Lee W. Wattenberg[1]

# Inhibition of Chemical Carcinogenesis

Current data suggest that chemical carcinogens have a significant role in the etiology of cancer in man. In this editorial, information will be presented concerning many compounds that have the capacity to inhibit the neoplastic effects of chemical carcinogens when administered either prior to exposure to the carcinogen or at the same time. Overall, this inhibition entails prevention of the active form of the carcinogen from reaching or reacting with the target site. Inhibition involving reversal of early phases of the carcinogenic process will not be included. This type of inhibition has been achieved with retinoids and has been reviewed by Sporn et al. (*1*). Prevention of neoplasia by blocking of the formation of carcinogens, as has been accomplished for nitroso compounds, likewise will not be dealt with (*2*). Most of the inhibitors to be discussed are synthetic compounds; some are constituents of natural products, including vegetables consumed by man (*3–5*). The inhibitors identified thus far show a great diversity of chemical structures, making it likely that we have only a limited knowledge of the total spectrum of compounds having this property. This diversity increases the probability that inhibitors have or could be made to have an impact on the response of humans to chemical carcinogens.

The following are some potential mechanisms of inhibition of chemical carcinogenesis:

1) Alteration of metabolism of the carcinogen:
   *a*) Decreased activation
   *b*) Increased detoxification
   *c*) Combination of (*a*) and (*b*)
2) Scavenging of active molecular species of carcinogens to prevent their reaching critical target sites in the cell
3) Competitive inhibition.

However, much of the information that has actually been obtained on inhibitors has come from empirical studies. Some inhibitors were originally identified as a result of work based on postulated mechanisms of inhibition. Even in these instances, as will become evident, there is frequently doubt about how correct the postulated mechanism really is. Thus the amount of phenomenologic data is considerable, but solid information on mechanisms of inhibition is scarce. As a result, the organization of the following presentation is somewhat arbitrary and is formed around groups of inhibitors having certain common features. After discussion of 8 such groups, some general considerations of problems and potentials of compounds that inhibit chemical carcinogenesis will be studied.

## INHIBITORS OF CHEMICAL CARCINOGENESIS

### Phenolic Antioxidants and Ethoxyquin

The use of antioxidants as possible inhibitors of the chemical carcinogens has been based in general on the concept that the antioxidants may exert a scavenging effect on the reactive species of carcinogens, thus protecting cell constituents from attack. In early studies, wheat germ oil and $\alpha$-tocopherol were employed. Experiments showing positive and negative results have been published. Confirmatory reports on the positive experiments have not appeared, so that the implications of this work are not clear. These investigations as well as our own experience with $\alpha$-tocopherol, which has not shown it to be inhibitory, have been summarized (*6*). However, under some appropriate conditions, an inhibitory effect possibly does occur.

During the past several years, studies have been performed with other antioxidants. The most extensive work of this type has been done with phenolic compounds, in particular, BHA and BHT. Inhibition occurs under a variety of experimental conditions and with a broad range of chemical carcinogens as shown in table 1. Several nonphenolic antioxidants inhibit chemical carcinogenesis. One of these is ethoxyquin, a widely used antioxidant commonly added to commercial animal feed.

Some studies of mechanism of inhibition by BHA and BHT of chemical carcinogenesis have been per-

Abbreviations used: BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; BP = benzo[*a*]pyrene; DMBA = 7,12-dimethylbenz[*a*]anthracene; DENA = diethylnitrosamine; FAA = *N*-2-fluorenylacetamide; N-OH-FAA = *N*-hydroxy-*N*-2-fluorenylacetamide; N-OH-FA = *N*-hydroxy-*N*-2-fluorenylamine; BP-4,5-oxide, BP-9,10-oxide, BP-7,8-oxide = oxides of BP; 3-HOBP = 3-hydroxybenzo[*a*]pyrene; DMH = *sym*-dimethylhydrazine; DMN = dimethylnitrosamine; MCA = 3-methylcholanthrene.

[1] Department of Laboratory Medicine and Pathology, Medical School, University of Minnesota, Minneapolis, Minn. 55455.

*Editor's note:* Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.

TABLE 1.—*Inhibition of carcinogen-induced neoplasia by phenols, lactones, and ethoxyquin*

| Carcinogen | Inhibitor [a] | Species | Site of neoplasm inhibited |
|---|---|---|---|
| BP | BHA (*7*), ethoxyquin [b] | Mouse | Lung |
| BP | BHA (*6*), BHT (*6*), coumarin, [b] α-angelicalactone [b] | Mouse | Forestomach |
| DMBA | BHA (*7*) | Mouse | Lung |
| DMBA | BHA (*6*), ethoxyquin (*6*) | Mouse | Forestomach |
| DMBA | BHA (*8*), BHT (*8*) | Mouse | Skin |
| DMBA | BHA (*6*), BHT (*6*), ethoxyquin (*6*), coumarin (*9*) | Rat | Breast |
| 7-Hydroxymethyl-12-methylbenz[*a*]anthracene | BHA (*7*) | Mouse | Lung |
| Dibenz[*a*,*h*]anthracene | BHA (*7*) | Mouse | Lung |
| DENA | BHA (*10*), ethoxyquin (*10*) | Mouse | Lung |
| 4-Nitroquinoline-*N*-oxide | BHA (*10*), ethoxyquin (*10*) | Mouse | Lung |
| Uracil mustard | BHA (*7*) | Mouse | Lung |
| Urethan | BHA (*7*) | Mouse | Lung |
| FAA | BHT (*11*) | Rat | Liver |
| N-OH-FAA | BHT (*11*) | Rat | Liver, breast |
| 4-Dimethylaminoazobenzene | BHT (*12*) | Rat | Liver |
| Azoxymethane | BHT (*13*) | Rat | Large intestine |

