INHIBITORS OF CHEMICAL CARCINOGENS

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A diverse group of compounds inhibit the action of chemical carcinogens when administered prior to and/or simultaneously with the carcinogen. The inhibitors include naturally-occurring constituents of foods as well as synthetic compounds introduced into the environment. Three general mechanisms of inhibition exist. The first, illustrated by disulfiram inhibition of dimethylhydrazine-induced neoplasia of the large bowel, is the direct blocking of enzymatic activation of the carcinogen to its reactive ultimate carcinogenic form. The second mechanism of inhibition entails the stimulation of a coordinated detoxification response which results in increased activity of detoxifying enzymes in the microsomes and also the cytosol. At least two subdivisions of this response occur. One, for which butylated hydroxyanisole is a prototype, shows enhanced activity of some microsomal enzymes but not aryl hydrocarbon hydroxylase (AHH). However, it does have a rapidly active component which results in marked alteration of microsomal metabolism of benz(a)pyrene. Another, for which a prototypical inhibitor is β-naphthoflavone is characterized by induction of increased AHH activity. The third general mechanism of carcinogen inhibition entails the direct scavenging of reactive carcinogenic species by the inhibitor.

Evidence supporting the possibility that inhibitors play a role in the response of humans to carcinogens consists of three types. The first is the chemical diversity of the inhibitors and their actual occurrence in the environment. The second is the responsiveness of the detoxification systems, particularly those in the tissues of the major portals of entry, to the naturally-occurring or synthetic inhibitors. The third is a group of epidemiological studies which suggest that individuals consuming relatively large quantities of vegetables, a major source of naturally-occurring inhibitors, are at lower risk from gastrointestinal cancers.
INTRODUCTION

An increasing number and diversity of compounds have been found to inhibit the neoplastic effects of chemical carcinogens when administered prior to and/or simultaneously with the carcinogen. These inhibitors encompass a wide range of chemical structures (Figure 1). They include naturally-occurring constituents of foods, particularly vegetables and fruit. They also include synthetic compounds, some of which are food additives (Wattenberg, 1979a, b, c). The time relationships between administration of the inhibitors and carcinogens, as well as studies of mechanisms of inhibition, indicate that this group of inhibitors can act by preventing carcinogenic species from reaching or reacting with critical cellular targets. In essence, they exert a barrier function. Data will be presented indicating that in some instances inhibition of neoplasia occurs via a selective carcinogen inactivation mechanism whereas in other circumstances detoxification systems having the capacity to inhibit a wide range of carcinogens are involved.

The first group of inhibitors to be discussed is disulfiram and related compounds. These inhibitors represent an example of compounds which inhibit chemical carcinogenesis by directly preventing enzymatic activation of a carcinogen to its ultimate reactive form. The second group of inhibitors includes butylated hydroxyanisole (BHA) and related compounds. BHA is quite remarkable in the range of carcinogens it inhibits. A third group of inhibitors are those which have in common the capacity to induce increased aryl hydrocarbon hydroxylase (AHH) activity. The mechanisms of inhibition of carcinogenesis by BHA and related compounds and by inducers of increased AHH activity will be discussed subsequently. They are complex and appear to entail a coordinated detoxification response in which a number of enzymes, both microsomal and in the cytosol, are involved. Inhibition by the compounds which have the capacity to scavenge reactive carcinogenic species will also be discussed.

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Disulfiram and Related Compounds

Disulfiram and related compounds are of particular interest for their effects on carcinogen-induced neoplasia of the large intestine. Disulfiram, diethylthiocarbamate, and bis(methylxanthogen) when added to the diet profoundly inhibit large bowel neoplasia resulting from subcutaneous administration of symmetrical 1,2-dimethylhydrazine (DMH) (Wattenberg, 1975). Similar studies have been carried out with azoxymethane, an oxidative metabolite of DMH. Under comparable experimental conditions to those used with DMH, disulfiram has also been found to inhibit azoxymethane-induced neoplasia of the large intestine but to a considerably less extent than with DMH as the carcinogen (Wattenberg, 1975; Wattenberg et al., 1977a, b). Studies of 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 (Fiala et al., 1977). Work bearing on the question of
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- Butylated hydroxyanisole
- Butylated hydroxytoluene
- Ethoxyquin

- Coumarin
- p-Hydroxycinnamic acid
- Benzyloisothiocyanate

- Disulfiram
- Bis(ethylxanthogen)

- Carbon disulfide
- Cysteamine
- Sodium selenide

- Indole-3-acetonitrile
- Indole-3-carbinol
- 3,3'-Diindolymethane

- Quercetin pentamethylether
- β-Naphthoflavone
- Phenothiazine
whether the inhibitory function resides in the intact molecule of disulfiram or a metabolite of this compound has been carried out. These investigations have demonstrated that CS₂, a metabolite of disulfiram inhibits the oxidation of DMH and azoxymethane. CS₂ itself, has been found to inhibit DMH-induced neoplasia of the large intestine in the mouse (Wattenberg and Fiala, 1978). The carcinogen-inhibiting effects brought about by disulfiram and diethylthiocarbamate have drawn attention to the possibility that pesticides containing a carbon disulfide moiety in their chemical structure might have similar properties. A number of such pesticides have been used in agriculture. Two of these studied thus far have been found to inhibit DMH-induced neoplasia of the large bowel in mice. The compounds are ethylene bis(dithiocarbamate) manganese (Maneb) and bis(ethylxanthonogen) (Bexide) (Wattenberg et al, 1977b).

