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MECHANISMS OF CHEMICAL CARCINOGENESIS

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Elizabeth K. Weisburger[1]
Carcinogen Metabolism and Toxicology Branch, National Cancer Institute, Bethesda, Maryland 20014

INTRODUCTION

Despite the seemingly disparate endpoints, there are similarities between many drugs and chemical carcinogens when viewed from the basis of pharmacological or toxicological principles. Various biochemical reactions or metabolic pathways of chemical carcinogens are the same as those of other xenobiotics. Similar enzyme systems operate in both cases. Certain biochemical pathways, once assumed to be manifest only in carcinogens, have been discovered from some drugs, where some of the toxic side effects may be due to these metabolic transformations (1–4). Another possibility is that drugs can themselves be carcinogenic; this is more likely to hold for those employed in the chemotherapeutic treatment of neoplastic disease (5–7). Finally, there are several reports that drugs that contain secondary or tertiary amine structures may react with nitrite, formed endogenously, to yield nitrosamines, many of which are potent carcinogens in most species (8). Against this background, it should be recognized that just as one drug can influence the metabolism of another (9, 10), likewise the effects of carcinogens can often be altered by other chemical entities. These topics are mentioned in the following sections.

MECHANISMS OF ACTION OF CHEMICAL CARCINOGENS

Although a sizable level of research effort has been applied toward determining the mechanism of action of chemical carcinogens, there still have been no truly definitive leads in this area (11). The premise has evolved, however, that there must be some interaction between the chemical carcinogen and the informational molecules of the cell, namely DNAs, RNAs, or proteins. Current theories are based on the ability of the carcinogens, either themselves or their metabolites, to act as electrophiles (electron-deficient species) which attach to electron-rich or nucleophilic sites in

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nucleic acids, proteins, or other cellular macromolecules (12, 13). Although the functions of the proteins involved have not been determined (14), it was observed about 30 years ago that chemical carcinogens are covalently bound to cellular proteins (15). In this respect, carcinogens and drugs are similar since the pharmacological action of drugs generally depends on their binding to cellular constituents (16, 17). Attempts have been made to correlate the levels of protein binding from various carcinogens with the activities of these substances in vivo, but the data have not always been definitive.

To date, histidine, cysteine, lysine, tyrosine, tryptophan, and methionine are the amino acids that have been identified as having reacted with carcinogens (18, 19). Binding of an activated aromatic amine to methionine in rat liver protein was mediated, in part, through a sulfotransferase (20). There also have been intensive efforts to isolate and characterize the liver protein apparently involved in the binding of the carcinogen 4-dimethylaminoazobenzene. However, there has been no definitive outcome of these experiments (21, 22).

Therefore, since the nucleic acids are implicated as being more directly involved in the transfer of genetic information, recent efforts have concentrated on the covalent binding of chemical carcinogens or their activated forms with nucleic acids. These interactions have been studied from various aspects, such as the effects of the carcinogens on the nucleic acids themselves, the changes in physical characteristics, and the function of the carcinogen-altered nucleic acids (23–25).

In addition to the covalent binding of carcinogens to cellular macromolecules, other noncovalent binding of carcinogens may distort the nucleic acids sufficiently to disrupt their proper function. Intercalation or stacking of molecules of the carcinogen between the base-pairs in DNA is more likely to occur with compounds possessing a planar aromatic ring approximately the size of a base-pair, i.e. benzo[a]-pyrene. Another type may involve the insertion of the carcinogen perpendicular to the nucleotide bases of the DNA (24).

