Methylchrysenes as Probes for the Mechanism of Metabolic Activation of Carcinogenic Methylated Polynuclear Aromatic Hydrocarbons

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The effects of methyl substitution on polynuclear aromatic hydrocarbon (PAH) tumorigenicity are remarkable. For example, 5-methylchrysene (5-MeC) is far more tumorigenic on mouse skin than any of the other methylchrysene isomers or chrysene.[1] Benz[*a*]anthracene is weakly tumorigenic, but 7,12-dimethylbenz[*a*]anthracene (DMBA) is the strongest PAH carcinogen.[2] Many other examples are known. What is the molecular basis for these extraordinary effects? In this Account, we will summarize our current understanding of these structure–activity relationships, with methylchrysenes as examples.

PAH are ubiquitous in the human environment, since they are the products of incomplete combustion of organic matter. Methylated PAH are always components of environmental PAH mixtures.[3] Human exposure to PAH, through tobacco smoke or in certain occupational or urban environments, is associated with increased risks for respiratory tract cancer.[4] In order to evaluate the role of methylated PAH in cancer induction, it is important to understand the mechanisms by which they initiate tumors. This will lead to insights for cancer prevention.

Covalent modification of DNA is a key step in carcinogenesis.[5] PAH do not react covalently with DNA, but require metabolic activation.[6] Metabolites which are on the pathway to reaction with DNA are called proximate carcinogens, and those that react with DNA are termed ultimate carcinogens.[7] The latter are electrophiles. Extensive studies have provided convincing evidence that angular ring dihydrodiol epoxides in which one carbon terminus of the epoxide ring is in the bay region (bay-region dihydrodiol epoxides) are ultimate carcinogens of many PAH.[6,8,9] (The bay region of phenanthrene is the region between the 4 and 5 positions and the angular rings are the 1–4 and 5–8 rings). The bay-region dihydrodiol epoxides are formed metabolically by the sequence: PAH → arene oxide →

Table I
Representative Results of Assays for Tumor Initiating Activity on Mouse Skin of Chrysene, Benz[*a*]anthracene, and Benzo[*a*]pyrene and Their Monomethyl Derivatives[a]

parent ring system	methyl isomer	dose (nmol)	% of mice with skin tumors	skin tumors per mouse	ref
chyrsene	–	4,390	55	0.6	1
	1	4,130	30	0.3	1
	2	4,130	42	0.7	1
	3	4,130	70	1.3	1
		410	15	0.2	1
	4	4,130	35	0.5	1
	5	4,130	85	4.8	1
		410	100	5.5	1
		33	80	3.9	1, 11
	6	4,130	35	0.6	1
benz[*a*]-	–	400	23	0.3	12
anthracene	1	400	27	0.3	12
	2	400	7	0.1	12
	3	400	13	0.2	12
	4	400	10	0.1	12
	5	400	17	0.2	12
	6	400	33	0.6	12
	7	400	77	4.9	12
	8	400	40	1.0	12
	9	400	28	0.6	12
	10	400	13	0.2	12
	11	400	10	0.1	12
	12	400	52	1.0	12
benzo[*a*]pyrene	–	200	67	2.2	13
	1	200	80	3.6	13
	2	200	38	0.6	13
	3	200	76	2.6	13
	4	200	67	2.0	13
	5	200	27	0.4	13
	6	200	24	0.4	13
	7	200	0	0	13
	8	200	3	0.3	13
	9	200	0	0	13
	10	200	0	0	13
	11	200	90	5.6	13
	12	200	69	1.9	13

[a] A more complete compilation of tumorigenic activities of methyl- and dimethyl-substituted PAH can be found in ref 2.

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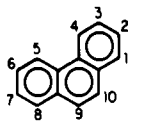
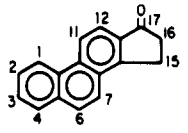
Chart I



