

Enzyme Induction and Dietary Chemicals as Approaches to Cancer Chemoprevention: The Seventh DeWitt S. Goodman Lecture¹

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ABSTRACT

Research on cancer chemoprevention is an important approach for decreasing both the incidence and number of deaths from cancer. The use of tamoxifen to prevent breast cancer, finasteride to prevent prostate cancer, and aspirin to prevent colon cancer are recent examples of cancer chemoprevention. This article describes research from my laboratory and related research from other laboratories on the effects of enzyme induction on chemical carcinogenesis as an approach to cancer chemoprevention, as well as studies on the inhibitory effects of curcumin, caffeine, (–)-epigallocatechin gallate (EGCG), and tea in animal models of carcinogenesis. The later substances appear to work, at least in part, by enhancing apoptosis in DNA-damaged cells or in tumors. The results of our studies and those of others provide a rationale for clinical trials on the potential chemopreventive effects of curcumin, caffeine, EGCG, and tea on the formation of cancer of the skin, mouth, esophagus, stomach, and colon in people with precancerous lesions and a high risk of developing these cancers.

It was pointed out that several compounds that are effective cancer chemopreventive agents in one experimental setting can enhance carcinogenesis in another experimental setting. These results suggest that it may be necessary to tailor the cancer chemopreventive regimen to individual subjects with known carcinogen exposures or to high cancer risk individuals with mechanistically understood pathways of carcinogenesis so that chemopreventive agents with known mechanisms of action can be better customized to the individual and selected on a more rational basis.

INDUCTION OF CARCINOGEN-METABOLIZING ENZYMES AS AN APPROACH TO CANCER CHEMOPREVENTION

Discovery of the Induction of Carcinogen-Metabolizing Enzymes

I joined the laboratory of James and Elizabeth Miller at the University of Wisconsin as a graduate student in September, 1952 and started work on the synthesis of a potential metabolite of β -naphthylamine. My efforts to become a synthetic chemist resulted in two explosions and were a dismal failure. Although I didn't know it at the time, this discouraging experience was extremely fortunate, because it led me to an exciting research program on the induction of liver microsomal enzymes as an approach to cancer chemoprevention, and to a research program on the pharmacological and toxicological significance of microsomal enzyme induction.

Received 8/21/03; accepted 8/28/03.

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¹ Research was supported in part by Research Contract No. PH 43-65-1066 from the National Institute of General Medical Sciences and by grants from the National Cancer Institute (CA49756, CA80759, and CA88961). This article is an expanded version of the Seventh DeWitt S. Goodman Lecture given in San Francisco, CA on April 10, 2002. It is dedicated to the memory of my graduate school mentors Drs. James A. Miller and Elizabeth C. Miller and to my former colleagues Drs. James Gillette, Alvaro Alvares and Shu-Jing (Caroline) Wei.

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After failing as a synthetic chemist, the Millers and I discussed research by H.L. Richardson *et al.* (1) who had reported that administration of 3-methylcholanthrene inhibited the hepatocarcinogenic activity of the aminoazo dye 3'-methyl-4-dimethylaminoazobenzene in rats. We discussed the possibility that treatment of rats with 3-methylcholanthrene might protect them by altering the metabolism of the dye. In late 1952 or early 1953, I started studies on the effects of treating rats with 3-methylcholanthrene and other polycyclic aromatic hydrocarbons on the hepatic *N*-demethylation and azo-link reduction of aminoazo dyes, metabolic pathways that resulted in noncarcinogenic products. I was delighted to find that a single i.p. injection of 3-methylcholanthrene caused a rapid and many-fold increase in hepatic azo dye *N*-demethylase and azo-link reductase activities (2, 3), and we provided strong evidence that treatment of rats with 3-methylcholanthrene had induced the synthesis of hepatic aminoazo dye *N*-demethylase and azo-link reductase (3). The induction of these enzyme systems by 3-methylcholanthrene administration, an early example of enzyme induction in mammals, is shown in Fig. 1. Structure-activity relationship studies showed that 3-methylcholanthrene, BP,³ dibenz(*a,h*)anthracene, and benz(*a*)anthracene were potent inducers of aminoazo dye metabolism, whereas pyrene had little or no activity (2, 3). The effects of the different polycyclic aromatic hydrocarbons to stimulate azo dye metabolism paralleled the effects of these compounds to inhibit azo dye carcinogenesis (2–4). Feeding 3-methylcholanthrene, BP, dibenz(*a,h*)anthracene, or benz(*a*)anthracene strongly inhibited the hepatocarcinogenicity of 3'-methyl-4-dimethylaminoazobenzene, whereas pyrene, which was inactive as an inducer of azo dye metabolism, did not inhibit azo dye carcinogenesis (Table 1; Ref. 4). Our research on the induction of azo dye-metabolizing enzymes provided a mechanistic explanation for the inhibitory effects of polycyclic hydrocarbons on azo dye carcinogenesis and provided an early example of mechanisms of cancer chemoprevention. These studies also placed into perspective the meaning of safety and benefit/risk ratio for the field of cancer chemoprevention. If I were a rat in an environment of carcinogenic aminoazo dyes that would with great certainty cause liver cancer and all that was available for protection was 3-methylcholanthrene, I would ingest the hydrocarbon, because it would save my life. Of course safer chemopreventive agents would be more desirable, and having a mechanistic understanding of cancer chemoprevention led to a search for safer and more effective chemopreventive agents.

In 1955, the Millers and I initiated research to determine whether polycyclic aromatic hydrocarbons that stimulated azo dye metabolism could also stimulate their own metabolism. In 1957, we reported that treatment of rats with BP, 3-methylcholanthrene, or several other polycyclic hydrocarbons induced the synthesis of hepatic BP hydroxylase activity (5). The stimulatory effect of BP administration on the hepatic metabolism of BP is shown in Fig. 2. Increased formation of 1- and 3-hydroxybenzo(*a*)pyrene (noncarcinogenic metabolites), as

³ The abbreviations used are: EGCG, (–)-epigallocatechin gallate; BP, benzo(*a*)pyrene; DMBA, 7,12-dimethylbenzo(*a*)anthracene; TCDD or dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; CYP, cytochrome P450; GST, glutathione *S*-transferase; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; IQ, 2-amino-3-methylimidazo(4,5-*f*)quinoline; Phip, 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine; PG, prostaglandin.

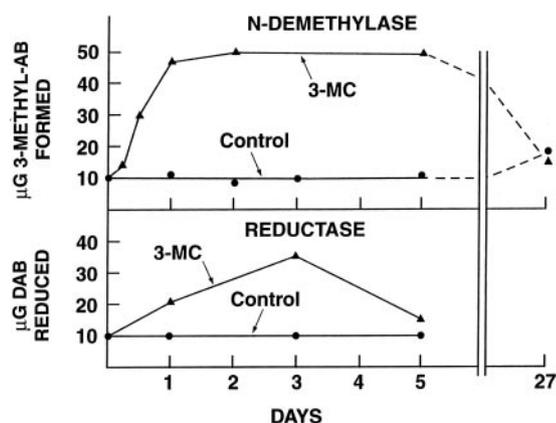


Fig. 1. Induction of hepatic aminoazo dye *N*-demethylase and reductase activities. Rats (50 g) were injected once i.p. with 1 mg of 3-methylcholanthrene (3-MC). *N*-Demethylase activity was determined in fortified liver homogenates by measuring the metabolism of 3-methyl-4-monomethylaminoazobenzene to 3-methyl-4-aminobenzene (3-methyl-AB). Reductase activity was determined by measuring the reduction of the azo linkage of 4-dimethylaminoazobenzene (DAB). Demethylase activity is expressed as μg of 3-methyl-AB formed/50 mg of liver/30 min. Reductase activity is expressed as μg of DAB reduced/30 mg of liver/30 min. Each point is the average of the activities for two or three rats. (Taken from Ref. 3.)

Table 1 Effect of polycyclic hydrocarbons on the induction of liver tumors by 3'-methyl-4-dimethylaminoazobenzene

Rats were fed 0.054% 3'-methyl-4-dimethylaminoazobenzene in the presence and absence of polycyclic hydrocarbons (0.123 millimoles/kg of diet). (Taken from Ref. 4.)

Experiment	Treatment	% of animals with liver tumors at 3 months
1	Control	75
	3-Methylcholanthrene	0
	Benzo(a)pyrene	0
	Dibenz(a,h)anthracene	0
2	Control	50
	3-Methylcholanthrene	0
	Benz(a)anthracene	0
	Pyrene	38

well as increased formation of 1,3-dihydroxybenzo(a)pyrene and additional polar metabolites were observed (5). The induction of BP hydroxylase occurred not only in the liver but also in several other organs of the rat such as the gastrointestinal tract, lung, kidney, skin, and placenta (6–11).

In 1957, I moved to the NIH in Bethesda and together with John Burns found that polycyclic aromatic hydrocarbons could induce the synthesis of drug-metabolizing enzymes, and that phenobarbital and several other drugs could also induce the synthesis of drug-metabolizing enzymes (12, 13). At the time we were studying the effects of drugs and polycyclic aromatic hydrocarbons as inducers of drug-metabolizing enzymes at the NIH, Herbert Remmer and Ryuichi Kato, working independently, also demonstrated a stimulatory effect of barbiturates and other drugs on drug metabolism (14–17). The selective induction of some drug-metabolizing enzymes but not others by polycyclic aromatic hydrocarbons and drugs provided a path that led to the discovery of multiple cytochromes P450 that metabolize large numbers of drugs, carcinogens, and steroid hormones (18). The pharmacological and toxicological significance of the induction of these enzymes has been described elsewhere (19–21).

Stimulatory Effects of Enzyme Inducers on the *in Vivo* Metabolism of Polycyclic Aromatic Hydrocarbons

Studies with BP. An early and important question during the course of our studies was whether the induction of carcinogen-metabolizing enzymes was paralleled by enhanced carcinogen metab-

olism *in vivo*. The induction of BP hydroxylase activity in rats was reflected *in vivo* by enhanced metabolism of BP, decreased blood and tissue concentrations of this carcinogen, and enhanced biliary excretion of its metabolites (22–24). 3-Methylcholanthrene, DMBA, and BP are potent inducers of BP hydroxylase activity, and pretreatment of rats with these compounds p.o. once daily for 2 days before an i.v. injection of tritiated BP on day 3 stimulated the disappearance of [^3H]BP from blood and decreased the concentration of [^3H]BP in various tissues (22). The effect of pretreatment of rats with 3-methylcholanthrene, DMBA, or BP to lower the concentration of BP in blood and fat at 45 min after an i.v. injection of [^3H]BP is shown in Table 2. Treatment of rats with pyrene or anthracene had little or no stimulatory effect on monooxygenase activity (3, 25) or on the *in vivo* metabolism of [^3H]BP (Table 2). Because the primary route of elimination of BP metabolites in rats is excretion into the bile, we investigated the effects of enzyme inducers on the metabolism of BP to biliary excretion products. Pretreatment of rats with nonradioactive BP for 2 days before an i.v. injection of [^{14}C]BP stimulated the excretion of metabolites of [^{14}C]BP into the bile (23). Seven min after the i.v. injection of 300 μg of [^{14}C]BP, the concentration of radioactive BP metabolites in the bile of pretreated rats was ~ 20 -fold higher than the concentration of [^{14}C]BP metabolites in the bile of control rats (23). The stimulatory effect of BP on its own metabolism is also illustrated by the decrease in tissue concentration of this compound that occurs when it is given chronically. At 24 h after a single oral dose of 1 mg of [^3H]BP to adult rats, the concentration of this hydrocarbon in fat was 249 ng/g, whereas 24 h after seven daily doses of 1 mg of [^3H]BP its concentration in fat was only 24 ng/g (22).

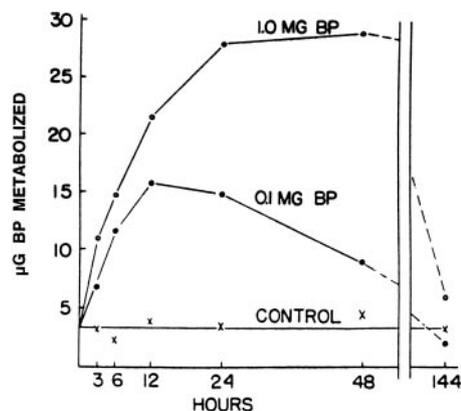


Fig. 2. Induction of hepatic BP hydroxylase activity. Rats (50 g) were injected once i.p. with 0.1 or 1 mg of BP. Homogenate from 50 mg of liver was incubated with 50 μg of BP for 12 min. (Taken from Ref. 5.)

Table 2 Effect of pretreatment of rats with polycyclic hydrocarbons on the metabolism of tritiated BP

In experiment 1, female rats weighing 175–185 g were treated orally with 20 mg/kg of BP once daily for 2 days. An equimolar amount of 3-methylcholanthrene (MC), DMBA, pyrene, or anthracene was administered in experiment 2. The day after the last dose of polycyclic hydrocarbon, rats were given an i.v. injection of 10 μg of [^3H]BP, and the animals were killed 45 min later. The data represent the mean \pm SE from three to six values where each value was obtained from 3 rats. (Taken from Ref. 22.)

Experiment	Pretreatment	Concentration of [^3H]BP in:	
		Blood, ng/ml	Fat, ng/g
1	Control	2.6 \pm 0.2	21.6 \pm 6.6
	BP	0.4 \pm 0.1	4.1 \pm 0.8
2	Control	2.9 \pm 0.3	15.0 \pm 3.5
	MC	0.5 \pm 0.0	4.3 \pm 0.7
	DMBA	0.7 \pm 0.1	5.4 \pm 0.6
	Pyrene	2.5 \pm 0.1	18.9 \pm 0.7
	Anthracene	2.3 \pm 0.3	16.9 \pm 2.5

Oral administration of several polycyclic hydrocarbons found in cigarette smoke to pregnant rats increased the hydroxylation of BP and the *N*-demethylation of 3-methyl-4-monomethylaminoazobenzene by fetal liver, maternal liver, and placenta (24). Studies were initiated to determine whether enzyme induction enhanced the *in vivo* metabolism of BP in pregnant rats and whether pretreatment with an enzyme inducer before administration of [³H]BP could decrease the concentration of the carcinogen in maternal tissues and in the fetus. Rats pregnant for 16 days were given oral doses of 20 mg of nonradioactive BP per kg or inactive vehicle once daily for 3 days. Twenty-four h after the last dose, the rats were given 20 mg of [³H]BP per kg p.o. and killed 2 h later. The concentrations of [³H]BP in the fetus, placenta, and maternal lung were markedly lower, and the concentrations of metabolites of [³H]BP were higher if pregnant animals had been pretreated with BP (24). Indeed, enzyme induction increased the ratio of the concentration of [³H]BP metabolites to that of [³H]BP by 84-, 19-, and 185-fold, respectively, in the fetus, placenta, and maternal lung (Table 3).

Studies with DMBA. Administration of DMBA to rats causes mammary cancer and massive necrosis of the adrenal gland (26–28). It was found that pretreatment of rats with any of several polycyclic hydrocarbons or aromatic azo derivatives protected rats from adrenal injury or mammary cancer by a subsequent dose of DMBA (28–32), and the protective effects of the various compounds were associated with the induction of menadione reductase [NAD(P)H: quinone reductase] in the soluble fraction of liver homogenate (30, 33, 34). In additional studies, we found that treatment of rats with the protective polycyclic aromatic hydrocarbons or aromatic azo derivatives enhanced by several-fold the hepatic metabolism of DMBA as well as the additional metabolism of monohydroxymethyl metabolites of DMBA to highly polar metabolites (35, 36). A stimulatory effect of polycyclic hydrocarbons on the hepatic metabolism of DMBA was also reported by Jellinck and Goudy (37, 38). The stimulatory effect of treatment of rats with 3-methylcholanthrene on the hepatic metabolism of DMBA and its monohydroxymethyl metabolites was paralleled by enhanced *in vivo* metabolism of DMBA (36). Treatment of rats with 3-methylcholanthrene 24 h before [³H]DMBA administration resulted in a markedly decreased concentration of tritiated hydrocarbon in the adrenal gland, fat, and mammary gland (Table 4). More detailed examination of the adrenal gland and fat revealed that pretreatment of the rats with 3-methylcholanthrene before the administration of DMBA decreased the concentrations of DMBA, 7-hydroxymethyl-12-methylbenz(*a*)anthracene, and 12-hydroxymethyl-7-methylbenz(*a*)anthracene in these tissues (36). Our results and those by Jellinck and Goudy (37, 38) indicated that the induction of enzymes that metabolize DMBA and its monohydroxymethyl metabolites played an important role in decreasing the adrenal toxicity and carcinogenicity of DMBA in rats pretreated with polycyclic hydrocarbons and aromatic azo derivatives.

Table 3 Effect of enzyme induction in pregnant rats on the metabolism of [³H]BP *in vivo*

Rats pregnant for 16 days were given 20 mg of nonradioactive BP per kilogram orally once daily for 3 days. Twenty-four h after the last dose, the rats were given an oral dose of 20 mg of [³H]BP per kilogram and were killed 2 h later. The tissue concentrations of unchanged [³H]BP and its radioactive metabolites were measured. Each value represents the mean ± SE from four rats. (Taken from Ref. 24.)

