ments of these interactions often have much in common with the treatment of CT perturbations outlined above. While there may be some intuitive value in distinguishing "through-bond" and "through-space" interactions, it may be useful to replace them with the idea that induced dipole moments modify the "shape" of the electronic wave functions in spatial regions of maximum donor–acceptor orbital overlap. Induced dipole moments are directional, and this feature suggests the possibility of some tendency of the perturbations to favor charge or energy transfer along one spatial direction over any others. Although the basis of this inference is a simple heuristic model, the idea is interesting enough to warrant some experimental study. It could be important in understanding electron transport in complex systems.

Progress in the study of electronic effects in electron-transfer and energy-transfer reactions has been dramatic in the past decade, but many of the important fundamental issues are only beginning to be articulated and few implications of the patterns of behavior discussed above have been explored. This article has developed some of these issues. Further intensive studies are likely to lead to a detailed understanding of electron transport in biologically important protein systems. Other novel implications seem likely, such as the manipulation of the probability of photochemical upconversion (i.e., the annihilation of two excited states to produce a high-energy photon). The area of research should continue to provide challenges for significant experimental and theoretical study. Maybe we will even get the orbitals back into electron-transfer chemistry.

The contributions of many co-workers and colleagues have been noted in the references. Much of the work and many of the ideas presented in this article were generated or stimulated during a collaboration with Dr. T. Ramasami a few years ago. Dr. Carolyn L. Schwarz provided a great deal of help with the evolution and critiquing of the manuscript. Much of the research from my laboratory was partially supported by the National Institutes of Health and the National Science Foundation. Wayne State University provided appreciable support for this work throughout its course.

Intercalation and Binding of Carcinogenic Hydrocarbon Metabolites to Nucleic Acids

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Two central dogmas underlie modern research in chemical carcinogenesis. The first is Miller's hypothesis[1] that the active forms of most carcinogens are electrophilic intermediates formed metabolically. The second is the assumption that the initiating step in the induction of tumors is the covalent binding of the active carcinogen species to a cellular macromolecule, generally presumed to be DNA. While there exists abundant evidence that formation of carcinogen–DNA adducts can result in mutations that lead ultimately to the induction of cancer, the details of the process at the molecular–genetic level remain obscure.

Ronald G. Harvey was born in Ottawa, Canada. He received a B.S. in biology from UCLA in 1952. Following military service and two years as project leader at Sinclair Research Laboratories, Harvey, IL, he completed his academic studies at The University of Chicago, where he received a Ph. D. in chemistry in 1960. He subsequently joined the faculty of the Ben May Institute of The University of Chicago. Except for a year of postdoctoral study with D. H. R. Barton at Imperial College, University of London, his academic career has been entirely at The University of Chicago, where he is now Professor. His major research interests include the chemistry of polycyclic hydrocarbons, the mechanisms of chemical carcinogenesis, and novel synthetic methods.

Nicholas E. Geacintov was born in France and was educated at Syracuse University (New York State College of Forestry), where he received a Ph.D. in 1961 in polymer science. After postdoctoral work at the Polytechnic Institute of Brooklyn with Gerald Oster, he became interested in photochemistry. His primary interests at the present time involve applications of molecular spectroscopic techniques in various fields of biophysical chemistry, including the mechanisms of interactions of polycyclic aromatic mutagens and carcinogens with nucleic acids. He has been a member of the Chemistry Department at New York University since 1969.

This Account reports recent advances in understanding the mechanism of interaction with nucleic acids of the active metabolites of one class of carcinogens, the polycyclic aromatic hydrocarbons (PAHs). That PAHs potentially play an important role in human cancer is suggested by their widespread environmental prevalence,[2,3] their relatively high tumorigenic potency, and their broad spectrum of activity in animal tissues.[4] Significant levels of benzo[*a*]pyrene and other carcinogenic PAHs are present in urban air, in auto exhaust, and in many common foods.[2,3] As a class, the PAHs rank second only to mycotoxin mold metabolites, e.g., aflatoxin, in relative carcinogenic potency. Moreover, PAHs are uniquely capable of selectively inducing diverse tumors in animal tissues, including mammary carcinoma, leukemia, sarcoma, etc., dependent upon the experimental conditions employed.[4] PAHs also offer significant advantages as model compounds for re-