Bis(ethylxanthonogen) has a feature that makes it of special interest. The molecule does not contain nitrogen. Structurally related pesticides have been shown to form nitrosamines, representing a hazard not existing with bis-(ethylxanthongen). A second relationship between disulfiram and nitrosamines has been reported by Schmahl et al. (1976). Disulfiram influences the organotrophy of diethyl nitrosoamine (DENA) and dimethyl nitrosoamine (DMNA). In the case of DENA, disulfiram added to the diet inhibits liver tumor formation but enhances neoplasia of the esophagus. With DMNA, suppression of neoplasia of the liver again is found but there is an increase in tumors of the paranasal sinuses.

**Phenolic antioxidants and ethoxyquin**

Investigations of the inhibitory effects of two phenolic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been carried out with a number of chemical carcinogens. These two antioxidants are of interest in that they are widely employed as food additives. Inhibition occurs under a variety of experimental conditions and with a broad range of carcinogens as will be seen in Table 1. Recently the naturally-occurring plant phenols, p-hydroxycinnamic acid and o-hydroxycinnamic acid, have also been found to be inhibitors (Wattenberg, 1979b). Ethoxyquin, a non-phenolic antioxidant, also inhibits chemical carcinogens.

A diversity of experimental models and administration schedules have been used in the experiments listed in Table 1. In a typical one, the inhibitor is fed in the diet for about a week prior to an initial administration of the carcinogen and the feeding is continued until all doses of the carcinogen have been given. One variation on this format is for the inhibitor to be administered either by oral intubation or parenterally prior to each administration of the carcinogen. A second variation is to include both the inhibitor and carcinogen in the diet. All of these time relationships between inhibitor and carcinogen administrations are those to be expected from inhibitors which enhance carcinogen detoxification or in some other manner prevent the active form of the carcinogen from reaching or reacting with critical cellular target sites.