phenanthrene

+B effect 1,4-diMe; 4,10-diMe
-P effect 4,9-diMe

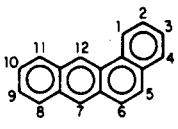


15,16-dihydrocyclopenta[a]-phenanthren-17-one

11-Me

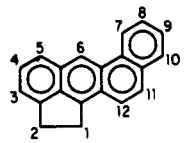
chrysene

5-Me; 5,11-diMe
5,12-diMe



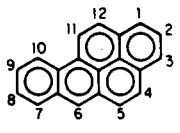
benz[a]anthracene

+B effect 12-Me; 7,12-diMe
-P effect 5,7-diMe, 5,7,12-triMe



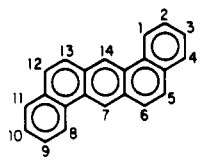
cholanthrene

3,6-diMe
3,11-diMe



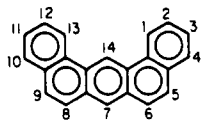
benzo[a]pyrene

11-Me
6-Me



dibenz[a,h]anthracene

+B effect 7,14-diMe
-P effect



dibenz[a,j]anthracene

7,14-diMe

dihydrodiol → dihydrodiol epoxide. Although the bay-region dihydrodiol epoxide hypothesis has been useful in helping to understand the mechanisms of tumor initiation by PAH, it does not by itself explain the effects of methyl substitution on tumorigenesis. All the methylchrysenes and chrysene can form bay-region dihydrodiol epoxides, but only 5-MeC is highly tumorigenic. As we will show, this is due partially to the unique properties of a bay-region dihydrodiol epoxide having a methyl group and epoxide ring in the same bay region.

Structural Requirements Favoring Tumorigenicity of Methylated PAH

The structural requirements favoring mouse skin tumorigenicity of methylated alternant PAH are a bay-region methyl group and free peri position, both adjacent to an unsubstituted angular ring.[10] (The peri positions adjacent to the angular rings of phenanthrene are carbons 9 and 10). The structures of the PAH in which the effects of methyl substitution have been most extensively studied are illustrated in Chart I, and some representative tumorigenesis data are summarized in Table I.

In all of these systems, the compounds with a methyl group in a bay region adjacent to an unsubstituted angular ring are more tumorigenic than are their parent PAH (+B effect). With the exception of 12-methylbenz[*a*]anthracene, all of the +B compounds are also more tumorigenic than any other methyl or dimethyl isomers tested.[1,2,10,12-19] The +B effect is well established in the phenanthrene, chrysene, cyclopenta[*a*]-phenanthrene, benzo[*a*]pyrene, cholanthrene, and dibenzanthracene systems.[1,10,13-19] However, in the nonalternant hydrocarbon benzo[*b*]fluoranthene, methyl substitution in the bay region adjacent to the angular ring does not enhance tumorigenicity.[20]

The peri position adjacent to the appropriate angular ring must be unsubstituted, or tumorigenicity decreases. This "–P effect" has been clearly demonstrated in the phenanthrene, chrysene, benz[*a*]anthracene, 3-methylcholanthrene, and benzo[*a*]pyrene systems.[10,13,15,21-24] Substitution in the appropriate angular