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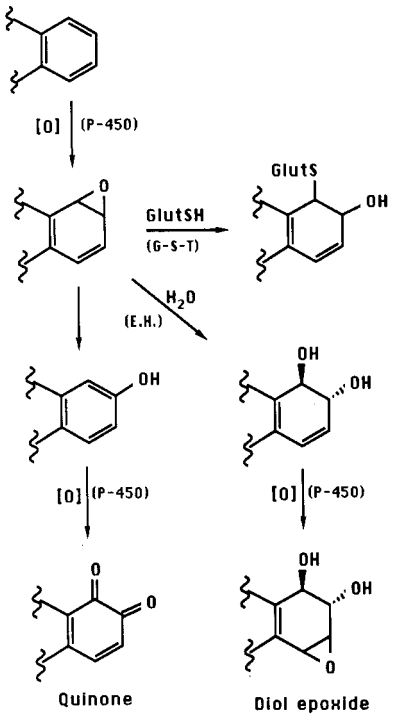

Figure 1. Metabolism of PAHs by P-450 microsomal enzymes. GlutSH = glutathione; G-S-T = glutathione-*S*-transferase; E.H. = epoxide hydrase.

search. In particular, their strong ultraviolet absorption and fluorescence allow detection of low levels of PAH compounds without the need for isotopic labels. Moreover, the structure–activity relationships of PAHs have been extensively investigated, and the findings provide a useful tool to probe mechanism.

Metabolic Activation and DNA Binding

Polycyclic hydrocarbons require metabolic activation to express their biological potential. Metabolism occurs principally on the microsomes of the endoplasmic reticulum catalyzed by the mixed-function oxygenase enzymes.[5] The primary metabolites are relatively unstable arene oxide intermediates (Figure 1).[6] The less stable arene oxides rearrange spontaneously to phenols, and the predominant phenolic isomers formed are predictable by molecular orbital theory.[7] The more stable arene oxide metabolites survive sufficiently long to undergo hydration catalyzed by epoxide hydrase to yield *trans*-dihydrodiols or to add glutathione catalyzed by glutathione-*S*-transferase. The phenols and dihydrodiols are excreted principally as their water-soluble glucuronic and sulfate esters, while the glutathione conjugates undergo degradation to mercapturic acid derivatives. Further oxidative metabolism of the phenols and dihydrodiols yields quinones,[8] diol epoxides, and other products. The diol epoxides are generally not

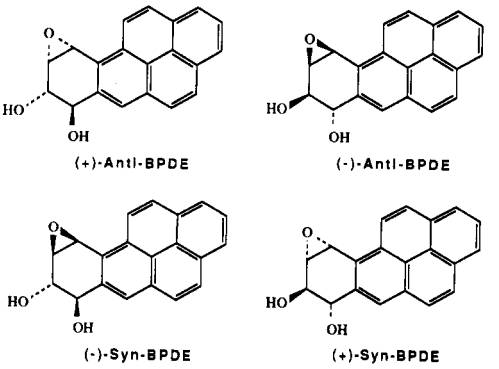

Figure 2. Structures and absolute stereochemistry of the anti and syn diastereomers of the benzo[*a*]pyrene *trans*-7,8-dihydrodiol 9,10-epoxide (BPDE).

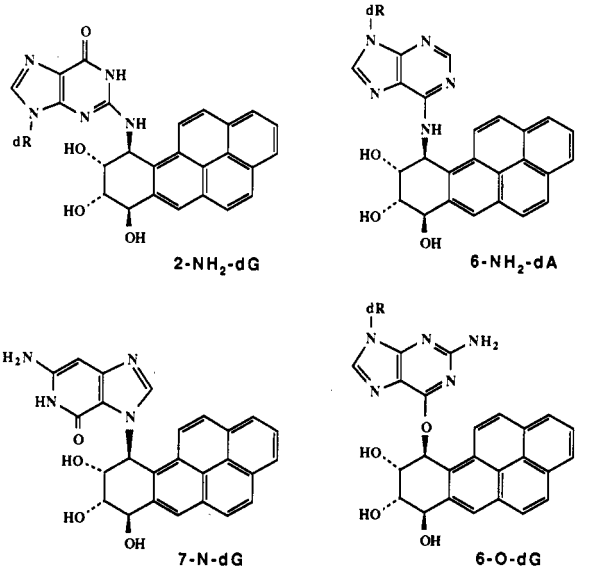

Figure 3. Structures of the major (2-NH_2-dG) and minor nucleoside adducts formed by the covalent binding to DNA.

isolable due to the facility of their reactions with water and other nucleophiles. However, there is substantial evidence implicating them as the principal carcinogen metabolites of PAHs.