In addition to inhibition of carcinogenesis, BHA has been found to inhibit mutagenesis resulting from administration of known carcinogens and some additional mutagenic compounds in which the capacity to produce neoplasms
<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>Antioxidant</th>
<th>Species</th>
<th>Site of neoplasm inhibited</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benz[a]pyrene</td>
<td>BHA, ethoxyquin</td>
<td>Mouse</td>
<td>Lung</td>
<td>Wattenberg 1973</td>
</tr>
<tr>
<td>Benz[a]pyrene</td>
<td>BHA, BHT, p-hydroxy-cinnamic acid</td>
<td>Mouse</td>
<td>Foregut</td>
<td>Wattenberg 1972a</td>
</tr>
<tr>
<td>7,12-Dimethylbenz[a]anthracene</td>
<td>BHA</td>
<td>Mouse</td>
<td>Lung</td>
<td>Wattenberg 1973</td>
</tr>
<tr>
<td>7,12-Dimethylbenz[a]anthracene</td>
<td>BHA, ethoxyquin</td>
<td>Mouse</td>
<td>Foregut</td>
<td>Wattenberg 1972a</td>
</tr>
<tr>
<td>7,12-Dimethylbenz[a]anthracene</td>
<td>BHA, BHT</td>
<td>Mouse</td>
<td>Skin</td>
<td>Slaga and Bracken 1977</td>
</tr>
<tr>
<td>7,12-Dimethylbenz[a]anthracene</td>
<td>BHA, BHT, ethoxyquin</td>
<td>Rat</td>
<td>Breast</td>
<td>Wattenberg 1972a</td>
</tr>
<tr>
<td>7-Hydroxy-12-methylbenz[a]anthracene</td>
<td>BHA</td>
<td>Mouse</td>
<td>Lung</td>
<td>Wattenberg 1973</td>
</tr>
<tr>
<td>Diben[a],[b]anthracene</td>
<td>BHA</td>
<td>Mouse</td>
<td>Lung</td>
<td>Wattenberg 1973</td>
</tr>
<tr>
<td>Diethylnitrosamine</td>
<td>BHA, ethoxyquin</td>
<td>Mouse</td>
<td>Lung</td>
<td>Wattenberg 1972b</td>
</tr>
<tr>
<td>4-Nitroquinoline-N-oxide</td>
<td>BHA, ethoxyquin</td>
<td>Mouse</td>
<td>Lung</td>
<td>Wattenberg 1972b</td>
</tr>
<tr>
<td>Uracil mustard</td>
<td>BHA</td>
<td>Mouse</td>
<td>Lung</td>
<td>Wattenberg 1973</td>
</tr>
<tr>
<td>Urethane</td>
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<td>Lung</td>
<td>Wattenberg 1973</td>
</tr>
<tr>
<td>Methylazoxymethanol acetate</td>
<td>BHA</td>
<td>Mouse</td>
<td>Large intestine</td>
<td>Wattenberg and Sparbins 1979a</td>
</tr>
<tr>
<td>trans-5-Amino-3-[2-(5-nitro-2-furyl)-vinyl]-1,2,4-oxadiazole</td>
<td>BHA</td>
<td>Mouse</td>
<td>Foregut, lung lymphomas</td>
<td>Bueiding et al 1978</td>
</tr>
<tr>
<td>Beta-Propiolactone</td>
<td>BHA</td>
<td>Mouse</td>
<td>Foregut</td>
<td>Wattenberg 1979c</td>
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<tr>
<td>N-2-Fluorenylacetamide</td>
<td>BHT</td>
<td>Rat</td>
<td>Liver</td>
<td>Ulland et al 1973</td>
</tr>
<tr>
<td>N-Hydroxy-N-2-fluorenylacetamide</td>
<td>BHT</td>
<td>Rat</td>
<td>Liver, breast</td>
<td>Ulland et al 1973</td>
</tr>
<tr>
<td>4-Dimethylaminolazobenzene</td>
<td>BHT</td>
<td>Rat</td>
<td>Liver</td>
<td>Frankfurt et al 1967</td>
</tr>
<tr>
<td>Azoxymethane</td>
<td>BHT</td>
<td>Rat</td>
<td>Large intestine</td>
<td>Weisburger et al 1977</td>
</tr>
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</table>
has not been established as yet. Two procedures have been employed, both of which entail use of Salmonella typhimurium tester strains TA-100 and TA-98. The first is a host mediated procedure in which the organisms are introduced into the peritoneal cavity. The test compound is administered and BHA is added to the diet. An alternate method entails determination of the effect of dietary BHA on excretion of mutagenic metabolites of test compounds. BHA was found to inhibit host mediated mutagenesis from BP, hycanthone, methionidazole, metrifonate, praziquantel, and mebendazole. BHA also reduced the excretion of mutagenic metabolites of these six compounds as well as diazepam (Batzinger et al., 1978).

The phenol most extensively studied for mechanism of inhibition has been BHA. This compound is quite remarkable in terms of the range of carcinogens, as well as mutagens, it inhibits. Studies carried out thus far indicate that an important mechanism of this inhibition resides in the capacity of BHA to cause enhanced detoxification. Mice which have been fed BHA for a period of one to two weeks at dose levels used for carcinogen inhibition experiments have been employed for studies of this nature. The microsomal monoxygenase system was the initial one shown to be altered. This alteration could be demonstrated by incubating benzo(a)pyrene (BP) with liver microsomes, cofactors required for mixed function oxidase activity, and added DNA. Under these conditions, reactive metabolites of BP bind to DNA. If liver microsomes from mice fed BHA are employed, approximately one half as much binding of BP metabolites to DNA occurs, as compared to incubation with control microsomes (Speier and Wattenberg, 1975). High pressure liquid chromatography (HPLC) studies of metabolites of BP occurring on incubating this carcinogen with microsomes from mice fed BHA as compared to controls likewise show changes. Two metabolic alterations are found that could result in inhibition of carcinogenesis. The first is a decrease in epoxidation of BP, which is an activation process, and the second is an increase in formation of 3-hydroxybenzo(a)pyrene, a metabolite of detoxification (Lam and Wattenberg, 1977).

Subsequent work has focused on the effects of BHA on the activities of conjugating enzymes. The initial work of this nature showed that addition of BHA to diets results in large increases in glutathione-S-transferase activity. Four substrates were employed, i.e., 1,2-dichloro-4-nitrobenzene, 1-chloro-2,4-dinitrobenzene, p-nitrobenzylchloride, and Δ⁴-androstene-3,17-dione. After 12 days of feeding BHA, the glutathione-S-transferase activity as determined with the first three of these substrates was increased over 10X that of control animals; a fivefold increase was found with the fourth substrate. Ethoxyquin also induced and increased glutathione-S-transferase activity but was about half as effective as BHA (Benson et al., 1978). In related investigations it was found that the administration of BHA produced an increase in the levels of reduced thiols in liver, kidney, lung and duodenum (Benson et al., 1978).