[a] *Numbers in parentheses* = reference numbers.
[b] Wattenberg LW: Unpublished observations.

formed. One set has been aimed at the determination of the mechanism of inhibition by BHA of BP-induced neoplasia. BP is metabolized by the microsomal mixed-function oxidase system which acts on a wide variety of xenobiotic compounds, including polycyclic aromatic hydrocarbons. Reactive metabolites as well as detoxification products are produced. The effects of administration of BHA on microsomal metabolism of BP in female A/HeJ mice has been studied with experimental conditions similar to those in which BHA inhibits neoplasia due to this carcinogen. Incubation of BP and DNA with liver microsomes from the BHA-fed mice results in approximately half the binding of BP metabolites to DNA as compared to that found with microsomes from control mice (*14*). Investigations have been undertaken to determine if the BP metabolites that are formed when this carcinogen is incubated with liver microsomes prepared from BHA-fed mice differ from those formed in the controls. Of major interest were the effects of BHA feeding on BP-epoxide formation. Formation of BP-4,5-oxide, which can be measured directly by high-pressure liquid chromatography, was reduced with microsomes from BHA-fed mice. BP-9,10-oxide and BP-7,8-oxide cannot be measured directly because of instability. However, data based on summation of diols and phenols resulting, respectively, from the enzymatic and spontaneous conversions of these oxides indicate that they are present in reduced amounts in the microsomal incubations from BHA-fed mice. The major metabolite in microsomal incubations from BHA-fed and control mice was 3-HOBP. This metabolite constituted a significantly higher percentage of the total metabolites formed when BP was incubated with microsomes from BHA-fed mice, as compared to the percentage in control mice. Thus BHA administration causes two metabolic alterations that could result in an inhibitory effect on BP-induced carcinogenesis: 1) a decrease in epoxidation, which is an activation process, and 2) an increase in 3-HOBP, a metabolite of detoxification (*15*).

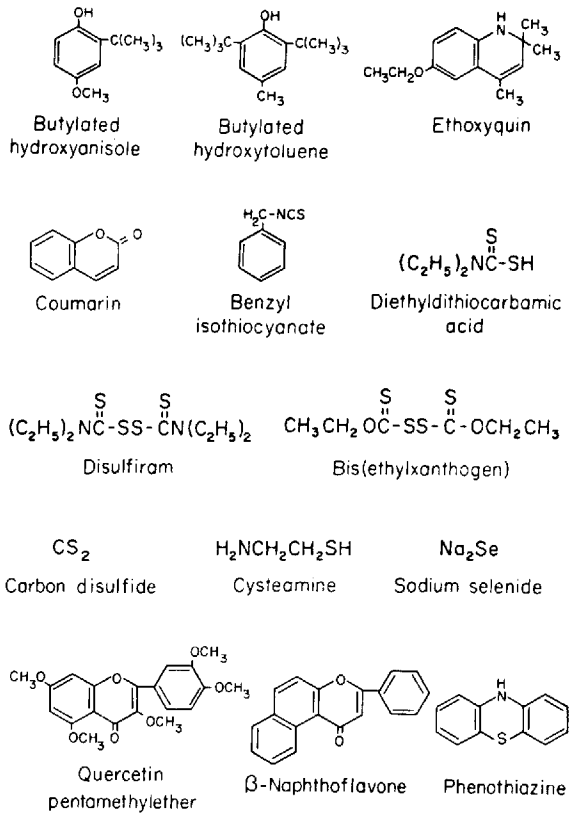
Studies of the mechanism of inhibition of polycyclic hydrocarbon-induced skin carcinogenesis by phenolic antioxidants have been performed by Slaga and Bracken (*8*). In this work, BHA or BHT was applied topically. The animals were killed 3 or 12 hours later, and the binding of [$^3$H]BP or [$^3$H]DMBA to DNA was determined in epidermal homogenates. Both carcinogens showed approximately half as much binding to DNA in homogenates from mice that had received BHA or BHT 3 hours before death than in homogenates from control mice. The inhibition of binding was still apparent at 12 hours but was a lesser order of magnitude.

An early investigation aimed at determining the mechanism of inhibition of carcinogenesis of FAA and N-OH-FAA by BHT was done by Grantham et al. (*16*) who found that administration of BHT in the diet increased excretion of each carcinogen in the urine. This higher level of excretion was accounted for chiefly by glucuronic acid conjugates. Animals receiving BHT showed lower levels of radioactivity in blood, liver, and liver DNA 48 hours after injection with labeled carcinogen. Thus BHT appears to increase detoxification of FAA and N-OH-FAA by enhancing conjugation and thereby reducing the pool of metabolites available for activation reactions.

At the present time BHA probably is the most versatile and least toxic inhibitor of chemical carcinogenesis that has been identified. Although BHT also inhibits a variety of carcinogens, unfortunately it has some noxious properties, including tumor promotion (*17*). An important difference between the two compounds is the nature of the substitution para to the hydroxyl group (text-fig. 1). In BHT this position has a methyl group that oxidizes to toxic metabolites. In BHA, a methoxy group is present in the para position. If metabolism occurs, it is a hydrolysis. The resultant phenolic group can be conjugated, which facilitates the excretion of the compound.

