

# Dietary Modifiers of Carcinogenesis

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Dietary components express a wide range of activities that can affect carcinogenesis. Naturally occurring substances in foods have been shown in laboratory experiments to serve as dietary antimutagens, either as bioantimutagens or as desmutagens. Dietary desmutagens may function as chemical inactivators, enzymatic inducers, scavengers, or antioxidants. Dietary components may also act later in the carcinogenic process as tumor growth suppressors. Examples of dietary factors acting in each of these stages of carcinogenesis are presented, and potential anticarcinogens such as the carotenoids, tocopherols, phenolic compounds, glucosinolates, metal-binding proteins, phytoestrogens, and conjugated linoleic acid are discussed. Individual foods typically contain multiple potential anticarcinogens. Many of these substances can influence carcinogenesis through more than one mechanism. Some substances exhibit both anticarcinogenic and carcinogenic activity *in vitro*, depending on conditions. Epidemiologic research indicates that high fruit and vegetable consumption is associated with lower cancer risk. Little research has focused on the effects of single substances or single foods in man. Realization of the potential of foodborne substances to reduce the human burden of cancer will only be achieved with better measurement of dietary exposures and funding of multidisciplinary research in this area commensurate with its importance. — Environ Health Perspect 103(Suppl 8):177–184 (1995)

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## Introduction

Few persons doubt that diet plays a large role in carcinogenesis. Doll and Peto (1) estimated in 1981 that diet can account for up to 70% of all avoidable cancers. Recent reevaluations have not challenged the relative importance of diet (2). Knowledge of the actual active substances and mechanisms of effect remain limited. We are still at a stage comparable to three blind men attempting to determine the physical nature of an elephant, with a relatively small group of scientists examining specific aspects of carcinogenesis in relative isolation. Realization of the potential of dietary prevention of cancer will require major investments in four areas. One of these is the identification of the substances in foods that can be protective. The promise and current knowledge in this area are reviewed later. Second, there is an acute need for the development of nutrition and cancer research units that promote multidisciplinary approaches toward research on diet and cancer. A critical mass of scientists working together could spearhead scientific

advancement across basic biochemical and epidemiologic research axes. A third important area is that of exposure measurement. The Achilles heel of diet and cancer epidemiology is the underdeveloped state of tools to measure or estimate these exposures. Cognitively smart dietary assessment tools that succeed in capturing complete information from large proportions of the population of interest are sorely needed. The development of biomarkers of dietary exposures could help but not eliminate the need for subject assessment (3). Accurate information on the long-term quantitative exposure levels and habitual diets of individuals is fundamental to scientific advancement. Finally, we need to invest in programs that educate and stimulate individuals to select healthier diets based on up-to-date knowledge of preventive foods.

The past two decades have witnessed a major effort to discover which elements of our diet affect carcinogenesis and by what mechanisms. Initial interest focused on carcinogenic effects of diet. More recent research includes increased focus on dietary prevention of cancer. Epidemiologic research remains the most powerful tool for determining the role of nutrition in the etiology of cancer in human populations. The reasons for this are related to the need for measurement of long exposures to active substances in the diet, long lags between exposure to risk factors and disease, ethical constraints on human experimentation, and the plethora of dietary factors of interest. Such complications, which may translate into problems with

measurement error, lag-time uncertainty, collinearity, and weak single-factor associations in epidemiologic studies, are reviewed elsewhere (4).

This paper surveys current knowledge regarding anticarcinogenic agents in foods. Potential mechanisms of action serve as a framework for discussion of epidemiologic findings for specific agents. General weaknesses of epidemiologic studies on diet and cancer are addressed and strategies to overcome them are proposed.

## Proposed Mechanisms for Dietary Anticarcinogens

Laboratory studies provide most of our information about potential mechanisms of action of dietary anticarcinogens. A review of the many laboratory studies on potential mechanisms of anticarcinogens reveals that many chemicals possess multiple modes of action. In Table 1, dietary components known to exert some form of anticarcinogenic activity are presented, along with their mechanisms of action. Most anticarcinogens also show potentially detrimental effects such as mutagenicity, comutagenicity, cocarcinogenicity, or tumor promotion under certain circumstances. In addition, both mutagens and antimutagens have been found in most well-studied whole foods.