There have been many discussions of the consequences of changes in DNA or RNA (12, 13, 18, 23, 25–28). Furthermore, there has been much effort to correlate various effects on the nucleic acids with the carcinogenicity of the compounds in question. These may include the degree of alkylation at various positions, the stability of the altered DNA or RNA, extent of repair of the altered nucleic acids, and attempts to correlate a greater degree of alkylation at a certain position with an organ-specific action of the carcinogen. The matter is complicated further by the occurrence of substitution at many positions. The positions in the bases that are attacked by the reactive intermediates differ depending on the parent carcinogen. Polycyclic aromatic hydrocarbons preferred the amino group in guanine and to a lesser extent those in adenine or cytosine (29–31). With aromatic amides, arylamidation at the 8-position of guanine occurred (13, 19). Nitrogen mustards, β-propiolactone, and dialkylnitrosamines were attached mainly at the 7-position of guanine, but other positions were also affected (32–34). Nitrosoalkylureas, besides forming 7-alkylguanines, also attacked the O-6 of guanine. To some extent, alkylation of O-6 was considered as more nearly correlated with the carcinogenic effect than N–7 alkylation (23, 28, 35). A recent publication has reported that N-nitrosoethylurea can alkylate all the oxygens in the bases of nucleic acids, namely O-2 of cytosine, O-2 and O-4 of uracil, O-6 of guanine, and O-2 and O-4 of thymine (36).

Philosophically, the question is whether alkylation in all these positions is related to the processes of carcinogenesis or whether alkylation at some of these positions may represent a random unrelated process. Obviously, this development requires further study to determine which of these events are relevant and which are not relevant.

In general, chemical carcinogens may be divided into two broad classes, those that act directly and those that require metabolic activation. Direct acting carcinogens include nitrogen or sulfur mustards, methyl methanesulfonate, ethylene imines, diepoxybutane, β-propiolactone, bis-(chloromethyl)ether, dimethylcarbamyl chloride, propane sultone, or other chemically reactive compounds. Because of their reactivity in an aqueous environment, N-nitrosoureas are also considered as direct acting carcinogens. Those requiring activation encompass most of the environmentally important carcinogens—polycyclic aromatic hydrocarbons, aromatic amines, amino azo dyes, aflatoxins, other naturally occurring carcinogens, and others.

Direct Acting Carcinogens

These compounds, because of their alkylating or acylating capability, react with the nucleophilic sites on proteins, nucleic acids, or other cellular macromolecules without the necessity for metabolism. For example, methyl methanesulfonate or β-propiolactone acts mainly at the 7-position of guanine in DNA or RNA, yielding 7-methylguanine or 7-(2-carboxyethyl)-guanine, respectively, after hydrolysis of the nucleic acid and characterization of the individual bases (32, 33). Other positions that were affected to a lesser extent were the N-1, N-3, and N-7 of adenine and N-3 of cytosine (34, 37). Reaction with proteins also occurred, but the products were not characterized (33).

A different type of carcinogen, 7-bromomethylbenz[a]anthracene and its 12-methyl analogue, are activated halogen compounds with a polycyclic aromatic nucleus. When reacted with nucleosides or nucleic acids in vitro, they formed adducts on the amino groups of the guanine, adenine, or cytosine residues. Similar compounds were characterized in the DNA isolated from mouse skin after exposure to the parent bromo derivatives (31).

The actual potency of activated compounds in animals may be considerably less than anticipated as a result of factors such as solubility, the steric configuration of the molecules involved, or reactivity with water or like molecules before a more critical target is reached.

Compounds Requiring Activation

Most chemical carcinogens are converted by metabolic activation to electrophiles which in turn react with nucleophiles in the cell (13). The metabolic pathways and the activated molecules differ, depending on the parent compounds (38).

Aromatic hydrocarbons, for example, are metabolized by means of nonspecific monooxygenases of the P450 family, located in the endoplasmic reticulum or microsomal fraction of cells, especially the liver (4). Current evidence points toward a diol epoxide as being the active form for the following reasons: (*a*) high mutagenic activity of certain diol epoxides, (*b*) reaction of synthetic diol epoxide with DNA to afford a product that coincided with that isolated from cells treated in culture with diol epoxide (39).

The product from reaction of a benzo[a]pyrene diol epoxide and DNA in vitro has been characterized (29). The hydrocarbon nucleus was attached to the amino group on the 2-position of guanosine, similar to the situation with 7-bromomethylbenz(a)anthracene (31) or the 5,6-epoxide of 7,12-dimethylbenz[a]anthracene (30).