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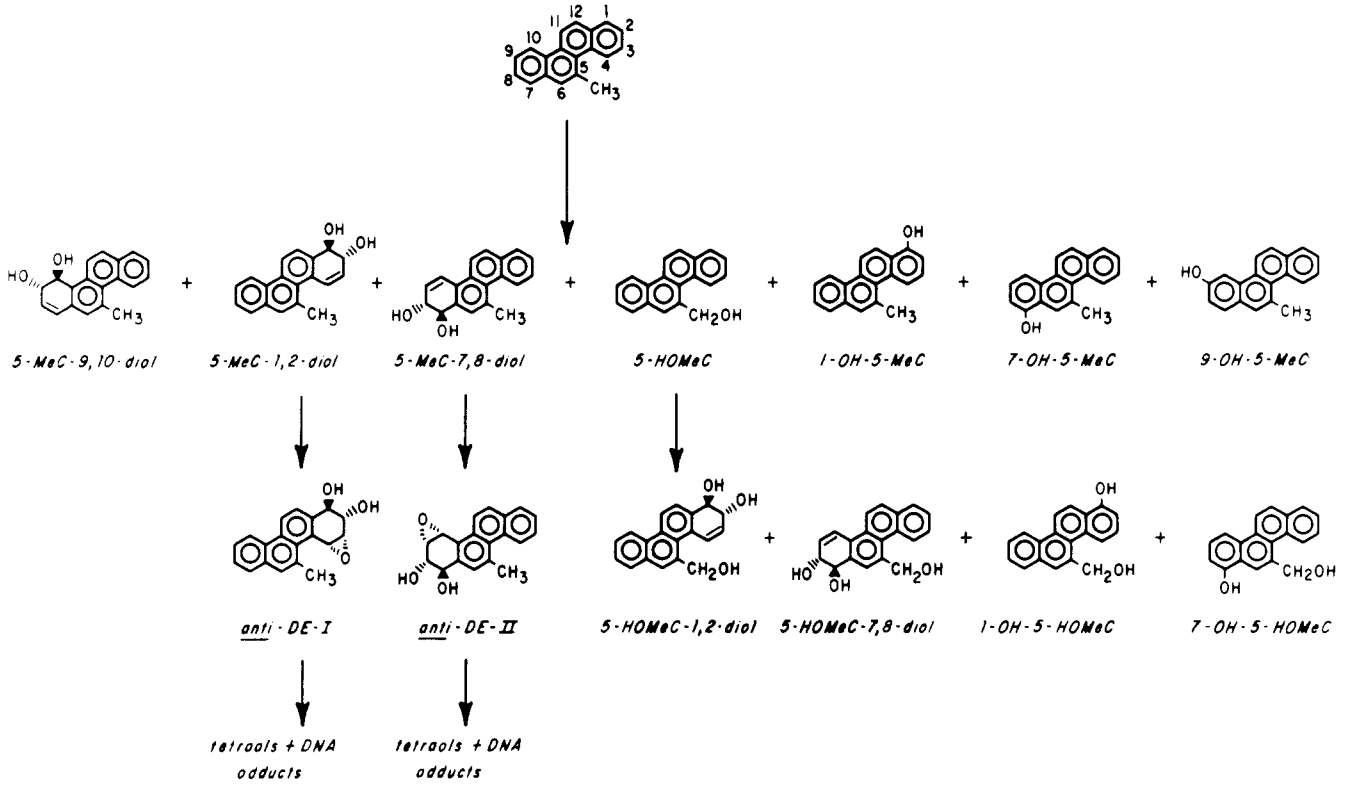

Figure 1. Identified metabolites of 5-MeC. Syn isomers of the dihydrodiol epoxides are also formed (ref 39). Glucuronide, sulfate, and glutathione conjugates of 5-MeC metabolites have been detected (ref 38 and unpublished data).

ring also decreases tumorigenicity (see Table I).

Metabolic Activation of 5-MeC

Our studies have focused on 5-MeC because of its unique tumorigenicity among the MeC isomers. 5-MeC is also structurally unique because it has two bay regions, one of which contains a methyl group. An understanding of the metabolic activation of 5-MeC, which fulfills the structural requirements favoring tumorigenicity of methylated PAH, will provide insights on the factors controlling the effects of methyl substitution on tumorigenicity of other PAH. In this section, we describe experimental approaches which have indicated that a major pathway of metabolic activation of 5-MeC is formation of *anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-5-MeC (*anti*-DE-I).

Fluorine-Probe Studies. Our initial approach focused on the synthesis and bioassay of a series of fluorinated 5-MeC derivatives. We assumed that fluorine substitution would inhibit enzymatic oxidation at the position to which it was attached. Thus, if a fluorinated 5-MeC were less active than was 5-MeC, the results would suggest that the position where fluorine was substituted might be involved in metabolic activation. This approach is well-known in medicinal chemistry and has been used in studies of the metabolic activation of other PAH.[25-32] Seven monofluoro-5-MeC were synthesized and tested for tumorigenicity on mouse skin.[21,33,34] The results clearly showed that 1-F-5-MeC, 3-F-5-MeC, and 12-F-5-MeC were less tumorigenic than was 5-MeC. In contrast, 6-F-5-MeC, 7-F-5-MeC, 9-F-5-MeC, and 11-F-5-MeC were not less tumorigenic than was 5-MeC.[21,34] These results indicated that the 1–4 angular ring of 5-MeC was involved in its metabolic activation.