Translating the large and complex body of knowledge on potential mechanisms into predictions of the health consequences of specific dietary interventions in humans is a great challenge. In the following section, we briefly review the mechanisms by which anticarcinogenic agents present in

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Abbreviations used: ATBC,  $\alpha$ -tocopherol  $\beta$ -carotene; DMBA, dimethylbenz[*a*]anthracene.

**Table 1.** Selected dietary anticarcinogens and their postulated mechanisms.

Compound	Mechanism						References
	Bioantimutagen	Desmutagen				Tumor growth suppressor	
		Direct chemical inactivater	Enzyme modulator	Binding scavenger	Antioxidant		
Ascorbic acid (vitamin C)	-	-	X	-	X	X	(27,63)
BHA (2(3) <i>tert</i> -butyl-4-hydroxyanisole)	-	-	X	-	X	-	(64)
Calcium (with vitamin D)	-	X	-	-	-	X	(65)
Carotenoids ( $\beta$ -carotene)	-	-	-	-	X	X	(34,35)
Conjugated linoleic acid	-	-	-	-	-	X	(60)
Coumarins	-	-	X	-	-	-	(15)
Dithiolthiones	-	-	X	-	-	-	(66,30)
Dietary fiber	-	-	-	X	-	-	(67)
Flavonoids (see phenolics)	-	-	X	-	X	X	
Folic acid	-	-	-	-	-	X	(68)
Genistein (see phytoestrogens, phenolics)	-	-	X	-	X	X	
Indoles (indole-3-carbinol)	-	-	X	-	-	X	(15,69)
Inositols (inositol hexaphosphate)	-	-	-	-	X	-	(31)
Isothiocyanates (benzyl isothiocyanate)	-	-	X	-	-	X	(15,70)
Lignins (curcumin/turmeric, sesamol)	-	-	-	-	X	-	(71)
Omega-3 fatty acids	-	-	-	-	-	X	(61)
Organosulfur compounds:Allium sp. (see also indoles, isothiocyanates)	-	-	X	-	X	X	(18)
Phenolics (flavonoids, tannins, catechins)	X	-	X	-	X	X	(12,31,43,50)
Phytates (phytic acid)	-	-	-	-	X	-	(73)
Phytoestrogens (from isoflavones, lignans)	-	-	?	-	?	X	(31)
Porphyrins (chlorophyllin)	-	-	-	X	X	X	(24)
Protease inhibitors	-	-	-	-	-	X	(74)
Retinoids (vitamin A, retinol) (see also carotenoids)	-	-	-	-	-	X	(75,76)
Saponins	-	-	-	-	-	X	(31)
Selenium	-	-	-	-	X	X	(57,77)
Terpenoids:noncarotenoid (monoterpenoids, limonoids)	-	-	X	-	-	-	(70,72)
Tocopherols (vitamin E)	-	X	-	-	X	-	(78)
Vanillin	X	-	-	-	-	-	(12)

the human diet may act. Anticarcinogens will be organized by mode of action, adapted from the schemata found in a recent review of antimutagenic agents in the diet (5). Detailed information on the various test systems used to detect antimutagenicity is provided in other recent works (6–8). In addition to antimutagens, we will include agents that act postinitiation and refer to these as tumor growth suppressors. While prevention of progression of late-stage events to neoplasms by both macro- and micronutrient manipulation has been demonstrated, this review focuses on specific compounds found in the diet.

**Dietary Antimutagens**

Components of the diet can act at any of the various stages of carcinogenesis. Those that block mutagenesis prior to tumor development can be considered antimutagens. Antimutagens in the diet can be broadly classified into two groups, the bioantimutagens and the desmutagens, the former acting on DNA and the latter not affecting genetic material directly (9).

**Bioantimutagens.** The bioantimutagens are naturally occurring substances that reduce mutant yield by acting on the DNA repair or replicative processes. These compounds act after a DNA adduct has formed but before the DNA lesion is fixed into a mutation. Because these compounds alter the mutation process, they have also been dubbed true antimutagens (9). Bioantimutagens exhibit several specific modes of action in the bacterial test systems commonly used for screening. They may *a*) inhibit the induction of strand-on-strand DNA repair, reducing replication of mutated strands; *b*) in cells containing mutations, make the “proofreading” in repair more like that seen in normal cells; or *c*) accelerate the recombination strand-on-strand repair rate, thus reducing the number of mutated strands (10).