A second conjugating enzyme investigated is UDP-glucuronyl transferase. Studies carried out under similar conditions to those for glutathione-S-transferase showed that feeding a diet containing BHA results in slightly greater than a fourfold increase in liver UDP-glucuronyl transferase activity using p-aminophenol as the substrate (Cha and Bueding, 1979). BHT also has been
shown to induce an increase in activity of this enzyme (Grantham et al., 1973). In addition to the two conjugating enzymes, the feeding of a diet containing BHA causes an increase in epoxide hydratase and glucose-6-phosphate dehydrogenase activities (Cha and Bueding, 1979).

The increases in enzyme activities described above have been observed in animals fed a diet containing BHA for at least three days. Little data are available for shorter time intervals. These are inducible systems and it would be anticipated that a substantial period of time would be required for the activity to increase. No increase in liver glutathione-S-transferase activity occurs for at least six hours subsequent to BHA administration (Wattenberg and Sparmins, 1979b). However in the case of liver microsomal metabolism of BP, changes can be found as early as two hours after a single oral administration of BHA. At that time the microsomal metabolism of BP to metabolites binding to DNA has been reduced to one-half that found with microsomes from control animals (Speier et al., 1978). The reduction is of the same order of magnitude as in mice fed BHA for a week or more. Changes in the BP metabolite pattern similar to those found after prolonged feeding occur four hours after a single administration of BHA, the earliest time interval studied thus far (Speier et al., 1978). A point of considerable importance is that no increase in the overall metabolisms of BP, as determined by HPLC, occurs at any time interval after administration of BHA either by oral intubation or in the diet. Likewise studies of BP metabolism using the determination of AHF activity do not show an increase (Lam and Wattenberg, 1977; Speier et al., 1978).

A perusal of the enzyme activities increased by BHA reveals that they include microsomal enzymes (UDP-glucuronyl transferase and epoxide hydratase) and also enzymes located in the cytosol of the cell (glutathione-S-transferase and glucose-6-phosphate dehydrogenase). This diversity of enzyme inductions suggests a relationship to observations made by Poland et al. on the inducing effects of some xenobiotic compounds chemically different from BHA. These include 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), polycyclic aromatic hydrocarbons, and beta-naphthoflavone (BNF). Their mechanism of induction has been extensively studied using the extremely potent compound TCDD (Poland and Kende, 1977). Work carried out by these investigators have demonstrated that TCDD binds to a cytoplasmic receptor. This complex enters the nucleus and causes the induction of increased activity of major detoxification systems including UDP-glucuronyl transferase, epoxide hydratase and glutathione-S-transferase (Poland et al., 1979). Induction of increased AHF activity also occurs as does the activity of a number of other enzymes (Poland and Kende, 1977). (Fig. 2). The sequence of events has similarities to that existing with steroids in which a steroid binds to a cytoplasmic receptor, the complex enters the nucleus and a coordinated series of biochemical changes ensue.

Methylcholanthrene (MC), BP, benzo(a)anthracene and BNF have been found to be competitive inhibitors for the binding of TCDD to the cytoplasmic receptor. Many of the same enzyme inductions are produced by these compounds as with TCDD suggesting that all four compounds act through the same receptor. This is not the case for phenobarbital, diphenylhydantoin, phenyl-
Figure 2: (a.) Coordinated induction by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3-methylcholanthrene (MC), benzo[a]pyrene (BP), or beta-naphthoflavone (BNF) of some enzymes important for the detoxification and excretion of polycyclic aromatic hydrocarbons and related compounds. (b.) Coordinated response to BHA administration.
butazone and pregnenolone-16-carbonitrile all of which also induce increased activity of hepatic microsomal enzymes (Poland and Kende, 1977).

BHA evokes a coordinated group of reactions, many involving the same enzymes as with TCDD. These enzymes have the overall effect of enhancing detoxification. We would postulate that this coordinated response to BHA is likely to be the result of a similar series of events to that found with TCDD, although differences in magnitude and diversities of enzymes induced clearly exist (Fig. 2). Identification of a putative cytoplasmic receptor for BHA has not been accomplished. It is probable that it would differ from that for TCDD and related compounds. If one assumes that at least some potentially toxic xenobiotic chemicals induce a coordinate enzyme response which enhances their own detoxification and that of chemical related compounds, then the differences between the coordinated responses to a phenol such as BHA as contrasted to compounds such as polycyclic aromatic hydrocarbons, TCDD, and BNF are understandable. Thus phenols contain a hydroxyl group which is available for conjugation. Increased activity of conjugating systems would be the main requirement for detoxification and excretion of these compounds. An increase in AHH activity is not necessary for this purpose. In contrast a compound such as MC or other polycyclic aromatic hydrocarbons require the presence of a polar group or groups in order for conjugation to occur. Thus an increase in AHH activity as well as increased activity of conjugating enzymes is necessary for detoxification and excretion. In Figure 2 differences in the coordinated enzyme responses are depicted.