Tissue	Pretreatment	[³ H]BP (ng/g)	[³ H]BP metabolites (ng equivalents/g)	Ratio of [³ H]BP metabolites to [³ H]BP
Fetus	Control	657 ± 115	525 ± 49	0.80
	BP	11 ± 2	742 ± 113	67
Placenta	Control	1994 ± 266	364 ± 78	0.18
	BP	253 ± 29	851 ± 138	3.4
Maternal lung	Control	4129 ± 652	93 ± 47	0.02
	BP	353 ± 36	1315 ± 216	3.7

Table 4 Effect of 3-methylcholanthrene (MC) pretreatment on the concentration of DMBA and its metabolites in tissues of rats given [³H]DMBA

In experiments 1 and 2, rats pregnant for 15 days were injected i.p. with 5 mg of MC at 24 h before the oral administration of 20 mg of [³H]DMBA, and the rats were killed 2 or 4 h later. Pregnant rats were used in experiments 1 and 2 to obtain sufficient mammary tissue for analysis. In experiment 3, nonpregnant female rats were injected i.p. with 5 mg of MC at 24 h before the oral administration of 20 mg of [³H]DMBA, and the rats were killed 2 h later. Total radioactivity was determined in the various tissues. Each value represents the average and SE from 5 rats per group. More detailed studies indicated that pretreatment of the rats with MC before administration of DMBA decreased the concentration of DMBA and its hydroxymethyl metabolites in the adrenal gland and fat (see text). (Taken from Ref. 36.)

Experiment	Tissue	Treatment	Hours after DMBA administration	Hydrocarbon (nmol/gm tissue)
1	Adrenal	Corn oil	2	51.0 ± 3.4
	Adrenal	MC		21.1 ± 1.4
	Mammary	Corn oil	2	19.5 ± 2.0
2	Mammary	MC	2	7.3 ± 0.8
	Adrenal	Corn oil		4
	Adrenal	MC	4	17.8 ± 1.0
	Mammary	Corn oil	4	63.4 ± 7.3
	Mammary	MC		8.8 ± 0.6
3	Adrenal	Corn oil	2	66.4 ± 4.8
	Adrenal	MC		24.3 ± 3.2
	Fat	Corn oil	2	36.2 ± 4.1
	Fat	MC		10.7 ± 2.2

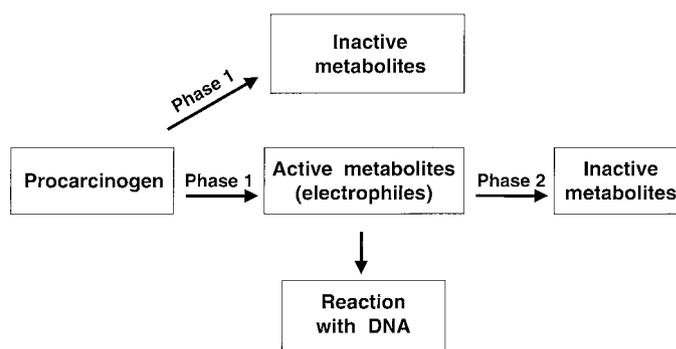


Fig. 3. Metabolism of procarcinogens to inactive and active metabolites by Phase I and Phase II enzymes. Phase I enzymes (predominantly the CYP family of enzymes) metabolize procarcinogens to inactive and active metabolites. Phase II enzymes inactivate electrophilic active metabolites formed by Phase I enzymes, and they also conjugate inactive metabolites (e.g., by glucuronidation, sulfonation, etc.) thereby enhancing their excretion in urine and bile.

Effects of Inducers of Phase I Monooxygenase Enzymes on Chemical Carcinogenesis

Most environmental carcinogens are initially metabolized by the CYP family of enzymes (Phase I monooxygenase enzymes) to biologically inactive metabolites as well as to chemically reactive electrophilic metabolites that covalently bind to specific sites on DNA to initiate a carcinogenic response (Fig. 3). The active metabolites may undergo additional metabolism by Phase I enzymes or by Phase II enzymes (e.g., GST, UDP glucuronosyltransferase, epoxide hydrolase and NAD(P)H: quinone reductase) to biologically inactive products (Fig. 3). Induction of either Phase I monooxygenases or Phase II enzymes can enhance the detoxification of carcinogens. Although Phase I enzymes metabolize procarcinogens to biologically active and inactive products, induction of Phase I enzymes is usually but not always associated with inhibition of carcinogenesis.

Topical applications or i.p. injections of nontoxic doses of TCDD 3 days before the topical application of DMBA enhanced the oxidative metabolism of DMBA by epidermal homogenates and caused a 90% inhibition in the tumor-initiating activity of DMBA in mice (39, 40). Covalent binding of topically applied DMBA to epidermal DNA was also decreased (39). Pretreatment of mice with TCDD also inhibited

the tumor-initiating activity of 3-methylcholanthrene and BP, as well as the tumorigenic activity of the ultimate carcinogenic metabolite of BP (bay-region diol epoxide; Ref. 39). The inhibitory effects of pretreatment of mice with TCDD on the tumor-initiating activities of DMBA, 3-methylcholanthrene, and BP on mouse skin were associated with large increases in epidermal hydrocarbon hydroxylase activity without increases in epidermal epoxide hydrolase or GST activities and with only a modest increase in epidermal UDP-glucuronosyltransferase activity (40). The results of these studies suggested that treatment of mice with nontoxic doses of TCDD enhanced monooxygenase-dependent inactivation of polycyclic aromatic hydrocarbons in the epidermis.

Treatment of rodents with inducers of microsomal monooxygenases provided protection from the carcinogenic effects of BP (39–42), DMBA (31, 32, 42–46), 2-acetylaminofluorene (4, 47, 48), 4-dimethylaminostilbene (49), urethane (50), aflatoxin B₁ (51, 52), diethylnitrosamine (48, 53), and aminoazo dyes (1, 2, 4, 48, 54, 55). Examples of inducers of liver microsomal monooxygenases that inhibit chemical carcinogenesis include 3-methylcholanthrene and other polycyclic aromatic hydrocarbons (1, 2, 4, 49), β -naphthoflavone (41, 44, 52), indole-3-carbinol (42, 56, 57), TCDD (39, 40), polychlorinated biphenyls (48), 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT; Ref. 46), phenobarbital (47, 51), and spironolactone (45). Phenothiazine and some closely related derivatives are potent inducers of hepatic and extrahepatic BP hydroxylase activity, and they inhibit DMBA-induced adrenal necrosis (58). The inhibitory effect of 3-methylcholanthrene administration on the carcinogenicity of 2-acetylaminofluorene was associated with an increased ratio of the metabolism of 2-acetylaminofluorene to inactive ring hydroxylated products relative to the formation of the carcinogenic *N*-hydroxylated metabolite *in vivo* as measured by the urinary excretion of these metabolites (59). In another study, rats were injected with 3-methylcholanthrene once daily for 2 days before the oral administration of carbon 14-labeled IQ. Pretreatment with 3-methylcholanthrene before administration of IQ markedly decreased the formation of IQ-DNA adducts in the liver, colon, small intestine, kidneys, bladder, heart, and lung (60). The results of this study suggested that 3-methylcholanthrene had induced CYP1A1/1A2 and had enhanced the ring hydroxylation of IQ (inactivation pathway) to a greater extent than the *N*-hydroxylation activation pathway.

Because many chemical carcinogens are metabolized by CYP enzymes to noncarcinogenic as well as to proximate and ultimate carcinogenic metabolites, the effects of inducers of these enzymes on the carcinogenicity of a chemical will depend on the effects of the inducer on the ratio of metabolism of the carcinogen to inactive and active metabolites by Phase I enzymes as well as on the levels of Phase II enzymes (*e.g.*, GST, epoxide hydrolase, NAD(P)H:quinone reductase, and UDP-glucuronosyl transferase) that inactivate chemically reactive metabolites. Because treatment of rodents with appropriate inducers of monooxygenases usually inhibits the carcinogenicity of chemical carcinogens *in vivo*, it is likely that these inducers enhance the *in vivo* detoxification of carcinogens to a greater extent than their activation.

Sometimes inducers of detoxifying enzymes also inhibit metabolic activation pathways. Treatment of rainbow trout with high doses of β -naphthoflavone, indole-3-carbinol, or with the condensation products of indole-3-carbinol induces the CYP1A family thereby enhancing the hydroxylation of aflatoxin B₁ to aflatoxin M₁ (inactivation pathway), but these inducers also inhibit the 8,9-epoxidation of aflatoxin B₁ (activation pathway; Refs. 61, 62). The inhibitory effect of low dose levels of the inducers on the action of aflatoxin B₁ in rainbow trout is predominantly by inhibition of the epoxidation pathway rather than by induction of enhanced detoxification (61, 62). Although inducers of Phase I enzymes usually inhibit chemical car-

cinogenesis, the stimulatory effect of phenobarbital administration on the hepatocarcinogenicity of safrole (63) is an example of an inducer of Phase I enzymes that enhances carcinogenesis.

The complexities of the effects of microsomal enzyme inducers on the carcinogenicity of chemicals was pointed out in studies with a pharmacogenetic model for transplacental carcinogenesis in noninducible or inducible pregnant mice where half of the fetuses were susceptible to induction of polycyclic hydrocarbon metabolism by CYP1A1 inducers and the other half were refractory to induction (64, 65). Although pretreatment of inducible pregnant mice with β -naphthoflavone (a noncarcinogenic inducer of CYP1A1) protected the fetuses from 3-methylcholanthrene-induced lung tumors observed later in the offspring, pretreatment of noninducible pregnant mice with β -naphthoflavone increased the carcinogenicity of 3-methylcholanthrene but only in the inducible fetuses (65). The results indicate that inducibility in both the mother and the fetus are important factors for determining whether an inducer of polycyclic hydrocarbon metabolism inhibits or enhances transplacental carcinogenesis by 3-methylcholanthrene (reviewed in Ref. 66).

Inhibition of Phase I monooxygenases can also inhibit chemical carcinogenesis apparently by inhibiting the metabolic activation of certain procarcinogens to a greater extent than their metabolic detoxification (67–70). The importance of Phase I enzymes for the metabolic activation and carcinogenicity of BP was emphasized recently by the studies of Shimizu *et al.* (71). These investigators demonstrated that mice lacking the aryl hydrocarbon receptor did not have detectable basal or BP-inducible levels of *CYP1A1* gene expression, and these mice were also refractory to BP-induced s.c. or epidermal carcinogenesis presumably because they were unable to metabolically activate BP (71).

In an additional study, Uno *et al.* (72) found that *CYP1A1* homozygous knockout mice treated with BP had impaired elimination of BP from the serum and higher levels of BP-DNA adducts in the liver than *CYP1A1* heterozygous knockout mice treated with BP. These investigators also showed that TCDD pretreatment of mice with either genotype enhanced the elimination of BP from the serum and lowered the levels of hepatic BP-DNA adducts. These results indicate that enzymes other than CYP1A1 are induced by TCDD and can play a role in the detoxification and elimination of BP.

Polycyclic aromatic hydrocarbons in cigarette smoke and β -naphthoflavone are potent inducers of cytochromes P450 (CYP1A1/1A2) that hydroxylate aflatoxin B₁ to aflatoxin M₁, (an inactivation pathway; Refs. 73–75). Feeding rats β -naphthoflavone, which stimulates the hepatic metabolism of aflatoxin B₁ to aflatoxin M₁, inhibits the hepatocarcinogenic activity of aflatoxin B₁ (52). In accord with these observations, epidemiology studies suggest that cigarette smokers who are heavily exposed to aflatoxin B₁ in their daily diet have a decreased risk of liver cancer when compared with nonsmokers who are also exposed to aflatoxin B₁ (76). If these studies are confirmed, it may be possible to isolate from tobacco smoke potent nontoxic inducers of aflatoxin B₁ detoxification that would be useful chemopreventive agents in people exposed to high levels of aflatoxin B₁. Alternatively, other inducers of the CYP1A family such as indole-3-carbinol, β -naphthoflavone, β -apo-8'-carotenal, astaxanthin, or canthaxanthin could be considered for additional studies. The later three compounds are of interest because of a recent report that indicates that treatment of rats with these carotenoids induced increased levels of hepatic CYP1A1/1A2, increased the hepatic hydroxylation of aflatoxin B₁ to aflatoxin M₁, and inhibited aflatoxin B₁-induced DNA damage and the formation of preneoplastic foci (77). Although the above studies suggest that an elevated level of CYP1A1/1A2 protects animals and possibly humans from the hepatocarcinogenic effects of aflatoxin B₁, additional studies are needed to determine whether or not

an increased level of CYP1A1/1A2 is associated with decreased carcinogenicity of aflatoxin B₁ in humans.

Many clinically useful drugs are inducers of CYP monooxygenases in humans, and it is likely that these drugs will also influence the metabolism and carcinogenicity of environmental carcinogens. Examples of inducers of oxidative drug metabolism in humans include phenobarbital (inducer of the CYP2B and 3A families), rifampicin (inducer of the CYP3A and 2C families), clotrimazole (inducer of CYP3A4), omeprazole (inducer of the CYP1A family), phenytoin (inducer of the CYP2C family), ethanol (inducer of CYP2E1), and the herbal antidepressant St. John's wort (inducer of CYP3A4). Epidemiology studies are needed to determine whether individuals who are heavily exposed to environmental carcinogens have an altered risk when they are also taking enzyme-inducing drugs.

Although the CYP monooxygenases metabolize chemical carcinogens to biologically inactive metabolites and to chemically reactive ultimate carcinogens, both kinds of metabolites can undergo additional metabolism by Phase II enzymes such as GST, NAD(P)H:quinone reductase (DT-diaphorase), epoxide hydrolase, and UDP-glucuronosyltransferase resulting in the inactivation and elimination of chemically reactive ultimate carcinogens so that elevated levels of Phase II enzymes help protect organisms from chemical carcinogens and from other toxic effects of electrophiles. The importance of GST for the detoxification of polycyclic aromatic hydrocarbons was emphasized by a study indicating enhanced DMBA-induced skin carcinogenesis in mice lacking the π class of GST when compared with DMBA-induced skin carcinogenesis in wild-type mice (78). The importance of GST was also pointed out by a study indicating that the (+)-7 β ,8 α -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo-(a)pyrene-DNA adduct level in the lungs of smokers is correlated with the *GSTM1*O* genotype, and this genotype appears to be a risk factor (79). Although many individuals may have sufficient GST and other Phase II enzymes to inactivate ultimate carcinogens that are formed during the metabolism of procarcinogens by Phase I enzymes, some individuals may benefit from increased levels of the Phase II enzymes.

Effects of Inducers of Phase II Enzymes on Chemical Carcinogenesis

Shortly after the discovery of the induction of hepatic monooxygenases and azo dye reductase, it was found that some inducers of Phase I enzymes such as polycyclic aromatic hydrocarbons and phenobarbital also induced increased levels of a Phase II enzyme (UDP-glucuronosyltransferase; Refs. 80–82). In accord with these observations, treatment of humans with phenobarbital decreased the serum level of unconjugated bilirubin (a substance eliminated from the body by glucuronidation), and jaundice was ameliorated in children with hyperbilirubinemia who were treated with phenobarbital (83–85). Another early example of the induction of a Phase II enzyme was provided by Huggins and his colleagues who demonstrated the induction of hepatic menadione reductase [NAD(P)H:quinone reductase] in rats by a variety of polycyclic aromatic hydrocarbons and aromatic azo compounds (30, 33, 34). The induction of hepatic menadione reductase in rats by the various compounds was associated with inhibitory effects of these compounds on DMBA-induced adrenal necrosis and mammary cancer (30, 33, 34). Subsequent studies have shown that many inducers of Phase I enzymes also induce Phase II enzymes.

During the 1960s and 1970s, Lee Wattenberg (86, 87) pioneered in research that identified several classes of inhibitors of chemical carcinogenesis, and some of the inhibitors enhanced the oxidative metabolism of carcinogens. It was shown by Ulland *et al.* (88) and Wattenberg and his colleagues (89–93) that treatment of rodents with

several polyphenolic antioxidants present in our diet inhibited chemical carcinogenesis.

During 1978–1980, the laboratories of Paul Talalay and Ernst Bueding reported on an important and novel type of induction of xenobiotic metabolism by certain antioxidants (94–97). They found that treatment of mice with butylated hydroxyanisole, butylated hydroxytoluene, and/or ethoxyquin resulted in increased levels of hepatic GST, epoxide hydrolase, and NAD(P)H:quinone reductase (94–97). These were the first reports on the induction of Phase II enzymes by antioxidants. Subsequent research by Talalay and his colleagues identified a large number of synthetic and dietary chemicals that are strong inducers of Phase II enzymes (98, 99), and many of these substances are electrophilic Michael reaction acceptors (99, 100). Many inducers of Phase II enzymes that inactivate ultimate carcinogens also induce enzymes that result in increased cellular levels of antioxidants (*e.g.*, glutathione and other substances) that protect cells from oxidative stress, and this response may be an important component of the effect of Phase II enzyme inducers to protect animals from chemical carcinogens (98, 101). Additional evidence for the protective effects of increased levels of Phase II enzymes against oxidants and electrophiles is discussed in a recent review by Talalay *et al.* (102).