The structures of the isomeric *anti*- and *syn*-diol epoxide derivatives of benzo[*a*]pyrene are shown in Figure 2. In the anti diastereomer (*anti*-BPDE) the benzylic hydroxyl group and the epoxide oxygen atom are on opposite faces of the molecule, whereas in the syn isomer (*syn*-BPDE) these groups are on the same face. Since each diastereomer may exist as a pair of enantiomers, four stereoisomers of BPDE are possible. Methods for the stereospecific syntheses of *anti*- and *syn*-BPDE were developed in our laboratories[11] and by Jerina's group.[12]

The principal clue that led to the identification of diol epoxides as the carcinogenic forms of PAHs was their covalent binding to nucleic acids. Initially, Borgen et al.[13] observed that the 7,8-dihydrodiol of benzo[*a*]pyr-

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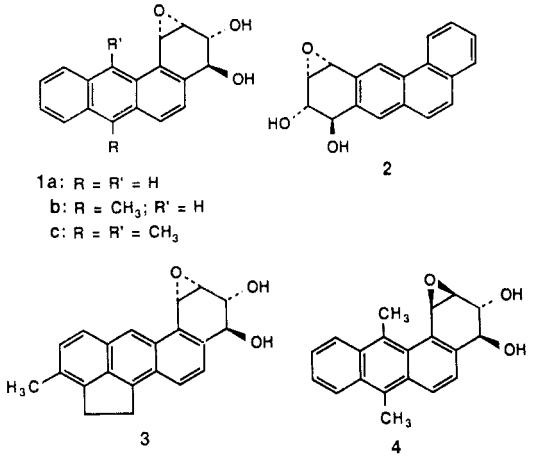

Figure 4. Active metabolites of BA, MBA, and DMBA.

ene was metabolized by rat liver microsomes to a reactive intermediate that bound covalently to DNA. Sims et al.[14] proposed a diol epoxide structure of unspecified stereochemistry. In collaboration with Brookes and Weinstein we found that the major product of reaction of synthetic *anti*-BPDE with poly-G following degradation to the nucleoside level was identical with the principal product of metabolism and binding of benzo[*a*]pyrene to DNA and RNA in rodent, bovine, and human cells.[15] This adduct was shown by chemical evidence and by Fourier transform NMR and mass spectral analysis to be a guanosine derivative covalently linked to the 2-NH_2 group (Figure 3).[16] Its absolute configuration was assigned by the exciton chirality dichroism method and shown to be derived from (+)-*anti*-BPDE.[17] Minor nucleic acid bound products arising from reaction of *anti*-BPDE on 6-NH_2-dA, 7-N-dG, and 6-O-dG have subsequently also been characterized,[18] and evidence has been obtained for alkylation of phosphate groups.[19] Small but significant quantities of *syn*-BPDE adducts, including adducts formed by reaction with adenine and cytosine residues, have also been detected, but not thoroughly characterized.[20]

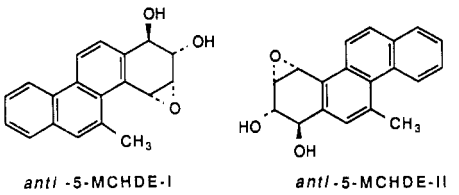

Figure 5. The isomeric *anti*-diol epoxide metabolites of 5-methylchrysene.

Additional evidence for the importance of diol epoxide metabolites comes from mutagenicity and tumorigenicity assays. Although *anti*- and *syn*-BPDE are both strongly mutagenic in bacterial and mammalian cells, *anti*-BPDE shows generally greater activity than *syn*-BPDE in most tests.[21] *anti*-BPDE also shows higher activity in the induction of malignant transformation of mouse fibroblasts,[22] in the inhibition of replication of bacterial viruses,[23] and as a carcinogen on mouse skin and in newborn mouse lung.[24,25]

Similar studies with other PAHs support the hypothesis that analogous diol epoxide metabolites are also the principal active carcinogenic forms of these PAHs. In most cases, the evidence rests primarily on metabolism studies and comparison of the mutagenicities and tumorigenicities of the possible isomeric dihydrodiol and diol epoxide metabolites obtained synthetically.[26] The findings are in agreement with predictions of molecular orbital theory that bay region diol epoxides are more reactive than their isomers.[27] In the case of benz[*a*]anthracene (BA), the *trans*-3,4-dihydrodiol which is the metabolic precursor of the diol epoxide structurally analogous to BPDE (Figure 4: **1a**) was shown to be more mutagenic and more tumorigenic than BA or the four other possible dihydrodiol isomers.[28] The BA diol epoxide **1a** was 5 times more active than BA as a skin tumor initiator, whereas the other diol epoxide isomers were less active than BA.[28] The DNA adducts formed from the metabolism of BA were found to arise mainly from the non-benzo-ring *anti*-diol epoxide isomer (**2**), with only a small percentage deriving from **1a**.[26,29] Both **1a** and **2** react preferentially at the 2-NH_2-dG site in nucleic acids, although **1a** is somewhat more reactive.[30] The weak carcinogenicity