The alteration of the microsomal monooxygenase system which is observed two hours after BHA administration is an interesting finding in terms of its temporal occurrence. It is possible that a totally coordinated detoxification system would have an early component to protect the organism until inducible enzymes have been produced. The rapid alteration in the microsomal monooxygenase system following BHA administration may represent such an event. If this is in fact the case then there may be some relationship between BHA, its putative cytoplasmic receptor, and the microsomal monooxygenase system which overall results in alteration of the monooxygenase system as contrasted to a simple direct interaction of BHA with the monooxygenase system. At present, these are theoretical considerations which require experimental exploration. The microsomal monooxygenase system is a complicated system which activates a number of carcinogens as well as detoxifying them. Competing pathways are frequently involved. The implications of this complexity are discussed in the following section.

**Inducers of increased microsomal monooxygenase activity**

A number of studies have demonstrated that it is possible to protect against chemical carcinogens by administration of inducers of increased microsomal monooxygenase activity. The carcinogens inhibited include BP, DMBA, N-2-fluorenylacetamide, 3'-methyl-4-dimethylaminoazobenzene, 4-dimethylaminostilbene, urethane, aflatoxin and active agent of Bracken Fern (Wattenberg, 1977, 1979a,b). A wide range of compounds will induce increased microsomal monooxygenase activity. Inducing activity also is present in plants
including vegetables consumed by man. The inducing compounds used for obtaining inhibition in early experiments were polycyclic aromatic hydrocarbons. Subsequently, inducers with less noxious properties were found to be equally effective. Two groups of naturally occurring inducers, i.e. flavones and indoles, are inhibitors of chemical carcinogens.

**Flavones.** The study of flavones as possible inhibitors of chemical carcinogens was undertaken as a result of data which showed that a number of compounds belonging to this chemical family induce increased AH activity using BP as the substrate. Flavone itself is a moderately potent inducer. Hydroxylation reduced inducing activity but corresponding methoxy compounds were active. The vast majority of naturally-occurring flavones are poly-hydroxy derivatives. A small number contain only methoxy groups. Two of these, tangeretin (5,6,7,8,4′-pentamethoxyflavone) and nobiletin (5,6,7,8,3′,4′-hexamethoxyflavone) were active inducers of increased AH activity (Wattenberg et al., 1968).

An example of inhibition of chemical carcinogenesis by flavones is a study in which the effects of addition of compounds of this family to diets was determined on neoplasia of the lung due to BP. The flavones chosen for study were BNF (5,6-benzo flavone), quercetin pentamethyl ether, and rutin (3,3′,5,7-pentahydroxyflavone-3-rutinoside). BNF, a synthetic compound, is the most potent flavone found thus far in its capacity to induce increased AH activity. Quercetin pentamethyl ether is a moderately potent inducer of increased AH activity. This compound is synthetic and was used as a substitute for tangeretin which could not be obtained in sufficient quantity for carcinogen-inhibition studies. Both are pentamethoxy flavones and have similar inducing capacities. Rutin is a naturally-occurring compound with very weak AH-inducing activity. The three flavones were added to the diet fed A/He mice. These animals as well as those fed a control diet were challenged with BP given by oral intubation. BNF caused almost total inhibition of pulmonary adenoma formation, quercetin pentamethyl ether reduced the number of these neoplasms by half. The number of adenomas in animals fed rutin and the control diet were similar. Thus the inhibitory effects on BP-induced neoplasia paralleled the potency of the three flavones in inducing increased AH activity. In other experiments in which the only flavone employed was BNF, this compound inhibited DMBA-induced mammary tumor formation in the rat, and BP-initiated epidermal neoplasia in the mouse (Wattenberg and Leong, 1968; Wattenberg and Leong, 1970).

**Indoles.** Indole-3-carbinol, 3,3′-diindolylmethane and indole-3-acetonitrile occur in edible cruciferous vegetables such as Brussels Sprouts, cabbage, cauliflower and broccoli which are consumed by large numbers of individuals. These three indoles have been studied for their effects on BP and DMBA-induced neoplasia in rodents. When added to the diet, all three indoles inhibit BP-induced neoplasia of the forestomach. Addition to the diet also has been found to inhibit BP-induced pulmonary adenoma formation. In other experiments, indole-3-carbinol and diindolylmethane was found to inhibit DMBA-
induced mammary tumor formation in female Sprague-Dawley rats, but indole-3-acetonitrile was inactive in this experimental model (Wattenberg and Loub, 1978).