The importance of Phase II enzymes for inactivating chemical carcinogens was highlighted in a study with *nrf2* transcription factor-deficient mice (103). *Nrf2* binds to the antioxidant response element and is necessary for the induction of Phase II enzymes. Constitutive hepatic and gastric activities of GST and NAD(P)H:quinone reductase, and the induction of these enzymes by oltipraz were markedly reduced in *nrf2*-deficient mice when compared with wild-type mice (103). These results are in agreement with *in vivo* studies indicating that *nrf2*-deficient mice are more sensitive to BP-induced gastric tumors than wild-type mice, and they are refractory to oltipraz-induced induction of Phase II enzymes and to oltipraz-induced inhibition of BP-induced gastric neoplasms (103). It would be of interest to determine whether induction of cytochromes P450 by TCDD, or a polycyclic hydrocarbon in *nrf2*-deficient mice or in GST-deficient mice would inhibit BP and DMBA-induced carcinogenesis as was observed in wild-type mice. Recent studies indicate that sulfhydryl groups of Keap1 are sensors regulating the induction of Phase II enzymes and that inducers of Phase II enzymes disrupt the cytoplasmic complex between actin-bound protein Keap1 and *nrf2*, thereby releasing *nrf2* to migrate to the nucleus and stimulate the transcription of genes for Phase II enzymes (104).

Two of the most widely studied inducers of Phase II enzymes are oltipraz (an antischistosomal drug) and sulforaphane (an isothiocyanate in broccoli; Refs. 105–108). Pretreatment of rats with sulforaphane inhibited DMBA-induced mammary cancer (108), and treatment of rats with oltipraz inhibited aflatoxin B₁-induced liver cancer (109). Oltipraz has undergone extensive evaluation as a cancer chemopreventive agent, and it was shown to be an effective anticarcinogen in >12 animal models (110). Oltipraz-induced decreases in aflatoxin B₁-induced liver cancer in rats was associated with decreased levels of aflatoxin-N-guanine adducts in the liver and urine, and this provided a useful biomarker (110).

Although sulforaphane and oltipraz are inducers of Phase II enzymes, both of these compounds may modulate carcinogen metabolism by other mechanisms. The administration to rats of certain isothiocyanates related to sulforaphane inhibits some CYP enzymes, whereas others are induced (111, 112). Both oltipraz and sulforaphane are strong *in vitro* inhibitors of human cytochromes P450 1A2 and 3A4 (113, 114), and oltipraz has also been reported to induce increased levels of CYP1A2-associated dealkylation of methoxyresorfin in rat liver (115). In normal volunteers, administration of a single

125 mg dose of oltipraz inhibited CYP1A2 activity as measured by studies on the *in vivo* metabolism of caffeine (116). Although many literature reports speak of monofunctional Phase II enzyme inducers, most inducers of Phase II enzymes also inhibit or induce Phase I CYP enzymes.

A recent intervention study in people heavily exposed to aflatoxin B₁ in China indicated that treatment of these individuals with 125 mg of oltipraz daily for 4 weeks increased the urinary excretion of aflatoxin B₁-mercapturic acid (glutathione conjugate of aflatoxin B₁), which is an inactivation product of aflatoxin B₁-8,9-oxide (117, 118). Formation of aflatoxin M₁ (a hydroxylated CYP-dependent inactivation product of aflatoxin B₁) was not affected (118). In contrast to these observations, administration of 500 mg of oltipraz once a week for 4 weeks decreased the urinary excretion of aflatoxin M₁, presumably by inhibiting CYP-dependent hydroxylation (or by enhancing the further metabolism of aflatoxin M₁), and the urinary excretion of aflatoxin B₁-mercapturic acid was not affected (118). The results indicated that the effects of oltipraz administration on aflatoxin B₁ metabolism in humans depended on the dosing regimen.

Potential Problems Associated with the Use of Enzyme Induction as an Approach to Cancer Chemoprevention

Although the induction of CYP enzymes that metabolize carcinogens usually inhibits carcinogenesis in experimental animals (presumably because detoxification pathways are enhanced to a greater extent than activation pathways), sometimes carcinogenesis is enhanced as occurs for the effect of phenobarbital administration on safrole carcinogenesis (63). Some enzyme inducers that inhibit carcinogenesis when given together with a carcinogen are tumor promoters when given after the carcinogen (*e.g.*, phenobarbital, indole 3-carbinol, and DDT; Refs. 47, 119–126).

Another potential problem associated with the use of enzyme induction as an approach to cancer chemoprevention is that enzyme inducers can stimulate the metabolism and alter the action of therapeutic drugs thereby leading to drug interactions in patients, and the metabolism and action of important normal body constituents (such as vitamin D, arachidonic acid, thyroid hormone, and steroid hormones) may also be modified. Although the scientific community is searching for monospecific inducers of Phase II enzymes, several inducers of Phase II enzymes (*e.g.*, butylated hydroxyanisole, sulforaphane, oltipraz, and some related compounds) can also induce or inhibit Phase I enzymes (111–114, 127).

The current state of our knowledge indicates that the selective induction of carcinogen-detoxifying enzymes (Phase I and/or Phase II enzymes) may be a useful approach for inhibiting carcinogenesis in individuals or populations at high unavoidable risk because of exposure to high levels of specific carcinogens, but this approach may not be useful in the general population. The possibility that enzyme inducers that increase cellular antioxidant defense systems may have a broad protective effect in the general population is a promising approach that requires additional research. Careful benefit/risk assessments need to be made before and during clinical trials with chemopreventive inducers of Phase I and/or Phase II enzymes.

Effects of Dietary Changes on the Metabolism of Foreign Compounds in Humans

Because chemical carcinogens are metabolized by the same enzyme systems that metabolize drugs, studies on the effects of environmental factors that influence drug metabolism in humans may lead to new approaches for stimulating the detoxification or inhibiting the metabolic activation of environmental carcinogens. Increasing the ratio of protein to carbohydrate in the diet or ingestion of charcoal broiled

beef or cabbage and brussels sprouts increased the oxidative metabolism of several drugs (128–132), and feeding cabbage and brussels sprouts also enhanced the glucuronidation of acetaminophen (a Phase II biotransformation; Ref. 133).

Other studies have shown that switching people from their home diet to a semisynthetic diet caused a 57% decrease in the CYP-dependent oxidative dealkylation of 7-ethoxycoumarin in the intestinal mucosa, but NADPH-cytochrome *c* reductase and 1-naphthol glucuronyltransferase activities were not affected (134). These results indicate that certain CYP-dependent oxidations in the intestinal mucosa are very sensitive to dietary changes.

In an additional study, feeding a brussels sprouts and broccoli-containing diet to human subjects for 12 days enhanced the metabolism of caffeine (CYP1A2 substrate) and decreased the urinary excretion of unchanged 2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline (MeIQx) and PhIP ingested in a cooked meat meal. The results suggested that the vegetable diet may have enhanced the metabolism of these heterocyclic amines (135).

Ingestion of watercress, which contains a high level of phenethyl isothiocyanate, increased the urinary levels of 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (NNAL) and its *O*-glucuronide [metabolically inactivated metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)] in smokers (136). This treatment also increased the glucuronidation of cotinine and *trans*-3'-hydroxycotinine in smokers, and the glucuronidation of *trans*-3'-hydroxycotinine correlated with the glucuronidation of 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (137). In other studies, ingestion of watercress inhibited the oxidative metabolism of acetaminophen and chlorzoxazone suggesting an inhibitory effect on the activity of CYP2E1 (138, 139). Watercress administration, however, had no effect on acetaminophen glucuronidation (138). These results suggest that ingestion of watercress has a selective stimulatory effect on some but not all of the UDP-glucuronosyltransferase enzymes and that ingestion of watercress also inhibits CYP2E1. It is apparent from these studies that the effects of watercress ingestion on xenobiotic metabolism are complex.

A single glass of grapefruit juice increased the oral bioavailability of felodipine, nifedipine, and several other drugs that are metabolized by CYP3A4 presumably by inhibiting the first pass metabolism of these drugs in the gastrointestinal tract and/or liver (140, 141). Certain furanocoumarins in grapefruit juice have been suggested as possible contributors to the inhibitory effect of grapefruit juice on drug metabolism. It was shown that administration of grapefruit juice three times a day for 6 days increased the area under the plasma concentration-time curve for felodipine and caused a 62% decrease in the concentration of CYP3A4 in the small bowel epithelium without influencing the concentration of CYP1A1 or CYP2D6 in the small bowel or the hepatic CYP3A4 activity as measured by the [¹⁴C-*N*-methyl] erythromycin breath test (141).

The studies described above indicate that dietary changes can influence the metabolism of foreign compounds including environmental carcinogens in humans. It is likely that these environmental modulators of foreign compound metabolism can influence the action of environmental carcinogens, and additional research may provide more leads for dietary cancer chemopreventive regimens.

Effects of Liver Microsomal Enzyme Inducers on Steroid Metabolism and Hormone-Related Cancers

Effects of Phenobarbital and Other Drugs on Steroid Metabolism. In studies that started 40 years ago (142), we found that treatment of rats with phenobarbital or other drugs, or with certain halogenated hydrocarbon insecticides increased liver microsomal monooxygenase activity for the metabolism of estradiol, estrone,

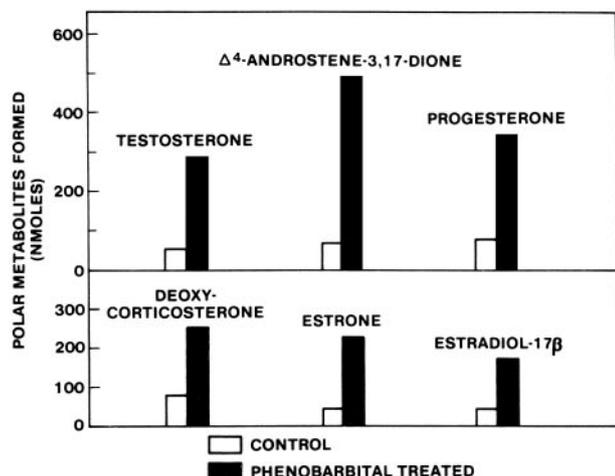


Fig. 4. Stimulatory effect of phenobarbital treatment on steroid hydroxylation by rat liver microsomes. Immature rats were given i.p. injections of 37 mg of sodium phenobarbital per kg of body weight twice daily for 4 days. The animals were killed the following day, and liver microsomes were prepared and incubated with ¹⁴C-labeled steroid in the presence of NADPH. Formation of polar metabolites with the chromatographic mobility of hydroxylated substrate was measured. (Taken from Refs. 142, 144, 149.)

deoxycorticosterone, progesterone, Δ^4 -androstene-3,17-dione, and testosterone (reviewed in Ref. 143). The stimulatory effect of treatment of rats with phenobarbital on the metabolism of these steroids to polar hydroxylated metabolites by liver microsomal enzymes is shown in Fig. 4. The stimulatory effects of liver microsomal enzyme inducers on steroid metabolism were paralleled *in vivo* by a decreased action of the steroids. Pretreatment of rats with phenobarbital or several other inducers of liver microsomal enzymes decreased: (a) the uterotrophic effects of estradiol, estrone, and certain oral contraceptive steroids (144–147); (b) the anesthetic effects of progesterone and deoxycorticosterone (148, 149); and (c) the growth promoting effects of testosterone on the seminal vesicles (150, 151).

Additional studies from our laboratory showed that treatment of female rats with phenobarbital stimulated the liver microsomal metabolism of estradiol to 2-, 4-, 6 α -, 6 β -, and 14 α -hydroxyestradiol, whereas treatment with 3-methylcholanthrene stimulated the 6 α -, 7 α -, and 15 α -hydroxylation of estradiol (152). Treatment of rats with dexamethasone stimulated the liver microsomal metabolism of estradiol to 2-, 4-, 6 β -, 7 α -, and 14 α -hydroxyestradiol, and the formation of several nonpolar unidentified metabolites of estradiol was also enhanced (152). At least a dozen different metabolites of estradiol were formed by liver microsomes from female rats treated with different inducing agents (152). Recent studies indicate that chronic administration of sodium phenobarbital (0.05% in the drinking water) inhibits the formation of estradiol-induced mammary tumors in ACI rats⁴ and the formation of estrogen-dependent spontaneous mammary tumors in C3H/OuJ mice.⁵

Administration of phenobarbital or other inducers of liver microsomal monooxygenases stimulated the 6 β -hydroxylation of cortisol in guinea pig liver microsomes (153), enhanced the urinary excretion of 6 β -hydroxycortisol in humans (154–159), increased the urinary excretion of polar metabolites of testosterone in humans (160), decreased the effectiveness of a synthetic glucocorticoid in steroid-dependent asthmatics (161), and stimulated the metabolism of synthetic estrogens and progestational steroids contained in oral contraceptives (162). Anticonvulsants and rifampicin are examples of

drugs that stimulate the metabolism of the oral contraceptive, ethinylestradiol, in humans (162–164), and unwanted pregnancies have occurred in women taking oral contraceptives simultaneously with anticonvulsants or rifampicin (discussed in Ref. 147). It will be of considerable interest to determine whether people treated chronically with phenobarbital, anticonvulsant mixtures, or other inducers of the CYP enzymes have a decreased risk of hormone-dependent cancers such as cancers of the endometrium, breast, and prostate.

Effects of Indole-3-Carbinol and TCDD on Steroid Metabolism. Chronic administration of indole-3-carbinol [a constituent of cruciferous vegetables that is converted to the Ah receptor agonist indolo(3,2-b)carbazole in the stomach] stimulates the 2-hydroxylation of estradiol and inhibits the formation of spontaneous mammary tumors in female C3H/OuJ mice (165–168). Another study indicated an inhibitory effect of administration of indole-3-carbinol on the formation of spontaneous uterine tumors in Donryu rats (169). The development of DMBA- or *N*-nitrosomethylurea-induced mammary tumors (both of which are highly dependent on endogenous estrogens; Ref. 170) in female Sprague Dawley rats was also inhibited by chronic treatment with indole-3-carbinol (57). Additional studies by Michnovicz, Bradlow, and their colleagues (171, 172) showed a stimulatory effect of indole-3-carbinol administration on the 2-hydroxylation of estradiol in humans, and they suggested that indole-3-carbinol may be an effective chemopreventive agent for breast cancer in women (173, 174). However, the use of indole-3-carbinol as a chemopreventive agent against estrogen-dependent human cancers through induction of estrogen 2-hydroxylation should receive more careful evaluation because indole-3-carbinol is a promoter of liver tumors in initiated animals (120–126). In addition, (a) some studies suggest that total catechol estrogen production is positively associated with an increased risk of breast cancer in women (175–177); (b) indole-3-carbinol [a prodrug for the formation of the potent Ah-receptor agonist, indolo(3,2-b)carbazole; Ref. 178], as well as other Ah-receptor agonists such as TCDD, have been reported to increase the rates of formation of both 2- and 4-hydroxyestradiol in liver and in estrogen target cells (166, 179–181); and (c) 4-hydroxyestradiol has carcinogenic activity when administered to Syrian hamsters or newborn mice, whereas 2-hydroxyestradiol has very low or no carcinogenic activity (182–185). Although treatment of animals or humans with indole-3-carbinol to stimulate the 2-hydroxylation of estradiol may be an effective way of inhibiting estrogen-induced carcinogenesis, and studies in this area should be encouraged, the significance of the tumor promoting activity of indole-3-carbinol and its stimulatory effect on the formation of the potentially toxic 4-hydroxyestradiol should receive more attention before its widespread use in humans.

Previous studies showed that treatment of animals with TCDD (a potent environmental Ah receptor agonist with strong monooxygenase-inducing activity) resulted in a marked antiestrogenic effect (186–188), but the blood level of estradiol was not altered (188). Additional studies revealed a marked stimulatory effect of treating cultured MCF-7 human breast cancer cells with TCDD on the 2-, 4-, 6 α -, and 15 α -hydroxylation of estradiol by microsomes from these breast cancer cells, and the 2-, 6 α -, and 15 α -hydroxylation of estradiol (estrogen inactivation pathways) were quantitatively the most important pathways that were stimulated (>90% of the total metabolites formed; Ref. 179). The stimulatory effect of TCDD on oxidative estradiol metabolism in cultured MCF-7 cells was associated with an inhibitory effect of TCDD on estradiol-induced formation of transformed foci and plasminogen activator activity in these cells (189, 190). Other studies indicated that administration of TCDD to mice injected with MCF-7 breast cancer cells inhibited the growth of these explants (190), presumably by enhancing the metabolic inactivation of estrogen in the breast cancer cell explants and/or in host tissues. These

⁴ S. Mesia-Vela, K. Reuhl, A. H. Conney, and F. Kauffman, unpublished observations.