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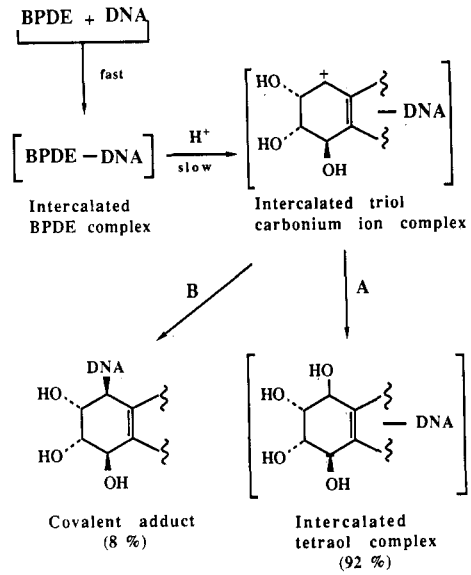

Figure 6. Mechanism of interaction of *anti*-BPDE with DNA.

of BA appears to result from its preferential metabolism to **2** rather than to the more biologically active isomer **1a**.

Metabolism of the BA analogues 7-methyl-BA (MBA), 7,12-dimethyl-BA (DMBA), and 3-methylcholanthrene (3-MC) affords considerably higher proportions of the dihydrodiol precursors of the corresponding bay region diol epoxide metabolites (Figure 4: **1b**, **1c**, **3**).[26] The considerably greater carcinogenic potency of these PAHs relative to BA appears to be partially due to inhibition of metabolism in the terminal ring by substitution in the meso region, resulting in increased formation of the bay region diol epoxides. The evidence from biological and DNA binding studies[26] supports the diol epoxide metabolites **1b**, **1c**, and **3** as the active forms of these carcinogens. In the case of DMBA, Dipple and co-workers have shown that a substantial proportion of the DNA adducts arise from the *syn*-diol epoxide isomer **4** and that a higher than usual level of adenosine adducts is formed in addition to 2-NH_2-dG adducts.[31]

In the chrysene series, the 5-methyl derivative, which like DMBA possesses a methyl group in a bay region, is considerably more carcinogenic than chrysene itself.[32] Although metabolism affords dihydrodiols in both terminal rings approximately equally,[33] the major DNA-bound adduct in mouse skin is *anti*-5-MCHDE-I (Figure 5), which has the methyl group in the same bay region as the epoxide function.[34] *anti*-5-MCHDE-I is also more biologically active than the related syn isomer or the diol epoxide isomers in the alternative bay region (5-MCHDE-II).[35] The principal site of attack of *anti*-5-MCHDE-I on DNA is 2-NH_2-dG.[34]

Mechanism of DNA Binding

Elucidation of the mechanism of interaction of reactive PAH metabolites with nucleic acids is crucial to understanding PAH carcinogenesis. Spectroscopic studies of the reaction of *anti*-BPDE with native calf thymus DNA are consistent with a mechanism (Figure 6) involving initial rapid intercalation (complete in <5 ms) of the diol epoxide between the base pairs of DNA.[36-41] This complex is detectable by a red shift (10 nm) in its UV absorption maximum and its negative linear dichroism spectrum,[39,40] both of which are consistent with an intercalated structure in which the plane of the pyrene chromophore lies parallel to the DNA base pairs. This complex undergoes rate-determining protonation to yield an intercalated triol carbonium ion intermediate which decomposes to products via two pathways. The major path (A), which accounts for >90% of BPDE, is *hydrolysis* to yield tetraols which also physically associate with DNA. The minor, more biologically important, path (B) is *covalent binding* to DNA.[42] While the overall rate of reaction is dependent upon pH, temperature, ionic strength, solvent, and other factors, the ratio of A/B is independent of these variables.[36] This mechanism is also consistent with the prediction of theoretical molecular modeling studies.[43]

Further evidence for the role of intercalation is provided by experiments with a series of 1-alkylbenzo[*a*]pyrenes varying in size from methyl to *tert*-butyl.[44] Tumorigenicity on mouse skin was found to be maximum for the methyl analogue and decreased markedly with increasing group size. Mutagenicity in both hamster V79 cells and bacterial cells showed a similar relation. Since no significant differences are observed in the extents of activation of these PAHs to carcinogenic metabolites, the decrease in biological activity with increasing group size is most likely a consequence of steric interference with intercalation of the diol epoxide metabolites into the DNA helix. Other factors such as differential rates of repair and stability of adducts may also be involved. Studies of the physical binding of the same series of PAHs to DNA confirm that the extent of intercalative binding is inversely related to the steric requirements of the alkyl groups in the 1-position.[45]