BHA is of interest because of its extensive use as an additive in food for human consumption. Studies of mice have been done in which BHA was added to the

diet with BP, a carcinogen widely encountered in the environment. At a concentration of 5 mg BHA/g diet, the carcinogenic effect of 1 mg BP/g diet on the forestomach of the mouse is inhibited. In the United States, the human consumption of BHA is of the order of several milligrams a day. If one assumes that the results of animal experiments hold for man, this amount of the antioxidant could be important in the inhibition of the effects of chronic exposure to low doses of carcinogens, the type of exposure that is most likely to occur in human populations.




TEXT-FIGURE 1.—Some compounds inhibiting chemical carcinogenesis.

## Disulfiram and Related Compounds

Experimental studies of the capacity of disulfiram (Antabuse; tetraethylthiuram disulfide) and some related compounds to inhibit chemical carcinogenesis have been done with the same experimental models employed for the phenolic antioxidants (table 2). These sulfur-containing compounds are potent inhibitors of BP-induced neoplasia of the forestomach. Work aimed at elucidation of the mechanism of disulfiram inhibition has shown that administration of disulfiram in the diet reduces binding of [$^3$H]BP and [$^{14}$C]BP to DNA, RNA, and protein of the forestomach (*18*). Disulfiram and related compounds are interesting in their effects on carcinogen-induced neoplasia of the large intestine (*3, 19, 20*). When added to the diet, disulfiram, diethyldithiocarbamate, and bisethylxanthogen profoundly inhibit large bowel neoplasia resulting from sc administration of DMH. Work has been done with azoxymethane, an oxidative metabolite of DMH, which also produces neoplasia of the large intestine. Under experimental conditions comparable to those used with DMH, disulfiram has also been found to inhibit azoxymethane-induced neoplasia of the large intestine but to a considerably lesser extent than with DMH as the carcinogen (*19*).

Studies bearing on the mechanism of inhibition of neoplasia of the large bowel by DMH and azoxymethane have shown that disulfiram inhibits the oxidation of both of these carcinogens in vivo (*27–29*). Work concerning the question of whether the inhibitory function resides in the intact molecule of disulfiram or a metabolite of this compound has been done (*28, 29*). These investigations have demonstrated that $CS_2$, a metabolite of disulfiram, inhibits the oxidation of DMH and azoxymethane. The data obtained suggest that $CS_2$ may be the chemical species responsible for the inhibitory action of disulfiram and related compounds. Others have reported that incubation of microsomes with $CS_2$ in the presence of NADPH results in covalent binding of the sulfur to the microsomes, with an accompanying decrease in cytochrome $P_{450}$ as measured spectroscopically

TABLE 2.—*Inhibition of carcinogen-induced neoplasia by sulfur-containing compounds*

| Carcinogen | Inhibitor[a] | Species | Site of neoplasm inhibited |
|---|---|---|---|
| BP | Disulfiram (*18, 21*), bisethylxanthogen (*19*), 2-chloroallyl diethyldithiocarbamate (*19*), *S*-propyl dipropylthiocarbamate (*19*) | Mouse | Forestomach |
| BP | Benzyl isothiocyanate (*22*), phenethyl isothiocyanate (*22*) | Mouse | Forestomach |
| DMBA | Disulfiram (*19*), benzyl isothiocyanate (*22*), phenethyl isothiocyanate (*22*), phenyl isothiocyanate (*22*), benzyl thiocyanate (*22*), cysteamine (*23*) | Rat | Breast |
| 1,2-Dimethylhydrazine | Disulfiram (*3, 19, 20*), diethyldithiocarbamate (*3*), bisethylxanthogen (*19*), carbon disulfide[b] | Mouse | Large intestine |
| Azoxymethane | Disulfiram (*19*) | Mouse | Large intestine |
| FAA | 1-Naphthylisothiocyanate (*24*) | Rat | Liver, ear duct |
| Ethionine | 1-Naphthylisothiocyanate (*24*) | Rat | Liver |
| 3′-Methyl-4-dimethylaminoazobenzene | 1-Naphthylisothiocyanate (*25*) | Rat | Liver |
| 4-Dimethylaminoazobenzene | 2-Naphthylisothiocyanate (*26*) | Rat | Liver |

[a] *Numbers in parentheses*=reference numbers.
[b] Wattenberg LW, Fiala ES: Unpublished observations.

(*30, 31*). When incubated with microsomes under comparable conditions (*31*), several thiono-sulfur-containing compounds, including disulfiram and diethyldithiocarbamate, produce a similar decrease in cytochrome $P_{450}$. This finding raises the possibility that thiono-sulfur-containing compounds as a group may have the potential to modify cytochrome $P_{450}$ so as to alter the microsomal metabolism of DMH, azoxymethane, and possibly other carcinogens in a manner that decreases their carcinogenicity.

The carcinogen-inhibiting effects induced by disulfiram and diethyldithiocarbamate have drawn attention to the possibility that a number of widely used pesticides having dithiocarbamate or thiocarbamate groups might have similar properties; several have been tested. Two of these, i.e., *S*-propyl dipropylthiocarbamate (Vernolate) and 2-chloroallyl diethyldithiocarbamate, when added to the diet, inhibited BP-induced neoplasia of the forestomach of the mouse. An additional pesticide, bisethylxanthogen (Bexide) also exerted an inhibitory effect in this test system. Thus far, bisethylxanthogen is the only one of these pesticides studied for its effects on DMH-induced neoplasia of the large bowel. Its inhibitory potency is similar to that of disulfiram (*19*).