⁵ B. T. Zhu and A. H. Conney, unpublished observations.

results indicate that Ah receptor agonists such as TCDD, indolo(3,2-b)carbazole (derived from indole-3-carbinol in cruciferous vegetables), and polycyclic aromatic hydrocarbons in tobacco smoke or charcoal broiled foods may decrease estrogen action in target cells because of markedly enhanced estrogen metabolism to inactive or poorly active estrogen metabolites. It should be noted that enhanced formation of 2-hydroxyestradiol may result in increased formation of 2-methoxyestradiol, a potential methylated metabolite of 2-hydroxyestradiol that has potent antiangiogenesis and anticarcinogenic activity (reviewed in Ref. 191). Selective modulation of estrogen metabolism in target tissues or cells may have profound biological effects without influencing the blood or urinary levels of estrogens and their metabolites (192).

It is of interest that although prolonged administration of TCDD to female rats enhanced the incidence of liver tumors, this treatment inhibited the formation of spontaneous tumors in the uterus, mammary gland, and pituitary (193), which are all target tissues for estrogen action. Whether the inhibitory effect of TCDD on the formation of spontaneous tumors in estrogen target tissues is caused by its strong stimulatory effect on the metabolic inactivation of estradiol in liver or in target tissues is worthy of additional investigation. Careful dose-response studies with TCDD are needed to determine whether the beneficial or toxic effects predominate at low dose levels of TCDD. Although TCDD has numerous adverse effects in humans, epidemiology studies in a population accidentally exposed to TCDD and other chemicals in Servasio, Italy suggested a decreased risk of breast cancer in these individuals in a 10-year follow-up study but not in a 20-year follow-up study (194–196). Additional research on the effects of exposure to TCDD on endometrial and breast cancer is needed.

Effects of Cigarette Smoking on Steroid Metabolism. Early studies from our laboratory showed that cigarette smoking (exposure to polycyclic aromatic hydrocarbons and other inducers) stimulated the oxidative metabolism of xenobiotics (10, 197–199). More recent studies showed that cigarette smoking increased the level of CYP1A1 in the placenta (200) and stimulated the placental 15 α -hydroxylation of estradiol (201), which is an inactivation pathway of estradiol metabolism that is stimulated in liver microsomes from rats treated with 3-methylcholanthrene (152). Cigarette smokers also have enhanced *in vivo* 2-hydroxylation of estradiol (202, 203), and some studies indicate that smokers have lower serum or urinary levels of estradiol and estrone (204–207), as well as lower urinary excretion of 16 α -hydroxyestradiol (an estrogenic metabolite of estradiol) than nonsmokers (208). Enhanced metabolic inactivation of estradiol in cigarette smokers may explain why cigarette smokers have an increased risk of osteoporosis (209–211) and a decreased risk of endometrial cancer (209, 211–214). Although the above studies suggest that cigarette smoking may inhibit endometrial cancer by enhancing the formation of 2- and 15 α -hydroxyestradiol (nonestrogenic metabolites), and by decreasing the formation of 16 α -hydroxyestradiol (an estrogenic metabolite), several investigators have not observed decreased serum levels of estradiol in cigarette smokers (208, 215, 216). The possibility that cigarette smoking enhances the metabolism of estradiol to inactive or protective metabolites in the liver or in the endometrium and breast is an area that needs additional research. Enhanced metabolism of estradiol to 2-hydroxyestradiol followed by additional metabolism of 2-hydroxyestradiol to 2-methoxyestradiol, possibly in target tissues, would be expected to inhibit estradiol-induced carcinogenesis and could occur in the absence of a decrease in the serum level of estradiol (191, 192).

Although epidemiology studies consistently indicate that cigarette smokers have a decreased risk of endometrial cancer (209, 211–214), studies to determine whether cigarette smoking alters the risk of breast cancer have been inconclusive (217). A recent study suggests a

dual/competing effect of cigarette smoking on breast cancer risk (218). It was observed that the risk of breast cancer was increased in parous women who started smoking within 5 years of menarche or in nulliparous women who smoked ≥ 20 cigarettes daily for ≥ 20 pack-years (218). In contrast with these observations, postmenopausal women whose body-mass index increased from age 18 and who started to smoke after a full-term pregnancy had a decreased risk of breast cancer (218). These observations suggested that in older women, the antiestrogen effects of cigarette smoking may modulate the increased risk seen with an increase in body weight (218). These possible competing effects of cigarette smoking on breast cancer risk are discussed additionally in a commentary by Russo (219).

In a recent study, it was found that cigarette smoking was associated with a decreased risk of breast cancer in women with a *BRCA1* or *BRCA2* mutation (220), and this report was discussed additionally in a commentary by Baron and Haile (221). In contrast to these observations in women with a *BRCA1* or *BRCA2* mutation, in a cohort study of high-risk breast cancer families in the general population it was found that cigarette smoking was associated with an increased risk of breast cancer (222). Although cigarette smoking is clearly associated with substantial increases in the risk for lung cancer, bladder cancer, oral cancer, osteoporosis, and cardiovascular disease, the available evidence suggests that cigarette smoking increases the risk for breast cancer in some women and decreases the risk for breast cancer in other women. Overall, the risks of cigarette smoking in the general population greatly exceed potential benefits, and all efforts to discourage people from smoking should continue.

In early studies, exposure of dogs to cigarette smoke was reported to stimulate the hepatic 6 β -hydroxylation of testosterone, decrease the serum level of testosterone, and to decrease the size of the prostate gland (223). It was also reported that human cigarette smokers had a decreased risk of benign prostate hypertrophy (224). In more recent studies, cigarette smoking was not found to be associated with an altered risk of benign prostate hypertrophy (225) or prostate cancer (226–228), but cigarette smoking was reported to be associated with an increased risk of fatal prostate cancer (227, 229). Another report indicated that cigarette smoking was positively related to benign prostate hypertrophy but only in men who smoke ≥ 35 cigarettes per day (230). More research is needed to resolve the conflicting reports on the effects of cigarette smoking on benign prostate hypertrophy and prostate cancer.

Metabolism of Xenobiotics by Intestinal Microorganisms

It is well known that many foreign chemicals can be metabolized by microorganisms in the gastrointestinal tract. The kinds of microorganisms differ in different individuals and can also differ in the same individual depending on the diet. Induction of enzymes that inactivate potentially toxic chemicals in the intestinal microflora, and the development and use of stable microorganisms that detoxify foreign chemicals in the gastrointestinal tract are additional approaches for enhancing the metabolic inactivation of carcinogens and other toxic chemicals.

INHIBITORY EFFECTS OF DIETARY CHEMICALS ON CARCINOGENESIS: STUDIES WITH CURCUMIN, CAFFEINE, EGCG, AND TEA

Epidemiology studies indicate that a substantial portion of human cancer in the United States is related to dietary factors (231, 232). Although it has been difficult from the epidemiology studies to identify specific dietary components that are inhibitory, the available evidence suggests that people who eat large amounts of fruit and

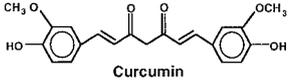
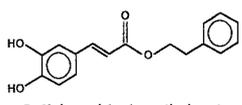
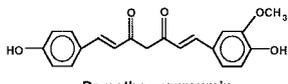
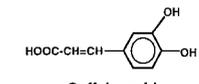
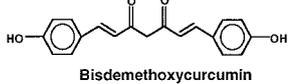
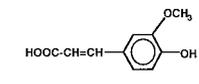
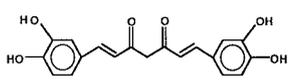
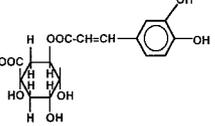
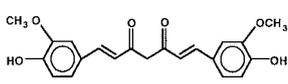
Compound	Effect observed	Compound	Effect observed
 Curcumin	++++	 Caffeic acid phenethyl ester (CAPE)	++++
 Demethoxycurcumin	++++	 Caffeic acid	+
 Bisdemethoxycurcumin	+	 Ferulic acid	+
 Bisdemethylcurcumin (dicatechol)	-	 Chlorogenic acid	++
 Tetrahydrocurcumin	+ to ++		

Fig. 5. Structures and inhibitory effects of curcumin and some analogues on tumor promotion by TPA. (Taken from data described in the text and from Refs. 257, 261, 263, 264.)

vegetables have a lower risk of several kinds of cancer (233–237). These observations in human populations pointed out the importance of identifying dietary substances that modulate carcinogenesis. The pioneering research of Wattenberg *et al.* (reviewed in Refs. 86, 238, 239) that demonstrated cancer chemopreventive activity for a large number of dietary chemicals in animals and the work by Sporn and his colleagues (reviewed in Ref. 240) on the chemopreventive effects of retinoids fostered the field of cancer chemoprevention. In recent years, there has been an increased emphasis on dietary modulators of carcinogenesis, and work in this field is being pursued in many laboratories. Our early research on diet and cancer focused on dietary inhibitors of nitrosamine formation (241–244), dietary inhibitors and activators of carcinogen-metabolizing enzymes (245), dietary antagonists of ultimate carcinogens (246–248), and effects of dietary factors on the *in vivo* metabolism of foreign compounds in humans by Phase I and Phase II reactions (128–133). More recently, we have studied the effects of curcumin, caffeine, EGCG, and tea on carcinogenesis in experimental animals, and these studies are described below.

Studies with Curcumin

Inhibitory Effect of Curcumin and Some of Its Analogues on Tumor Promotion by TPA. Curcumin is the major yellow pigment in turmeric, curry, and mustard, and it is obtained from the rhizome of the plant *Curcuma longa* Linn. The ground dried rhizome of this plant (turmeric) has been used for centuries for the treatment of inflammatory diseases (249, 250), and curcumin is currently in wide use as a spice, food preservative, and yellow coloring agent for foods, drugs, and cosmetics.

Several studies indicated that compounds that possess antioxidant or anti-inflammatory activity inhibit TPA-induced tumor promotion in mouse skin. Because curcumin was reported to possess both antioxidant and anti-inflammatory activity (251–256), we studied the effect of topical applications of curcumin on TPA-induced tumor promotion on mouse skin, and we also studied the effect of curcumin as a

potential inhibitor of tumor initiation by polycyclic aromatic hydrocarbons. Studies in our laboratory showed that topical application of curcumin inhibited TPA-induced tumor promotion as well as BP- and DMBA-induced tumor initiation on mouse skin (257, 258). These were the first studies to demonstrate an inhibitory effect of curcumin on carcinogenesis. In additional studies, we also found that topical application of curcumin inhibited TPA- or arachidonic acid-induced inflammation (mouse ear edema), as well as TPA-induced increases in hydrogen peroxide formation, ornithine decarboxylase activity, the synthesis of mRNA for ornithine decarboxylase, DNA synthesis, and hyperplasia in mouse skin (257, 259–261).

We examined the potential inhibitory effects of topical applications of several structural analogues of curcumin on TPA-induced tumor promotion in mouse skin. The structures of the compounds tested and the results obtained are shown in Fig. 5. Among the compounds tested, demethoxycurcumin (~17% of commercial grade curcumin) and bisdemethoxycurcumin (~3% of commercial grade curcumin) are naturally occurring substances present in the rhizome of *Curcuma longa* Linn and in turmeric. Caffeic acid phenethyl ester is a constituent of the propolis of honeybee hives, and caffeic acid, ferulic acid, and chlorogenic acid are widespread constituents of fruits and vegetables. Chlorogenic acid accounts for 6–8% of the dry weight of the coffee bean (262). The results of our studies indicate that curcumin, demethoxycurcumin, and caffeic acid phenethyl ester are highly active inhibitors of TPA-induced tumor promotion on mouse skin (Fig. 5; Refs. 257, 261, 263, 264). Bisdemethoxycurcumin, tetrahydrocurcumin, caffeic acid, ferulic acid, and chlorogenic acid were less active than curcumin (257, 263), and bisdemethylcurcumin (a dicatechol synthesized by Dr. Toshihiko Osawa at Nagoya University, Nagoya, Japan) was inactive (Fig. 5). Commercial grade curcumin had the same inhibitory effect as pure curcumin on TPA-induced tumor promotion (263).

Examination of antioxidant activities for several of the curcumin derivatives that were tested as potential inhibitors of tumor promotion indicated that the antioxidant activities of these compounds when

Table 5 Inhibitory effect of dietary curcumin on azoxymethane (AOM)-induced colon carcinogenesis in mice

CF-1 female mice were injected s.c. with AOM (10 mg/kg body weight) once weekly for 6 weeks. The mice were fed AIN 76A diet or curcumin in AIN 76A diet for 2 weeks before the first AOM administration and until the end of the experiment. The mice were killed 27 weeks after the last dose of AOM, and colon tumors were counted. Data are expressed as the mean \pm SE from 34–56 mice per group. (Taken from Ref. 270.)

Dietary inhibitor	Adenomas per mouse	Adenocarcinomas per mouse	Tumor volume per tumor (mm ³)	Tumor volume per mouse (mm ³)
Control diet	4.91 \pm 0.56	0.72 \pm 0.15	11 \pm 2	64 \pm 14
0.5% Curcumin	2.47 \pm 0.49	0.31 \pm 0.11	6 \pm 1	19 \pm 5
2.0% Curcumin	2.15 \pm 0.48	0 \pm 0	3 \pm 1	7 \pm 2

evaluated *in vitro* did not parallel their activities as inhibitors of TPA-induced tumor promotion *in vivo*. The *in vitro* studies indicated that curcumin, demethoxycurcumin, and bisdemethoxycurcumin were equally potent inhibitors of iron-stimulated lipid peroxidation in rat brain homogenate and rat liver microsomes (265). In other studies, the relative activities of curcumin derivatives as *in vitro* inhibitors of lipid peroxidation were tetrahydrocurcumin > curcumin > demethoxycurcumin > bisdemethoxycurcumin (266–268). In addition, bisdemethylcurcumin (a dicatchol) was found to have potent antioxidant activity *in vitro* (studies by T. Osawa and his colleagues). It was apparent from these studies that the *in vitro* antioxidant activities of several curcumin derivatives did not predict their activities as inhibitors of TPA-induced tumor promotion. Although *in vitro* studies indicated that bisdemethylcurcumin and tetrahydrocurcumin were more potent antioxidants than curcumin, it was found that tetrahydrocurcumin was less active than curcumin as an inhibitor of TPA-induced tumor promotion *in vivo*, and bisdemethylcurcumin was inactive (Fig. 5).

Studies with potential inhibitors of carcinogenesis in the TPA-induced tumor promotion model on mouse skin may be useful in predicting the cancer chemopreventive potential of agents in a variety of epithelial tissues such as the gastrointestinal tract, esophagus, oral mucosa, lung, breast, prostate, and bladder. Absorption, distribution, and pharmacokinetic studies after oral administration will help in the selection of compounds for additional study.

Inhibitory Effects of Dietary Curcumin on Colon, Duodenal, Stomach, Esophageal, and Oral Carcinogenesis. In 1992–1994, we reported that 0.5–2% dietary curcumin inhibited the formation of azoxymethane-induced focal areas of colonic dysplasia and colon tumors, *N*-ethyl-*N'*-nitrosoguanidine-induced duodenal tumors and BP-induced forestomach tumors in mice (269, 270), and Tanaka *et al.* (271) reported an inhibitory effect of 0.05% dietary curcumin on 4-nitroquinoline-1-oxide-induced carcinogenesis of the tongue in rats. Dietary curcumin was also shown to inhibit chemically induced colon cancer in rats (272, 273), esophageal carcinogenesis in rats (274), glandular stomach carcinogenesis in rats (275), and spontaneous intestinal tumors in the Min/+ mouse (276, 277). The inhibitory effect of dietary curcumin on the formation of azoxymethane-induced colon tumors was associated with enhanced apoptosis in the tumors (278). Application of curcumin three times a week to the buccal pouch of DMBA-initiated Syrian golden hamsters inhibited oral carcinogenesis, and greater effects were observed when topical applications of curcumin were combined with p.o. administered green tea (279). In an additional study, p.o. administered or topically applied curcumin or turmeric together with DMBA inhibited DMBA-induced oral cancer in Syrian hamsters (280).

In our studies, feeding 0.5% or 2% curcumin in the diet to CF-1 mice inhibited the number of azoxymethane-induced colon adenomas per mouse by 50% and 56%, respectively, and the number of colon adenocarcinomas per mouse was inhibited by 57% and 100%, respectively (Table 5). Feeding curcumin also had a marked inhibitory effect on the size of the tumors (Table 5). An inhibitory effect of curcumin on colon carcinogenesis was observed when the compound was fed

either during the initiation or postinitiation phase of azoxymethane-induced colon carcinogenesis (270).

Although p.o. administered curcumin has poor bioavailability, and only low or nonmeasurable blood levels were observed (277), this route of administration inhibits chemically induced skin and liver carcinogenesis (281, 282). Oral administration of curcumin also inhibits the initiation of radiation-induced mammary and pituitary tumors (283–285), as well as diethylstilbesterol-induced tumor promotion in the mammary glands of rats initiated with radiation (286). In the later study, tetrahydrocurcumin was observed in the serum. It is likely that biologically active metabolites of curcumin, such as tetrahydrocurcumin, are responsible for the systemic chemopreventive activity observed.