Identification of 5-MeC Metabolites. Identified metabolites of 5-MeC are illustrated in Figure 1. The general pattern of 5-MeC metabolism is similar to that of other PAH.[34-41] The initial oxidation products are likely to be arene oxides, which can be hydrated to dihydrodiols or can rearrange nonenzymatically to phenols. As in the metabolism of chrysene, dihydrodiol formation in the angular rings is preferred;[42] metabolites

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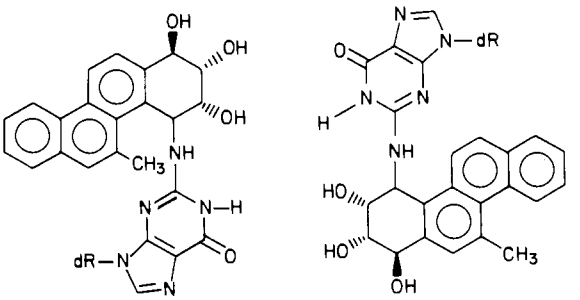
of 5-MeC resulting from oxidation at the 5,6- and 11,12-bonds have not been detected. Initial oxidation at the 3,4-position is also not favored, presumably due to the methyl group. 5-Hydroxymethylchrysene (5-HOMeC) is further oxidized to dihydrodiols and phenols.[36] 5-MeC-1,2-diol and 5-MeC-7,8-diol are of special interest because they are the precursors to the bay-region dihydrodiol epoxides *anti*-DE-I and *anti*-DE-II which lead to DNA adduct formation in mouse skin.[37,38] The syn dihydrodiol epoxides are also formed in mouse skin.[39] Glucuronide and sulfate conjugates of the dihydrodiols and phenols have been observed in mouse skin in vivo.[38]

Tumorigenicity of Metabolites. Identification of *anti*-DE-I as an Ultimate Carcinogen. Mouse skin tumorigenicity assays were carried out to determine which metabolites were involved in metabolic activation. The results clearly showed that 5-MeC-1,2-diol was more tumorigenic than was 5-MeC, but that 5-MeC-7,8-diol was less tumorigenic than was 5-MeC. 5-MeC-9,10-diol was inactive.[11,43] 5-HOMeC had comparable tumorigenic activity to that of 5-MeC.[36] All of the metabolically formed hydroxy-5-MeC were tumorigenic, but none was as active as 5-MeC. These results indicated that 5-MeC-1,2-diol was a likely major proximate carcinogen of 5-MeC. The high activity of 5-MeC-1,2-diol was not unexpected since it is the precursor to a bay-region dihydrodiol epoxide. However, the higher tumorigenicity of 5-MeC-1,2-diol than of 5-MeC-7,8-diol was intriguing since the latter could also form a bay-region dihydrodiol epoxide.

In the next step, the tumorigenic activities of the corresponding dihydrodiol epoxides were tested.[11] These assays were carried out in mouse skin and in newborn mice. The newborn mouse lung is more sensitive to dihydrodiol epoxides than is mouse skin.[8] The results demonstrated that *anti*-DE-I was more tumorigenic than was *anti*-DE-II. The newborn mouse assays clearly showed that *anti*-DE-I was exceptionally tumorigenic, with higher activity than the 5-MeC-diols or 5-MeC. Taken together, these data provide strong evidence that a major activation pathway is 5-MeC → 5-MeC-1,2-diol → *anti*-DE-I. The results show that a bay-region dihydrodiol epoxide, *anti*-DE-I, with the methyl group and epoxide ring in the same bay region, is a potent tumorigen. In contrast, no significant tumorigenicity was observed for *anti*-DE-II which is also a bay-region dihydrodiol epoxide but does not have the epoxide ring and the methyl group in the same bay region.

The results of these bioassays agreed remarkably well with the results of the structure-activity studies of fluorinated 5-MeC. Substitution of fluorine at the 1- and 3- positions should block formation of *anti*-DE-I; the inactivity of 1-F-5-MeC and 3-F-5-MeC can be explained on this basis.[34] The low activity of 12-F-5-MeC can also be explained by inhibition of 5-MeC-1,2-diol formation (see section on –P effect below).[44] In contrast, 7-F-5-MeC and 9-F-5-MeC had tumorigenic activities similar to that of 5-MeC because 5-MeC-7,8-diol does not contribute significantly to 5-MeC tumorigenesis. Thus, the fluorine probe studies provide strong supportive evidence for the hypothesis that *anti*-DE-I is a major ultimate carcinogen of 5-MeC. Similar results were obtained in fluorine probe studies of 5-HOMeC, indicating that it is activated via its 1,2-dihydrodiol-3,4-epoxide.[36]

DNA Adduct Formation from 5-MeC and Its Dihydrodiol Epoxides. Since *anti*-DE-I appeared to be a major ultimate carcinogen of 5-MeC, its reactions with DNA were investigated, and compared to those of *anti*-DE-II. Reaction of each racemic dihydrodiol epoxide with DNA gave at least 7 adducts, but in each case one adduct predominated. These adducts were



identified by their spectral properties.[45] Their structural features are similar to those observed for other PAH dihydrodiol epoxide–DNA adducts[2].