An example of a bioantimutagen is vanillin, present in vanilla beans, which appears to enhance postreplication recombinational repair under certain conditions (11). This results in a decrease in the number of mutants recovered after exposure of

a cell culture to mutagen. Vanillin has been tested *in vitro* using a variety of cell types, from *Escherichia coli* to mammalian cells, and against a wide variety of physical and chemical agents, such as *N*-nitroso compounds, heterocyclic amines, and small nonplanar alkylators. The antimutagenicity of vanillin is not universal. Review of 31 *in vitro* assays found that vanillin reduced mutagenicity in 17 and showed no effect in 5; in 9 assays, however, mutagenicity was enhanced (12).

In addition to the complexity of the antimutagenicity exhibited in *in vitro* test systems, many of the bioantimutagens can exhibit genotoxicity under certain testing conditions (13). The effectiveness of many of these compounds in blocking carcinogenicity in animals and man has not been adequately studied.

**Desmutagens.** Desmutagens encompass all agents that affect mutagenicity through mechanisms other than DNA repair or replication. These mechanisms include enzyme induction, mutagen scavenging, and blocking of mutagen activation. As

Table 1 reflects, many more desmutagens are known than bioantimutagens. Like bioantimutagens, they suppress the appearance of mutants under assay conditions. Unlike bioantimutagens, however, they create this effect without directly affecting the genetic material (5). This can occur through alteration of the survival of mutant cells or by altering the dose of mutagen delivered to normal cells. The net effect is a reduction of altered DNA levels per cell. The next few sections discuss several specific mechanisms of action for desmutagens.

**Chemical Inactivators and Enzymatic Modulators.** Chemical inactivators and enzymatic modulators are agents that prevent the formation of mutagens or their activation to more potent forms (5). Chemical inactivators can act directly to inhibit the formation of active carcinogenic compounds, such as the conversion of nitrosamines from nitrite in the stomach. Modulators can act through enzyme systems by inducing phase I or phase II enzymes or by altering the balance of different enzyme activities. These agents range from ascorbic acid to glucosinolates from cruciferous vegetables and the allium compounds found in garlic.

The anticarcinogenic potential of cruciferous vegetables has been related to two sets of compounds found exclusively in these vegetables: dithiolthiones and glucosinolates. Dithiolthiones are among the strongest inducers of phase II enzymes, including quinone reductase, glutathione transferase, and glutathione reductase (13). They have been found to decrease colon and liver cancer in rats but increase DNA damage in mice. The glucosinolates are hydrolyzed into isothiocyanates and indole derivatives, both of which have also been implicated as stimulators of mixed-function oxidase activity through the P450 cytochrome system. Isothiocyanates have been shown to reduce mammary and pulmonary tumorigenesis in rats and mice (14–16). They are also phase II enzyme inducers.

Indole 3 carbinol, when administered simultaneously with aflatoxin in animal studies, reduces aflatoxin B<sub>1</sub> binding to DNA. When the indole is given preinitiation, liver carcinogenesis is also reduced (17). However, if administered later, carcinogenesis is enhanced. This illustrates the complexity of the interaction of dietary compounds in a particular test system.

Garlic extract and many of its components exhibit activity against a wide range of chemical mutagens and radiation (18).

Increased glutathione S-transferase activity in one or more organs has been shown in mice and rats administered garlic components such as diallyl sulfide and allyl-methyltrisulfide. Evidence of effects on mixed-function oxidase and P450 enzyme systems has also been reported. In addition to effects on enzymes, it appears that garlic compounds may scavenge radicals and inhibit promotion—mechanisms considered later in this review (18).

