def.	↑	(10)
	Rats	FANFT	Ret. palmitate	NE	(10)
Lung	Rats	3MC	Vit. A def.	↑	(47)
	Rats	3MC	Ret. acetate	NE	(47)
	Hamsters	BP-FeO_3	Retinyl acetate	↑	(63)
Trachea	Hamsters	MNU	Vit. A analogues	↑	(67)
Liver	Rats	AFB_1	Vit. A def.	↑	(48)
	Rats	DMAB	Ret. acid	↓	(13)
	Hamsters	BOP	Vit. A analogues	↑	(3)
	Rats	Aza	Vit. A analogues	↑ NE	(34)
Colon	Rats	AFB_1	Vit. A def.	↑ NE	(49)
	Rats	MNNG	Vit. A def.	↓	(46)
	Rats	DMH	13 cis ret. Ac	↓	(49)
	Rats	MNU	Vit. A analogues	NE	(62)
Pancreas	Hamsters	BOP	Vit. A analogues	↑ NE	(3)
	Rats	Aza	Vit. A analogues	↓	(34)

[a] Def., deficiency, ret. Ac., retinoic acid, NE, negative.

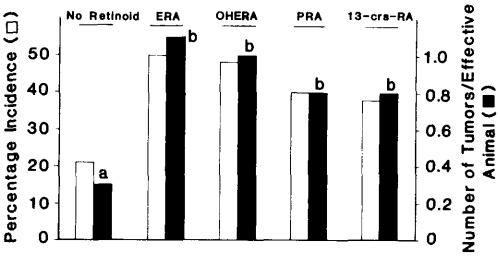

FIG. 1. The effects of dietary retinoids on BOP-induced pancreatic carcinogenesis in male hamsters. Each bar represents groups of 24 to 40 male hamsters. Retinoids were fed at levels from 0.4 to 1.0 mmol/kg diet. All surviving hamsters were killed at 40 weeks after treatment with 40 mg BOP/kg body weight at 8 weeks of age. Results χ^2 tests are indicated by the letters on bars a < b ($P < 0.05$). Experimental details were published (3).

to inhibit carcinogenesis was first documented in studies by Mirvish *et al.* [as reviewed in (40)]. Sodium nitrite, when given in the drinking water, and morpholine, when administered in the diet, react to form *N*-nitrosomorpholine, which induces liver cancer in rats (40). The simultaneous administration of dietary sodium ascorbate (22.7 g/kg) resulted in a 1.7-fold increase in the time required to induce liver tumors (40). However, ascorbate did not influence liver tumor induction by preformed *N*-nitrosomorpholine (40).

The influence of vitamin C on carcinogenesis by a number of other preformed carcinogens is shown in Table II. These results indicate that vitamin C can influence carcinogenesis in a variety of ways, depending upon the carcinogenesis model and on whether vitamin C is administered as the acid or the sodium salt. Skin carcinogenesis in ultraviolet light-treated mouse was inhibited by vitamin C (14); however, subcutaneous 3MC-induced tumors in guinea pigs were either enhanced by or not influenced by vitamin C treatments of scorbutic animals (2, 55).

Tracheal and nasal cancer induced in hamsters by *N*-nitrosodiethylamine (DEN) and cigarette smoke (19), and lung cancer induced by dimethylnitrosamine (DMN) in mice (40) were all inhibited in animals fed vitamin C. Mammary carcinogenesis by DMBA was not influenced by vitamin C supplements in the drinking water (1).

Divergent effects of vitamin C were observed in colon carcinogenesis by DMH in the rat. Results from one laboratory indicated an