These major adducts were also identified in mouse skin, after treatment with [^{3}H]5-MeC. The ratio of the *anti*-DE-I adduct to the *anti*-DE-II adduct was 3 to 1, 4–48 h after application of [^{3}H]5-MeC.[37,38] The predominance of the *anti*-DE-I adducts over the *anti*-DE-II adducts parallels the results described above which indicate that *anti*-DE-I, but not *anti*-DE-II, is a major ultimate carcinogen of 5-MeC. Some possible reasons for the preferential formation of *anti*-DE-I adducts were investigated.[38] The extents of formation of 5-MeC-1,2-diol and 5-MeC-7,8-diol in mouse epidermis were the same. Differences in metabolism of 5-MeC to these two potential proximate carcinogens would therefore not account for the observed differences in DNA adduct formation. The comparative metabolism of the dihydrodiols to *anti*-DE-I and *anti*-DE-II in mouse skin has not been examined. On steric grounds, preferential formation of *anti*-DE-II would be expected. Studies of the reactivity of *anti*-DE-I and *anti*-DE-II with DNA in vitro indicated that *anti*-DE-I reacted more extensively. This could be partially responsible for the higher in vivo levels of *anti*-DE-I–DNA adducts, and was investigated in more detail as described below. Since the ratio of the major *anti*-DE-I–DNA adduct to the *anti*-DE-II–DNA adduct was constant over the 4–48-h period investigated, differential repair of the 2 adducts would not seem to be involved in their differing levels.

These results indicated that dihydrodiol epoxide reactivity with DNA could play a role in the preferential formation of *anti*-DE-I DNA adducts in vivo. To examine this in more detail, the rates of hydrolysis and extents of DNA binding of *syn*- and *anti*-DE-I, *syn*- and *anti*-DE-II, and *anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrochrysene (*anti*-chrysene-DE) were investigated.[46] The rates of hydrolysis of the dihydrodiol

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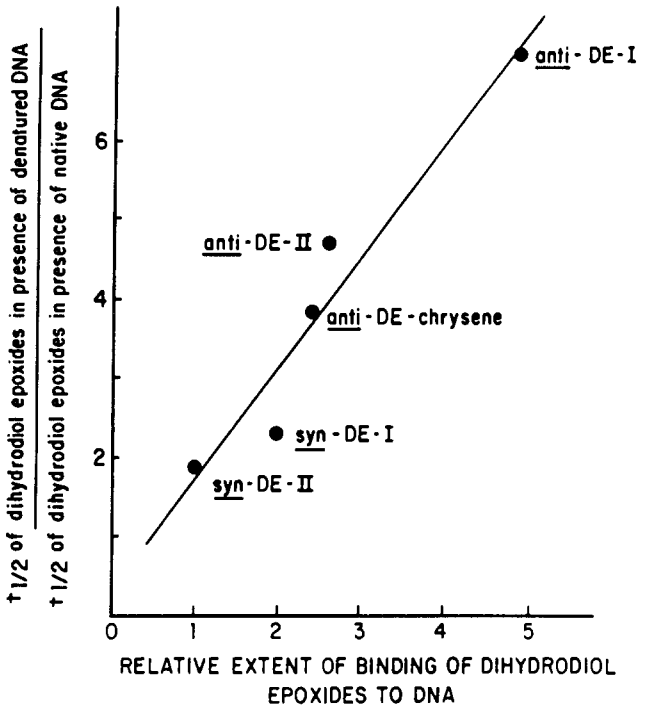

Figure 2. Plot of the ratio of the half-lives of dihydrodiol epoxides in the presence of denatured DNA to their half-lives in the presence of native DNA vs. their extents of covalent binding to calf thymus DNA.