The enzyme inducers as a class have some promising characteristics. They usually act against a broad range of mutagens. In addition, enzyme inducers need not necessarily be present simultaneously with a promutagen to be effective in blocking its activity. Unfortunately, these compounds exhibit deleterious effects under certain circumstances. For example, active compounds in cruciferous vegetables appear to act as cocarcinogens in some rodent models (19,20). Similarly, the inducers of P450 may be beneficial in one circumstance and not in another, as this enzyme system is involved not only in detoxification but also in the activation of some carcinogens (5). An additional complication is that many of the compounds that induce phase II enzymes may be carcinogenic at high concentrations. This is thought to arise from the electrophilic nature of most phase II inducers (21,22). While the overall effect of enzyme inducers in the diet is likely to be anticarcinogenic, assessing their effect on specific carcinogens of interest demands careful evaluation.

**Scavengers (Non-O<sub>2</sub>).** Scavengers bond with mutagens to render the mutagen incapable of reacting with DNA. The mutagen generally remains intact during this process (5). One class of chemicals that forms complexes with mutagenic compounds is the porphyrins, including chlorophyllin (23). Chlorophyllin inhibits the mutagenicity of a variety of dietary mixtures as well as individual large planar mutagens (e.g., aflatoxin B<sub>1</sub>, benzo[*a*]pyrene) *in vitro*. It has little or no effect against small nonplanar carcinogens (5). Additionally, the urinary mutagenicity of humans ingesting cooked ground beef has been lowered with co-administration of chlorophyllin (23). Chlorophyllin has been shown to complex with polycyclic compounds, thereby blocking their mutagenic potential (24). As with the other categories discussed so far, porphyrins do not appear to be universally benign. Chlorophyllin, for example, tested positive as a tumor promoter in the rat–dimethylhydrazine colon carcinogenesis

model (25). One would expect that for compounds in this category to be effective, they should be co-administered with the mutagen. This may make these agents less effective for dietary preventive purposes than the enzymatic inactivators, particularly for certain scavengers that are not normally consumed with most meals.

**Antioxidants and Free Radical Scavengers.** Antioxidants exert their effect by donating electrons to unstable oxygen species generated from endogenous processes or formed as a result of radiation or chemical exposure. This property could protect against cancer in many ways. Preempting oxidative attack on chemical bonds at other points in the cell, including DNA helices, may reduce the incidence of mutations arising from oxidant-induced DNA lesions. Blocking the stimulation of cell division attributed to oxidants may also lead to a reduction in the rate of mutation (26). Beyond antimutagenic effects, antioxidants help to maintain the physical integrity of cell membranes and normal production of membrane-derived cellular regulatory agents. They may also prevent collateral damage caused by free radicals produced by cells of the immune system during attack on foreign materials. Vitamin C and carotenoids possess well-documented immuno-stimulatory effects that may stem, at least partly, from the antioxidant properties just described.

Ascorbic acid (vitamin C) is an example of a water-soluble extracellular antioxidant with intriguing dual properties. On one hand, it quenches singlet oxygen and assorted free radicals; on the other hand, it preserves the function of other antioxidant compounds, notably vitamin E, by reducing them back to active form after they have quenched free radicals (27). Carotenoids such as  $\beta$ -carotene and lycopene illustrate lipid-based antioxidants.

A major advantage of the antioxidants is that they are generally effective against a wide range of mutagens, both exogenous and endogenous. While most have no known major adverse effects, their redox potential may facilitate some pro-oxidant activity. In the presence of iron, for example, ascorbic acid can enhance carcinogenesis under experimental conditions (28). A further interesting property of antioxidants is that they may exhibit antipromotional as well as antimutagenic activity through inhibition of oxidant-stimulated cell division (29). Such activity overlaps with that of the final category of anticarcinogens, substances that suppress tumor growth.

### Tumor Growth Suppressors

Tumor growth suppressors exert their effects at late stages in the carcinogenic process, i.e., postinitiation. The substances in this category express a wide range of effects, including interference with promotion (when coadministered with a known tumor promoter) or interference with the ability of the tumor to grow and invade other tissues. Protease inhibitors found in soybeans, grains, and other vegetables are examples of this class of substances (30). They are believed to work by interfering with proteases used by neoplastic cells for destruction of the extracellular matrix, cellular detachment, and invasion of metastatic cells into new sites. Further examples of tumor inhibition are provided by phytoestrogens and garlic oil. Phytoestrogens bind to estrogen receptors, blocking the binding of other more potent estrogens without stimulating cellular growth (31). Garlic oil significantly reduces skin tumor yield in rodents when coadministered with promoting agents (18).