The epoxide pathway is not unique with hydrocarbons. Other carcinogens such as aflatoxin B_1 (40) and some halogenated olefins as vinyl chloride (41) appear to be activated through this route. Despite the similarity in activation pathways, neither of these two compounds reacts with nucleic acids as do the polycyclic aromatic hydrocarbons. The major product from DNA and activated aflatoxin B_1 was characterized as 2,3-dihydro-2-(N-guanyl)-3-hydroxyaflatoxin B_1 where the guanine and hydroxyl functions were in a *trans* configuration (42). In this respect aflatoxin B_1 resembled the dialkylnitrosamines or β-propiolactone in its attachment to DNA. Vinyl chloride, after metabolic activation, apparently adds to the N-1 and the 6-amino group of adenosine, forming a new ring (43).

Furthermore, epoxidation is not limited to carcinogens, for epoxide metabolites of several useful drugs have been identified (44–46). However, these epoxides were not mutagenic whereas some of those from carcinogens were potent frameshift agents (46). The effect of carcinogenic aromatic amines or amides and amino azo dyes, as well as their precursors, is to attach an arylamino or arylamido moiety at the C-8 of guanine in nucleic acids. This process is apparently mediated through an active ester of an N-hydroxy derivative (19, 47).

Dialkylnitrosamines are oxidized enzymatically to a monoalkylnitrosamine that decomposes to furnish a carbonium ion, R^+ (18, 28). This activated form, as mentioned, alkylates various positions of the nucleic acid bases (34, 35).

The reactions of other types of carcinogens with nucleic acids have not been elucidated so well as those mentioned here. There remains the problem of whether the altered bases, as identified, are actually important for carcinogenesis by all the compounds mentioned previously, or whether less abundant or unidentified products may be significant. To solve this problem there will be an additional need to characterize further the functional properties of cells that are susceptible to various chemical carcinogens. One approach that has been suggested is a kinetic study of the rate of elimination of alkylated bases from the DNA of tissues of varying susceptibility (48). In any event, further investigation is necessary on the sequence of cellular and molecular events associated with the process of neoplastic transformation.

METABOLIC ACTIVATION OF CARCINOGENS

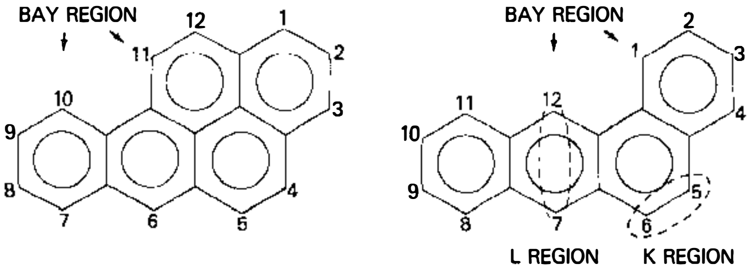
Polycyclic Aromatic Hydrocarbons

Cancer in humans was linked with exposure to soot, a mixture of polycyclic aromatic hydrocarbons and other combustion products, over 200 years ago. Isolation and structural identification of some of the active compounds in soots, tars, and similar materials was accomplished almost 50 years ago. However, sizable advances in knowledge of the metabolic activation of the aromatic hydrocarbons have occurred in the past few years (49), largely because of improved techniques for the synthesis or separation of these compounds and their derivatives (50–52).

From attempts to correlate the chemical reactivity of certain regions of the polycyclic aromatic hydrocarbons with their carcinogenic activity there came the concept of the K- and L-region, terms introduced to designate areas of high electron density (53). The K-region is an area that has the greatest double bond-like character, such as the 9,10-bond of phenanthrene, the 5,6-bond of benz[a]anthracene, or the 4,5-bond of the ubiquitous benzo[a]pyrene (Figure 1). The L-region was equivalent to the 9 and 10 positions of phenanthrene. In many aromatic hydrocarbons there was a rough correlation between high reactivity in the K-region and carcinogenicity. Therefore it was assumed that any addition of cellular constituents to a hydrocarbon occurred at the K-region, leaving other areas free for metabolic reactions (53).

More recently, it has been proposed that the carcinogenicity of a polycyclic aromatic hydrocarbon is due to metabolic activation of benzo ring epoxides where the epoxide is located near the "bay" region of the hydrocarbon (54) (Figure 1). The regions between the 1- and 12-positions of benz[a]anthracene, the 10- and 11-positions of benzo[a]pyrene, or the 1- and 12-positions of 7-methylbenz[a]anthracene would all qualify as bay regions.