epoxides did not correlate with their DNA binding properties. When the hydrolyses were carried out in the presence of denatured DNA, a rate enhancement of 1.1- to 2.5-fold was observed. In the presence of native DNA the enhancement was 2- to 17-fold. The ratios of the rates of hydrolysis in the presence of native DNA to the rates of hydrolysis in the presence of denaturated DNA did correlate with the extents of DNA binding (Figure 2). These results suggest that physical association of the dihydrodiol epoxide with DNA precedes reaction, as has been observed with B[*a*]P-7,8-dihydrodiol-9,10-epoxides.[47-49] It is of interest that the greatest rate enhancement and highest extent of binding were observed for *anti*-DE-I. This parallels the greater in vivo DNA binding and tumorigenicity of *anti*-DE-I compared to *anti*-DE-II and the other dihydrodiol epoxides. A unique structural feature of *anti*-DE-I is the presence of a methyl group in the same bay region as the epoxide ring. Among the six MeC isomers, only 5-MeC can form such a metabolite.

Relationship of Metabolic Activation Pathways to the +B Effect

The +B effect is the key structural requirement leading to high methylated PAH tumorigenicity. 5-MeC is a useful model for studying this effect because it has two bay regions but only one of them has a methyl group. Thus, comparisons of the chemical and biological properties of the dihydrodiols and dihydrodiol epoxides that can form in the two angular rings of 5-MeC provides insight into the mechanism of the +B effect. As described above, these studies have shown that the high tumorigenicity and reactivity with DNA of *anti*-DE-I can explain the +B effect in the MeC system.

While these comparative studies of 5-MeC activation via the DE-I and DE-II pathways provide an intramolecular probe for the mechanism of the +B effect, comparison of the metabolic activation of 5-MeC and 6-MeC provides an intermolecular probe.[40] The structural difference between 5-MeC and 6-MeC is that the former contains a bay-region methyl group and the latter does not, and of course 5-MeC is a strong tumorigen whereas 6-MeC is not. Comparison of the metabolism of [^{3}H]6-MeC and [^{3}H]5-MeC in mouse skin showed that [^{3}H]6-MeC-1,2-diol was the major metabolite of the former and that its concentration was greater than that of [^{3}H]5-MeC-1,2-diol, produced from [^{3}H]5-MeC. In fact, 1,2-dihydrodiol formation was the major pathway of [^{3}H]6-MeC metabolism in mouse skin. Thus the bay-region methyl group of 5-MeC did not enhance 1,2-dihydrodiol formation relative to 6-MeC. 5-MeC-1,2-diol was significantly more tumorigenic than was 6-MeC-1,2-diol. Metabolic studies showed that the conversions in mouse skin of the two dihydrodiols to tetraols, as a monitor for dihydrodiol epoxide formation, were similar. Comparison of the extents of DNA adduct formation from [^{3}H]5-MeC and [^{3}H]6-MeC in mouse skin showed that dihydrodiol epoxide–DNA adducts from [^{3}H]5-MeC exceeded those from [^{3}H]6-MeC by about 20-fold. Taken together, these results present a picture similar to that obtained by the comparative studies of the 5-MeC-1,2-diol vs. 5-MeC-7,8-diol pathways. They indicated that the strong tumorigenicity of 5-MeC is due to the unique reactivity and tumorigenicity of *anti*-DE-I. It can be concluded from these studies that the +B effect results from the unique properties of such bay-region dihydrodiol epoxide metabolites.

Bay-region dihydrodiol epoxides having a methyl group and epoxide ring in the same bay region are important in the metabolic activation of other methylated PAH. Studies on 11-methylcyclopenta[*a*]-phenanthrene-17-one have shown that its 3,4-dihydrodiol 1,2-epoxide is an ultimate carcinogen.[50] Comparative studies of the metabolic activation of the various methylcyclopenta[*a*]phenanthren-17-one isomers have led to conclusions similar to those described above, although differences in repair of DNA adducts from the 11- and 12-methyl isomers also seem to be involved.[51,52] Investigations of the metabolic activation of DMBA have clearly shown that the 3,4-dihydrodiol 1,2-epoxide metabolite, which has a methyl group and epoxide ring in the same bay region, is involved in the formation of major DNA adducts in mouse skin. Adducts are formed from both the *anti*- and *syn*-dihydrodiol epoxides and it has been suggested that the *syn*-adducts, which result mainly from interaction with deoxyadenosine, may be partially responsible for the unique tumorigenicity of DMBA.[53-56]

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X-ray crystallographic studies of methylated PAH having a bay-region methyl group have shown that distortions from normal PAH geometry occur.[57] In 5,12-diMeC and 5-MeC, in-plane and out-of-plane distortions occur to accomodate the methyl group in the bay region. The in-plane distortions result from widening of the bay region and the out-of-plane distortions from torsion about the bay-region bonds. Out-of-plane distortions are greater in DMBA than in 5-MeC. The extent to which these distortions may play a role in the +B effect is not known, but based on the discussion above it would be expected that distortions in the dihydrodiol epoxide metabolite would be of greatest importance.