In summary, dietary anticarcinogens act via several mechanisms: DNA repair and replication, chemical or enzymatic inactivation, scavenging of mutagens, antioxidant activity, and tumor inhibition. As more data accumulate, it is becoming apparent that many foods contain more than one compound and that many compounds exhibit more than one mechanism (Table 1). The anticarcinogenic picture painted by laboratory studies is often complex, making prediction of the effect on living humans impossible without epidemiologic findings.

### Evidence for Association of Cancer with Specific Food Components

Epidemiologic studies examining relationships between the intake of fruits and vegetables provide consistent evidence of protective effects. A review of the 156 studies by Block (32) examining intakes of fruits and vegetables showed lower rates of cervical, ovarian, lung, esophageal, oral cavity, larynx, pancreatic, stomach, colon, rectal, bladder, and breast cancers in people who consumed fruits or vegetables relatively more frequently than those who did not. While indicating the wisdom of general advice to eat more fruits and vegetables, use of such wide dietary constructs precludes more specific recognition of actions and interactions. Only recently, however, has epidemiology begun to turn from broadly defined food categories to explore the active ingredients in these

foods. Research now should focus on the isolation of specific agents in fruits and vegetables (as well as in other foodstuffs) that impact cancer risk.

### Vitamins and Related Compounds

Specific substances from plant sources that have come under scrutiny because of their known physiologic importance, high consumption levels, and antioxidant potential include carotenoids, tocopherols (vitamin E), and ascorbic acid (vitamin C). The carotenoid family includes over 500 members, the best known of which is the provitamin,  $\beta$ -carotene.

**Carotenoids.** The rich array of conjugated double bonds characteristic of carotenoids inspires interest in their potential as free radical scavengers. The range of antioxidant activity exhibited by individual carotenoids in the laboratory varies greatly. Research has concentrated largely on  $\beta$ -carotene, although some other carotenoids (e.g., lycopene, lutein) show greater singlet oxygen-quenching potential (33).

Studies of the effects of  $\beta$ -carotene on tumor formation in animals yield inconsistent results. Krinsky (34,35) presents an overview of experimental findings on the effects of  $\beta$ -carotene on chromosome breaks, sister chromatid exchange, and tumor growth. He attributes the mixed results to difficulties in assuring significant uptake of carotenoids in most animal models. It is also unclear whether  $\beta$ -carotene or its retinoic derivatives are acting in these tests. The administration of carotenoids has, however, shown impressive results in the hamster, including decreased incidence and even regression of tumors.

Evidence from human studies is more consistent. Block (32) notes that at least 32 studies have examined relationships between consumption of foods rich in carotenoids and/or  $\beta$ -carotene levels in serum and lung cancer risk, with 30 of these studies showing a protective relationship. In a similar review, Canfield et al. (36) relate that a statistically significant inverse association between lung cancer and estimated dietary  $\beta$ -carotene intake appears in 16 published studies, while 7 studies show a similarly significant association for  $\beta$ -carotene measured in serum. The same reviewers note that 5 of 6 studies found significant associations of cervical dysplasia with low dietary and/or serum  $\beta$ -carotene levels. In addition,  $\beta$ -carotene administration was efficacious against oral leukoplakia in at least three clinical trials (37,38).

More recent findings muddy the picture.  $\beta$ -Carotene, whether alone or in combination with vitamins C and E, failed to reduce the recurrence of colon polyps in a 3-year clinical trial (39). In addition, Finnish smokers receiving  $\beta$ -carotene supplements for 5 to 8 years showed no reduction in lung cancer risk—in fact, risk increased, particularly among older subjects (40). The ability to draw firm conclusions from either study is limited by methodological considerations. In both studies, the supplementation period was short and the supplementation may have come too late in the neoplastic process to influence the outcome. More positive results were observed in supplementation studies on gastric cancer, wherein mixtures of  $\beta$ -carotene and vitamin E reportedly lowered rates of occurrence (41).