Bisethylxanthogen has a feature that makes it of particular interest: The molecule does not contain nitrogen (text-fig. 1). Structurally similar dithiocarbamate pesticides have been shown to form nitrosamines, representing a hazard not occurring with bisethylxanthogen. A second relationship between disulfiram and nitrosamines has been reported recently (*32*): Disulfiram influences the organotrophy of DENA and DMN. In the case of DENA, disulfiram added to the diet inhibits liver tumor formation but enhances neoplasia of the esophagus. With DMN, neoplasia of the liver is again suppressed, but tumors of the paranasal sinuses are increased.

## Organic Isothiocyanates and Organic Thiocyanates

With studies of the inhibitory capacities of sulfur-containing antioxidants, experiments with benzyl isothiocyanate, phenethyl isothiocyanate, and benzyl thiocyanate have been performed (*22*). These three compounds are naturally occurring constituents of edible cruciferous plants (*33, 34*). Phenyl isothiocyanate, a fourth compound included in some experimental work, is synthetic. The inhibitory effects of these compounds are presented in table 2. 1-Naphthylisothiocyanate and 2-naphthylisothiocyanate have both been shown to suppress the neoplastic effects of azo dyes on the liver. Of interest are studies showing that 1-napthylisothiocyanate alters cytochrome $P_{450}$ in a manner comparable to that of thiono-sulfur-containing compounds (*30*).

## Lactones

Coumarins are constituents of a wide variety of plants, including vegetables, used for human consumption (*35*). The administration of coumarin by oral intubation inhibits DMBA-induced mammary tumor formation (*9*). Additional studies have been done with BP-induced neoplasia of the forestomach as the test system. When added to the diet, coumarin, 6-nitrocoumarin, and $\alpha$-angelicalactone inhibited forestomach tumor formation (Wattenberg LW: Unpublished observations).

## Selenium Salts

Inhibition of chemical carcinogenesis by selenium salts has been reported. In an initial paper, the experimental system employed consisted of initiation of epidermal neoplasia with DMBA followed by promotion with croton oil; sodium selenide added to the croton oil suppressed the development of skin tumors (*36*). In subsequent work, MCA was repeatedly applied to the skin. Again, addition of sodium selenide inhibited epidermal neoplasia. In a further experiment, mice were placed on a selenium-deficient diet (Torula yeast) without supplements or with added sodium selenide or sodium selenite. BP was applied to the skin daily to produce epidermal neoplasia. A slight inhibition was found with both of the selenium salts (*37*).

Recently, the addition of sodium selenite to the drinking water has been shown to inhibit large bowel neoplasia in the rat, which results from administration of DMH or methylazoxymethanol acetate (*38, 39*). In additional works, selenium was found to reduce mutagenicity of FAA, N-OH-FAA, and *N*-hydroxy-*N*-2-fluorenylamine for the *Salmonella typhimurium* T-1538 histidine mutant (*39*). Evidence has been presented showing an inverse relationship between *a*) the amount of selenium in soil and forage crops and *b*) human cancer death rates in the United States and Canada in 1965. Likewise, an inverse relationship between human blood levels of selenium and human cancer death rates in several cities has been reported (*40*).

## Inducers of Microsomal Mixed-Function Oxidase Activity

Several studies have demonstrated that protection against chemical carcinogens by the administration of inducers of increased microsomal mixed-function oxidase activity is possible (table 3). The inducers employed have varied from compounds such as polycyclic hydrocarbons, which are noxious agents, to chemicals such as flavones, which have little toxicity (*41, 42*). In early studies (*43, 44*), administration of polycyclic hydrocarbon inducers inhibited the occurrence of hepatic cancer resulting from feeding 3′-methyl-4-dimethylaminoazobenzene. Likewise, polycyclic hydrocarbon inducers can markedly reduce the incidence of tumors of the liver, mammary gland, ear duct, and small intestine in rats fed FAA or 7-fluoro-*N*-2-fluorenylacetamide (*44*).

More recently, studies have been done in which protection against the carcinogenic effects of a number of other carcinogens has been observed (table 3). Considerable work has been done with two polycyclic hydrocarbon carcinogens, DMBA and BP. With the use of the pulmonary adenoma test system in the mouse, flavone inducers have been shown to inhibit lung tumor formation resulting from oral administration of DMBA or BP (*41, 42*). An experimental model that has been

TABLE 3.—*Inhibition of carcinogenesis by inducers of increased microsomal enzyme activity*

| Carcinogen | Inducer[a] | Species | Organ |
|---|---|---|---|
| 3'-Methyl-4-dimethylaminoazobenzene | Polycyclic hydrocarbons (*43, 44*), α-benzene hexachloride (*45*), polychlorinated biphenyls (*46*) | Rat | Liver |
| FAA | Polycyclic hydrocarbons (*44*), polychlorinated biphenyls (*45*) | Rat | Liver, breast, small intestine |
| 4-Dimethylaminostilbene | Polycyclic hydrocarbons (*47*) | Rat | Ear duct |
| Urethan | Phenobarbital (*48–50*), chlordane (*50*) | Mouse | Lung |
| BP | β-Naphthoflavone (*42*), quercetin pentamethylether (*42*) | Mouse | Lung |
| BP | β-Naphthoflavone (*42*) | Mouse | Skin |
| DMBA | β-Naphthoflavone (*41*) | Mouse | Lung |
| DMBA | β-Naphthoflavone (*41*), phenothiazines (*51*), polycyclic hydrocarbons (*52, 53*) | Rat | Breast |
| Aflatoxin | Phenobarbital (*54*) | Rat | Liver |
| Bracken fern | Phenothiazine (*55*) | Rat | Small intestine, bladder |

[a] *Numbers in parentheses* = reference numbers.

widely used in studies of the effects of inducers of increased mixed-function oxidase activity has been mammary tumor formation in rats given DMBA. Several different types of inducers administered prior to DMBA will inhibit tumor formation. These include: polycyclic hydrocarbons (*52, 53*), phenothiazines (*51*), and flavones (*41*).