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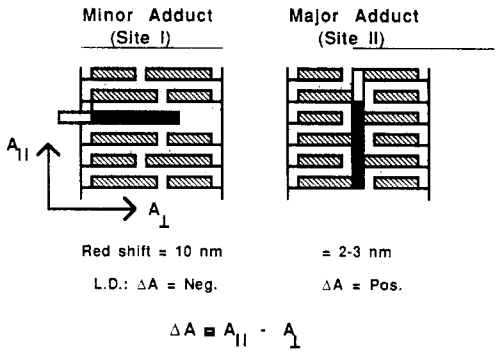

Figure 7. Schematic representation of the structures of the principal adducts formed on covalent binding of *anti*-BPDE to DNA.

However, the relation between intercalation and the levels of formation of covalent adducts appears to be complex and dependent upon both PAH molecular structure and reaction conditions.[46] Furthermore, while covalent binding of BPDE occurs preferentially at dG sites, the formation of physical intercalation complexes is favored at alternating dA-dT sequences.[47] An analysis of these effects suggests that the ability of a given PAH diol epoxide to form covalent adducts with DNA in a cellular environment depends on its ability to form physical complexes with DNA at any sequence;[46] the formation of such complexes prevents these molecules from undergoing chemical reactions with other cellular nucleophiles. It has been suggested that the PAH diol epoxide molecules may diffuse from one binding site to another on the DNA molecule, so that the BPDE molecules initially associated with dA-dT sites may eventually react with dG or other critical site.[46]

Structures of DNA-Bound Adducts

The structures of the covalent adducts formed by *anti*-BPDE with DNA are of two types, designated "site I" and "site II" according to their spectroscopic properties. The site I adducts, which are relatively minor products, are characterized by a negative linear dichroism (LD) spectrum and a 10-nm red shift in the ultraviolet absorption spectrum. The site II adducts exhibit a positive LD and a smaller 2–3-nm red shift in the ultraviolet region. Geacintov et al. proposed an intercalated structure for the site I adduct and for the site II adduct, an externally bound conformation with the aromatic moiety residing in the minor groove with its long axis inclined at an angle of ~35° to the DNA helix.[38,48] The properties of these adducts are represented schematically in Figure 7. These structures are consistent with the findings of subsequent studies utilizing a variety of physicochemical techniques, including optical detection of magnetic resonance,[49] flow dichroism,[37] and electric field pulse-induced fluorescence polarization techniques.[50] However, on the basis of fluorescence quenching experiments Hogan et al.[51] proposed an alternative interpretation of the spectral data according to which the pyrene ring system of the major adduct is intercalated within a bent region of the DNA double helix, forming a wedge-shaped intercalation complex. Support for this structure was provided by fluorescence quenching experiments with oxygen.[52]

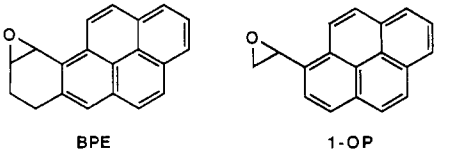

Figure 8. 9,10-Epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPE) and 1-oxiranylpyrene (1-OP).

In view of these discrepancies, we reinvestigated the fluorescence characteristics of the *anti*-BPDE–DNA adducts in greater detail.[53] Fluorescence decay experiments showed the presence of at least three different fluorescence decay components, which were used to interpret acrylamide and oxygen fluorescence quenching curves quantitatively. The two shorter decay times (1.4 and 6 ns) were attributed to covalently bound BPDE chromophores. These experiments suggest that a major fraction of the adducts are solvent accessible (site II) and that only a small fraction is inaccessible (site I). A third unusually long-lived, strongly fluorescent component (200 ns) is also present in variable ratio. This product was shown to be tetraol formed by UV-induced hydrolysis of the covalent adducts. This previously unrecognized photodecomposition accounts for most of the discrepancies in the prior literature data and its interpretation by various investigators. When this is taken into account, the findings are most satisfactorily interpreted in terms of the site I and II structures originally proposed.