Different foods contribute different levels of carotenoids. Carrots contain primarily  $\beta$ -carotene, broccoli contains lutein and  $\beta$ -carotene. Tomatoes and crustaceans are rich sources of lycopene. Raw guava and watermelon also provide large concentrations of lycopene. Lutein can be found in very high amounts in chicory, chives, kale, collards, cress, and other greens. The major sources of carotenes in the American diet are carrots, tomatoes, sweet potatoes, yellow squash, spinach, and cantaloupe (42). These six foods account for 70% of carotenoids in the diet. Romaine lettuce, broccoli, spinach and iceberg lettuce contribute approximately another 10%. The carotenoids deserve more focused research attention to clarify their activities and more closely examine the effects of carotenoids other than  $\beta$ -carotene.

**Tocopherols.** The natural tocopherols include eight compounds that are commonly known as vitamin E.  $\alpha$ -Tocopherol is the predominant component. Most vitamin E is derived from vegetable oils, nuts, sunflower seeds, and whole grains.

A number of studies have examined the relationship between  $\alpha$ -tocopherol or total vitamin E levels in blood with cancer. The majority of these studies is consistent with a protective association for all cancers as a group; specific cancer types with evidence of protective association include gastrointestinal, lung, colon, breast, cervical, and oral cancers. Many studies reporting associations for vitamin E reported similar associations for one or more other fruit- and vegetable-associated antioxidants as well. It is notable that the previously mentioned clinical trial of  $\beta$ -carotene's effect on recurrence of colon polyps also included trials of

vitamin C and E supplementation, with similarly negative results (39). While the Alpha-Tocopherol Beta-Carotene (ATBC) Trial did not show a protective association of vitamin E with lung cancer, prostate cancer mortality was significantly lower in the vitamin E-supplemented group (40).

### Phenolic Compounds

The phenolic compounds include simple phenols, phenolic acids, hydroxycinnamic acid and its derivatives, and flavonoids. The most biologically active phenolic substances are thought to be the flavonoids, the proanto- and anto-cyanins, and the catechins. Flavonoids provide an extreme example of disagreement between laboratory-based test systems. Evidence of bacterial mutagenicity implicates flavonoids as potential carcinogens, yet *in vivo* studies yield little evidence of carcinogenicity. Rodent-based studies involving administration of known carcinogens provide evidence of protection against epithelial and colon tumorigenesis by flavonoids (43). Animal experiments also indicate anticarcinogenic activity for several specific catechins. Tea catechins, for example, reduce tumorigenesis and tumor growth in mice (44).

Phenolic compounds could affect carcinogenesis through a number of mechanisms. These compounds may scavenge carcinogens or free radicals. They may also block generation of reactive oxygen species. Epicatechin gallate, for example, inhibits free radical chain reactions of cell membrane lipids and can influence mutagenicity and DNA-damaging activity (45). Some flavonoids bind to estrogen receptors. It has been argued that by this binding they act on the regulation of gene transcription and may protect against estrogen-related cancers (46). Phenolic compounds, in general, affect phase II enzymes. It remains unclear whether such induction by phenolic compounds affects carcinogenesis *in vivo* (47). Phenolic compounds may also reduce cellular proliferation through the modulation of protein kinase C activity. A few phenolics may possess bioantimutagenic properties (12).

Estimates of the amounts consumed daily vary widely. Based on the average diet of a Dutch population, tea provides the greatest amounts of flavonoid (61%); onions and apples are the next greatest sources, providing 13 and 10%, respectively (48). Tea leaves may have catechin concentrations that represent up to 30% of the dry weight of the tea leaf (45). Allium vegetables (leeks, shallots, scallions, garlic,

and onions) range in their flavonol content from none to more than 1 g/kg of vegetable. Shallots have uniformly high concentrations, but onions range widely, with no measurable amounts in white onions and high levels in yellow onions (49).

The mechanisms hypothesized as influencing carcinogenicity have hardly been studied in epidemiologic efforts directed at specific phenolic compounds. One exception is a recent Dutch study (50) in which estimated dietary intake of flavonoids showed no association with lung, gastrointestinal, or all-cause cancer mortality incidence in a male cohort followed for 5 years. The modest size and short follow-up period of the study greatly limit its power, however.

Despite the wealth of studies on food groups such as fruits and vegetables and cancer, there is a dearth of studies on active ingredients in foods. This is largely because of the lack of reliable edited food compositional databases for phenolic compounds.