For some time after the carcinogenicity of the polycyclic aromatic hydrocarbons was discovered it was thought that these compounds were active as such because (*a*) small quantities caused tumors at the site of application and (*b*) any metabolites isolated were less effective than the parent compounds. However, based on metabolism studies with simple aromatic hydrocarbons such as phenanthrene or naphthalene, Boyland (55) proposed that hydrocarbons were metabolized through an epoxide (arene oxide) intermediate. This activated intermediate could account for the isolation of phenols, dihydrodiols, premercapturic acids, and mercapturic acids as urinary metabolites of aromatic hydrocarbons, depending on whether the epoxide



BENZO [a] PYRENE

BENZ [a] ANTHRACENE

Figure 1 Structures of benzo[a]pyrene and benz[a]anthracene, showing K, L, and bay regions.

rearranged nonenzymatically or reacted enzymatically with water or glutathione (4, 49, 56–58). However, phenols apparently can also be produced by the alternate pathway of oxygen insertion (59). Eventually, synthetic methods were developed for arene oxides (60–62) and further studies indicated that these compounds were transient metabolic intermediates (4, 63).

Contrary to the K-region hypothesis (53), when various arene oxides were tested by painting on mouse skin, the K-region epoxides of benzo[a]pyrene and other aromatic hydrocarbons had less activity than did certain non-K-region oxides (64) or else had negligible activity (65–67). On the other hand, the K-region epoxides were highly active as mutagens for bacteria (68) or mammalian cells in culture (69, 70). In addition they were more active than the parent hydrocarbons in producing malignant transformation of mouse prostate fibroblasts or of hamster embryo cells in culture (71–74). The discrepancy in results between in vivo and in vitro systems has not been explained. Inactivation of the arene oxide by enzyme systems, premature reaction, or inability to reach the target cells in the animal may have been partly responsible.

Further investigations on the metabolic activation of hydrocarbons such as benzo[a]pyrene have mainly been done with in vitro systems, thus allowing direct isolation or testing of metabolites without the secondary effects occurring in vivo (74, 75). Improved separation systems have also facilitated such efforts (50, 76).

Similar to other xenobiotics, the aromatic hydrocarbons are metabolized by an inducible monooxygenase of the cytochrome P450 family, a subject reviewed several years ago by Gillette et al (77). If induced by polycyclic hydrocarbons such as 3-methylcholanthrene, a cytochrome P448 is the relevant enzyme (78, 79). Any epoxide initially formed through the P448 is a substrate for glutathione-S-transferase (80, 81) or epoxide hydratase (hydrase) (58, 80, 82), leading to mercapturic acids and precursors, and dihydrodiols, respectively.

From experiments in vitro with metabolites, DNA adducts, and presumed intermediates, the premise grew that not only was epoxide formation necessary for activation of a polycyclic hydrocarbon to a carcinogen but also the presence of the dihydrodiol moiety (39). The potent carcinogenicity of the 7,8-dihydrodiol of benzo[a]pyrene, equivalent to that of benzo[a]pyrene (83, 84), provided evidence that this dihydrodiol was probably an intermediate toward the final activated carcinogen. The extreme mutagenic activity of the 7,8-dihydrodiol-9,10-epoxide of benzo[a]pyrene toward mammalian cells in culture (85) and its reactivity with DNA (19, 39, 86) thus point toward the diol epoxide as an ultimate carcinogenic form of benzo[a]pyrene (65). This latter compound may be formed by a second oxidation step on the 7,8-dihydrodiol, itself resulting from the initial oxidation of benzo[a]pyrene and the action of epoxide hydratase. The stereochemical configuration of the dihydrodiol-epoxide from benzo[a]pyrene has been elucidated. Basically the 9,10-oxide and the 8-hydroxy group are both behind the plane of the ring, anti to the 7-hydroxy group (85, 87). Additional evidence for the stereospecific nature of the reactions and the specificity of the enzyme systems has been reported (88). Liver microsomes from 3-methylcholanthrene-treated rats or a purified cytochrome P448 system converted the major part of (–)*trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene to the transient but metabolically important diol epoxide. Microsomes from control or phenobarbital-treated rats were less stereospecific in the conversions of the dihydrodiol (88).