TABLE II. SUMMARY OF THE INFLUENCE OF VITAMIN C ON CHEMICAL CARCINOGENESIS

Site	Species	Agent	Vitamin C route (dosage)	Effects	Reference
Skin	Mouse	UV	Diet (100 g/kg)	↓	(14)
	Guinea pig	3MC	Varied	NE	(55)
	Guinea pig	3MC	ip	↑	(2)
Nose	Hamster	DEN + cigarette smoke	Diet (10 g/kg)	↓	(19)
Trachea	Hamster	DEN + cigarette smoke	Diet (10 g/kg)	↓	(19)
Lung	Mouse	DMN	Diet (23 g/kg)	↓	(40)
Mammary	Rat	DMBA	Water (2.5 g/kg)	NE	(1)
Colon	Rat	DMH	Diet (2.5–10 g/kg)	↓	(54)
	Rat	DMH	Diet (50 g/kg)	↑	(59)
	Mouse	DMH	Diet (<0.5 g/kg)	↓	(30)
	Rat	MNU	Diet (2.5–10 g/kg)	NE	(54)
Kidney	Rat	DMH	Diet (2–10 g/kg)	↓	(54)
	Hamster	estradiol		↓	(33)
	Hamster	DES		↓	(33)
Urinary bladder	Rat	BBN	Diet (50 g/kg) Ascorbic acid	NE	(18)
		BBN	Diet (50 g/kg) Sodium ascorbate	↑	(17)
		MNU	Diet (50 g/kg) Sodium ascorbate	↑	(23)

inhibition of carcinogenesis with 2–10 g/kg vitamin C (54), while an enhancement of carcinogenesis was observed in a study using 50 g/kg (59). DMH carcinogenesis was reduced in mice that consumed diet containing <0.5 g/kg of vitamin C (30) and MNU-induced colon cancer in rats was not influenced by dietary vitamin C (54). Renal carcinogenesis induced by DMH in rats (54) or estrogens in hamsters (33) was inhibited by vitamin C.

A most interesting influence of vitamin C on the promotion of urinary bladder carcinogenesis was investigated in more recent studies. Urinary bladder carcinogenesis induced by MNU (23) or BBN (17) in rats was promoted when sodium ascorbate was fed at the 50 g/kg level following carcinogen treatment. However, no influence of ascorbic acid fed at the same level was observed on BBN-induced cancer (18). This and other studies have led to the speculation that the sodium salts of certain organic acids act as promoters of urinary bladder cancer (18).

Thus, vitamin C is believed to play an important role in preventing nitrosamine formation. However, the involvement of this vitamin in inhibiting carcinogenesis by preformed carcinogens varies with the different carcinogen and vitamin C treatment protocols.

Effects of vitamin E on carcinogenesis. Like vitamin C, vitamin E (tocopherols) was demonstrated to inhibit nitrosation (37, 38, 40, 69). One very important difference between vitamin E effects in this regard, and those of vitamin C is that vitamin E inhibition of nitrosation occurs in the lipid compartment, while vitamin C is effective only in the aqueous phase (40, 69). There is little direct epidemiological evidence to support a role for vitamin E in preventing human cancer; however, vitamin E intake is higher among individuals who consume diets high in vegetables and this may account for some of the effects of vegetable consumption that are inversely correlated with some cancer rates.

The effects of vitamin E treatment on chemical carcinogenesis have been evaluated in a number of models, as shown in Table III. Skin tumorigenesis initiated by DMBA and promoted by 12-O-tetradecanoyl-phorbol-13-acetate (TPA) was inhibited in mice treated topically with vitamin E prior to the TPA treatment (52), while subcutaneous tumors induced by 3,4,9,10-dibenzpyrene (DBP) were not influenced by dietary vitamin E (15). Carcinogenesis induced in the hamster cheek pouch by painting DMBA was inhibited in two laboratories by the oral administration of

TABLE III. SUMMARY OF THE INFLUENCE OF VITAMIN E ON CHEMICAL CARCINOGENESIS

Site	Species	Agent	Vitamin E route (dosage)	Effect	Reference
Skin	Mouse	DMBA + TPA	Topical (17 mg 2× wk)	↓	(52)
Skin	Mouse	DBP	Diet (25–50 g/kg)	NE	(15)
Cheek pouch	Hamster	DMBA	Oral (7 IV 2× wk)	↓	(77)
			Oral (10 IV 2× wk)	↓	(60)
Forestomach	Mouse	DMBA	Diet (10 g/kg)	↓	(76)
Colon	Mouse	DMH	Diet (0.6 g/kg)	↓	(12)
	Mouse	DMH	Diet (40 g/kg)	↑	(74)
	Rat	DMH	Vit. E def.	↑ or ↓	(68)
Mammary gland	Rat	DMBA	ig (unclear)	↓	(76)
		DMBA	Diet (30 mg/kg)	NE (low fat)	(27)
				↓ (high fat)	(27)
		DMBA	Diet (50 mg/kg)	NE (low Se)	(22)
				↓ (high Se)	(22)
		DMBA	Diet (2 g/kg)	NE	(31)