Relation between DNA Adduct Structure and Bioactivity

Is there any relation between the activities of various PAHs as carcinogens and either their facility of reaction with DNA or the types of adducts they form? To obtain information on this question, we examined the reactions with DNA of a series of PAH epoxide derivatives of benzo[*a*]pyrene (BP), benz[*a*]anthracene (BA), and chrysene, representing a range of carcinogenic activity. Through our synthetic program we have available the oxidized metabolites of a wide range of PAHs.[54] The BP analogues BPE and 1-OP (Figure 8) lack the hydroxyl groups of *anti*-BPDE, and both are strong mutagens but weak carcinogens.[55] In the BA series, BA shows only borderline activity, while its alkyl-substituted derivatives 3-MC and DMBA are highly potent carcinogens.[4,56] Chrysene is inactive as a carcinogen,

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while 5-MCH is equipotent with BP.[32]

Comparison of the reactions with DNA of 1-OP and *anti*-BPDE reveals that hydrolysis of both compounds is catalyzed by DNA via rapid formation of an intercalation complex.[57] The binding constant for physical binding of 1-OP with DNA (K = 4000 M^{-1}) is virtually identical with that for *anti*-BPDE. Electronic interaction between the pyrenyl moiety and the nucleic acid bases as measured by K is the dominant factor in the complexation, and the hydroxyl groups of *anti*-BPDE appear to play a minor role.[58] The percentage of PAH molecules that bind covalently to DNA (f_{cov}) are also similar for 1-OP and BPDE (8%). However, in contrast to the *anti*-BPDE–DNA adduct, which exhibits a positive LD signal characteristic of an externally bound adduct, the 1-OP–DNA adduct has a negative LD, indicative of an intercalated conformation. Similar studies of the covalent adducts formed by BPE with DNA also show a negative LD, indicative of a site I conformation.[38]

Since the DNA adducts formed by 1-OP and BPE are both site I while the adduct formed by BPDE is mainly site II, these findings suggest that steric interactions of the hydroxyl groups of the latter with the DNA helix act as a driving force for the rearrangement of the DNA-bound BPDE intermediate from its intercalated form into its final externally bound conformation. These results also suggest that the marked difference in carcinogenic potency between BPDE and its analogues 1-OP and BPE may be related to the difference in the conformations of their DNA adducts.

Similar studies of the reaction of the 3-MC *anti*-diol epoxide (**3**) with DNA show that both the mechanism of reaction and the structures of the adducts formed closely resemble those of *anti*-BPDE.[59] However, some notable differences are also evident. Thus, K and the overall rate constant for the reaction (k_3) are both about one-fourth those of BPDE, while the extent of covalent binding (f_{cov}) is almost triple that of BPDE. Moreover, unlike all of the other PAH diol epoxides studied so far, the conformation of the noncovalent DNA complex of **3** does not appear to be intercalative in nature. The alkyl groups in **3** (compared to **1a**) apparently hinder its insertion between the base pairs of DNA. Since 3-MC is a more potent carcinogen than BP, these findings suggest that f_{cov} may relate more directly to tumorigenicity than either K or k_3. It is probably also significant that the adducts formed by 3-MC and BPDE are both predominantly site II.

The BA diol epoxide derivative reacts with DNA by a similar mechanistic pathway.[60] The values of K and k_3 are similar to those for BPDE, while f_{cov} is higher and close to that for the 3-MC analogue. The reaction of the BA derivative with DNA affords a mixture of site I and site II adducts. Although BA is a weak carcinogen, its relatively high levels of covalent binding and formation of a site II adduct resembles the more potent carcinogens BP and 3-MC. The weak activity of BA appears to be more a consequence of its relatively low level of metabolic activation to the appropriate diol epoxide intermediate than to the type or quantity of the DNA adducts it forms. Similar studies with the DMBA diol epoxide (**4**) have been hampered by the chemical instability of the synthetic DMBA diol epoxide.[61]

Metabolism of 5-MCH affords the dihydrodiol precursors of the two possible bay region diol epoxides (5-MCHDE-I and -II) approximately equally.[33] 5-MCHDE-I, which has the methyl group in the same bay region as the epoxide function, is the major DNA-bound metabolite formed in mouse skin.[34] This isomer is also the most tumorigenic.[35] Both isomers have been synthesized[62] and their reactions with DNA investigated.[63] For 5-MCHDE-I the values of K and k_3 are approximately one order of magnitude less than those for BPDE under similar conditions of ionic strength. The lower value of K is consistent with the smaller aromatic ring system of the 5-MCH derivative. For 5-MCHDE-II the value of K is half that of isomer I. More significantly, the extent of covalent binding of 5-MCHDE-I to DNA as measured by f_{cov} is essentially identical with that for BPDE, while f_{cov} for 5-MCHDE-II is only one-third this value. Thus the methyl group in the bay region of 5-MCHDE-I actually enhances the extent of covalent binding to DNA, despite its likelihood of steric interference with bonding. Nor does the methyl group appear to affect the nature of the adduct formed, since the structures of the DNA-bound adducts formed by both isomers were shown to be predominantly 2-alkylguanine derivatives[34] with site II conformations.[59,64] The findings of 5-MCHDE-I with regard to the structure of the adduct and the extent of covalent binding closely resemble those for BPDE, consistent with the equivalent carcinogenic potency of the parent PAHs.