The original rationale for the use of the three indoles is based on their ability to alter microsomal monooxygenase activity. All three compounds induce increased activity of this system towards BP and also with other substrates such as phenacetin, 7-ethoxycoumarin and hexobarbital (Loub et al., 1975; Pantuck et al., 1976). The most potent inducer is indole-3-carbinol and the least active is indole-3-acetonitrile. In experiments in which neoplasms occur at a site distant from that of administration of the carcinogen, as with mammary tumor formation resulting from oral administration of BP, inhibitory potency paralleled the potency of the indole in inducing increased AHH activity. Indole-3-acetonitrile, a weak inducer of microsomal monooxygenase activity, does not inhibit DMBA-induced mammary tumor formation and is considerably less active than indole-3-carbinol in inhibiting BP-induced pulmonary adenoma formation. However in the case of the BP-induced neoplasia of the foreestomach, a different situation exists. Although indole-3-carbinol is a more potent inducer of increased AHH activity than indole-3-acetonitrile in this tissue, the two compounds inhibit tumor formation to the same degree. The effect of administration of indoles on the metabolite pattern of carcinogens has not yet been determined. It is possible that in the foreestomach, in contrast to other tissues, indole-3-acetonitrile is particularly favorable in terms of deviating metabolism of BP towards detoxification. The fact that in inhibition of foreestomach tumor formation, indole-3-acetonitrile comes into direct contact with target tissue also must be considered. Studies comparable to those employing indole-3-acetonitrile have been carried out with phenyl acetonitrile and octanenitrile in order to determine whether inhibition of BP-induced foreestomach neoplasia was a general property of nitriles. This is not the case. These two nitriles did not exert an inhibitory effect. Since indole-3-acetonitrile is the most abundant of the indoles found in many edible cruciferous vegetables, elucidation of its mechanism of inhibition is of particular importance.

Hazards of inducers. Having presented information which shows a protective effect from administration of inducers of increased microsomal monooxygenase activity, it is important to discuss possible hazards from inducers. It has been demonstrated for many chemical carcinogens that the microsomal monooxygenase system converts these compounds to a proximate carcinogenic form. However, frequently there is a competing detoxification pathway or pathways. The classic example of this is the aromatic amines. With these compounds, ring hydroxylation results in detoxification whereas hydroxylation of the nitrogen is an activation reaction (Miller and Miller, 1969). Thus, administration of inducers of increased monooxygenase activity in these instances may result in a relatively greater proportion of the carcinogen being detoxified rather than activated to a carcinogenic metabolite. An additional consideration is that inducers of increased AHH activity frequently induce increased activity of conjugating enzymes. This could enhance detoxification as has been discussed in the section on phenolic antioxidants and ethoxyquin.
In spite of the above considerations, the complexity of the microsomal monoxygenase system is sufficiently great so as to make it possible that under some circumstances an induction of increased activity of one or more of its cytochrome P-450 species would enhance carcinogenesis. Such may be the case with saforol. Administration of phenobarbital in the drinking water of rats concurrently fed saforol in the diet results in a greater number of tumors of the liver that in animals not receiving the phenobarbital (Wislocki et al., 1977). However phenobarbital is a compound with multiple biological actions which lends an element of uncertainty as to the mechanism by which it enhances the carcinogenic response to saforol. One of these biological actions is its capacity to act as a tumor promoting agent. When phenobarbital is administered subsequent to the hepatocarcinogens 2-acetylaminofluorene, diethylaminoethane and 2-methyl-N,N-dimethyl-4-aminazoazobenzene, it increases the neoplastic response (Peraino et al., 1973; Peraino et al., 1977; Kitagawa et al., 1979). A somewhat similar situation exists for BHT. This phenol, like phenobarbital, can induce increased microsomal monoxygenase activity. Also like phenobarbital, when BHT is given subsequent to carcinogens, it can enhance a neoplastic response. Administration of BHT subsequent to urethane increases the number of pulmonary adenomas formed (Witschi, 1977). If administered after 2-acetylaminofluorene, an increase in hepatic tumors is observed (Peraino, 1977). The neoplasia enhancing effects of phenobarbital and BHT represents a hazard which requires evaluation with respect to other compounds having the capacity to induce increased microsomal monoxygenase activity. It remains to be determined whether tumor promotion is a related or unrelated characteristic of a particular class or classes of inducers.