vitamin E (60, 77), and induction of forestomach tumors in mice by DMBA was also inhibited (76). Divergent effects of vitamin E on colon carcinogenesis by DMH in mice were reported. Lower dietary levels inhibited colon carcinogenesis in one laboratory (12), while high doses enhanced colon carcinogenesis in another (74). Vitamin E deficiency was shown to enhance the initiation of colon carcinogenesis by DMH, but to inhibit the growth of tumors in a recent study (68).

Interesting effects of vitamin E administration were observed in rat mammary gland carcinogenesis induced by DMBA, as shown in Table III. Ip and colleagues (22, 27) demonstrated that low doses of dietary vitamin E (30–50 mg/kg) were ineffective in preventing mammary carcinogenesis, and King and McKay showed that a higher dietary dose (2 g/kg) (31) was also not inhibitory. Ip's laboratory found that the vitamin E was effective when fed with a high fat diet (27) or in conjunction with selenium supplements (22) and that selenium supplements were less effective when given in a vitamin E deficient diet (28). Intragastric administration of vitamin E inhibited mammary cancer in a separate study (76). The potential interaction between vitamin E and other nutrients is particularly noteworthy, since recent epidemiological results have suggested that selenium may protect against cancer risk when vitamin E intake is low (8, 56).

Effects of selenium on carcinogenesis. Two years ago an excellent symposium on "selenium and carcinogenesis" was presented by the American Institute of Nutrition at the 68th Annual Meeting of the Federation of American Societies for Experimental Biology. The papers from this symposium have been published (11, and following papers), and at this time I wish only to point out some recent studies, two of which are not yet published, showing that high dietary selenium can promote cancer. The studies summarized in Table IV indicate that high dietary selenium has been shown to consistently inhibit mammary (24, 25, 36, 71) and colon (4, 29, 64) carcinogenesis, that some inhibition was reported in a lung carcinogenesis model (4), that either inhibition or enhancement was observed in the liver (9, 20, 32) and skin (50, 57; Pelling, Bresnick, and Birt, preliminary results), depending upon the carcinogen employed, and that there have been reports of no effect on tracheal cancer (73) and of enhanced pancreatic cancer (Birt *et al.*, submitted) in hamster models.

The effects of selenium on BOP-induced pancreatic carcinogenesis in hamsters are shown in Figs. 2 and 3. Supplemental selenium at the 2.5 ppm level increased the pancreatic carcinoma yield in male hamsters fed a high

TABLE IV. SUMMARY OF THE INFLUENCE OF SELENIUM ON CHEMICAL CARCINOGENESIS

Site	Species	Agent	Effect	Reference(s)
Skin	Mice	uv light	↓	(50)
	Mice	3MC	↓	(57)
	Mice	α-Pyrene	↓	(57)
	Mice	DMBA + TPA	↑	(Pelling *et al.*, unpublished)
Liver	Rat	DMAB	↓	(9)
	Rat	AAF	↓	(20)
	Rat	AFB_1	↑	(32)
Trachea	Hamster	MNU	NE	(73)
Lung	Rat	BOP	↓	(4)
Mammary gland	Mice	DMBA	↓	(36)
	Rat	DMBA	↓	(24, 25)
	Rat	MNU	↓	(71)
Colon	Rat	DMBA	↓	(29)
	Rat	BOP	↓	(4)
	Rat	AOM	↓	(64)
Pancreas	Hamster	BOP	↑	(Birt *et al.*, submitted)

fat diet, but not in those fed a low fat diet. Selenium supplementation with 2.5 ppm selenium from sodium selenite did not influence pancreatic carcinogenesis in female hamsters, while feeding selenium from seleno-DL-methionine elevated the yield of pancreatic carcinomas in females.