Inhibitory Effect of Curcumin on Arachidonic Acid Metabolism. Several inhibitors of arachidonic acid metabolism inhibit TPA-induced inflammation and tumor promotion, and it is thought that arachidonic acid metabolites are important for TPA-induced inflammation and tumor promotion in mouse skin. Because of the possibility that curcumin might inhibit carcinogenesis in mouse skin by inhibiting arachidonic acid metabolism, we evaluated the effect of curcumin on the metabolism and action of arachidonic acid in the epidermis. We found that topical application of curcumin inhibited arachidonic acid-induced ear inflammation in mice (257, 287). Studies on the metabolism of arachidonic acid by mouse epidermis revealed that curcumin (10 μ M) inhibited the epidermal metabolism of arachidonic acid to 5- and 8-hydroxyicosatetraenoic acid by 60% and 51%, respectively (lipoxygenase pathway), and the metabolism of arachidonic acid to PGE₂, PGF_{2 α} , and PGD₂ was inhibited 70%, 64%, and 73%, respectively (cyclooxygenase pathway) (Ref. 287). In another study, dietary administration of 0.2% curcumin to rats inhibited azoxymethane-induced colon carcinogenesis, and decreased colonic and tumor phospholipase A₂, phospholipase C γ 1, and PGE₂ levels (272). In this study, dietary curcumin also decreased enzyme activity in colonic mucosa and tumors for the formation of PGE₂, PGF_{2 α} , PGD₂, 6-keto PGF_{1 α} , and thromboxane B₂ via the cyclooxygenase system, and production of 5(S)-, 8(S)-, 12(S)-, and 15(S)-hydroxyicosatetraenoic acid via the lipoxygenase pathway of arachidonic acid metabolism was also inhibited. More recent studies have shown that curcumin is a strong inhibitor of cyclooxygenase 2 activity as well as an inhibitor of cyclooxygenase 2 mRNA formation (288, 289).

Synergistic Effects of Curcumin on All-trans Retinoic Acid- and 1 α ,25-Dihydroxyvitamin D₃-Induced Differentiation. Suh *et al.* (290) suggested the use of HL-60 cell differentiation as a bioassay that is useful for the discovery of novel cancer chemopreventive agents in natural products. We considered the possibility that some of the cancer chemopreventive effects of curcumin may be related to an effect of this compound on cellular differentiation, and we investigated the effect of curcumin on differentiation in the human promyelocytic HL-60 leukemia cell model system. We also investigated the effects of combinations of curcumin together with 1 α ,25-dihydroxyvitamin D₃ or all-*trans* retinoic acid on differentiation in HL-60 cells. Treatment of HL-60 cells with 10 μ M curcumin for 48 h resulted in small increases in differentiation as measured by the proportion of

Table 6 Effect of curcumin and all-trans retinoic acid on the differentiation of HL-60 cells as measured by nitroblue tetrazolium reduction and Mac-1 expression

HL-60 cells were treated with curcumin (CUR) and all-trans retinoic acid (RA) for 48 h, and nitroblue tetrazolium (NBT)-positive or Mac-1-positive cells were measured. The numbers in parentheses were corrected for background values from control samples. Each value represents the average of three experiments. (Taken from Ref. 291.)

Treatment	% NBT-positive cells	% Mac-1-positive cells
Control	3.0	5.6
CUR 1 μM	4.6 (1.6)	8.6 (3.0)
CUR 10 μM	13.9 (10.9)	16.5 (10.9)
RA 10 nM	12.9 (9.9)	16.0 (10.4)
RA 100 nM	20.6 (17.6)	17.4 (11.8)
RA 10 nM + CUR 10 μM	79.4 (76.4)	64.7 (59.1)
RA 100 nM + CUR 1 μM	47.1 (44.1)	34.8 (29.2)
RA 100 nM + CUR 10 μM	76.3 (73.3)	71.9 (66.3)

Table 7 Effect of curcumin and 1 α ,25-dihydroxyvitamin D₃ on the differentiation of HL-60 cells as measured by nitroblue tetrazolium reduction and Mac-1 expression

HL-60 cells were treated with curcumin (CUR) and 1 α ,25-dihydroxyvitamin D₃ (VD₃) for 48 h, and nitroblue tetrazolium (NBT)-positive or Mac-1-positive cells were measured. The numbers in parentheses were corrected for background values from control samples. Each value represents the average of three experiments. (Taken from Ref. 291.)

Treatment	% NBT-positive cells	% Mac-1-positive cells
Control	7.8	5.5
CUR 1 μM	12.6 (4.8)	9.8 (4.3)
CUR 10 μM	17.8 (10.0)	16.1 (10.6)
VD ₃ 100 nM	19.1 (11.3)	33.0 (27.5)
VD ₃ 100 nM + CUR 1 μM	34.2 (26.4)	53.9 (48.4)
VD ₃ 100 nM + CUR 10 μM	54.0 (46.2)	74.8 (69.3)

cells that reduced nitroblue tetrazolium and expressed Mac 1 (Tables 6 and 7; Ref. 291). Synergistic induction of differentiation as measured by the above markers was observed when 1–10 μM curcumin was combined with 10–100 nM all-trans retinoic acid or with 100 nM 1 α ,25-dihydroxyvitamin D₃ (Tables 6 and 7; Ref. 291). Combinations of all-trans retinoic acid and curcumin stimulated differentiation predominantly to granulocytes, whereas combinations of 1 α ,25-dihydroxyvitamin D₃ and curcumin stimulated differentiation predominantly to monocytes (291). Independent studies by Sokolowski *et al.* (292) have also shown a synergistic effect of curcumin and 1 α ,25-dihydroxyvitamin D₃ on differentiation in HL-60 cells, and they suggest the importance of NF- κ B inhibition for this effect. In additional studies, Amir *et al.* (293) have shown a synergistic effect of lycopene in combination with 1 α ,25-dihydroxyvitamin D₃ on differentiation in HL-60 cells. It is possible that many dietary chemicals such as curcuminoids, carotenoids, and other substances in fruits, vegetables, and other edible plants prevent human cancer in part by synergizing with endogenously produced stimulators of differentiation such as all-trans retinoic acid, 1 α ,25-dihydroxyvitamin D₃, and butyrate. More research is needed to test this hypothesis.

Translational Studies with Curcumin. The results of our studies and those by others indicating an inhibitory effect of curcumin on oral, skin, stomach, esophageal, and colon carcinogenesis in experimental animals suggest that curcumin may be a useful cancer chemopreventive agent in high cancer risk patients with actinic keratoses, oral leukoplakia, Barrett's esophagus, colon polyps, or precancerous lesions in the stomach, as well as in individuals who have had a colon cancer or skin cancer removed.

Studies with Tea, Caffeine, and EGCG

Stimulatory Effect of Tea Administration on the Liver Microsomal Metabolism of Foreign Compounds. Oral administration of green tea or black tea to rats has a selective inducing effect on the level of certain liver microsomal CYP enzymes (294–296), and the administration of a green tea polyphenol fraction increases the activity of certain Phase II enzymes (297). Studies by Sohn *et al.* (294)

indicated that oral administration of green tea or black tea to rats for 6 weeks stimulated by several-fold the activity of methoxyresorufin dealkylase (marker of CYP1A2) in liver microsomes. The dealkylation of ethoxyresorufin (marker of CYP1A1) and pentoxyresorufin (marker of CYP2B1) was increased to a much smaller extent, and the demethylation of N-nitrosodimethylamine (marker of CYP2E1) and erythromycin (marker of CYP3A4) was not affected. In the study by Sohn *et al.* (294), the administration of tea caused only a small increase in UDP-glucuronosyltransferase activity, and there was no effect on GST activity. In an additional study, it was shown that oral administration of green tea, black tea, or caffeine to rats increased the level of methoxyresorufin dealkylase activity and the level of CYP1A2 in liver microsomes, but the decaffeinated teas were inactive (296). This study indicated that caffeine was the major tea constituent responsible for the induction of methoxyresorufin dealkylase activity and for the elevated level of CYP1A2 (296). Because caffeine 3-demethylation is catalyzed predominantly by CYP1A2 (298), it is likely that caffeine administration enhances its own metabolism *in vivo*. The possibility that caffeine induces its own metabolism may explain why heavy coffee drinkers are relatively resistant to the effects of caffeine (299–301) and why restriction of caffeine intake in heavy coffee drinkers for 2 weeks decreased the elimination rate constant for caffeine (302). Additional studies are needed to determine more fully the effects of tea and coffee administration on the metabolism of foreign compounds and endogenous substrates in humans.

Inhibitory Effects of Tea and Tea Constituents on the Mutagenic and Carcinogenic Actions of Chemical Carcinogens. In early *in vitro* studies, we found that ellagic acid and other plant polyphenols

Table 8 Inhibitory effects of oral administration of green and black tea on the formation of UVB-induced keratoacanthomas and squamous cell carcinomas in DMBA-initiated SKH-1 mice

Female SKH-1 mice (30/group) were treated topically with 200 nmol of DMBA. One week later the mice were treated with gradually increasing concentrations of tea leaf extracts as drinking fluid for 6 days and full-strength teas (1.25 g tea leaves/100 ml hot water; ~4 mg tea solids/ml) for an additional 8 days before and during treatment with UVB (30 mJ/cm²) twice weekly for 31 weeks. (Taken from Ref. 317.)

Treatment	Tea solids (mg/ml)	% mice with keratoacanthomas	No. of keratoacanthomas per mouse	% mice with carcinomas	No. of carcinomas per mouse
Water control		97	6.33	33	0.60
Green tea	4.0	43	1.37	7	0.07
Black tea	4.4	35	1.35	4	0.04
Decaf. green tea	3.6	77	1.90	17	0.17
Decaf. black tea	3.9	67	1.73	17	0.20

Table 9 Effect of oral administration of green tea, decaffeinated green tea, or caffeine on UVB-induced complete carcinogenesis

In experiment 1, female SKH-1 mice were treated with UVB (30 mJ/cm²) twice weekly for 40 weeks, and the animals were killed 4 weeks later. Water, tea leaf extracts (1.25 gm tea leaf/100 ml hot water; ~4 mg tea solids/ml), and caffeine (0.36 mg/ml) were administered as the drinking fluid until the animals were sacrificed. Each value is from 24–30 mice. In experiment 2, female SKH-1 mice were treated with UVB (30 mJ/cm²) twice weekly for 45 weeks. Lyophilized teas (9 mg tea solids/ml) were administered as the drinking fluid. Each value is from 27–30 mice.

Experiment	Treatment	Tea solids (mg/ml)	Number of keratoacanthomas per mouse	Number of carcinomas per mouse
1	Water	—	5.75 ± 1.04	1.58 ± 0.32
	Green tea	4.0	2.21 ± 0.46 ^a	0.86 ± 0.23 ^b
	Decaf. green tea	3.6	4.58 ± 0.64	1.66 ± 0.30
	Caffeine	0.36	1.81 ± 0.44 ^a	1.00 ± 0.20 ^c
2	Decaf. green tea + caffeine	4.0	2.53 ± 0.43 ^a	0.57 ± 0.12 ^a
	Water	—	0.89 ± 0.24	0.93 ± 0.28
	Green tea	9.0	0.29 ± 0.09 ^a	0.19 ± 0.09 ^a
	Decaf. green tea	9.0	2.37 ± 0.46 ^a	1.44 ± 0.24 ^c

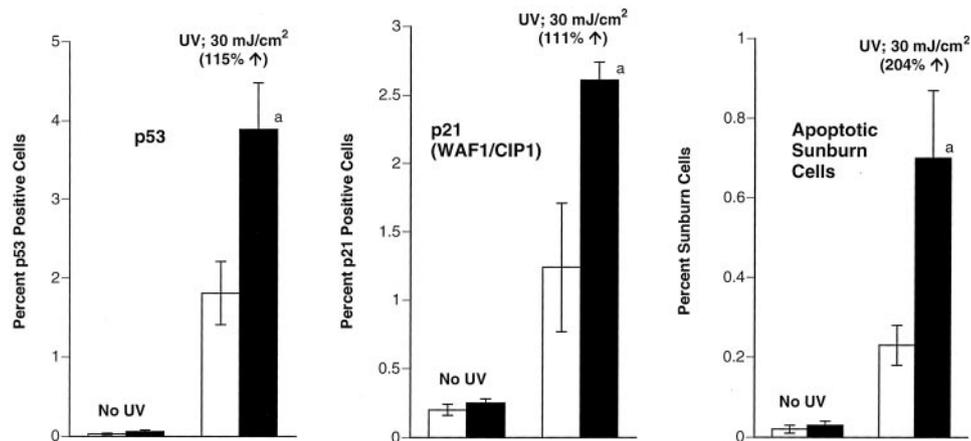
Values ± SE that are statistically different from the corresponding water group are indicated. (Taken from Ref. 318.)

^a P < 0.01.

^b P < 0.05.

^c P < 0.10.

Fig. 6. Effect of oral administration of green tea on UVB-induced increases in p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells in the epidermis of mice. Female SKH-1 mice were treated with water (□) or 0.6% green tea (6 mg of tea solids/ml; ■) as their sole source of drinking fluid for 2 weeks. The mice were then treated with UVB (30 mJ/cm²) and were killed 10 h later. Each value represents the mean ± SE from five mice. ^aStatistically different from the water group treated with UVB (*P* < 0.05). (Taken from Ref. 319.)



such as tannic acid and hydroxylated flavonoids in tea were potent inhibitors of the mutagenic activity of the bay-region diol epoxide that is an ultimate carcinogenic metabolite of BP (246–248). The mechanism of inhibition was formation of an ether adduct between the diol epoxide and the plant phenol (303). *In vivo* studies indicated an inhibitory effect of these plant phenols on the carcinogenic action of the diol epoxide in mice (304, 305), but the plant phenols were only weakly active or inactive when tested for inhibitory effects on the tumorigenic activity of BP (304). Additional studies showed a strong inhibitory effect of green tea, black tea, or certain polyphenolic tea catechins on the mutagenicity of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (306). Although the inhibitory effect of tea polyphenols on cytochrome P-450-dependent metabolism of carcinogens is well known, the use of tea or tea constituents as inhibitors of the action of ultimate carcinogens is a relatively unexplored area.

In a key study, Yoshizawa *et al.* (307) showed an inhibitory effect of topical applications of EGCG (a major catechin in green and black tea) on tumor promotion by teleocidin on mouse skin initiated previously with DMBA, and Fujita *et al.* (308) showed an inhibitory effect of oral administration of EGCG on *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine-induced duodenal carcinogenesis. These studies stimulated great interest worldwide on the potential cancer chemopreventive effects of tea and tea constituents. Tea and some of its constituents have a broad spectrum of cancer chemopreventive activity that has been observed by many investigators in several organ systems in experimental animals (reviewed in Refs. 309–312). Oral administration of green tea, black tea, EGCG, or a green tea polyphenol fraction to rodents has been reported to inhibit chemically-induced carcinogenesis in many organs including esophagus, forestomach, glandular stomach, duodenum/small intestine, colon, lung, liver, and pancreas. Oral administration of EGCG was also shown to inhibit the formation of spontaneous liver cancer in mice (313), and administration of a green tea polyphenol fraction inhibited the formation of spontaneous prostate cancer in mice (314).

Inhibitory Effects of Tea and Caffeine on UVB-Induced Carcinogenesis. In early studies, Wang *et al.* (315) reported an inhibitory effect of oral administration of a green tea polyphenol fraction⁶ on UVB-induced tumorigenesis. In subsequent studies and with histological characterization of tumors, we found that oral administration of green tea, black tea, or the decaffeinated teas to DMBA-initiated SKH-1 mice inhibited UVB-induced formation of keratoacanthomas and squamous cell carcinomas (Table 8; Refs. 316, 317). In these studies, administration of the regular or decaffeinated teas decreased the percentage of mice with

tumors and the number of tumors per mouse (Table 8). In addition, tumors from DMBA-initiated mice treated with UVB and tea were smaller than tumors from initiated mice treated only with UVB (317). In other experiments with a complete carcinogenesis model (chronic UVB treatment without DMBA initiation), oral administration of green tea inhibited UVB-induced tumor formation, but decaffeinated green tea was either inactive (at moderate dose levels) or actually enhanced the tumorigenic effects of UVB (at a high dose level; Table 9; Ref. 318). Oral administration of caffeine had a strong inhibitory effect on UVB-induced complete carcinogenesis, and adding caffeine to decaffeinated green tea restored its inhibitory activity (Table 9). These results indicated that caffeine is an important constituent of tea that is responsible for its inhibitory effect on UVB-induced complete carcinogenesis.