For benzo[a]pyrene it thus is obvious that the K-region is less likely to be involved in the carcinogenic process than the bay region (54). Likewise, preliminary results with other polycyclic hydrocarbons indicate that non-K-region metabolic activation is probably more important than that on the K-region (89–93).

In addition to the studies mentioned on metabolic activation of aromatic hydrocarbons, there have been other proposals that have much less experimental evidence to substantiate them. The first implicates one-electron oxidation as a mechanism (94), the other activation by hydroxymethylation (95, 96). However, these theories have less credence in view of the impressive evidence for the arene oxide mechanism of activation of polycyclic hydrocarbons.

Aromatic Amines and Aminoazo Dyes

The carcinogenicity of aromatic amines to humans was suspected almost 90 years ago. However, it was only in the 1930s that experiments in animals confirmed the carcinogenic activity of certain aminoazo dyes and aromatic amines (97, 98). The fact that large quantities of such substances were needed to elicit a response, coupled with the apparent lack of a local action, indicated that a metabolic product was probably responsible for the carcinogenicity of such compounds. This premise was only substantiated after a long period of effort on isolation and identification of metabolites of various aromatic amines and azo dyes (99–102). Since *o*-hydroxy-2-naphthylamines, some of the numerous metabolites of 2-naphthylamine (100), were apparently carcinogenic by implantation in mouse bladder, it was suggested that the *o*-hydroxy metabolites of aromatic amine carcinogens were the activated entities (103). However, animal tests did not confirm this opinion, for the ring-hydroxylated derivatives of 2-naphthylamine or N-2-fluorenylacetamide (2-acetylaminofluorene) were less active carcinogens than the parent compounds. Eventually a new type of urinary metabolite of N-2-fluorenylacetamide was identified where hydroxylation had occurred on the amido nitrogen (19, 104, 105). This new compound was more carcinogenic than the parent amide in most species, was often active locally, and was effective in species such as the guinea pig which usually did not respond to the original amide. It thus seemed that N-hydroxy-N-2-fluorenylacetamide was an activated form more closely related to the ultimate carcinogen. The same evidence held for other carcinogenic aromatic amines or amides.

However, further reaction of the N-hydroxy compounds was needed to produce an ultimate carcinogen, capable of reacting with nucleic acids or proteins (19, 105, 106). Activated esters of an N-hydroxy amide such as the sulfate, acetate, glucuronide, or even phosphate were implicated (19, 105, 107). There is substantial evidence for such forms, namely the strong electrophilic nature of such esters and their reactivity with tissue nucleophiles (13, 108, 109), and the identity of products formed from these in vitro reactions with those isolated in vivo. In addition, the action of the carcinogenic amide or N-hydroxyamide was inhibited by simultaneous administration of acetanilide or *p*-hydroxyacetanilide which deplete or trap the sulfate necessary for enzymic formation of the activated sulfate esters (110). These results plus the carcinogenicity (111), high mutagenicity (112, 113), and effects on DNA repair (114, 115) of the activated esters all point toward such compounds as intermediates in the carcinogenic process.

Furthermore, in some species the levels of liver sulfotransferase, one of the activation enzymes, could be roughly correlated with the carcinogenicity of N-2-fluorenylacetamide in these species (19, 116). However, the mammary gland or the sebaceous gland of the ear duct were lacking in sulfotransferase (117) but were still susceptible to the carcinogenic action of aromatic amides. Thus sulfotransferase may be implicated for activation in the liver but not other organs.

The systems involved in these various activation steps of an arylamine or amide involve an initial N-oxidation by an inducible P450 monooxygenase (77, 118). There is some evidence for a relationship between induction of arylhydrocarbon hydroxylase and N-acetylarylamine N-hydroxylase (119). Oxidation is followed by activation via sulfotransferase, acyltransferase, glucuronyl transferase, or other enzymes (19, 107). Further enzymatic action may convert the more stable of such conjugates to less stable but more reactive entities (120). The acetate esters of N-hydroxyamides might also be formed via one electron oxidation of the parent N-hydroxyacetylamine, followed by dismutation of the nitroxide thus formed to a nitroso compound and the N-acetoxyarylamide (121). There is also an acetyltransferase that transfers an acetyl group in the N-hydroxyacetylarylamine to form an N-acetoxyhydroxylarylamine (107, 122), a reactive material that readily forms nucleic acid adducts in vitro (107, 122, 123).