Relationship of Metabolic Activation Pathways to the -P Effect

The conformation of angular ring dihydrodiols is important in determining their tumorigenic properties. Angular ring dihydrodiols with the hydroxyl groups in the diequatorial conformation are generally converted readily to the corresponding bay-region dihydrodiol epoxides. However, dihydrodiols with their hydroxyl groups in the diaxial conformation are frequently not converted enzymatically to the corresponding dihydrodiol epoxides and/or the dihydrodiol epoxides are not exceptionally tumorigenic.[8,58] When a methyl group occupies the peri position adjacent to an angular ring, as in 6-methylbenzo[*a*]pyrene or 5,12-diMeC, the conformation of the angular ring dihydrodiol will be diaxial because of the steric bulk of the methyl group. A fluorine atom has the same effect. Thus, it has been suggested that a peri-methyl group can inhibit tumorigenicity by altering the conformation of the dihydrodiol precursor to a bay-region dihydrodiol epoxide from diequatorial to diaxial. Studies of 6-methylbenzo[*a*]pyrene and 6-fluorobenzo[*a*]pyrene metabolism have provided convincing evidence that a conformational change of the 7,8-dihydrodiol from diequatorial to diaxial is partially responsible for their lower tumorigenic activities compared to benzo[*a*]pyrene.[30,59,60]

A second possible explanation for the -P effect is that a peri-methyl group may inhibit formation of the dihydrodiol in the adjacent angular ring. Comparative metabolic studies of the strong tumorigen 5,11-diMeC and the inactive peri-substituted 5,12-diMeC provide support for this hypothesis.[61] These experiments indicated that 5,12-diMeC was preferentially metabolized at its 7,8-positions compared to its 1,2-positions. The ratio of 7-hydroxy-5,12-diMeC to 1-hydroxy-5,12-diMeC was as high as 100 to 1, and formation of 5,12-diMeC-7,8-diol greatly exceeded formation of 5,12-diMeC-1,2-diol. In 5,11-diMeC, the 1,2-dihydrodiol was formed. These results strongly suggest that a shift in metabolism from the 1,2- to the 7,8-bond is the basis for the lower tumorigenicity of 5,12-diMeC compared to 5-MeC and 5,11-diMeC. 7,8-Dihydrodiol formaton is also inhibited in 6-methylbenzo[*a*]pyrene compared to benzo[*a*]pyrene.[60] Comparative studies of [^{3}H]5-MeC and [^{3}H]12-F-5-MeC metabolism have been carried out in mouse skin.[44] Whereas the ratio of [^{3}H]5-MeC-1,2-diol to [^{3}H]5-MeC-7,8-diol was 1:1, the ratio of [^{3}H]12-F-5-MeC-1,2-diol to [^{3}H]12-F-5-MeC-7,8-diol was 1:68. These results provide an explanation for the -P effect in 12-F-5-MeC.

Prospects for Further Research

With some exceptions, the basic relationships between structure and tumorigenicity among methylated PAH are now reasonably well-understood. This is not the case for the methylated nonalternant PAH. However, further research is required before the detailed steps of methylated PAH activation leading to tumor initiation can be fully understood. For example, methylated PAH such as 5-MeC and DMBA form multiple DNA adducts in target tissues. Which adducts are responsible for tumor initiation? Do certain adducts activate protooncogenes, as has been suggested for the *syn*-deoxyadenosine adducts of DMBA?[55] What are the structural features, if any, which may favor oncogene activation by methylated PAH? The stereochemical details of PAH dihydrodiol epoxide–DNA interactions are important in determining biological activity but these details are not well understood for methylated PAH. In addition, very little is known about methylated PAH distribution, pharmacokinetics, and excretion particularly as conjugated metabolites. Most metabolic studies have been carried out in vitro or in particular tissues such as breast or skin in vivo, but the picture in the whole animal is not clear.