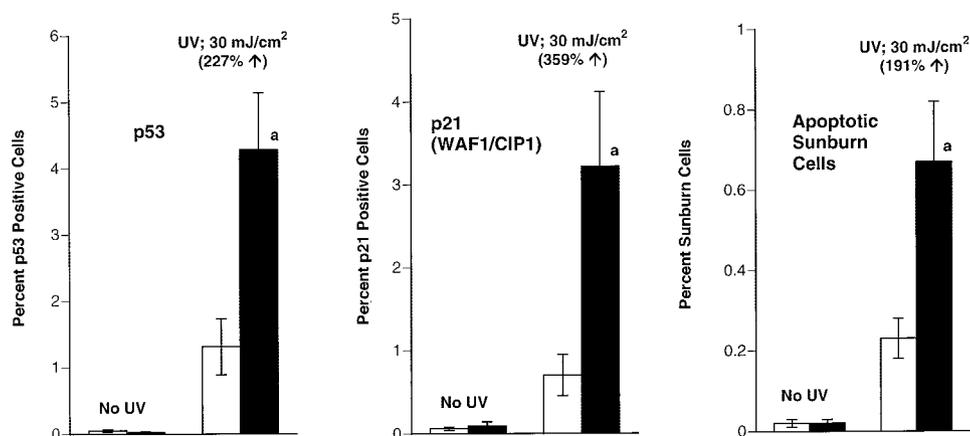
Stimulatory Effect of Tea and Caffeine on UVB-Induced Increases in Epidermal p53, p21(WAF1/CIP1), and Apoptotic Sunburn Cells in SKH-1 Mice. Treatment of mice with UVB causes an adaptive increase in epidermal wild-type p53, p21(WAF1/CIP1), and apoptosis, which results in the death of cells with too much DNA damage to be adequately repaired. This response to UVB was magnified in animals pretreated with green tea or caffeine (319). Although treatment of mice with green tea or caffeine in the absence of UVB did not influence epidermal wild-type p53, p21(WAF1/CIP1), or apoptosis, oral administration of 0.6% green tea (6 mg tea solids/ml), or caffeine (0.44 mg/ml; concentration of caffeine that is present in 0.6% green tea) as the sole source of drinking fluid for 2 weeks before a single application of 30 mJ/cm² of UVB enhanced the UVB-induced increase in the number of epidermal p53-positive cells, enhanced the UVB-induced increase in the number of epidermal p21(WAF1/CIP1)-positive cells, and enhanced the UVB-induced increase in the number of apoptotic sunburn cells at 10 h after exposure to UVB (Figs. 6 and 7; Ref. 319).

Although pretreatment of mice with p.o. administered green tea or caffeine for 2 weeks enhanced UVB-induced increases in apoptosis in the epidermis, these treatments had little or no effect on UVB-induced increases in bromodeoxyuridine incorporation into epidermal DNA or on UVB-induced increases in the mitotic index (319).

A single topical application of caffeine (1.2 mg/100 μ l acetone) immediately after exposure of SKH-1 mice to UVB (30 mJ/cm²) enhanced the UVB-induced increase in apoptosis at 6 h by 100%, and topical applications of caffeine immediately after exposure to UVB, and 30 and 120 min later enhanced UVB-induced increases in apoptotic sunburn cells at 6 h by 162% (320), but topical application of caffeine in the absence of UVB treatment did not increase apoptosis (320). In contrast to our studies with caffeine, topical application of EGCG immediately after UVB did not enhance UVB-induced apo-

⁶ The polyphenol fraction also contained caffeine (personal communication from Z. Y. Wang).

Fig. 7. Effect of oral administration of caffeine on UVB-induced increases in p53-positive cells, p21 (WAF1/CIP1)-positive cells, and apoptotic sunburn cells in the epidermis of mice. Female SKH-1 mice were treated with water (□) or caffeine (0.44 mg/ml; ■) as their sole source of drinking fluid for 2 weeks. The mice were then treated with UVB (30 mJ/cm²) and were killed 10 h later. Each value represents the mean ± SE from five mice. ^aStatistically different from the water group treated with UVB (*P* < 0.05). (Taken from Ref. 319.)



ptosis (320). Although homozygous p53 knockout mice have a poor apoptotic response to UVB (321), topical application of caffeine immediately after exposure of these mice to UVB resulted in increased epidermal apoptosis indicating that caffeine can stimulate a UVB-induced p53-independent apoptotic pathway.⁷

Inhibitory Effects of Oral Administration of Tea and Caffeine on Carcinogenesis in Mice Treated Previously with UVB (Initiated High-Risk Mice): Relationship to Decreased Tissue Fat. In another series of studies, we exposed SKH-1 mice to UVB (30 mJ/cm²) twice a week for 20–22 weeks, and then UVB irradiation was stopped. The mice were tumor-free, but they had epidermal hyperplasia and a high risk of developing skin tumors during the next several months in the absence of additional treatment with UVB (“high-risk mice”; Ref. 322). This is a useful animal model that may be comparable with humans exposed previously to moderate/high levels of sunlight during childhood who have a high risk of developing skin cancers later in life even in the absence of continued heavy sunlight exposure. We found that oral administration of green tea or black tea to these initiated high-risk mice inhibited the formation of malignant and nonmalignant tumors, and the tumors that were observed were smaller in size than those observed in mice that were not treated with tea (Table 10; Ref. 322).

In our studies, we found that treatment of high-risk mice with the regular teas, the decaffeinated teas plus caffeine, or only caffeine decreased the size of the parametrial fat pads and the thickness of the dermal fat layer. Oral administration of green tea or black tea (6 mg tea solids/ml) for 23 weeks to high-risk mice in the absence of continued treatment with UVB had no effect on body weight but decreased the number of tumors per mouse by 66–68%, the size of the parametrial fat pads by 32–54%, the thickness of the dermal fat layer away from tumors by 39–46%, and the thickness of the dermal fat layer directly under tumors by 49–53% (Table 11; Ref. 323). Administration of the decaffeinated teas had little or no effect on any of these parameters, and adding caffeine (equivalent to the amount in the regular teas) to the decaffeinated teas restored their inhibitory effects (Table 11; Ref. 323). Administration of caffeine alone (0.44 mg/ml) did not effect body weight but decreased the number of tumors per mouse by 61%, the size of the parametrial fat pads by 56%, the thickness of the dermal fat layer away from tumors by 40%, and the thickness of the dermal fat layer directly under tumors by 49% (Table 11; Ref. 323). A representative illustration of the effect of p.o. administered green tea on the thickness of the dermal fat layer away from tumors and under tumors is shown in Fig. 8. Although only the

regular teas, caffeine, or the decaffeinated teas plus caffeine decreased tumor multiplicity in high-risk mice (Table 11), tumor size (per tumor) was decreased by administration of the regular teas, the decaffeinated teas, or caffeine.

An additional conclusion that can be made from Table 11 is that the dermal fat layer is much thinner under tumors than away from tumors in all of the experimental groups. For instance, in high-risk mice given only water as their drinking fluid for 23 weeks, the average thickness of the dermal fat layer away from tumors was 162 μ m but was only 60 μ m directly under tumors (Table 11). In high-risk mice given 0.6% green tea for 23 weeks, the average thickness of the dermal fat layer away from tumors was 100 μ m but was only 28 μ m directly under tumors (Table 11). These results suggest that tumors may be using near-by dermal fat as a source of energy and/or that tumors may be secreting agents that enhance the breakdown of dermal fat.

When the 179 mice with skin nodules described in Table 11 were analyzed histologically for tumors, 152 of these mice had a total of 689 tumors, and 27 mice had no tumors. The relationship between the thickness of the dermal fat layer away from tumors (possible surrogate for total body fat levels) in individual mice and the number of tumors per mouse in all 179 mice is shown in Table 12. Fourteen mice with a very thin dermal fat layer (≤ 50 μ m) away from tumors had an average of only 1.6 ± 0.7 tumors/mouse whereas 7 mice with a thick dermal fat layer (>250 μ m) away from tumors had 7.4 ± 1.8 tumors/mouse. Regression analysis was performed with data from all 179 mice to assess the relationship between the thickness of the dermal fat layer away from tumors for each mouse and the number of tumors per mouse. There was a highly significant positive linear association between the number of tumors per mouse and the thickness of the dermal fat layer away from tumors (*P* = 0.0001; Table 12; Ref. 323).

The relationship between the thickness of the dermal fat layer away from tumors and the number of tumors per mouse in animals that received only noncaffeinated beverages (water and decaffeinated teas; 72 mice) was also evaluated. For mice ingesting only noncaffeinated beverages, there was still a highly significant positive linear association between the number of tumors per mouse and the dermal fat thickness away from tumors (*P* = 0.009; Ref. 323).

Although regression analysis of data from all 179 mice examined histologically indicated a statistically significant relationship between the thickness of the dermal fat layer (away from tumors) and average tumor size per mouse (*P* = 0.034), this relationship was much weaker than the relationship between the thickness of the dermal fat layer and the number of tumors per mouse (Table 12).

⁷ Y-P. Lu, Y-R. Lou, X-H. Li, J-G. Xie, and A. H. Conney, unpublished observations.

Table 10 Effect of oral administration of green or black tea on tumor formation in mice treated previously with UVB (initiated high-risk mice)

Female SKH-1 mice were treated with UVB (30 mJ/cm²) twice a week for 22 weeks. One week later, these initiated mice (30/group) with no observable tumors were given green or black tea (6 mg tea solids/ml) as their sole source of drinking fluid for 23 weeks. Each value is the mean ± SE. (Taken from Ref. 322.)

Treatment	Keratoacanthomas			Squamous cell carcinomas		
	Percentage of mice with tumors	Tumors per mouse	Volume per tumor (mm ³)	Percentage of mice with tumors	Tumors per mouse	Volume per tumor (mm ³)
Water	79	4.00 ± 0.65	2.8 ± 0.5	61	1.82 ± 0.41	40 ± 20
Green tea	46	1.25 ± 0.34	0.5 ± 0.1	39	0.68 ± 0.22	13 ± 4
Black tea	56	1.26 ± 0.38	0.4 ± 0.1	44	0.70 ± 0.19	13 ± 7

Effects of Topical Applications of Caffeine or EGCG on Carcinogenesis and Apoptosis in UVB-Pretreated High-Risk Mice.

SKH-1 hairless mice were irradiated with UVB twice weekly for 20 weeks, and treatment with UVB was stopped. These tumor-free mice that had a high risk of developing skin tumors during the next several months were then treated topically with caffeine (6.2 μmol) or EGCG (6.5 μmol) once daily 5 days a week for 18 weeks in the absence of additional treatment with UVB. Topical applications of caffeine to these mice decreased the number of nonmalignant and malignant skin tumors per mouse by 44 and 72%, respectively, and topical applications of EGCG decreased the number of nonmalignant and malignant tumors per mouse by 55 and 66%, respectively (Table 13; Ref. 324). Topical applications of caffeine or EGCG not only decreased the number of tumors per mouse, but tumor size was also decreased (324). Immunohistochemical analysis showed that topical applications of caffeine or EGCG increased apoptosis as measured by the number of caspase 3-positive cells in nonmalignant skin tumors by 87 or 72%, respectively, and in squamous cell carcinomas by 92 or 56%, respectively, but there was no effect on apoptosis in nontumor areas of the epidermis (Table 14; Ref. 324). Topical applications of caffeine or EGCG had a small inhibitory effect on proliferation in nonmalignant tumors as measured by bromodeoxyuridine labeling (16–22% inhibition), and there was also a similar but nonsignificant inhibitory effect on proliferation in malignant tumors (324). In summary, topical applications of caffeine or EGCG inhibited carcinogenesis in UVB-

pretreated high-risk mice, and this treatment with EGCG or caffeine had a selective stimulatory effect on apoptosis in nonmalignant and malignant skin tumors in the absence of an apoptotic effect in nontumor areas of the epidermis. A small inhibitory effect of topical applications of EGCG or caffeine on proliferation in tumors was also observed. The results suggest a need for translational studies to determine whether topical applications of caffeine or EGCG can inhibit sunlight-induced skin cancer in humans.

EGCG increases apoptosis in various tumor cell lines, and tumor cells appear to be more sensitive to EGCG-induced apoptosis than their normal counterparts (325). Although the mechanism by which EGCG induces apoptosis in tumor cells remains to be elucidated, some proposals have already been made. These include inhibition of NF-κB (326, 327), activation of a tumor necrosis factor α-mediated signaling pathway (328), cell cycle arrest at G₀/G₁ (327, 329), or G₂-M (328), and EGCG binding to Fas, presumably on the cell surface, to trigger Fas-mediated apoptosis (330).

Several studies indicate that low millimolar concentrations of caffeine and other methylxanthines sensitize cultured cancer cells to the toxic effects of radiation or certain chemotherapeutic agents (331–335) possibly by blocking normal checkpoint control of the cell cycle and allowing replication of the caffeine-treated cells (336). It was suggested that caffeine-induced checkpoint defects may be caused by an inhibitory effect of caffeine on ATM and ATR kinase activities that are needed for the phosphorylation of p53 and other proteins that are

Table 11 Effect of oral administration of tea, decaffeinated tea, and caffeine on tumorigenesis, the size of the parametrial fat pads and the thickness of the dermal fat layer in SKH-1 mice treated previously with UVB (high-risk mice)

Female SKH-1 mice were treated with UVB (30 mJ/cm²) twice a week for 22 weeks, and UVB administration was stopped. These tumor-free initiated mice had a high-risk of developing skin tumors during the next several months, and they were treated for 23 weeks with 0.6% lyophilized green tea (GT, 6 mg tea solids/ml), black tea (BT, 6 mg tea solids/ml), or decaffeinated green or black tea (DGT or DBT, 6 mg tea solids/ml) as their sole source of drinking fluid. Other mice received 0.044% caffeine (CF, 0.44 mg/ml) or 0.6% lyophilized decaffeinated teas plus 0.044% caffeine. Tumors were classified by histological evaluation of skin samples from the 179 mice with skin nodules, and calculations of tumors/mouse included data from all 217 mice alive at the end of the experiment. The data for total tumors include papillomas, keratoacanthomas, and squamous cell carcinomas.

The combined length of the two parametrial fat pads from all 217 mice alive when the experiment was terminated was measured and graded as follows: 1 (<1 cm), 2 (1–2 cm), or 3 (>2 cm). The thickness of the dermal fat layer was measured in 5–10 representative nontumor areas of the skin (>0.5 cm away from tumors) from 19–28 mice per group (total 179 mice) using a light microscope with an ocular micrometer and 100-fold magnification. The 152 tumor-bearing mice were used for calculations of the thickness of the dermal fat layer under tumors.

The values represent the mean ± SE, and the values in parentheses represent percentage decrease compared with the drinking water positive control group. Statistical evaluation of differences from the water-positive control group was done by the Dunnett's T test. The data for total tumors includes a small number of squamous cell papillomas. (Taken from Ref. 323.)

Treatment	Number of mice	Keratoacanthomas (tumors per mouse)	Squamous cell carcinomas (tumors per mouse)	Total tumors (tumors per mouse)	Size of fat pads (relative units)	Thickness of dermal fat layer away from tumors (μm)	Thickness of dermal fat layer under tumors (μm)
Water	28	4.00 ± 0.47	1.82 ± 0.30	6.18 ± 0.71	1.41 ± 0.11	162 ± 9	60 ± 4
0.6% GT	28	1.25 ± 0.47 ^a (69)	0.68 ± 0.30 ^b (63)	2.00 ± 0.71 ^a (68)	0.64 ± 0.11 ^a (54)	100 ± 11 ^a (39)	28 ± 6 ^a (53)
0.6% BT	27	1.30 ± 0.48 ^a (68)	0.70 ± 0.31 ^c (61)	2.11 ± 0.72 ^a (66)	0.95 ± 0.11 ^b (32)	88 ± 10 ^a (46)	31 ± 6 ^a (49)
0.6% DGT	27	2.93 ± 0.48 (27)	1.85 ± 0.31 (0)	4.96 ± 0.72 (20)	1.62 ± 0.11 (0)	152 ± 10 (6)	55 ± 4 (8)
0.6% DBT	26	2.88 ± 0.49 (28)	1.58 ± 0.31 (13)	4.62 ± 0.74 (25)	1.23 ± 0.12 (13)	150 ± 10 (8)	51 ± 4 (14)
0.044% CF	27	1.70 ± 0.48 ^a (57)	0.63 ± 0.31 ^b (65)	2.41 ± 0.72 ^a (61)	0.62 ± 0.11 ^a (56)	98 ± 10 ^a (40)	31 ± 6 ^a (49)
DGT + CF	25	1.04 ± 0.50 ^a (74)	0.92 ± 0.32 (50)	2.00 ± 0.75 ^a (68)	0.61 ± 0.12 ^a (57)	105 ± 11 ^a (35)	29 ± 6 ^a (51)
DBT + CF	29	0.72 ± 0.46 ^a (82)	0.38 ± 0.30 ^a (79)	1.17 ± 0.70 ^a (81)	0.89 ± 0.11 ^a (37)	87 ± 11 ^a (47)	24 ± 7 ^a (61)

^a P < 0.01.

^b P < 0.05.

^c P < 0.1.