Preliminary results in an ongoing study indicate that when 2 or 4 ppm selenium from sodium selenite is fed, the promotion of mouse skin carcinogenesis is enhanced in the SENCAR mouse (Pelling, Bresnick, and Birt, preliminary observations). A dose–response elevation in tumor number has been observed in DMBA-initiated mice fed the high selenium diets prior to initiation with DMBA and during promotion with TPA.

These results, which show that high dietary selenium can enhance carcinogenesis in animals, lead us to be cautious in administering selenium to humans with the aim of preventing cancer.

Conclusions. The vitamins and minerals discussed here for their potential in inhibiting carcinogenesis can enhance, as well as inhibit, tumor induction or development. However, because of the brevity of this paper, it was not possible to discuss the differences in experimental design which may have caused some of the variations in response. In summary, scientists must continue to search for other dietary factors which may inhibit cancer, or for combinations of such factors which may prevent a wider range of tumors, without enhancing cancer at any site. The epidemiological (8, 56) and experimental (26, 28, 72) data suggesting that selenium may interact with vitamin E and vitamin A in preventing cancer are encouraging, and similar lines of research should be vigorously pursued with other combinations of nutrients.

The data also suggest that the vitamin A, C, and E content of vegetables probably cannot explain the association of vegetable consumption with reduced cancer rates and the search for other inhibitors in vegetables should be continued and strengthened. It is possible that other components of vegetables interact with certain of these vitamins or selenium to improve their ability to inhibit cancer.

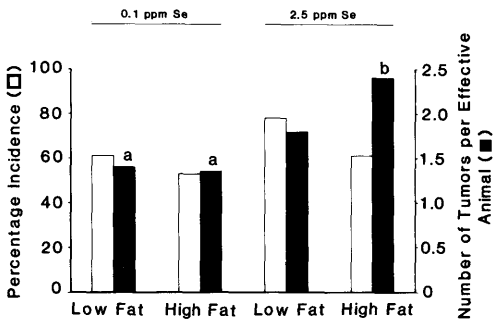

FIG. 2. The effects of dietary selenium fed with low (6%) and high (24%) fat diets on BOP-induced pancreatic carcinogenesis in male hamsters. Each bar represents groups of 18–23 male hamsters. Selenium as sodium selenite was fed from 4 weeks before BOP treatment in a low-fat diet. High-fat diet began 1 week after the final BOP dosage. All surviving hamsters were killed 70 weeks after the first of four weekly BOP treatments (5 mg/kg body wt) began at 8 weeks of age. Results of χ^2 tests are indicated by the letters on bars a < b ($P < 0.05$). Experimental details are being published (Birt *et al.*, submitted).

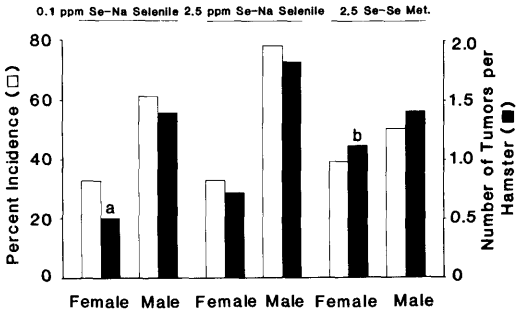

FIG. 3. The effects of the form of dietary selenium on BOP-induced pancreatic carcinogenesis in male and female hamsters. Each bar represents groups of 16–23 hamsters. Selenium as sodium selenite was fed from 4 weeks before BOP treatment in a low-fat diet. High-fat diet began 1 week after the final BOP dosage. All surviving hamsters were killed 70 weeks after the first of four weekly BOP treatments (5 mg/kg body wt) began at 8 weeks of age. Results of χ^2 tests are indicated by the letters on bars a < b ($P < 0.05$). Experimental details are being published (Birt *et al.*, submitted).