### Glucosinolates

Glucosinolates are present exclusively in vegetables of the family *Cruciferae*, especially in the genus *Brassicaceae*. This includes cabbages, broccoli, brussels sprouts, and cauliflower. Differences in cabbage consumption have been associated with differences in colon and breast cancer mortality across Europe (51).

The modulation of carcinogenesis by the consumption of Brassica vegetables has been investigated in laboratory animals challenged with carcinogens. The comparison of tumor development in animals with and without cabbage supplements provides evidence of an effect on the production of some tumors. Mammary tumors, particularly in mice and rats, seem to be reduced upon the addition of 5 to 20% of cabbage, by weight, to the diet (22,52).

The glucosinolate content of foods varies from species to species, from crop to crop, and from lab to lab. The amount of thiocyanate, for example, in various Brassica ranges as much as 7-fold within a species. Their average concentrations in milligram per kilogram fresh weight vary up to 4-fold between species. Among the subspecies, late sowing and younger samples show a larger percentage of acetonitrile extracts from these vegetables, ranging from 4.6 to 15.6% of dry weight (53).

A number of activities related to glucosinolates and dithiolthiones have been reviewed earlier. Another proposed action is related to the Brassica component

indole-3-carbinol, which acts as a modulator of estrogen metabolism in a way that reduces carcinogenesis. The 2-hydroxyestrone shows minimal estrogenic activity, whereas the 16-hydroxyestrone is both genotoxic and exerts full estrogenic potency (54). The *in vivo* effect of many compounds implicated in the carcinogenic process on this pathway has been demonstrated (55). Indole-3-carbinol enhances urinary excretion of 2-OH estradiol metabolites, whereas carcinogens such as dimethylbenz[*a*]anthracene and benzo[*a*]pyrene reduce the 2-OH estradiol metabolite levels. Alcohol and the polyunsaturated fatty acids linoleic and arachidonic, on the other hand, increase the excretion of 16-OH metabolites. The chemoprotective effects of indoles have received little attention.

### Metals and Metal-binding Proteins

Extracellular metal-binding proteins may act as antioxidants by rendering metals needed to catalyze oxidant-releasing reactions unavailable. They may also quench free radicals directly. Prominent extracellular metal-binding proteins include lactoferrin, transferrin, ceruloplasmin, and haptoglobins.

Selenium forms the centerpiece of the best known and most heavily studied metalloenzyme antioxidant system, selenium glutathione peroxidase. Other less well-characterized selenoenzymes may play equal or greater roles as antioxidants *in vivo*, however (56,57).

Decreased tissue selenium levels have been reported in case-control studies of many cancer types. In most studies it is unclear whether selenium levels fell prior to or following disease. A recent prospective study found a significant inverse relationship of toenail selenium concentration and lung cancer in a Dutch cohort (58). The relationship was much stronger among subjects with low estimated dietary vitamin C intakes, and somewhat stronger in the low  $\beta$ -carotene subgroup. Other, extracellular metal-binding proteins have received less attention in human population studies than have selenoenzymes.

### Phytoestrogens

Given the established associations between estrogens and certain cancers (breast, endometrial, ovarian), the theoretical importance of phytoestrogens is clear. Promotional activity could be expected for some cancers such as estrogen-sensitive breast tumors and inhibition for others

such as prostate tumors. The potential impact of phytoestrogens is tempered by their low potency relative to estradiol. In theory, the competition of low-potency phytoestrogen with high-potency estradiol for estrogen receptors may have a net anti-estrogenic effect. A Singapore case-control study attributed an inverse association between breast cancer and consumption of soy products to phytoestrogens (59). Phytoestrogens often belong to classes of compounds with more general anticarcinogenic effects. They may act through mechanisms typical of other anticarcinogens in those classes in addition to estrogen receptor-mediated antipromotional effects. The heavily researched phytoestrogen genistein, for example, is a member of the flavonoid family and shares antioxidant and antipromotional characteristics associated with that family of compounds. While substantial *in vitro* experimental data is available for some phytoestrogens, epidemiologic studies specifically targeting phytoestrogens are currently lacking.