In addition to these activation pathways, other systems tend to deactivate N-hydroxy compounds. Reduction back to the parent amine or amide can occur, either through a mammalian liver fraction or intestinal bacteria (105). Isomerization to *o*-hydroxyarylamides is another route leading to alteration of N-hydroxyamides (105, 124).

Carcinogens that are activated in a similar fashion include 4-nitroquinoline-1-oxide and several nitrofurans, some of which are antibacterial agents. These compounds are reduced in vivo to the more active hydroxylamino derivatives (106, 125, 126).

During initial studies on the carcinogenic aminoazo dyes it was thought that certain ring positions might be involved in the process of carcinogenesis (101). Later it became apparent that aminoazo dyes were activated in the same fashion as aromatic amines. For dyes carrying N-alkyl groups, oxidative dealkylation, followed by N-oxidation and esterification, offered an explanation for the binding of aminoazo dyes to proteins and nucleic acids, both in vitro and vivo (19, 47, 127).

Since the discovery of N-hydroxylation of aromatic amides, it has been mentioned that N-hydroxy intermediates of drugs of the arylamide type may be responsible for some of their toxic effects (1, 17, 38). Identification of N-hydroxy metabolites of various useful drugs substantiates this hypothesis (2, 3, 128). One of these, N-hydroxyphenacetin, was further activated by formation of a glucuronide or sulfate ester, similar to N-2-fluorenylacetamide (129).

However, the presence of the N-hydroxy group in a molecule is not sufficient for carcinogenicity; phenylhydroxylamine and N-hydroxysuccinimide, among others, are not carcinogenic (19, 105). Nevertheless, it may be provident to limit indiscriminate use of drugs that are N-hydroxylated in vivo in view of the toxic, mutagenic, and possible carcinogenic activities of many N-hydroxy compounds.

N-Nitroso Compounds

N-Nitroso compounds, including N-nitrosamines, -amides, -ureas, or -urethans, have a greater potential than other carcinogens to affect all species, including those resistant to some other carcinogens. The toxic effects of dimethylnitrosamine in humans were first noted in 1937, but the carcinogenicity to experimental animals was not reported until 1956 (130). Several extensive reviews on nitroso compounds cover structure-activity relationships, organotropism of nitrosamines, and metabolic activation (18, 131–134). Presently it is considered that a typical dialkylnitrosamine such as dimethylnitrosamine is oxidized on one methyl group to yield (α-hydroxymethyl)methylnitrosamine which on heterolysis yields formaldehyde and an unstable monomethylnitrosamine (18, 131, 133). The unstable nitrosamine collapses with elimination of nitrogen and formation of the carbonium ion, CH_3^+, which serves to alkylate nucleic acids and proteins as discussed previously (18, 132, 135). Elimination of nitrogen also occurred with a nitrosoheterocyclic, affording an aliphatic diol which bound to nucleic acids (136). Nitrosoureas and amides often are unstable in aqueous solution, especially at higher pH and decompose spontaneously to form an alkylating moiety.

The oxidative demethylation of nitrosamines is accomplished by a liver microsomal cytochrome P450 enzyme (137), although the additional presence of NADPH-cytochrome *c* reductase and a phospholipid fraction gave the greatest demethylating activity (138, 139).

Although the initial metabolic intermediate, the α-hydroxymethylmethylnitrosamine is apparently too unstable to isolate, a stabilized derivative methyl(acetoxymethyl)nitrosamine, has been synthesized and tested. This compound was quite mutagenic (140) and a more active carcinogen than the parent dimethylnitrosamine (141, 142), substantiating the concept of α-hydroxylation as an activation pathway. Additional evidence for α-hydroxylation has been provided by the reduction in carcinogenicity of nitrosamines through α-deuterium substitution (143, 144). Breaking a carbon-hydrogen bond adjacent to the nitrosamino function may be a rate-limiting step in carcinogenesis by nitrosamines (144).