1. Abul-Hajj YJ, Kelliher M. Failure of ascorbic acid to inhibit growth of transplantable and dimethylbenzanthracene induced rat mammary tumors. Cancer Lett **17**:67–73, 1982.
2. Banic S. Vitamin C acts as a cocarcinogen to methylcholanthrene in guinea pigs. Cancer Lett **11**:239–242, 1981.
3. Birt DF, Davies MH, Pour PM, Salmasi S. Lack of inhibition by retinoids of bis(2-oxopropyl)-

nitrosamine-induced carcinogenesis in Syrian hamsters. Carcinogenesis **4**:1215–1220, 1983.
4. Birt DF, Lawson TA, Julius AD, Runice CE, Salmasi S. Inhibition by dietary selenium of colon cancer induced in the rat by bis(2-oxopropyl)nitrosamine. Cancer Res **42**:4455–4459, 1982.
5. Bollag W. Therapy of chemically induced skin tumor of mice with vitamin A palmitate and vitamin A acid. Experientia **27**:90–92, 1971.
6. Chaudhny AP, Singer L, Gorlin RS, Vickers RA. Effects of vitamin A deficiency on experimental carcinogenesis in submandibular glands of hamsters. J Dent Res **40**:327–330, 1961.
7. Chu EW, Malmgren RA. An inhibitory effect of vitamin A on the induction of tumors of forestomach and cervix in the Syrian hamster by carcinogenic polycyclic hydrocarbons. Cancer Res **25**:884–895, 1965.
8. Clark LC. The epidemiology of selenium and cancer. Fed Proc **44**:2584–2589, 1985.
9. Clayton CC, Baumann CA. Diet and azo dye tumors: Effect of diet during a period when the dye is not fed. Cancer Res **9**:575–582, 1949.
10. Cohen SM, Wittenberg JF, Bryan GT. Effect of avitaminosis A and hypervitaminosis A on urinary bladder carcinogenicity of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide. Cancer Res **36**:2334–2339, 1976.
11. Combs GF Jr. Selenium and carcinogenesis, introduction. Fed Proc **44**:2561–2562, 1985.
12. Cook MG, McNamara P. Effect of dietary vitamin E on dimethylhydrazine-induced colonic tumors in mice. Cancer Res **40**:1329–1331, 1980.
13. Dauod AH, Griffin AC. Effect of retinoic acid, butylated hydroxytoluene, selenium and sorbic acid on azo dye hepatocarcinogenesis. Cancer Lett **9**:299–304, 1980.
14. Dunham WB, Zuckerkandl E, Reynolds R *et al.* Effects of intake of L-ascorbic acid on the incidence of dermal neoplasms induced in mice by ultraviolet light. Proc Natl Acad Sci USA **79**:7532–7536, 1982.
15. Epstein SS, Andrea JJ, Forsyth J, Mantel N. The null effect of antioxidants on the carcinogenicity of 3,4,9,10-dibenzpyrene to mice. Life Sci **6**:225–233, 1967.
16. Forbes PD, Urbach F, Davies RE. Enhancement of experimental photocarcinogenesis by topical retinoic acid. Cancer Lett **7**:85–90, 1979.
17. Fukushima S, Imaida K, Sakata T, Okamura T, Shibata MA, Ito N. Promoting effects of sodium-L-ascorbate on two stage urinary bladder carcinogenesis in rats. Cancer Res **43**:4454–4457, 1983.
18. Fukushima S, Kurata Y, Shibata MA, Ikawa E, Ito N. Promotion by ascorbic acid, sodium erythorbate and ethoxyquin of neoplastic lesions in rats initiated with *N*-butyl-N-(4-hydroxybutyl)nitrosamine. Cancer Lett **23**:29–37, 1984.