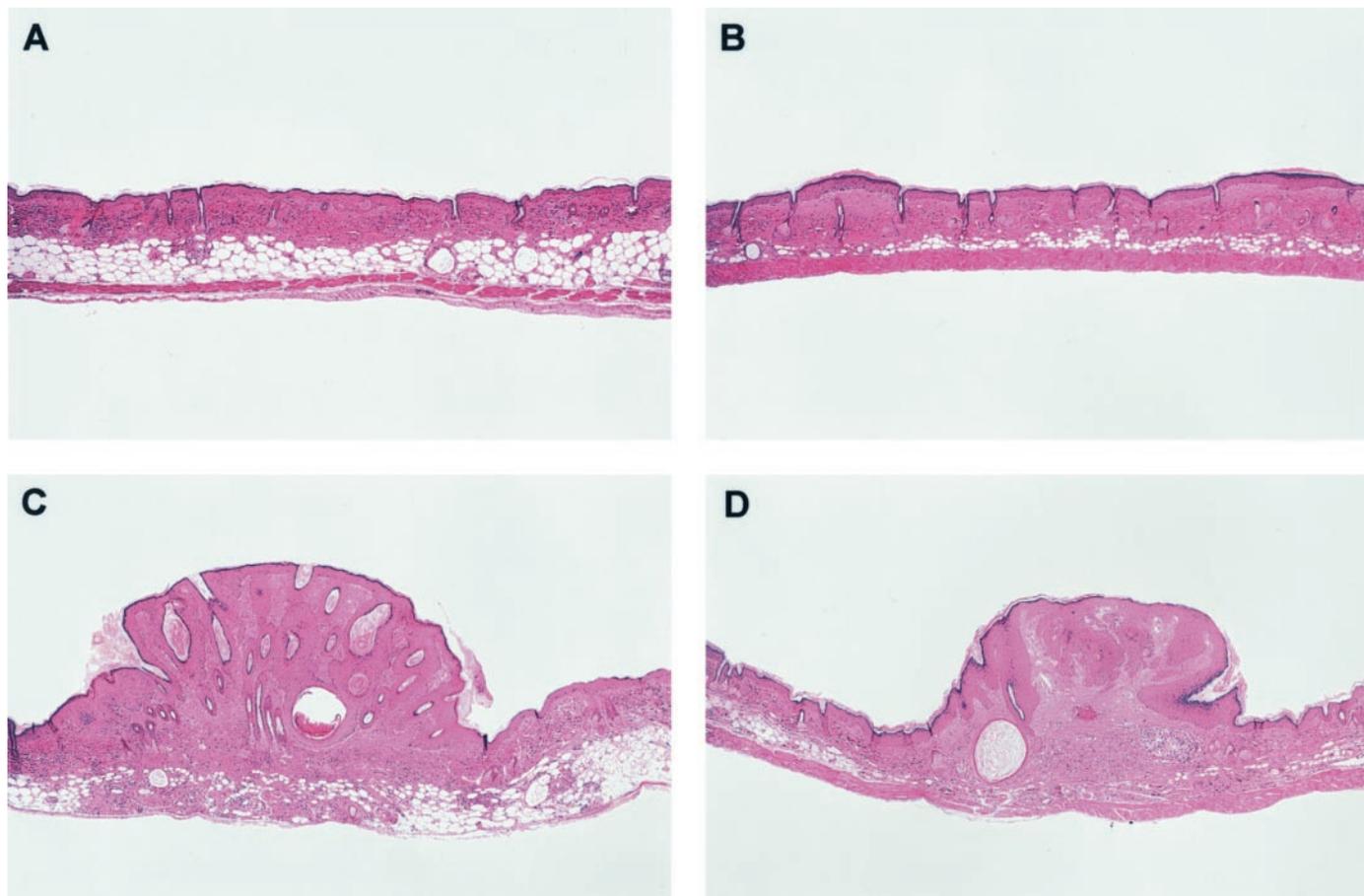


Fig. 8. Effect of oral administration of green tea to high-risk mice on the dermal fat layer (histological evaluation in representative mice). High-risk SKH-1 mice that were pretreated with UVB for 22 weeks received water (A and C) or 0.6% green tea (6 mg of tea solids/ml; B and D) as their sole source of drinking fluid for 23 weeks in the absence of additional UVB treatment. The dermal fat layer was examined histologically (100-fold magnification) in areas of the skin away from tumors (A and B) and in areas under keratoacanthomas (C and D). (Taken from Ref. 323.)

important for checkpoint control of the cell cycle (336). In another study, combinations of caffeine and radiation caused a synergistic effect on apoptosis in p53-defective cells via a p53-independent pathway (337). Recent studies by Nghiem *et al.* (338) with cultured human osteosarcoma U20S cells indicated that: (a) caffeine-induced inhibition of ATR (but not ATM) caused premature chromatin condensation and cell death; (b) that ATR (but not ATM) prevented

Table 12 Relationship between the thickness of the dermal fat layer away from tumors, the number of tumors/mouse, and the tumor size/mouse in mice receiving noncaffeinated or caffeinated beverages

UVB-pretreated high risk SKH-1 mice were given water, green tea (GT), black tea (BT), decaffeinated green tea (dGT), decaffeinated black tea (dBT), caffeine (CF), dGT + CF, or dBT + CF for 23 weeks as described in Table 11. The thickness of the dermal fat layer in areas away from tumors or in mice with no tumors was determined. The data were obtained from 28 mice in the water group, 21 mice from the GT group, 24 mice from the BT group, 22 mice from the dGT group, 22 mice from the dBT group, 22 mice from the CF group, 21 mice from the dGT + CF group, and 19 mice from the dBT + CF group (total, 179 mice). Each value represents the mean ± SE. (Taken from Ref. 323.)

Thickness of dermal fat layer away from tumors (μm)	No. of mice	No. of tumors/mouse ^a	Tumor diameter/mouse ^b (mm)
≤50	14	1.6 ± 0.7	0.70 ± 0.26
50–100	63	2.9 ± 0.4	1.18 ± 0.13
100–150	68	3.8 ± 0.6	1.26 ± 0.15
150–200	17	5.5 ± 1.0	1.51 ± 0.26
200–250	10	7.8 ± 1.4	1.56 ± 0.18
>250	7	7.4 ± 1.8	1.24 ± 0.15

^a *P* = 0.0001 (from the Pearson correlation coefficient) for the thickness of the dermal fat layer away from tumors versus the number of tumors/mouse for all 179 mice.

^b *P* = 0.034 (from the Pearson correlation coefficient) for the thickness of the dermal fat layer away from tumors versus the average tumor diameter/mouse for all 179 mice.

Table 13 Effect of topical applications of caffeine or EGCG on the formation of histologically characterized tumors in high-risk mice

High-risk UVB-pretreated SKH-1 mice (30/group) were treated topically with 100 μl acetone, caffeine (6.2 μmol) in 100 μl acetone, or EGCG (6.5 μmol) in 100 μl acetone once daily 5 days a week for 18 weeks. The numbers in parentheses represent percentage of inhibition. Each value represents the mean ± SE. (Taken from Ref. 324.)

Treatment	Nonmalignant tumors		Squamous cell carcinomas	
	Percentage of mice with tumors	Tumors per mouse	Percentage of mice with tumors	Tumors per mouse
Acetone	82	7.21 ± 1.28	64	1.18 ± 0.25
Caffeine	77 (6% ↓)	4.03 ± 0.76 ^a (44% ↓)	23 ^b (64% ↓)	0.33 ± 0.12 ^b (72% ↓)
EGCG	70 (15% ↓)	3.27 ± 0.67 ^b (55% ↓)	23 ^b (64% ↓)	0.40 ± 0.18 ^b (66% ↓)

^a *P* < 0.05.

^b *P* < 0.01.

premature chromatin condensation; and (c) that ATR prevented premature chromatin condensation via Chk-1 regulation. These investigators suggested that cancer cells with a disrupted G₁ checkpoint (such as loss of p53 function) should be sensitized to ATR inhibition and lethal premature chromatin condensation (338, 339). These investigators also pointed out that cancer cells with defective checkpoint control may be selectively affected by ATR inhibitors and suggested that ATR inhibitors that are more potent than caffeine may be useful agents for cancer therapy. The concepts developed by Nghiem *et al.* (338) on the selectivity of ATR inhibitors for p53-defective cells may help explain why caffeine has a selective apoptotic effect in UVB-

Table 14 Effect of topical applications of EGCG or caffeine on the induction of caspase 3-positive cells in tumors

High-risk mice (30 per group) were treated topically with acetone (100 μ l) or with EGCG (6.5 μ mol) or caffeine (6.2 μ mol) in 100 μ l acetone once daily 5 days a week for 18 weeks. Each value for the percentage of caspase 3 positive cells represents the mean \pm SE. (Taken from Ref. 324.)

Treatment	No. of nontumor areas or tumors examined	Percentage of caspase 3 positive cells	Percentage increase
Nontumor areas			
Control	370	0.159 \pm 0.015	—
EGCG	276	0.123 \pm 0.024	—
Caffeine	271	0.165 \pm 0.027	4
Nonmalignant tumors			
Control	202	0.229 \pm 0.017	—
EGCG	98	0.394 \pm 0.031 ^a	72
Caffeine	121	0.428 \pm 0.033 ^a	87
Carcinomas			
Control	33	0.196 \pm 0.022	—
EGCG	12	0.305 \pm 0.050 ^b	56
Caffeine	10	0.376 \pm 0.056 ^a	92

^a $P < 0.01$.

^b $P < 0.10$.

induced tumors (shown previously to have p53 mutations) but not in nontumor areas of the epidermis.

Inhibitory Effect of Tea on the Growth of Established Skin Tumors in Mice. In our initial chemoprevention studies with tea, we found that treatment of mice with green or black tea not only inhibited the formation of UVB-induced tumors, but tumor size was also decreased (316, 317). These observations suggested that administration of tea may have inhibited tumor growth. To test the possibility that tea inhibits the growth of well-established tumors, we generated papilloma-bearing animals by treatment of CD-1 mice with DMBA or UVB followed by twice weekly topical applications of TPA. After obtaining papilloma-bearing mice, TPA was stopped, and 2 weeks later we treated the tumor-bearing mice with green tea as their sole source of drinking fluid, or we injected a green tea polyphenol fraction or EGCG i.p. three times per week. The results of this study indicated that continuous oral administration of green tea, i.p. injections of a green tea polyphenol fraction three times a week, or i.p. injections of EGCG three times a week for 4–10 weeks inhibited the growth of

established skin papillomas (Figs. 9 and 10; Ref. 340). In some experiments, administration of green tea or tea constituents caused complete inhibition of tumor growth or tumor regression. Examination of data from 10 experiments done in our laboratory revealed that none of the 220 papilloma-bearing control mice given only water as their drinking fluid exhibited complete tumor regression, but complete tumor regression was observed in 14 of 346 papilloma-bearing mice (4%) that were treated with green tea in the drinking water or with i.p. injections of green tea constituents. These observations indicated that oral administration of green tea, i.p. administration of a green tea polyphenol fraction, or i.p. administration of EGCG inhibited the growth and/or caused the regression of established experimentally induced skin papillomas.

In another experiment, skin tumors were generated by treating SKH-1 mice with UVB (30 mJ/cm²) twice weekly for 22 weeks. UVB administration was stopped, and tumors were allowed to develop during the following 13 weeks. These tumor-bearing mice were then divided into two groups with an approximately equal number of tumors per mouse and with an approximately equal tumor size per mouse. One group received water, and the other group was treated with black tea (6 mg tea solids/ml) as its sole source of drinking fluid for 11 weeks. In this experiment, tumor growth as measured by an increase in tumor volume per mouse was inhibited by 70% (341). Histological examination revealed that tea-treated mice had a 58% decrease in the number of nonmalignant tumors (primarily keratoacanthomas) per mouse and a 54% decrease in the number of squamous cell carcinomas per mouse (Table 15). In addition, administration of black tea decreased the volume per tumor by 60% for nonmalignant tumors and by 84% for squamous cell carcinomas (341). Mechanistic studies with tumors from these mice revealed that administration of black tea decreased the bromodeoxyuridine labeling index in squamous cell papillomas, keratoacanthomas, and squamous cell carcinomas by 56%, 45%, and 35%, respectively (Table 16), and apoptosis (terminal deoxynucleotidyl transferase-mediated nick end labeling assay) was increased by 44%, 100%, and 95%, respectively (Table 17). Similar results were observed when apoptosis was measured by

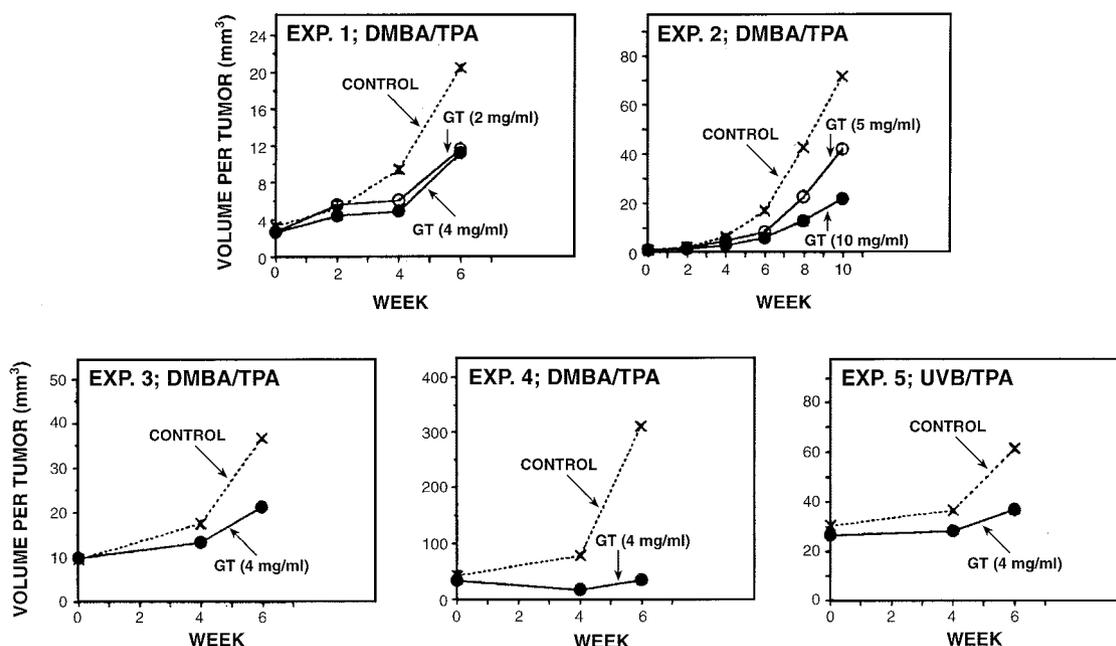


Fig. 9. Effect of oral administration of green tea on the growth and regression of skin papillomas in mice. Female CD-1 mice (6 weeks old) were initiated with DMBA or UVB and promoted with TPA as described elsewhere (340). One week after stopping TPA administration, tumor-bearing mice were treated with green tea as their sole source of drinking fluid for 6–10 weeks. The concentration of tea (expressed as mg of tea solids per ml) is indicated. (Taken from Ref. 340.)

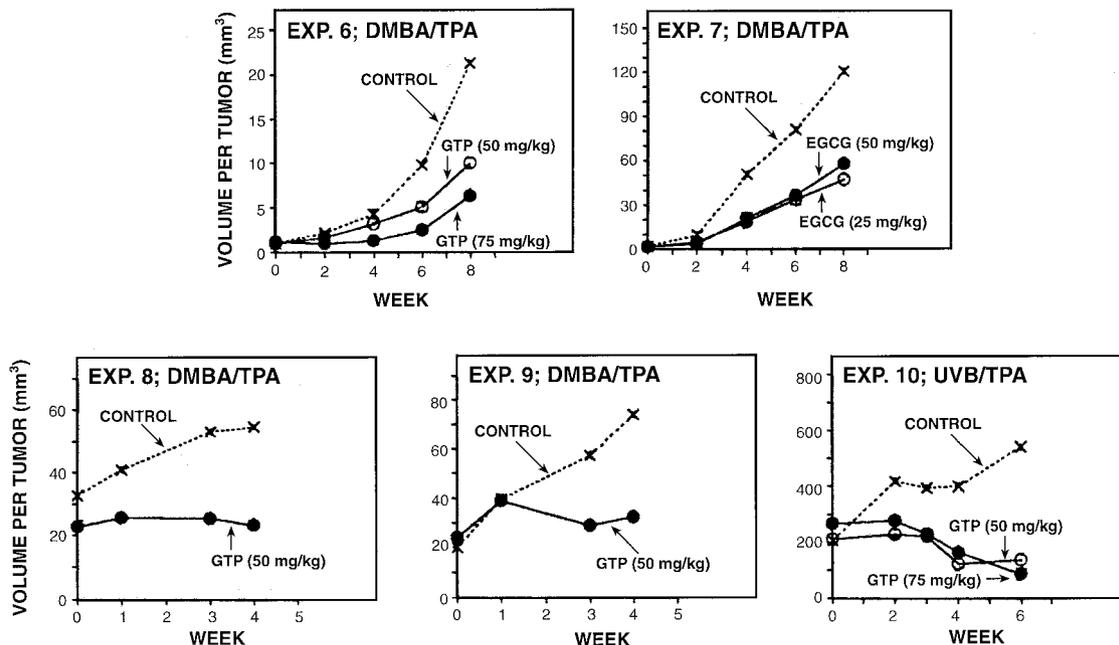


Fig. 10. Effect of i.p. administration of a green tea polyphenol fraction or EGCG on the growth and regression of skin papillomas in mice. Female CD-1 mice (6 weeks old) were initiated with DMBA or UVB and promoted with TPA as described elsewhere (340). One week after stopping TPA administration, tumor-bearing mice were treated with i.p. injections of a green tea polyphenol fraction or EGCG three times a week for 4–8 weeks. The amount of tea constituent administered for each injection is given in the figure. (Taken from Ref. 340.)

counting morphologically distinct apoptotic cells or when green tea was given instead of black tea.