**DISCUSSION**

A critical contribution to the field of chemical carcinogenesis has been the demonstration of common mechanisms of carcinogen activation and likewise the demonstration that the ultimate carcinogenic species reacting with cellular macromolecules have common features (Miller and Miller, 1974; Miller, 1978). The elucidation of these commonalities has provided a relatively simple organizational pattern for understanding the mechanisms of action of a large number of carcinogens, many with diverse chemical structures. The question arises as to whether some type of general organization of inhibitors also can be provided. It can, but not completely, and with substantial uncertainty. Three major catagories are evident. The first is a large one. Information currently available suggest that many of the inhibitors of chemical carcinogenesis identified thus far may act by stimulation of a coordinated response of detoxification systems. A coordinated response refers to a sequence of events in which the inhibitor binds to a cytoplasmic receptor which enters the nucleus and causes the activation of multiple detoxification mechanisms including those in microsomes and the cytosol. A prototype of this type of mechanism is depicted in Figure 2. It is not unlikely that most, and possibly all, of the compounds in the top two lines and in the bottom two lines of Figure 1 fall into this catagory. The support for this postulation is fragmentary at the present time. That for BHA has
been provided previously and is depicted in Figure 2. BHT is structurally similar to BHA, inhibits several of the same carcinogens as BHA, and like BHA has been found to enhance UDP-glucuronyl transferase activity (Grantham et al., 1973; Wattenberg, 1979a). If the assumption is made that there is a coordinated response of detoxification systems to certain related phenols then p-hydroxycinnamic acid and coumarin, which hydrolyzes in vivo to o-hydroxycinnamic acid, could fall into this category. Ethoxyquin and benzylisothiocyanate inhibit BP and DMBA induced neoplasia and both stimulate increased glutathione-S-transferase activity (Wattenberg, 1971; Benson et al., 1978; Wattenberg, 1979a; Wattenberg and Sparmins, 1979b). The compounds on the bottom two lines of Figure 1 all induce increased AHH activity and all inhibit neoplasia resulting from administration of BP or DMBA. BNF produces a coordinated enzyme response similar to MC and is a competitive inhibitor to the binding of TCDD to a cytoplasmic receptor (Poland and Kende, 1977). Data are not available for quercetin pentamethylether but the structural similarities of the two compounds make a comparable mechanism of action likely. In addition to inducing increased AHH activity, indole-3-carbinol, 3,3'-dindolylmethane, and indole-3-acetonitrile all induce an increase in glutathione-S-transferase activity (Loub et al., 1975; Wattenberg and Sparmins, 1979b).

If it is true that BHA, BHT, ethoxyquin, coumarin, p-hydroxycinnamic acid, benzylisothiocyanate, BNF, quercetin pentamethylether, indoles and phenothiazine all are effective by virtue of stimulating a coordinated detoxification response, differences in this response clearly exist. As is shown in Figure 2, the response to BHA and BNF differ very dramatically in that the former does not induce increased AHH activity whereas the latter does. One may anticipate other differences between inhibitors. These could be due to differences in receptors or differences in the property of a particular receptor when interacting with different ligands. However, the existence of a generic type of defense mechanism against chemical carcinogens, stimulated by highly diverse chemicals, and which responds in somewhat different ways depending upon the inhibitor employed provides an organizational framework for the study of many inhibitors of chemical carcinogens. One aspect of the overall composition of a coordinated response of detoxification systems which merits investigation, is the existence and nature of immediate or rapid events. The alteration of microsomal monooxygenase system two hours after BHA administration suggest the possibility that early events protecting the organism until inducible enzymes have been induced may occur.

The denoting of two other categories into which inhibitors might be placed would appear to be useful. Some inhibitory effects, such as those of disulfiram on DMH-induced neoplasia of the large bowel appear to be quite selective. They entail the blocking of a specific reaction or reactions required for carcinogen activation. Inhibitors of this type could be placed into one category with appropriate subdivisions. A third category exists which is of general nature. This category includes inhibitors which act directly with reactive carcinogenic species. They perform a scavenging function. Proof of their mechanism of action would consist of identification of adducts or expected reaction products. A number of physiological compounds act in this
manner (Miller and Miller, 1974). We are not aware of a definitive demonstration of any xenobiotic compound protecting against chemical carcinogenesis by this mechanism. However, data indicating that cysteamine inhibits DMBA-induced mammary tumor formation by a direct scavenging action is suggestive (Marquardt et al., 1974).

The identification of a substantial number of compounds having the capacity to inhibit the neoplastic effects of chemical carcinogens gives rise to two questions. The first is, “What is the current role that these compounds play in reducing the impact of chemical carcinogens on man?” The second is, “What is the optimal role that they could have?” The first of these questions can be rephrased in two additional ways which may have some conceptual value, i.e. “Is there a significant balance between carcinogens and anticarcinogenic agents which determines whether an individual will have cancer?”, and “To what extent do variations in cancer incidence amongst groups of individuals reflect differences in magnitude of exposure to carcinogens, and to what extent to protective agents?” Evidence suggesting that inhibitors do play a role in man is of three types: the nature of the inhibitory compounds themselves, mechanisms of inhibition, and epidemiological data. The inhibitors found thus far are very diverse in chemical structures. Many of these compounds are consumed by man. A number occur as natural constituents of vegetables. These compounds include: indoles, flavones, phenols, coumarins, selenium salts, and aromatic isothiocyanates. Others such as BHA are synthetic compounds consumed as food additives. The great chemical diversity of inhibitors indicates the likelihood that others exist and could play a significant and perhaps even more important role than those already identified.