19. Harada T, Kitazawa T, Maita K, Shirasu Y. Effects of vitamin C on tumor induction by diethylnitrosamine in the respiratory tract of hamsters exposed to cigarette smoke. Cancer Lett **25**:163–169, 1985.
20. Harr JR, Exon JH, Whanger PD, Weswig PM. Effect of dietary selenium on *N*-fluorenyl-acetamide (FAA) induced cancer in vitamin E supplemented rats. Clin Toxicol **5**:187–194, 1972.
21. Hennings H, Wenk ML, Donahoe R. Retinoic acid promotion of papilloma formation in mouse skin. Cancer Lett **16**:1–5, 1982.
22. Horvath PM, Ip C. Synergistic effect of vitamin E and selenium in the chemoprevention of mammary carcinogenesis in rats. Cancer Res **43**:5335–5341, 1983.
23. Imaida K, Fukushima S, Shirai T, Masui T, Ogiso T, Ito N. Promoting activities of butylated hydroxyanisole, butylated hydroxytoluene and sodium L-ascorbate on forestomach and urinary bladder carcinogenesis initiated with methylnitrosourea in F344 male rats. Gann **75**:769–775, 1984.
24. Ip C. Prophylaxis of mammary neoplasia by selenium supplementation in the initiation and promotion phases of chemical carcinogenesis. Cancer Res **41**: 4386–4390, 1981.
25. Ip C. Selenium mediated inhibition of mammary carcinogenesis. Biol Trace Element Res **5**:317–330, 1983.
26. Ip C. Chemoprevention of mammary tumorigenesis by a combined regimen of selenium and vitamin A. Carcinogenesis **2**:915–918, 1981.
27. Ip C. Dietary vitamin E intake and mammary carcinogenesis in rats. Carcinogenesis **3**:1453–1456, 1982.
28. Ip C. Attenuation of the anticarcinogenic action of selenium by vitamin E deficiency. Cancer Lett **25**: 325–331, 1985.
29. Jacobs MM, Jansson B, Griffin AC. Inhibitory effects of selenium on 1,2-dimethylhydrazine and methylazoxymethanol acetate induction of colon tumors. Cancer Lett **2**:133–138, 1977.
30. Jones FE, Komorowski RA, Condon RE. The effects of ascorbic acid and butylated hydroxyanisole in the chemoprevention of 1,2-dimethylhydrazine-induced large bowel neoplasms. J Surg Oncol **25**:54–60, 1984.
31. King MM, McCay PB. Modulation of tumor incidence and possible mechanisms of inhibition of mammary carcinogenesis by dietary antioxidants. Cancer Res **43**: 2485s–2490s, 1983.
32. LeBoeuf RA, Laishes BA, Hoekstra WG. Effects of dietary selenium concentration on the development of enzyme-altered liver foci and hepatocellular carcinoma induced by diethylnitrosamine or *N*-acetylaminofluorene in rats. Cancer Res **45**:5489–5495, 1985.
33. Liehr JG, Wheeler WJ. Inhibition of estrogen-induced renal carcinoma in Syrian hamsters by vitamin C. Cancer Res **43**:4638–4642, 1983.
34. Longnecker DS, Curphey TJ, Kuhlmann ET, Roebuck BD. Inhibition of pancreatic carcinogenesis by retinoids in azaserine treated rats. Cancer Res **42**:19–24, 1982.
35. Maiorana A, Gullino PM. Effect of retinyl acetate on