In summary, the results of our studies indicate that oral administration of tea, or topical applications of EGCG or caffeine during the development and growth of skin tumors enhances apoptosis and inhibits proliferation in the tumors (324, 341). Interestingly, the results of the topical application studies indicate selective apoptotic effects of caffeine and EGCG in tumors but not in nontumor areas of the skin (Table 14; Ref. 324). As indicated earlier, a similar apoptotic effect of caffeine was observed when it was applied topically to mice immediately after a single irradiation with UVB or when caffeine was given in the drinking water for 2 weeks before UVB irradiation, but caffeine administration did not enhance apoptosis in normal nonirradiated skin (319, 320).

The possibility that EGCG could inhibit the growth of human breast or prostate tumors *in vivo* was evaluated in athymic immunodeficient mice by Liao *et al.* (342) at the University of Chicago. In these studies it was found that treatment of athymic mice with daily i.p. injections of EGCG inhibited the growth of transplanted MCF-7 human breast cancer cells and LNCaP 104R human prostate cancer cells.

Epidemiology Studies with Tea. Although animal studies have shown that administration of tea or tea components inhibits carcinogenesis in many animal models, the results of studies on the relationship between tea ingestion and cancer in humans have been inconsistent. Of seven case-control studies on the relationship between green tea consumption and stomach cancer risk, four studies in Japan, China, and Taiwan showed a significant inverse association, two studies showed a nonsignificant inverse association, and one study showed a nonsignificant positive association (reviewed in Refs. 311, 343). In a recent large population-based prospective study in Japan by Tsubono *et al.* (343), no association between green tea consumption and stomach cancer risk was observed even after correcting for the possible confounding effects of cigarette smoking. Because cigarette smokers have enhanced metabolism of caffeine (Ref. 344; an important biologically active component of tea), and cigarette smokers also have decreased serum levels of zeaxanthin, β -cryptoxanthin, α -

carotene, α -tocopherol, retinol, and retinyl palmitate (Ref. 345; potential chemopreventive substances), even matching tea drinkers and non-tea drinkers for cigarette smoking status may not correct for the confounding effects of cigarette smoking. It is important in studies that analyze the relationship between tea ingestion and cancer risk to consider the potential confounding effects of cigarette smoking.

Early studies indicated that tea was a risk factor for esophageal cancer, but this was observed only in people who drank very hot tea (reviewed in Ref. 346). In a population-based case-control study, consumption of green tea was associated with a lower risk for esophageal cancer, especially among nonsmokers and nonalcohol drinkers (347). In this study, drinking “burning hot fluids,” including tea, soups, and other drinks was associated with a 5-fold increase in the risk of esophageal cancer.

In a recent nested case-control study, tea ingestion, as measured by prediagnostic urinary tea polyphenols, was associated with a decreased risk of gastric and esophageal cancer but only in people with low serum carotenes and after correction for *Helicobacter pylori* seropositivity (348). In this study, the protective effect of tea was greater in nonsmokers and in nonalcohol drinkers. The results of the above studies on tea and cancer point out important problems with confounding variables in epidemiology studies. It will be important in future epidemiology studies relating tea ingestion to the risk of stomach and esophageal cancer to carry out studies in nonsmokers and in nonalcohol drinkers, to determine the status of fruit and vegetable intake (*e.g.*, measure serum carotene levels or other suitable biomarkers in serum), to determine whether the individuals studied are infected with *H. pylori*, to determine tea ingestion status more accurately by urinary tea catechin measurements, to determine whether burning hot or cooler tea preparations were ingested, and to determine whether the individuals studied are taking inducers or inhibitors of xenobiotic metabolism, or are genotyped as poor or rapid metabolizers of tea constituents. The use of suitable biomarkers, and more detailed questionnaires and laboratory measurements should provide more definitive conclusions on the effects of tea ingestion on cancer risk.

Table 15 Effect of oral administration of black tea on the incidence and multiplicity of squamous cell papillomas, keratoacanthomas, and squamous cell carcinomas in tumor-bearing SKH-1 mice

Female SKH-1 mice (7–8 weeks old) were irradiated with UVB (30 mJ/cm²) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) with the same number and size of tumors per group were then treated with lyophilized black tea (6 mg tea solids/ml) or water as the drinking fluid for 11 weeks. The animals were killed and the tumors were characterized by histopathology studies. Each value is the mean ± SE, and the numbers in parentheses represent percentage of inhibition. (Taken from Ref. 341.)

Treatment	Number of mice	Squamous cell papillomas		Keratoacanthomas		Total nonmalignant tumors	
		Percentage of mice with tumors	Tumors per mouse	Percentage of mice with tumors	Tumors per mouse	Percentage of mice with tumors	Tumors per mouse
Water	15	33.3	0.53 ± 0.22	73.3	3.33 ± 0.80	80.0	3.87 ± 0.94
Black tea	14	14.3 (57)	0.21 ± 0.15 (60)	57.1 (22)	1.43 ± 0.47 (57)	64.3 (20)	1.64 ± 0.49 (58)

Translational Studies with Tea, Caffeine, and EGCG in High-Risk Individuals. The results of our studies and those by others in animals suggest that oral administration of tea, or topical applications of caffeine or EGCG may be useful cancer chemopreventive regimens for patients with actinic keratoses and for patients who have had a skin cancer removed. These individuals have a high risk for developing additional skin cancers. In addition, oral administration of tea, caffeine, or EGCG may be a useful cancer chemopreventive regimen for patients with oral leukoplakia, Barrett's esophagus, or precancerous lesions in the stomach. These are important areas for additional translational research.

INHIBITORS OF CARCINOGENESIS IN ONE EXPERIMENTAL MODEL MAY STIMULATE CARCINOGENESIS IN ANOTHER EXPERIMENTAL MODEL

There are several examples of cancer chemopreventive agents that are effective inhibitors in one experimental setting but have the opposite effect in another experimental setting.

Studies with Decaffeinated Tea

As indicated above, oral administration of moderate dose levels of regular or decaffeinated green tea inhibited UVB-induced carcinogenesis in mice pretreated with DMBA (Table 8; Refs. 316, 317). However, oral administration of moderate doses of decaffeinated green tea had little or no effect on UVB-induced complete carcinogenesis, and high dose levels of decaffeinated green tea enhanced UVB-induced complete carcinogenesis (Table 9; Ref. 318).

Studies with Caffeine

Although many studies indicated inhibitory effects of caffeine administration on carcinogenesis in animals (318, 322, 324, 349–357), some studies showed a stimulatory effect of caffeine administration on carcinogenesis (357–362). It was found that topical applications of caffeine inhibited TPA-induced tumor promotion (354) and cigarette smoke condensate-induced tumorigenesis (349) in mouse skin, and that oral or topical administration of caffeine also inhibited UVB-induced carcinogenesis in mouse skin (318, 322, 324, 351). In addition, s.c. injections of caffeine immediately after administration of urethane or 4-nitroquinoline-1-oxide inhibited the formation of lung tumors in mice (350, 353, 355), and the i.p. injection of caffeine three times a week inhibited the formation of spontaneous or urethane-induced pulmonary adenomas in strain A mice (352). In other studies, treatment of GR mice with caffeine in the drinking water (0.5 mg/ml) inhibited ovarian hormone-induced breast tumorigenesis (356), and 0.1% caffeine in the drinking fluid inhibited the formation of PhIP-induced mammary tumors in rats (357).

In contrast to the inhibitory effects of caffeine on carcinogenesis described above, administration of caffeine in the drinking water

(0.25–0.50 mg/ml) increased spontaneous or DMBA-induced breast tumorigenesis in mice (361, 362). In addition, treatment of rats with caffeine in the drinking water enhanced DMBA-induced breast carcinogenesis (359, 360) and PhIP-induced colon carcinogenesis (357). It was also observed that multiple topical applications of caffeine together with 4-nitroquinoline-1-oxide increased the tumorigenic effects of 4-nitroquinoline-1-oxide in mice pretreated with a single application of β-radiation (358). The results of these studies indicate that the effects of caffeine on carcinogenesis are complex, and whether caffeine inhibits or stimulates carcinogenesis depends on the experimental model used. More detailed mechanistic studies are needed to determine why caffeine inhibits carcinogenesis in some animal models and stimulates carcinogenesis in others.

Studies with Phenobarbital and Indole-3-Carbinol

Although administration of phenobarbital together with 2-acetylaminofluorene inhibits its hepatocarcinogenicity by enhancing metabolic detoxification, when phenobarbital is administered chronically after a short exposure to 2-acetylaminofluorene, phenobarbital is a promoting agent that enhances the formation of liver tumors (47). Similarly, administration of indole 3-carbinol before or together with certain carcinogens inhibits their carcinogenic activity (42, 57, 123), but chronic administration of indole 3-carbinol after a hepatocarcinogen functions as a tumor promoter and enhances the formation of liver cancer (120–126).

Studies with Vitamin E

Although some epidemiological studies suggest that low levels of vitamin E are associated with an increased risk of cancer and supplemental vitamin E may have cancer chemopreventive effects (363–366), other studies have failed to find an inhibitory effect of administration of vitamin E on cancer formation in human populations (367). In animal studies, topical applications of vitamin E inhibited DMBA-induced complete carcinogenesis in the hamster buccal pouch (368), DMBA-induced tumor initiation in mouse skin (369), TPA-induced tumor promotion in mouse skin (370), and UV-induced carcinogenesis in mouse skin (371). Although α-tocopherol was an effective inhibitor of UV-induced carcinogenesis, α-tocopherol acetate and α-tocopherol succinate were inactive (372). In contrast to the inhibitory effects of vitamin E on carcinogenesis described above, feeding high levels of vitamin E to rats stimulated 1,2-dimethylhydrazine-induced intestinal tumors when compared with rats fed a low level of vitamin E (373), and topical applications of vitamin E exerted tumor promoting activity in DMBA-initiated mice (374). In additional unpublished studies in our laboratory, topical applications of α-tocopherol 5 days a week to initiated high-risk mice treated previously with UVB for 5 months enhanced the formation of skin tumors. In addition, the growth of tumors transplanted into mice, rats, or chickens was stimulated when the animals received multiple injections of vitamin E (375, 376).

Table 15 Continued

Treatment	Number of mice	Squamous cell carcinomas		Total tumors	
		Percentage of mice with tumors	Tumors per mouse	Percentage of mice with tumors	Tumors per mouse
Water	15	53.3	1.40 ± 0.43	86.7	5.27 ± 1.22
Black tea	14	42.9 (20)	0.64 ± 0.25 (54)	71.4 (18)	2.29 ± 0.65 (57)

Studies with All-trans Retinoic Acid and 1α,25-Dihydroxyvitamin D₃

Topical applications of all-trans retinoic acid or 1α,25-dihydroxyvitamin D₃ strongly inhibited tumor promotion by TPA in mice initiated previously with DMBA (377–379). However, when all-trans retinoic acid or 1α,25-dihydroxyvitamin D₃ was administered topically twice a week together with DMBA using a complete carcinogenesis protocol, a markedly enhanced tumor response was observed (379–381).

Studies with β-Carotene

Because β-carotene is a strong antioxidant present in fruits and vegetables, it was tested as a potential chemopreventive agent in a population with a high risk of developing lung cancer. In contrast to expectations, it was found that daily supplementation with 30 mg of β-carotene increased the risk of lung cancer in smokers (382–384). These results could be analogous to the stimulatory effect of all-trans retinoic acid treatment on complete carcinogenesis by DMBA and may not have occurred in people who had stopped smoking. It is of interest that the stimulatory effects of β-carotene on lung cancer formation in smokers occurred to a greater extent in smokers who were also alcohol drinkers. Additional analysis of the β-carotene trial indicated that consumption of rosaceae fruit and cruciferae vegetables decreased the risk of lung cancer in the placebo arm of the trial, but

daily supplementation with 30 mg of β-carotene prevented the beneficial effects of fruits and vegetables (385).

In contrast to the adverse effects of β-carotene in smokers described above, in subjects who neither smoked nor drank alcohol, daily supplementation with 25 mg of β-carotene inhibited the recurrence of colorectal adenomas (386). However, β-carotene administration increased the risk for the recurrence of colorectal adenomas in people who smoked and drank alcoholic beverages (386). These studies point out the complexities of cancer chemoprevention studies in human populations and indicate some potential confounders.

TAILORING CANCER CHEMOPREVENTIVE REGIMENS TO INDIVIDUAL NEEDS

As indicated above, some compounds may be extremely effective cancer chemopreventive agents in one experimental setting but enhance carcinogenesis in another experimental setting. It is likely that an optimal cancer chemopreventive regimen for smokers will be different from an optimal cancer chemopreventive regimen for former smokers or for people exposed to specific dietary carcinogens such as heterocyclic amines in broiled meat and fish or aflatoxin B₁ in mold-contaminated foods. Greater efforts should be made to understand mechanisms of cancer chemoprevention and to determine whether a potential chemopreventive agent is useful in many exper-

Table 16 Effect of oral administration of black tea on bromodeoxyuridine incorporation into the DNA of established papillomas, keratoacanthomas, and squamous cell carcinomas in SKH-1 mice

Female SKH-1 mice (7–8 weeks old) were irradiated with UVB (30 mJ/cm²) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) were then treated with lyophilized black tea (6 mg tea solids/ml) or water as the drinking fluid for 11 weeks. Bromodeoxyuridine (50 mg/kg) was injected i.p., and the animals were killed 1 h later. Bromodeoxyuridine positive cells in each area of focal epidermal hyperplasia and in each tumor were counted and expressed as the number of positive cells per 100 cells counted. Each value is the mean ± SE, and the numbers in parentheses represent percentage of inhibition. (Taken from Ref. 341.)

Treatment	Number of mice	Bromodeoxyuridine labeling index					
		Focal epidermal hyperplasia	Squamous cell papillomas	Keratoacanthomas	Nonmalignant tumors	Squamous cell carcinomas	Total tumors
Water	15	14.1 ± 1.5	33.9 ± 6.5	35.2 ± 2.9	35.0 ± 2.6	54.5 ± 4.3	40.2 ± 2.4
Black tea	14	14.3 ± 1.9 (-1)	15.0 ± 4.4 (56)	19.5 ± 2.4 (45)	19.0 ± 2.1 (46)	35.2 ± 4.2 (35)	23.5 ± 2.3 (42)

Table 17 Effect of oral administration of black tea on apoptosis in established papillomas, keratoacanthomas, and squamous cell carcinomas in SKH-1 mice

Female SKH-1 mice (7–8 weeks old) were irradiated with UVB (30 mJ/cm²) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) were then treated with lyophilized black tea (6 mg tea solids/ml) or water as the drinking fluid for 11 weeks. The apoptosis index (TUNEL assay) was determined by evaluating the number of cells stained and the intensity of staining. Each value is the mean ± SE, and the numbers in parentheses represent percentage of increase. (Taken from Ref. 341.)

Treatment	Number of mice	Apoptosis index (mean ± SE)					
		Focal epidermal hyperplasia	Squamous cell papillomas	Keratoacanthomas	Total nonmalignant tumors	Squamous cell carcinomas	Total tumors
Water	15	1.37 ± 0.22	1.16 ± 0.32	0.79 ± 0.10	0.84 ± 0.10	0.77 ± 0.19	0.82 ± 0.09
Black tea	14	2.15 ± 0.24 (57)	1.67 ± 0.44 (44)	1.58 ± 0.22 (100)	1.59 ± 0.19 (89)	1.50 ± 0.33 (95)	1.56 ± 0.17 (95)

imental settings or whether it is only useful in a limited number of experimental settings. There is a need to understand genetic, environmental, and lifestyle factors that influence carcinogenesis in humans, and to use this information to help in the selection of an appropriate cancer chemopreventive regimen in individuals with a high cancer risk. In many instances, it may be necessary to tailor cancer chemopreventive agents to individual subjects with known carcinogen exposures or to individuals at high risk for cancer with mechanistically understood pathways of carcinogenesis so that chemopreventive regimens can be customized to the individual and selected on a more rational basis.

ACKNOWLEDGMENTS

I thank my many colleagues, postdoctoral fellows, and students during the past 50 years for their outstanding contributions, and also Florence Florek for excellent help in the preparation of this manuscript.

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Cancer Res 2003;63:7005-7031.

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