The experiments listed in table 3 show a protective effect from administration of inducers of increased mixed-function oxidase activity; however, an apparently conflicting set of data should also be discussed. For many chemical carcinogens, the microsomal mixed-function oxidase system has been demonstrated to convert these compounds to a proximate carcinogenic form (*56*). An initial thought might be that if a compound is activated by an enzyme system to a noxious form, then enhancement of the activity of this system would result in greater damage to the organism. This phenomenon is true in situations involving a reversible effect in which a substantial threshold exists. Rapid activation could be important in achieving such a threshold. However, for chemical carcinogenesis, different conditions exist. In this instance, there appears to be either no threshold or a very low threshold (*57*). Thus one might anticipate that slow activation would result in an equal or even a greater carcinogenic effect than does rapid activation. With slow activation, wastage of activated species of carcinogen from cells would be less likely to occur from production of an excess amount over that most effective for the number of critical binding sites available at a particular time. In addition, active carcinogenic species would be present over a longer period and, therefore, would be more likely to exist at a critical time or times in the cell cycle.

Another factor that may be important in the explanation of carcinogen inhibition by induction of increased mixed-function oxidase activity is that in many instances this system subjects chemical carcinogens to detoxification reactions as well as to activation. The classic example of this double effect is found with the aromatic amines. With these compounds, ring hydroxylation results in detoxification, whereas hydroxylation of the nitrogen is an activation reaction (*58*). Thus administration of inducers of mixed-function oxidase activity in these instances may cause a relatively greater proportion of the carcinogen to be detoxified rather than activated to a carcinogenic metabolite. Changes in proportion of detoxified metabolites to carcinogenic metabolites could simply be the result of relative responses of the two pathways to the inducer. An alternative possibility suggested by the studies with BHA is that a more basic alteration in metabolism may occur, resulting in a changed metabolite pattern. In this instance, the change in metabolite pattern could be independent of the magnitude of induction and, as in the case of BHA, might occur without any overall increase in mixed-function oxidase activity. Further work is required to ascertain the mechanism of action of the compounds discussed in this section which have already been shown to have the combined properties of increasing mixed-function oxidase activity and inhibiting chemical carcinogenesis.

Phenobarbital induces increased mixed-function oxidase activity, an increase in endoplasmic reticulum, and liver enlargement. When administered prior to the carcinogen, it suppresses neoplasia (table 3). However, if it is given subsequent to the carcinogen, the neoplastic response may be enhanced (*59*). This cocarcinogenic effect represents a hazard that requires evaluation with respect to other inducers.

## Inhibitors of Microsomal Mixed-Function Oxidase Activity

If the activity of the microsomal mixed-function oxidase system were totally absent, carcinogens requiring activation by this system would not produce a neoplastic effect. Efforts have been made to inhibit chemical carcinogenesis by this mechanism. Studies of suppression of polycyclic hydrocarbon-induced neoplasia have been done with 7,8-benzoflavone (α-naphthoflavone), a potent inhibitor of microsomal mixed-function oxidase activity. In experiments in which DMBA was the carcinogen, epidermal neoplasia has been inhibited (*60*). A problem with exploitation of inhibition of mixed-function oxidase activity as a means of suppressing chemical carcinogenesis is that it would render the organism more susceptible to the noxious effects of xenobiotic

compounds detoxified by this system.

### Physiologic Trapping Agents

Active forms of chemical carcinogens bind to a wide variety of physiologic compounds. Their carcinogenic potential resides in such reactivity occurring at selective sites. However, if the carcinogenic agent is trapped by other cell nucleophiles, protection might occur. Numerous biochemical compounds contain nucleophilic groups. These have been discussed by Miller and Miller (*61*). A question arises as to whether the amount of one or more of such nucleophiles might be increased so as to protect against chemical carcinogens. A compound of particular interest in this regard is glutathione, which is an excellent trapping agent. However, the level of glutathione has been difficult to increase by experimental procedures. In contrast, a number of extrinsic factors, particularly administration of toxic compounds, can deplete glutathione. The levels and control mechanisms of protective cell nucleophiles could be of great importance in the response to chemical carcinogens. Work aimed at a fuller understanding and exploitation of this potential defense certainly is warranted.

## DISCUSSION

With the knowledge that inhibitors of chemical carcinogenesis exist, two questions arise. The first relates to the current role that these compounds have in reducing the impact of environmental carcinogens on man. The second is the optimal role that they might have. To assess their current role, additional information is required as to the full spectrum of compounds in the environment that have the capacity to inhibit carcinogens. A perusal of text-figure 1 and tables 1–3 shows that compounds with a broad range of chemical structures can inhibit chemical carcinogenesis. The diversity indicates that this capacity to inhibit does not reside in restricted chemical characteristics and suggests that other inhibitors that have not yet been identified almost certainly exist. The quest to identify these inhibitors is important in order to correctly account for their impact. Also of critical importance is a full elucidation of mechanisms of inhibition. Such an understanding could provide information that could assist in the identification of compounds in the environment that are inhibitors. In addition, mechanisms of inhibition that entail measurable biochemical parameters could provide a basis for assessment of the susceptibility of particular population groups or individuals to neoplasia from chemical carcinogens.