the incidence of mammary carcinomas and hepatomas in mice. J Natl Cancer Inst **64**:655–663, 1980.

36. Medina D, Shepherd F. Selenium-mediated inhibition of 7,12-dimethylbenz(a)anthracene-induced mouse mammary tumorigenesis. Carcinogenesis **2**:451–455, 1981.
37. Mergens WJ. Efficacy of vitamin E to prevent nitrosamine formation. Ann NY Acad Sci **393**:61–69, 1982.
38. Mergens WJ, Kamm JJ, Newmark HL, Fiddler W, Pensabene S. Alpha-tocopherol: Uses in preventing nitrosamine formation. In: Walker EA, Griciute L, Castegnaro M, Lyle RE, Davis W, eds. Environmental Aspects of *N*-Nitroso Compounds. Lyon, IARC, pp199–212, 1978.
39. Mettlin C, Graham S, Swanson M. Vitamin A and lung cancer. J Natl Cancer Inst **62**:1435–1438, 1979.
40. Mirvish SS. Inhibition of the formation of carcinogenic *N*-nitroso compounds by ascorbic acid and other compounds. In: Burchenal JH, Oettgen HP, eds. Cancer 1980: Achievements, Challenges, Projects. New York, Grune & Stratton, Vol. 1: pp557–587, 1981.
41. Miyata Y, Tsuda H, Matayoshi-Miyasato K, Fukushima S, Murasaki G, Ogiso T, Ito N. Effect of vitamin A acetate on urinary bladder carcinogenesis induced by *N*-butyl-n-(4-hydroxybutyl)nitrosamine in rats. Gann **69**:845–848, 1978.
42. Moon RC, Grubbs CJ, Sporn MB. Inhibition of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis by retinyl acetate. Cancer Res **36**:2626–2630, 1976.
43. Moon RC, Grubbs CJ, Sporn MB, Goodman DG. Retinyl acetate inhibits mammary carcinogenesis induced by *N*-methyl-n-nitrosourea. Nature (London) **267**:620–621, 1977.
44. Moon RC, McCormick DL. Inhibition of chemical carcinogenesis by retinoids. J Amer Acad Dermatol **6**:809–814, 1982.
45. Moon RC, McCormick DL, Becci PJ, Shealy YF, Frickel F, Paust J, Sporn MB. Influence of 15 retinoic acid amides on urinary bladder carcinogenesis in the mouse. Carcinogenesis **3**:1469–1472, 1982.
46. Narisawa T, Reddy BS, Wong C-Q, Weisburger JH. Effect of vitamin A deficiency on rat colon carcinogenesis by N-methyl-N-nitro-N-nitrosoguanidine. Cancer Res **36**:1379–1383, 1976.
47. Nettesheim P, Snyder C, Kim JCS. Vitamin A and the susceptibility of respiratory tract tissues to carcinogenic insult. Environ Health Perspect **29**:89–93, 1979.
48. Newberne PM, Rogers AE. Rat colon carcinomas associated with aflatoxin and marginal vitamin A. J Natl Cancer Inst **50**:439–448, 1973.
49. Newberne PM, Suphakarn V. Preventive role of vitamin A in colon carcinogenesis in rats. Cancer **40**: 2553–2556, 1977.
50. Overvad K, Thorling EB, Bjerring P, Ebbesen P. Selenium inhibits UV light induced skin carcinogenesis in hairless mice. Cancer Lett **27**:163–170, 1985.
51. Pedersen H, Wolf H, Kromann-Jensen S, Lund F, Hansen E, Rosenkilde-Olsen P, Sorensen BL. Administration of a retinoid as prophylaxis of recurrent non-invasive bladder tumors. Scand J Virol Nephrol **80**:121–123, 1984.
52. Perchellet JP, Owen MD, Posey TD, Orten DK, Schneider BA. Inhibitory effects of glutathione level-raising agents and D-α-tocopherol on ornithine decarboxylase induction and mouse skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate. Carcinogenesis **6**:567–573, 1985.
53. Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? Nature (London) **290**:201–208, 1981.
54. Reddy BS, Hirota N, Katayama S. Effect of dietary sodium ascorbate on 1,2-dimethylhydrazine- or methylnitrosourea-induced colon carcinogenesis in rats. Carcinogenesis **3**:1097–1099, 1982.
55. Russel WO, Ortega LR, Wynne ES. Studies on methylcholanthrene induction of tumors in scorbutic guinea pigs. Cancer Res **12**:216–218, 1952.
56. Salonen JT, Salonen R, Lappetelainen, R, Maenpaa, PM, Alfthan G, Puska P. Risk of cancer in relation to serum concentrations of selenium and vitamins A and E: Matched case control analysis. Brit Med J **290**: 417–420, 1985.
57. Shamberger RJ. Relationship of selenium to cancer. I. Inhibitory effect of selenium on carcinogenesis. J Natl Cancer Inst **44**:931–936, 1970.
58. Shekelle RB, Liu S, Raynor WJ, Lepper M, Maliza C, Rossof AH, Paul O, MacMillan Shryock A, Stamler J. Dietary vitamin A and risk of cancer in the Western Electric study. Lancet **2**:1185–1190, 1981.
59. Shirai T, Ikawa E, Hirose M, Witaya T, Ito N. Modification by five antioxidants of 1,2-dimethylhydrazine-initiated colon carcinogenesis in F344 rats. Carcinogenesis **6**:637–639, 1985.
60. Shklar G. Oral mucosal carcinogenesis in hamsters: Inhibition by vitamin E. J Natl Cancer Inst **68**:791–797, 1982.
61. Shklar G, Schwartz J, Grau D, Trickler DP, Wallace KD. Inhibition of hamster buccal pouch carcinogenesis by 13 cts retinoic acid. Oral Surg Oral Med Oral Pathol **50**:45–52, 1980.
62. Silverman J, Katayama S, Zelenakas K, Lauber J, Musser TK, Reddy M, Levenstein MJ, Weisburger JH. Effect of retinoids on the induction of colon cancer in F344 rats by N-methyl-N-nitrosourea or by 1,2-dimethylhydrazine. Carcinogenesis **2**:1167–1172, 1981.
63. Smith DM, Rogers AE, Newberne PM. Vitamin A and benzo(a)pyrene carcinogenesis in the respiratory tract of hamsters fed a semisynthetic diet. Cancer Res **35**:1485–1488, 1975.
64. Soullier BK, Wilson PS, Nigro ND. Effect of selenium