The available information on mechanisms of inhibition of chemical carcinogenesis has been discussed above. A important characteristic of these systems is that their activities can be changed by xenobiotic compounds occurring in the environment. Of some interest is the observation that in tissues of the major portals of entry of carcinogens (intestinal tract and lungs), the activity of the microsomal monoxygenase system in metabolizing at least some carcinogens appears to be almost totally determined by exogenous environmental factors (Wattenberg, 1970). Thus animals fed purified diets and kept in animal quarters with filtered air show almost no monoxygenase activity for polycyclic aromatic hydrocarbons and azo dyes. The implication of these findings is that the nature of the diet as well as other environmental exposures will determine the activity and characteristics of this important carcinogen metabolizing system in tissues which come into initial contact with chemical carcinogens.

An interesting source of naturally-occurring inducers of increased microsomal monoxygenase activity is vegetables. In experimental animals, cruciferous vegetables such as Brussels Sprouts, cabbage, cauliflower and broccoli have a moderately potent inducing effect on monoxygenase oxidase activity. Other vegetables such as alfalfa, spinach and celery have weak inducing activity (Wattenberg, 1971, 1972c). A study has been carried out in which diets containing large amounts of cabbage and Brussels Sprouts have been fed to normal human volunteers between 21 and 32 years of age. The
effects of this diet on the metabolism of antipyrine and phenacetin was studied. The results indicate that the test diets rich in vegetables enhanced the metabolism of both drugs (Pantuck et al., 1979).

Some support for the possibility that inhibitors of carcinogens do play a role in man is derived from epidemiological investigations. Several studies have been published which indicate that consumption of vegetables may diminish the risk from chemical carcinogens. One of the most dramatic of these is a case-control study by Saxon Graham et al., which shows an inverse correlation between the magnitude of consumption of cabbage and the occurrence of cancer of the colon (Graham et al., 1978). The relative risk in individuals with the highest consumption of cabbage as compared to those with little or no intake of this vegetable is about one-third. Several investigations have been published in which an inverse relationship has been found between magnitude of consumption of other vegetables including lettuce, celery and tomatoes and cancer of the stomach or precursor lesions in that organ (Haenszel et al., 1972; Haenszel et al., 1976). The reduced incidence of cancer in Seventh Day Adventists, a group which has a vegetarian diet, is well documented, and is in accord with the above (Phillips, 1975).

The group of epidemiological studies cited are of interest because of the occurrence of inhibitors of chemical carcinogenesis in vegetables. However, these studies cannot be considered as conclusive. At least two major areas of uncertainty exist. The first is a lack of evidence that the relatively high consumption of vegetables has actually enhanced the effectiveness of protective systems against chemical carcinogens in the individuals at risk. Obtaining quantitative data of this nature is critical for establishing firmly a relationship between dietary factors and risk. The second uncertainty is whether there might be important undefined correlates between magnitude of consumption of vegetables and aspects of lifestyle or diet which are the true variable altering the responses of the individual to carcinogens. These obviously are difficult problems and require solutions.

Considerations of the optimal role that compounds which prevent cancer-producing agents from reaching or reacting with critical target sites might play entails evaluations of their deliberate use. At present, it clearly would be premature to undertake such measures. 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 factors entailed in making decisions concerning the deliberate use of inhibitors of chemical carcinogenesis. For any normal group of individuals, a critical restraint is the possibility of toxicity. Inhibitors would have to be taken by individuals for many years in order to be effective. Even a low toxicity could outweigh any benefits. However, there are selected situations in which this formidable obstacle might be overcome. One specific instance is carcinogens within the gastrointestinal tract. In this case, it is conceivable that an inhibitor could be designed which would not be absorbed. Under these conditions, a compound with little or no toxicity might be available. The importance of considerations of this type is made more compelling by recent findings of
mutagenic substances in the feces (Varghese et al., 1977). If these mutagens are in fact carcinogens, efforts at finding effective inhibitors active within the large bowel might be warranted. In other sites, specific situations amenable to selective approaches could exist as well.

A conceivable basis for introduction of an inhibitor into the environment would be the acquisition of favorable data from epidemiological investigations. Such data should 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. such as tissues from the particular population group showing an increased capacity to detoxify carcinogens, would be important. In addition, there should be clear evidence of lack of toxicity from the inhibitor. Under these conditions, consideration of the use of the material bringing about the inhibition might be warranted. This in essence, is 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.

There do exist individuals who because of genetic or acquired characteristics are at increased risk from chemical carcinogens. Under these conditions, 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 a mechanism for allowing increased exposures to carcinogens or increasing tolerance levels to cancer-producing substances.

ACKNOWLEDGEMENTS

Investigations included in this presentation were supported by Public Health Service Grants CA-09599, CA-15638, and CA-14146 from the National Cancer Institute.

REFERENCES