### Conjugated Linoleic Acid and Omega-3 Fatty Acids

A mixed isomeric form of linoleic acid, conjugated linoleic acid, may act as a tumor inhibitor. Administration to carcinogen-exposed rats reportedly significantly reduced the incidence of mammary tumors (60). No human studies relating conjugated linoleic acid intakes to risk of disease are available. Turkey and red meats, milk, and cheese form the primary dietary sources of conjugated linoleic acid. These foods also serve as sources of simple linoleic or arachidonic acid, which have been implicated in tumor promotion.

Omega-3 fatty acid administration is generally associated with modest-to-significant tumor inhibition in animal models. Most studies employ a mix of omega-3 fatty acids rather than one specific type. Marine fish comprise the major dietary source of omega-3 fatty acids. Fish oil supplementation is associated with reduction of mammary tumorigenesis in rodent models (61). Human epidemiologic evidence is largely based on non-specific exposures such as fish consumption and contains equivocal results (62).

### Dietary Modulators: General Considerations and Research Needs

One crucial caveat in many studies of specific dietary factors is the uncertainty about whether an observed association is the

product of a carcinogen, an anticarcinogenic effect of the specific exposure in question, or of some other covarying exposure(s). This is particularly relevant for potential dietary anticarcinogens, since most occur largely in fruits and vegetables. Supplementation trials provide a way around the problem; over 25 intervention studies are now under way for various antioxidants, alone or in combination. The number of potential anticarcinogenic agents in the diet identified even in this brief review is clearly too large for specific trials of each agent, let alone all possible combinations.

Calculating exposures based solely on those foods that contain the highest concentrations of a substance may be misleading if other foods that contain relatively low concentrations of the substance are consumed in greater quantities. This situation can arise when complete food composition data are lacking. Data are often particularly spotty for the less publicized dietary factors. In general, information on concentrations of anticarcinogens in food is not widely available. Furthermore, reported species concentrations may not be predictive of the concentrations of samples from different cultivars. These considerations combine with more general problems such as reliable and precise measurement of portion size and frequency to point out the inherent limitations of studies based on questionnaire data. Such studies contribute the bulk of the epidemiologic literature on dietary agents and cancer to date.

No single compound is likely to affect all carcinogens. In addition, it may be the combination of different compounds and different mechanisms of those compounds that is responsible for the effects of food on cancer seen in epidemiologic studies. Further, we need to be concerned with undesirable side effects of compounds used for cancer prevention. Those compounds or foods that are most beneficial for adults in the quest to avoid cancer may not be appropriate in the diet of pregnant women and children. While the retinols, for example, appear to be beneficial for the prevention of cancer, some are also teratogens. We know little about the activities of these substances *in vivo* in combination with the other components of a normal diet. Only intensified research will reveal their true benefits for human health.

Research since Doll and Peto's suggestion that up to 70% of all cancers may be diet related (1) has certainly not diminished interest in dietary factors as potential keys to carcinogenesis. In fact, Austoker (2) recently

concluded that dietary modifications might potentially reduce overall cancer incidence by as much as two-thirds based on current evidence. What has become clearer in the intervening years is the complexity of the diet-cancer relationship. Research continues to introduce new compounds or new activities of old compounds as factors in the relationship. Furthermore, the discovery and understanding of interactions—positive and negative—between compounds active in the cancer arena have barely begun.

Only a few human cancers show a clear-cut association with specific dietary factors. Part of the problem undoubtedly lies in the poor quality of most of our exposure measures. Another part of the problem probably arises from failure to account for modifying or confounding factors (either by design or due to incomplete knowledge of the factors involved). In addition, logistical difficulties associated with the long latent period of most and the relative rarity of some types of cancer make prospective studies difficult. Much of the problem, however, also probably lies in the inherent nature of the association between diet and cancer, which is in fact not one association but a web of intermingled causal pathways. The challenge is to identify those factors that, individually or in tandem, are amenable to practical intervention. This will require research and provision of support commensurate with the extent of the problem. To return to the analogy of the blind men and the elephant, we may envision the elephant as the population at risk of diet-related cancer. The provision of glasses, in the form of better measurement tools for dietary exposures, would largely restore the vision of the blind men (and women) now groping with this puzzling animal. Thus unblinded, closer cooperation in the form of interdisciplinary research will further sharpen our view. Effective intervention to preserve the health and longevity of the elephant may then fall within our grasp.

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