on azoxymethane-induced intestinal cancer in rats fed high fat diet. Cancer Lett **12**:343–348, 1981.

65. Sporn MB, Squire RA, Brown CC, Smith JM, Wenk ML, Springer S. 13-Cis-retinoic acid: Inhibition of bladder carcinogenesis in the rat. Science **195**:487–489, 1977.
66. Squire RA, Sporn MB, Brown CC, Smith JM, Wenk ML, Springer S. Histopathological evaluation of the inhibition of rat bladder carcinogenesis by 13-cis retinoic acid. Cancer Res **37**:2930–2936, 1977.
67. Stinson SF, Reznik G, Donahoe R. Effect of three retinoids on tracheal carcinogenesis with N-methyl-n-nitrosourea in hamsters. J Natl Cancer Inst **66**:947–951, 1981.
68. Suniyoshi H. Effects of vitamin E deficiency on 1,2-dimethylhydrazine-induced intestinal carcinogenesis in rats. Hiroshima J Med Sci **34**:363–369, 1985.
69. Tannenbaum SR, Mergens W. Reaction of nitrite with vitamins C and E. Ann NY Acad Sci **355**:267–279, 1980.
70. Thompson HJ, Becci PJ, Brown CC, Moon RC. Effect of the duration of retinyl acetate feeding on inhibition of 1-methyl 1-nitrosourea induced mammary carcinogenesis in the rat. Cancer Res **39**:3977–3980, 1979.
71. Thompson MJ, Becci PJ. Selenium inhibition of N-methyl-n-nitrosourea-induced mammary carcinogenesis in the rat. J Natl Cancer Inst **65**:1299–1301, 1980.
72. Thompson MJ, Meeker LD, Becci PJ. Effect of combined selenium and retinyl acetate treatment on mammary carcinogenesis. Cancer Res **41**:1413–1416, 1981.
73. Thompson MS, Becci PJ. Effect of graded dietary levels of selenium on tracheal carcinomas induced by 1-methyl-1-nitrosourea. Cancer Lett **7**:215–219, 1979.
74. Toth B, Patil K. Enhancing effect of vitamin E on murine intestinal tumorigenesis by 1,2-dimethylhydrazine dihydrochloride. J Natl Cancer Inst **70**:1107–1111, 1983.
75. Wald N, Idle M, Boreham J. Low serum-vitamin A and subsequent risk of cancer. Lancet **2**:813–815, 1980.
76. Wattenberg LW. Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. J Natl Cancer Inst **48**: 1425–1430, 1972.
77. Weerapradist W, Shklar G. Vitamin E inhibition of hamster buccal pouch carcinogenesis: A gross, histologic, and ultrastructural study. Oral Surg **54**:304–312, 1982.
78. Welsch CW, DeHoog JV, Moon RC. Lack of an effect of dietary retinoids in chemical carcinogenesis of the mouse mammary gland: Inverse relationship between mammary tumor cell anaplasia and retinoid efficacy. Carcinogenesis **5**:1301–1304, 1984.