An important aspect of the evaluation of the role of inhibitors is a consideration of toxicity. Inhibitors currently identified have, to a greater or lesser extent, other biologic activities. A number have toxic properties. Some are even carcinogens or cocarcinogens. However, noxious properties clearly can be dissected away for the basic mechanisms of inhibition. Thus among phenolic inhibitors, BHA is considerably less toxic than BHT and is a more effective inhibitor. In early studies in which protection was obtained by induction of increased microsomal mixed-function oxidase activity, the potent carcinogen MCA was used. Flavones and phenothiazines cited in table 3 inhibit by inducing increased mixed-function oxidase activity, but they are not carcinogens. As more is learned about the basic requirements for inhibition, compounds with increasing inhibitory potency and fewer side effects probably can be found. This point should be stressed, since the currently available inhibitors are a first-generation group. Many have been discovered by chance, or if a particular mechanism was being explored, compounds that were readily available were used. More complete information should enable the design of inhibitors in which unnecessary biologic activities are pealed away, providing less toxic and more effective compounds.

Considerations of the optimal role that inhibitors of carcinogenesis might play entail evaluations of their deliberate use. At present, such a meaure would clearly be premature, because we simply do not have an adequate base of information. However, at a future time when more data are available on mechanisms of inhibition, diversity of inhibitors, and their toxicity, this course of action might be entertained. Accordingly, there would be some value in considering possible criteria that would have to be met prior to deliberate usage of inhibitors of chemical carcinogenesis. I believe that the following criteria are prerequisite to seriously considering deliberate exposure of human populations to a particular inhibitor of chemical carcinogenesis:

Populations with "normal" risk of exposure to chemical carcinogens:

1) The inhibitor has no toxicity or trivial toxicity and/or
2) Human population groups have been exposed inadvertently to the inhibitor (a "natural" experiment in man), and it has been shown to be effective in reducing the incidence of carcinogen-induced neoplasia in appropriate epidemiologic studies. In addition, no evidence of the toxic properties are associated with the inhibitor.

Populations with high risk of exposure to chemical carcinogens:

1) Use of an inhibitor for which there is evidence that the benefits outweigh risks.

For any normal group of individuals, a critical restraint is the possibility of toxicity. An inhibitor would have to be taken by individuals for many years to be effective, so that even a low toxicity could outweigh any benefits. However, selected situations occur in which this formidable obstacle might be overcome. One specific instance entails carcinogens within the gastrointestinal tract. In this instance, an inhibitor that would not be absorbed could be designed. Under such conditions, a compound with little or no toxicity might be available. The importance of these types of considerations is made real by recent findings of mutagenic substances in the feces (*62*). If these substances are in fact carcinogens, as well

as mutagens, efforts at finding effective inhibitors active within the large bowel might be warranted. In other sites, specific situations amenable to selective approaches might exist as well.

A second basis for introduction of an inhibitor into the environment would be the acquisition of favorable data from epidemiologic investigations. Such data would include firm evidence that a population group with a significant intake of a particular inhibitor has a diminished incidence of one or more neoplasms. Mechanistic data relating the intake of the inhibitor to carcinogen inhibition, i.e., tissues from the particular population group showing an increased capacity to detoxify carcinogens, would be important. In addition, lack of toxicity from the inhibitor should be clearly evident. Under these conditions, consideration of the use of the material bringing about the inhibition would be warranted. This set of circumstances, in essence, forms a natural or unplanned type of experiment. Depending on the magnitude of the inhibition and reliability of estimates of lack of adverse side effects, convincing data could be provided for deliberate use of the substance.

Population groups with elevated exposures to chemical carcinogens do exist. For individuals of this type, less rigid requirements for lack of toxicity of inhibitors might be justified. With regard to this possibility, an exceedingly important prohibition is that inhibitors should not be used as mechanisms for allowing increased exposures to carcinogens or for increasing tolerance levels to cancer-producing substances.

## REFERENCES


(1) Sporn MB, Dunlop NM, Newton DL, et al: Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Fed Proc 35:1332–1338, 1976

(2) Mirvish SS, Cardesa A, Wallcave L, et al: Induction of mouse lung adenomas by amines or urea plus nitrite and by N-nitroso compounds: Effects of ascorbate, gallic acid, thiocyanate, and caffeine. J Natl Cancer Inst 55:633–636, 1975

(3) Wattenberg LW: Inhibition of chemical carcinogenesis by antioxidants and some additional compounds. *In* Fundamentals of Cancer Prevention. Proceedings of the Sixth International Symposium of the Princess Takamatsu Cancer Research Fund (Magee PN, Takayama S, Sigimura T, et al, eds). Baltimore: University Park Press, 1976, pp 153–166

(4) Wattenberg LW, Loub WD, Lam LK, et al: Dietary constituents altering the responses to chemical carcinogens. Fed Proc 35:1327–1331, 1976

(5) Wattenberg LW: Inhibitors of chemical carcinogenesis. *In* Origins of Human Cancer, Cold Spring Harbor Symposium on Quantitative Biology. Saddle Brook, N.J.: American Book-Stratford Press. In press

(6) ———: Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. J Natl Cancer Inst 48:1425–1430, 1972

(7) ———: Inhibition of chemical carcinogen-induced pulmonary neoplasia by butylated hydroxyanisole. J Natl Cancer Inst 50:1541–1544, 1973

(8) Slaga TJ, Bracken WM: The effects of antioxidants on skin tumor initiation and aryl hydrocarbon hydroxylase. Cancer Res 37:1631–1635, 1977

(9) Feuer G, Kellen JA, Kovacs K: Suppression of 7,12-dimethylbenz[*a*]anthracene induced carcinoma by coumarin in the rat. Oncology 33:35–39, 1976

(10) Wattenberg LW: Inhibition of carcinogenic effects of diethylnitrosamine and 4-nitroquinoline-*N*-oxide by antioxidants. Fed Proc 31:633, 1972