In addition to oxidative dealkylation, longer-chain dialkylnitrosamines can be metabolized by ω-oxidation of the alkyl groups, often yielding compounds with very selective action. Thus N-nitrosobutyl-4-hydroxybutylamine and N-nitrosobutyl-3-carboxypropylamine are both specific bladder carcinogens (145). In contrast, the parent dibutylnitrosamine acts mainly as a bladder carcinogen only if injected subcutaneously (131). Similarly, dipropanolnitrosamine is a specific pancreatic carcinogen in hamsters (146).

The problem with nitrosamines does not stem from their deliberate addition to environments. It is rather their endogenous formation from nitrite and secondary or tertiary amines including some drugs, pesticides, or the like (147–150), or their formation by bacteria (151). On the other hand, bacteria can also degrade nitrosamines to the parent amine and nitrite (152). The reaction of nitrite with amines can be catalyzed by anions such as thiocyanate (153) or by substances like formaldehyde (154). In turn, nitrite is formed endogenously by bacterial reduction of dietary nitrate, present in most vegetable foods (155).

Many experiments on simultaneous administration of nitrite and amines or ureas to animals have demonstrated quite convincingly that formation of a nitrosamine or -urea did occur in vivo (150, 156, 157). However, the toxicity and carcinogenicity of combinations of nitrite and amines can be inhibited (157, 158). Ascorbic acid, its derivatives, and propyl gallate all react readily with nitrite, preventing the nitrosation reaction (159). In actuality there may be a balance between the endogenous formation and degradation of nitrosamines. Otherwise, cancer rates would be much higher than presently observed.

Other Carcinogens

The metabolic activation of other types of carcinogens, although thoroughly investigated, has not been so well delineated as those reviewed in previous sections of this chapter. Aflatoxins apparently are activated by oxidation to an epoxide (40), although various hydroxyaflatoxins, most of which may be detoxication products, have also been identified (160–162). The natural product safrole is hydroxylated on the propenyl side chain to 1'-hydroxysafrole. Acetylation to 1'-acetoxysafrole affords an activated ester, capable of cleavage to an electrophilic species (163). The actual amount of 1'-hydroxysafrole formed from safrole is very low, a partial explanation for the high doses and long exposure time needed to elicit the carcinogenicity of safrole. 1,2-Dimethylhydrazine, often used as a model intestinal carcinogen, is apparently oxidized through azomethane to azoxymethane (164). In turn azoxymethane, methylazoxymethanol, and the naturally occurring carcinogen, cycasin, share a common pathway leading to oxidation of one carbon to formaldehyde while the other carbon eventually provides a carbonium ion that alkylates nucleic acids or proteins (18, 165). Ethionine has a metabolic pathway like that of methionine (166). Urethan poses a dilemma; although N-hydroxyurethan is also carcinogenic, there is some question whether it is the actual activated metabolite (167). Recently it has been suggested that vinylene carbamate may be the metabolic intermediate responsible for the carcinogenicity of urethan (168), but experimental evidence will be required to confirm this hypothesis. As mentioned in a previous section, some halogenated aliphatics may be activated through an epoxide (169); carbon tetrachloride may form free radical derivatives that bind to hepatic lipids or proteins (170). With most of these carcinogens the first activation step involves metabolism by a mixed-function oxidase system.

INFLUENCE OF OTHER XENOBIOTICS ON CARCINOGENS

Environmentally, people are exposed to many compounds, including carcinogens such as benzo[a]pyrene from combustion products. Just as drugs influence the action of each other (17), the action of carcinogens can be influenced by other substances present in the environment (10).

Enhancing the effect of carcinogens, either by concurrent administration of other substances (cocarcinogenesis) or by administration after a limited course of treatment with a carcinogen (promotion), was reviewed several years ago (171, 172). Some cocarcinogens enhance the action of carcinogens by increasing absorption. A fair number of cocarcinogens thus have a lipophilic-hydrophilic nature (172) while others are derived from fatty acids or their esters (173–175) or are long-chain paraffins (176). It has been suggested that unsaturated fatty acid esters may have growth-stimulating activity or act as cellular receptors or effectors (177). Substances with irritant activity or those that induce enzymes converting the parent carcinogen to its activated form can also be cocarcinogens (172, 178, 179). The amino acid tryptophan has a cocarcinogenic effect on certain aromatic amines (99, 180), although the tryptophan metabolites may be the actual vectors. Thus, although there are some leads, in most cases the actual mechanisms by which noncarcinogens enhance carcinogenicity have not been elucidated.