While the number of examples studies to date is still small, a consistent picture has begun to emerge. Thus, all of the PAH epoxides, with the exception of **3**, appear to react with DNA via a common mechanistic path involving initial intercalation. Moreover, the diol epoxide metabolites of the most potent carcinogenic PAHs appear to be distinguished from their less biologically active counterparts by their relatively high levels of covalent binding to DNA and their tendency to afford relatively high ratios of externally bound site II adducts. The observed high levels of covalent binding of the bay region diol epoxide metabolites of highly carcinogenic PAHs agree with the prediction of the "bay region theory".[27] The orientation of the PAH moiety with respect to the DNA helix also appears to be important, optimum activity being associated with a configuration approximately parallel to the axis of the helix. While the extents of covalent binding of the active intermediates are determined partially by their inherent chemical reactivities, it is apparent that steric and electronic factors also play an important role in determining whether the reactive PAH metabolites are able

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to bond covalently to the appropriate base site in DNA in the intermediate complex.

Structure, Activity, and Intercalation

The spectroscopic studies discussed above support a mechanism for the covalent binding of most carcinogenic PAH metabolites to DNA which involves initial insertion of the reactive intermediate between the base pairs of the nucleic acid double helix.[65] If intercalation is an essential prerequisite for covalent binding leading to tumor induction, it may explain in part the observed PAH structure–activity relationships.[66] Intercalation imposes limitations of size and shape on the inserting molecules. For subsequent covalent bonding to take place, additional geometrical restrictions are imposed by the requirement for proper orientation and sufficiently close approach of the reacting electrophile (i.e., a triol carbonium intermediate) to an appropriate base site on DNA. The latter is most likely the 2-amino group of deoxyguanosine, since it is the primary site of attack of the diol epoxide derivatives of most carcinogenic PAHs investigated to date. However, other sites such as deoxyadenosine cannot be ruled out.

The structural requirements of optimum PAH carcinogenicity include 4–6 aromatic rings, an unsubstituted benzo ring capable of forming a bay region diol epoxide, and electron-donating groups, particularly methyl groups, in appropriate molecular sites.[66] The optimum size of the polycyclic ring system for activity approximates the dimensions of the nucleic acid base pairs and may be expected to provide maximum overlap between the PAH aromatic ring system and the base pairs in the DNA double helix. The physical association constant K provides a measure of this interaction for unsubstituted PAHs.

Methyl groups, because of their electron-donating properties, may be anticipated to increase K and further enhance electronic interaction between the fused aromatic ring system and DNA.[67] However, the position of substitution is also likely to be important. Metabolic activation is strongly inhibited by introduction of methyl groups into the benzo ring that undergoes enzymatic activation to a diol epoxide. For example, three of the four methylbenzo[*a*]pyrenes having a methyl group in the benzo ring are inactive as carcinogens, and the fourth exhibits only borderline activity.[68] Also, the introduction of methyl groups into peri positions adjacent to the benzo rings of carcinogenic PAHs, e.g., the 5-positions of BA or DMBA, generally results in a decrease in tumorigenic activity.[69] This "peri effect" is thought to result from a shift in the conformations of the benzo ring dihydrodiols and diol epoxides from diequatorial to diaxial due to steric interaction.[70,71] The most biologically active diol epoxides are diequatorial.[71] The lesser activity of the diaxial conformers may result from steric interference of the diaxial hydroxyl groups with intercalation and binding to DNA. Thus, the *anti*-diol epoxide of benzo[*e*]pyrene, which exists in the diaxial conformation, binds less effectively to DNA than *anti*-BPDE, which is diequatorial, and the structure of the adduct formed by the benzo[*e*]pyrene derivative is quasi-intercalated rather than site II.[72] Also, the less efficient binding to DNA of the *syn*-diol epoxides in comparison with the *anti* diol epoxides may be partially explicable by a similar steric effect, since the former show a greater preference for the diaxial conformation.[71]

The most dramatic effect of substitution on carcinogenic activity is the "bay region methyl effect". Introduction of a methyl group into a bay region nonbenzo-ring site often results in a remarkable enhancement of carcinogenic activity.[69,73] The best known examples are DMBA and 5-MCH, both of which are potent carcinogens, despite the fact that the parent PAHs exhibit minimal tumorigenicity. In the case of 5-MCH, the available evidence suggests that a major effect of the methyl group is to enhance the reactivity with DNA of the epoxide function in the same bay region.[69] This is somewhat surprising, since the presence of a methyl in the same bay as the epoxide ring undergoing reaction may be expected to interfere sterically with bond formation in this region. Evidently, steric destabilization of the epoxide function increases its reactivity sufficiently to overcome steric interference with covalent binding.