(11) Ulland BM, Weisburger JH, Yamamoto RS, et al: Antioxidants and carcinogenesis: Butylated hydroxytoluene, but not diphenyl-*p*-phenylenediamine, inhibits cancer induction by *N*-2-fluorenylacetamide and by *N*-hydroxy-*N*-2-fluorenylacetamide in rats. Food Cosmet Toxicol 11:199–207, 1973

(12) Frankfurt OS, Lipchina LP, Bunto TV, et al: The influence of 4-methyl-2,6-tertbutylphenol (Ionol) on the development of hepatic tumors in rats. Bull Exp Biol Med 8:86–88, 1967

(13) Weisburger EK, Evarts RP, Wenk ML: Inhibitory effect of butylated hydroxytoluene (BHT) on intestinal carcinogenesis in rats by azoxymethane. Food Cosmet Toxicol. In press

(14) Speier JL, Wattenberg LW: Alterations in microsomal metabolism of benzo[*a*]pyrene in mice fed butylated hydroxyanisole. J Natl Cancer Inst 55:469–472, 1975

(15) Lam LK, Wattenberg LW: Effects of butylated hydroxyanisole on the metabolism of benzo[*a*]pyrene by mouse liver microsomes. J Natl Cancer Inst 58:413–417, 1977

(16) Grantham PH, Weisburger JH, Weisburger EK: Effect of the antioxidant butylated hydroxytoluene on the metabolism of the carcinogens *N*-2-fluorenylacetamide and *N*-hydroxy-*N*-2-fluorenylacetamide. Food Cosmet Toxicol 11:209–217, 1973

(17) Witschi H, Williamson D, Lock S: Enhancement of urethan tumorigenesis in mouse lung by butylated hydroxytoluene. J Natl Cancer Inst 58:301–305, 1977

(18) Borchert P, Wattenberg LW: Inhibition of macromolecular binding of benzo[*a*]pyrene and inhibition of neoplasia by disulfiram in the mouse forestomach. J Natl Cancer Inst 57:173–179, 1976

(19) Wattenberg LW, Lam LK, Fladmoe A, et al: Inhibitors of colon carcinogenesis. Cancer. In press

(20) Wattenberg LW: Inhibition of dimethylhydrazine-induced neoplasia of the large intestine by disulfiram. J Natl Cancer 54:1005–1006, 1975

(21) ———: Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by several sulfur-containing compounds. J Natl Cancer Inst 52:1583–1587, 1974

(22) ———: Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. J Natl Cancer Inst 58:395–398, 1977

(23) Marquardt H, Sapozink M, Zedeck M: Inhibition by cysteamine-HCl of oncogenesis induced by 7,12-dimethylbenz-[*a*]anthracene without affecting toxicity. Cancer Res 34:3387–3390, 1974

(24) Sidransky H, Ito N, Verney E: Influence of α-naphthyl-isothiocyanate on liver tumorigenesis in rats ingesting ethionine and *N*-2-fluorenylacetamide. J Natl Cancer Inst 37:677–686, 1966

(25) Sasaki S: Inhibitory effects of alpha-naphthyl isothiocyanate on the development of hepatoma in rats treated with 3'-methyl-4-dimethylaminoazobenzene. J Nara Med Assoc 14:101–115, 1963

(26) Lacassagne A, Hurst L, Xuong MD: Inhibition par deux naphthoisothiocyanates de l'hépatocarcérogenèse produite, chez le rat par le p-diméthyl-amino-azobenzène (DAB). CR Soc Biol (Paris) 164:230–233, 1970

(27) Fiala ES, Bobotas G, Kulakis C, et al: Inhibition of 1,2-dimethylhydrazine metabolism by disulfiram. Xenobiotica 7:5–9, 1976

(28) Fiala ES, Bobotas G, Kulakis C, et al: The effects of disulfiram and related compounds on the in vivo metabolism of the colon carcinogen 1,2-dimethylhydrazine. Biochem Pharmacol. In press

(29) Fiala ES: Investigations into the metabolism and mode of action of the colon carcinogens 1,2-dimethylhydrazine and azoxymethane. Cancer. In press

(30) DeMatteis F: Covalent binding of sulfur to microsomes and loss of cytochrome P-450 during the oxidative desulfuration of several chemicals. Mol Pharmacol 10:849–854, 1974

(31) Hunter AL, Neal RA: Inhibition of hepatic mixed-function oxidase activity in vitro and in vivo by various thiono-sulfur-containing compounds. Biochem Pharmacol 24:2199–2205, 1975

(32) Schmähl D, Krüger FW, Habs M, et al: Influence of disulfiram

on the organotropy of the carcinogenic effect of dimethylnitrosamine and diethylnitrosamine in rats. Z Krebsforsch 85:271–276, 1976

(33) VIRTANEN AI: Some organic sulfur compounds in vegetables and fodder plants and their significance in human nutrition. Angew Chem [Engl] 1:299–306, 1962

(34) LICHTENSTEIN EP, STRONG FM, BORGAN DG: Identification of 2-phenylisothiocyanate as an insecticide occurring naturally in edible parts of turnips. Agriculture Fd Chem 10:30–33, 1962

(35) FEUER G: The metabolism and biological action of coumarins. Prog Med Chem 10:85–157, 1973

(36) SHAMBERGER RJ: Protection against cocarcinogenesis by antioxidants. Experientia 22:116, 1966

(37) ———: Relationship of selenium to cancer. I. Inhibitory effect of selenium on carcinogenesis. J Natl Cancer Inst 44:931–936, 1970