For mixtures of various carcinogens, enhancement was generally noted only when the target organ was the same for each component (181). Fairly often, there was no additional effect or even an inhibitory action (181, 182).

There is a wide range of compounds that can inhibit the action of chemical carcinogens (183–186). The overall picture is anomalous, for a substance that inhibits one carcinogen may potentiate the effect of another. One example is indole which enhances bladder tumors in hamsters from N-2-fluorenylacetamide (187) but inhibits those from dibutylnitrosamine (188). Furthermore, a compound may have a biphasic effect, inhibiting carcinogenicity on one regimen and enhancing it under another (189, 190).

Since metabolic activation is required in most cases, blocking the necessary enzyme systems may be one means to retard the action of carcinogens. This analogy seems to hold for aminoacetonitrile, which inhibits the oxidative dealkylation of dialkynitrosamines and delays the tumorigenicity of dimethylnitrosamine (191). Although phenobarbital reportedly induces a deethylase acting on diethylnitrosamine (192) and theoretically should enhance its effect, in animal tests the opposite action has been noted (193, 194). The steroid analogue, pregnenolone-16α-carbonitrile, apparently reduced the toxicity in vivo and the demethylation of dimethylnitrosamine in vitro. However, it did not affect the overall rate of metabolism of dimethylnitrosamine or the extent of binding to cellular macromolecules (195). Thus, results from in vitro studies may not be exactly comparable to those from animal tests.

Inhibition of chemical carcinogenesis can also be achieved through competition by structurally related compounds for the active binding sites in cellular macromolecules. Several noncarcinogenic aromatic hydrocarbons thus served to delay the tumorigenic action of 7,12-dimethylbenz[a]anthracene (183). Similarly simple aromatic amines or amides (acetanilide, *p*-hydroxyacetanilide, or chloramphenicol) decreased tumors from feeding N-2-fluorenylacetamide or some of the aminoazo dyes (196, 197).

However, structural similarity was not always required, for several sulfur-containing compounds decreased the tumor incidence from aromatic hydrocarbons and inhibited the binding of carcinogen to cellular protein and RNA (198, 199).

Of several protective vitamins, riboflavin acts against carcinogenic aminoazo dyes because it functions as part of an azo reductase which cleaves the azo linkage, yielding noncarcinogenic aromatic amines (186). Vitamins A and E reduce the carcinogenicity of several polycyclic hydrocarbons; the mechanism has not been determined (186). Vitamin A analogues or retinoids have a pronounced inhibitory effect on several carcinogens, including N-nitrosomethylurea which can yield an activated form spontaneously (200). The mechanism is unknown but retinoids may affect the development of the preneoplastic lesions rather than influence the carcinogen directly.

Among still other means to inhibit carcinogenesis, the one of greatest environmental importance is that of enzyme induction. If the induced enzymes increase the detoxification pathways of a carcinogen, its toxic and carcinogenic effects can be decreased. However, if the proportion of activated intermediate is greater, potentiation of carcinogenic action results (179). Many enzyme-inducing substances, including pesticides (201, 202), terpenes (203, 204), flavones (185), aromatic hydrocarbons (205), drugs such as phenobarbital or phenothiazine (185, 189, 193, 194), some antioxidants (190, 206), and various natural products (207–209) have shown inhibitory activity against various types of carcinogens. Of special interest are indoles and isothiocyanates present in cruciferous plants used as vegetables (209–211). Such compounds may aid in reducing the impact of human exposure to environmental carcinogens (210).

To summarize, most chemical carcinogens are initially converted through mixed function oxidases to activated forms. Further reaction leads to entities, often transient, which are electrophiles and react with cellular nucleophiles. Other environmental factors may enhance or inhibit the action of carcinogens; on balance there is a preponderance of the inhibitory processes.

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