While the effects of substitution in other molecular regions on PAH carcinogenicity are well documented,[66,68,69] their molecular basis is less well understood. However, the mechanism of PAH–DNA interaction discussed above provides some basis for understanding these effects. Thus, introduction of methyl or other groups into PAH molecules may be expected to inhibit activity, if it leads to steric interference with intercalation into DNA, blocks the proper orientation of the active intermediate in the reaction complex for bond formation, or interferes with adoption of a site II conformation. On the other hand, substituent groups may in principle enhance activity by blocking PAH metabolism in molecular sites leading to detoxification products, by sterically facilitating the optimum orientation of the reactive intermediate in the intercalated complex, by sterically interfering with detoxification of active metabolites, or by enhancing electronically the reactivity of the active metabolites. Data are lacking to

(65) While **3** appears to be an exception to the apparent rule that intercalation precedes covalent binding of carcinogenic diol epoxide metabolites to DNA, metabolism studies indicate that **3** accounts for only a small percentage of the metabolites of 3-MC which bind to DNA in cells. The 1-, 2-, and 3-hydroxy derivatives of **3** are also detected as DNA-bound metabolites.

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evaluate the relative importance of these factors.

The above arguments are predicated on the assumption that intercalation is an essential feature of the molecular mechanism of PAH–DNA interaction. While this seems reasonably probable in most of the cases examined to date, an alternative mechanistic pathway involving external binding without prior intercalation appears to be operative in the case of 3-MC[59,65] and cannot be entirely ruled out in other cases.

Future Directions

Recent advances have expanded our knowledge of the molecular mechanism of PAH carcinogenesis to include detailed information on the interaction of the active PAH metabolites with DNA. The experimental methods utilized in these studies can be extended in principle to a wider range of PAH derivatives to elucidate further the molecular basis of observed structure–activity relationships. One of the practical limitations of this approach is the multiplicity of adducts formed on various base sites by the direct alkylation of DNA. As a consequence, the findings represent an average of the structures present. An important direction for future research lies in the preparation of DNA sequences alkylated on specific base sites to gain more specific information on the molecular structures of the individual adducts formed.

Complete understanding of the mechanism of induction of tumors by PAHs and other chemical carcinogens requires the solution of many additional problems. Among the most important is determination of the specific sites on DNA which are essential for tumor induction. Alkylation of native double-stranded DNA in vitro takes place at numerous sites along the nucleic acid chain, with preferential binding in G–C-rich regions.[74] The sites of alkylation in vivo are restricted by the secondary structure of DNA and its association with chromatin. However, it is likely that alkylation at only a few sites is relevant to carcinogenesis. While there is evidence to suggest that oncogenes, such as the ras gene,[75] may be important regions of alkylation damage, the critical targets for tumor induction are unknown. Other important questions concern the nature of the specific mutations involved[76] and the role of repair processes in the mechanism of tumor induction.

While many problems remain, it appears that complete elucidation of the molecular mechanism of PAH carcinogenesis is now an attainable goal.

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Probing Radicals with Alcohol Thermolysis and Molecular Mechanics

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The difference between the layman and the synthetic organic chemist is that the former is often unwittingly engaged in the indiscriminate scission of C–C bonds in the form of fossil fuels, whereas the latter is more actively concerned with the selective formation of the same. What they have in common is that neither is directly interested in the energies of these vital bonds, this topic lying in the realm of the thermodynamicist and the physical organic chemist.

In fact, the homopolar dissociation energy of the C–C bond varies very considerably, 11 kcal mol^{-1} being enough to break it in the Gomberg dimer[1,2] and 88 kcal mol^{-1} being required to cleave ethane.[3] From Ziegler's early work[4] it is known that C–C bond dissociation is facilitated by bulky substituents to the bonded atoms and by groups that can stabilize the incipient radicals. Rüchardt and Beckhaus, more recently, have established quantitative relationships between thermal stability, strain, and resonance effects of substituents.[5] Despite surveys and compilations[3,6,7] going back almost 40 years, however, there is still much debate about the C–C and C–H bond energies of small alkanes and about the corresponding heats of formation of the derived alkyl radicals: for the *tert*-butyl radical, for example, in recent years $\Delta H_f°_{298}$ has been ascribed values in the

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