Almost all bioassays of methylated PAH have been carried out by topical application in mice or by subcutaneous or intravenous injection in rats. Nothing is known about the consequences of inhalation exposure to methylated PAH yet this is probably the major route of human exposure to these compounds. Although such assays are expensive, they could be informative if a few important methylated PAH were judiciously chosen for study. An added complication is the effects of other components of the mixtures in which the methylated PAH are always found. Some studies have been carried out on the effects of other PAH on the tumorigenicity and metabolism of methylated PAH;[62,63] more work in this area is needed. Cocarcinogens, promoters, and inhibitors of tumorigenesis can occur in the diet and in the general environment. More research is needed on their effects on methylated PAH tumorigenicity. In particular, the general conclusion that dihydrodiol epoxides are important ultimate carcinogens of methylated PAH suggests new approaches for the identification of naturally occurring or synthetic dihydrodiol epoxide scavengers, which may be effective in vivo. Extensive studies of this type are already underway for benzo[*a*]pyrene.[64,65]

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Approaches for the estimation of individual human susceptibility to methylated PAH need to be developed. With the basic metabolic patterns of the more tumorigenic members of the class having been established, it should be possible to develop sensitive assays to detect key metabolites or adducts in blood, urine, or exfoliated cells. Such assays are already available for benzo[*a*]pyrene–DNA adducts.[66] The biological significance of particular measurable adducts or metabolites, with respect to tumor formation, needs to be established in animal studies. These results can possibly be used to provide an index of human susceptibility to methylated PAH tumorigenesis.

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Nuclease Activity of 1,10-Phenanthroline–Copper Ion

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DNA exhibits conformational variability that is dependent on base composition and environmental conditions. Linear DNA can adopt at least three distinct double-stranded helical forms which retain the fundamental Watson–Crick A–T and G–C base pairings. These include the A, B, and Z structures. Humidity affects the relative stability of the A and B structures in DNA fibers while salt concentration, counterions, heat, and organic solvents can influence the relative stabilities of single-stranded and the three double-stranded structures in soluble DNA. In addition, closed circular DNAs assume superhelical conformations which alter the density of base pairs in a given linear sequence.[1]

Crystallographic analysis of the self complementary dodecamer 5′-CGCGAATTCGCG-3′ has enhanced the awareness of the possible sequence dependent variation of DNA even within a prevailing B format. Although the averaged helical parameters of the B helix correspond to those of the Watson–Crick structure, values of the helical twist, base plane roll, propellor twist, and torsion angle vary within the sequence.[2] Steric arguments emphasizing repulsive interactions across the major and minor groove of DNA have been proposed to account for these structural variations.[3,4]

What experimental methods can detect conformational variability in DNA? Does this conformational variability, if it exists, play a functionally significant role in modulating the expression and/or organization of DNA within a cell? Do proteins which decipher the genetic message alter the structure of DNA? The purposes of this Account are to review the chemistry of the oxidative nuclease activity of the 2:1 1,10-phenanthroline–cuprous complex, $(OP)_2Cu^+$, and its coreactant, hydrogen peroxide, and to present recent results which suggest this nuclease activity provides one useful avenue to approach the issues raised above.

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Discovery of Nuclease Activity

The nuclease activity of $(OP)_2Cu^+$ was discovered by our research group in 1979 while investigating the inhibition by 1,10-phenanthroline of the poly[d(A-T)] directed polymerization catalyzed by *E. coli* DNA polymerase I.[5] These studies were initiated on the premise that OP inhibition reflected an essential role for tightly bound zinc ion. Instead, we found that the inhibition required cupric ion and thiol.[6,7] Since preincubation of poly[d(A-T)] with OP, Cu^{2+}, and mercaptopropionic acid under aerobic conditions greatly enhanced the inhibition of the polymerization activity, a reaction with DNA was strongly suggested. Electrophoretic analysis of the incubation mixture revealed extensive depolymerization of the poly[d(A-T)] with complete correspondence between the reaction conditions which inhibited enzymatic activity and those which caused extensive degradation of the DNA. Ironically, subsequent work demonstrated that *E. coli* DNA polymerase I was not a zinc metalloenzyme.[8]

$(OP)_2Cu^+$ and H_2O_2 Are Coreactants

The efficiency of the nuclease reaction was striking since submicromolar levels of copper were sufficient to

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