(38) JACOBS MN, JANSSON B, GRIFFIN AC: Inhibitory effects of selenium on 1,2-dimethylhydrazine and methylazoxymethanol acetate induction of colon tumors. Cancer Lett. In press

(39) JACOBS MN: Inhibitory effects of selenium on 1,2-dimethylhydrazine and methylazoxymethanol colon carcinogenesis. Cancer. In press

(40) SHAMBERGER R, WILLIS C: Selenium distribution and human cancer mortality. Clin Lab Sci 2:211–221, 1971

(41) WATTENBERG LW, LEONG JL: Inhibition of the carcinogenic action of 7,12-dimethylbenz[*a*]anthracene by beta-naphthoflavone. Proc Soc Exp Biol Med 128:940–943, 1968

(42) ———: Inhibition of the carcinogenic action of benzo[*a*]pyrene by flavones. Cancer Res 30:1922–1925, 1970

(43) RICHARDSON HL, STEIN AR, BORSON-NACHT-NEBEL E: Tumor inhibition and adrenal histologic responses in rats in which 3′-methyl-4-dimethylaminoazobenzene and 20-methylcholanthrene were simultaneously administered. Cancer Res 12:356–371, 1952

(44) MILLER EC, MILLER JA, BROWN RR, et al: On the protective action of certain polycyclic aromatic hydrocarbons against carcinogenesis by aminoazo dyes and 2-acetylaminofluorene. Cancer Res 18:469–477, 1958

(45) THAMAVIT W, HIASA Y, ITO N, et al: The inhibitory effects of α-benzene hexachloride on 3′-methyl-4-dimethylaminoazobenzene and DL-ethionine carcinogenesis in rats. Cancer Res 34:337–340, 1974

(46) MAKIURA SH, AOE S, SIGIHARA S, et al: Inhibitory effect of polychlorinated biphenyls on liver tumorigenesis in rats treated with 3′-methyl-4-dimethylaminoazobenzene, *N*-2-fluorenylacetamide, and diethylnitrosamine. J Natl Cancer Inst 53:1253–1257, 1974

(47) TAWFIC HN: Studies on ear duct tumors in rats. II. Inhibitory effects of methylcholanthrene and 1,2-benzanthracene on tumor formation by 4-dimethylaminostilbene. Acta Pathol Jpn 15:255–260, 1965

(48) ADENIS L, VLAEMINCK MN, DRIESSENS J: L'adénome pulmonaire de la souris Swiss recevant de l'uréthane. 8. Action du phénobarbital. C R Soc Biol (Paris) 164:560–652, 1970

(49) SILVA EA: Da acaó inhibitória do prétratamento com fenobarbital sóbre a atividade carcinogenica pulmonar da uretana etilica em camundongos. Hosp Rio de Janeiro 71:1483–1493, 1967

(50) YAMAMOTO RS, WEISBURGER JH, WEISBURGER EK: Controlling factors in urethane carcinogenesis in mice: Effects of enzyme inducers and metabolic inhibitors. Cancer Res 31:483–486, 1971

(51) WATTENBERG LW, LEONG JL: Inhibition of 9,10-dimethylbenzanthracene (DMBA) induced mammary tumorigenesis by phenothiazines. Fed Proc 26:692, 1967

(52) HUGGINS C, LORRAINE G, FUKUNISHI R: Aromatic influences on the yields of mammary cancers following administration of 7,12-dimethylbenz[*a*]anthracene. Proc Natl Acad Sci USA 51:737–742, 1964

(53) WHEATLEY DN: Enhancement and inhibition of the induction by 7,12-dimethylbenz[*a*]anthracene of mammary tumours in female Sprague-Dawley rats. Br J Cancer 22:787–792, 1968

(54) MCLEAN AE, MARSHALL A: Reduced carcinogenic effects of aflatoxin in rats given phenobarbitone. Br J Exp Pathol 52:322–329, 1971

(55) PAMUKCU AM, WATTENBERG LW, PRICE JM, et al: Phenothiazine inhibition of intestinal and urinary bladder tumors induced in rats by bracken fern. J Natl Cancer Inst 47:155–159, 1971

(56) MILLER EC, MILLER JA: Biochemical mechanisms of chemical carcinogenesis. *In* The Molecular Biology of Cancer (Busch H, ed). New York: Academic Press, 1974, pp 377–342

(57) DIPAOLO JA, DONOVAN PJ, NELSON RL: In vitro transformation of hamster cells by polycyclic hydrocarbons: Factors influencing the number of cells transformed. Nature [New Biol] 230:240–242, 1971

(58) MILLER JA, MILLER EC: The metabolic activation of carcinogenic aromatic amines and amides. Prog Exp Tumor Res 11:273–301, 1969

(59) PERAINO C, FRY RJ, STAFFELDT E, et al: Effects of varying exposure to phenobarbital on its enhancement of 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat. J Natl Cancer Inst 33:2701–2705, 1973

(60) GELBOIN HV, WIEBEL F, DIAMOND L: Dimethylbenzanthracene tumorigenesis and aryl hydroxylase in mouse skin: Inhibition by 7,8-benzoflavone. Science 170:169–171, 1970

(61) MILLER JA, MILLER EC: Some current thresholds of research in chemical carcinogenesis. *In* Chemical Carcinogenesis (Ts'o PO, DiPaolo JA, eds). New York: Marcel Dekker, 1974, pp 61–86

(62) VARGHESE AJ, LAND P, FURRER R, et al: Evidence for the formation of mutagenic N-nitroso compounds in the human body. Proc Am Assoc Cancer Res 18:80, 1977