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Mechanism of Carcinogenesis of the Polycyclic Aromatic Hydrocarbons

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The carcinogenic activity of the benzo[a]pyrene 1, the 7,12-dimethylbenz[a]anthracene 2 and the 3-methylcholanthrene 3 is suggested to be determined by the electrophilic attack of the active oxygen, induced by the hydroxylating enzyme systems, on the most reactive substituting carbon atom(s). The cationic intermediate(s) with the charge mainly localized on a complementary, interrelated position(s) of the hydroxyl substituted position(s) reacts further with the cellular nucleophiles.

The electrophilic nature of the ultimate chemical carcinogens constitutes the common distinctive feature that correlates their different structures and allows us to understand their carcinogenicity. The formation of a covalent bond with the nucleophiles of the biological macromolecules, nucleic acids and proteins, appears to be the essential requirement in the primary process of carcinogenesis.

The mode of action and the criterion of carcinogenicity for the polycyclic aromatic hydrocarbons remain ambiguous until now. Pullman and Pullman[1] have associated the carcinogenic potency of these compounds with the presence of a chemically reactive phenanthrene double bond (K

region). They have proposed further that the primary process in cancer induction is marked by the addition of the K region to the cellular receptor.

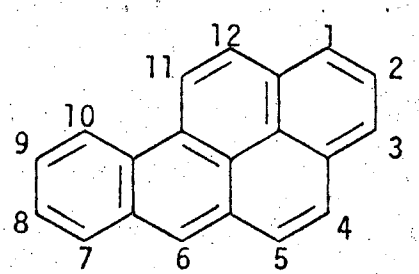
The discovery of the mutagenic effect of the heterocyclic acridines, explainable by virtue of an intercalation mechanism of DNA-acridine dye complexes,[2] suggested by analogy the idea that the carcinogenic action of the aromatic hydrocarbons might be attributed to a similar model of intercalation.[3] However, the interpretation of the results along these lines has been quite unsatisfactory.[4]

The covalent binding among DNA, RNA, proteins, and aromatic hydrocarbons,[5,6] after painting mice with these compounds and isolating their cellular macromolecules, provided new impetus to the idea that a chemical reaction is a necessary and probably crucial step in the cancer initiation. The reaction is presumably induced *in vivo* by the microsomal hydroxylating enzyme systems. In fact, the chemical linkage between DNA, or protein, with aromatic hydrocarbons has been obtained *in vitro* in the presence of rat liver microsomes.[7,8] The same binding between DNA and carcinogenic aromatic hydrocarbons has also been induced by hydrogen peroxide[9], with or without ferrous ion, and the ascorbic acid model hydroxylating systems.[10] These model hydroxylating systems produce electrophilic hydroxylation on the aromatic substrates and offer a comparison of some extent with the rat liver microsomal hydroxylating enzyme systems.[11] In addition, the electrophilic nature of the active oxygen produced by the hydroxylating enzymes is also partially corroborated by the general occurrence of the NIH shift.[12]

The electrophilic oxygen as activator has been considered by Dipple *et al.*[13] They have postulated as ultimate carcinogens for the unsubstituted and methyl-substituted aromatic hydrocarbons the carbonium ion corresponding to the open form of the K region epoxide and the benzyl carbonium ion, respectively.

A general evaluation of the metabolism *in vivo* and *in vitro* of the carcinogenic hydrocarbons reveals the hydroxylation to occur primarily on the most chemically reactive region(s). As a logical consequence, the carcinogenicity of these compounds might be pursued on the grounds of this observation.

The carcinogenic activity for the benzo[a]pyrene 1 has been related to the presence of three active substituting positions, *i.e.*,



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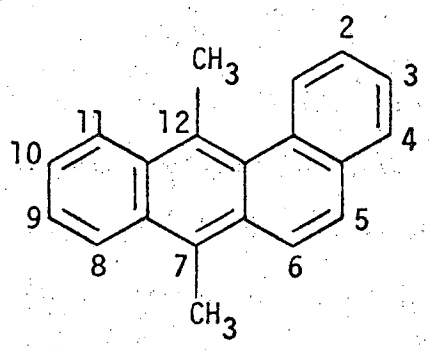
the 1-, 3-, and 6-position, both by the demonstration of direct reaction with cellular nucleophiles, and by exchange reactions with the hydrogen isotopes.[14] When the electrophilic active oxygen induced by the hydroxylating enzyme systems attacks the 6-position of 1, the cationic species produced is mainly localized on the two interrelated 1- and 3-carbon atom. If one of the two latter positions possesses a suitable configuration with respect to the cellular nucleophile, the covalently bound complex is formed. Conversely, the attack of the active oxygen on the 1- or 3-carbon atom determines

an overall localization of the cationic charge on the 6-position. This intermediate then reacts further with the cellular nucleophile.

The comparison of the very potent carcinogen 7,12-dimethylbenz[a] anthracene (DMBA) 2 with the less potent 7-methylbenz[a]anthracene and 12-methylbenz[a]anthracene and with the very weakly active



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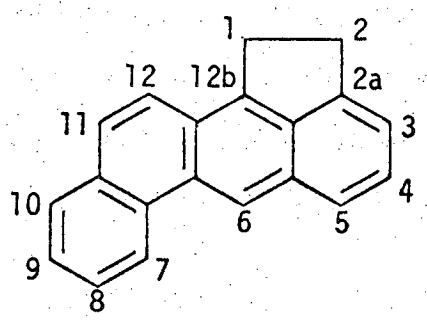
benz[a]anthracene hints that the presence of the methyl group(s) may be connected with the biological activity.

The nuclear magnetic resonance spectrum of DMBA in trifluoroacetic acid, boron trifluoride and water[15] shows a characteristic quartet corresponding to the protonation of both 7- and 12-positions. Furthermore, some of the products obtained by chemical oxidation of this hydrocarbon[16] indicate the 7- and 12-positions to be the reactive ones. The metabolism of the DMBA in the presence of rat liver homogenates[17,18] shows mainly the formation of 7-hydroxymethyl-12-methylbenz[a]anthracene and 12-hydroxymethyl-7-methylbenz[a]anthracene. Incidentally, the hydroxymethyl derivative is the major metabolite from p-methylphenylalanine in the presence of phenylalanine hydroxylase.[19] Some speculation concerning the formation of the hydroxymethyl derivative suggests as precursor intermediate the hydroxyl group

substituted on the same carbon atom at which the methyl group is substituted.[12]

The carcinogenic activity of the hydrocarbon is then probably determined by the initial electrophilic attack of the active oxygen on the 7- or 12-position with subsequent formation of active cationic intermediate(s). The cationic charge is mainly localized on a complementary, interrelated position(s) of the 7- or 12-position, which necessarily must be separated by an even number of carbon atoms. The reactive intermediate(s) likely attacks the cellular nucleophile.

An analogous situation is presented by the 3-methylcholanthrene 3, where the metabolism by rat liver homogenates reveals predominantly the presence of 1- and 2-hydroxyl derivatives. This presumably indi-



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cates, by analogy with DMBA, the highest substituting reactivity of the 12b- and 2a-carbon atom. In this case, the interrelated position(s), which must be separated by an even number of carbon atoms from these two active ones, are probably the same either for the 12b- or 2a-position. Experiments displaying the presence of the complementary positions for dimethylbenz[a]anthracene and 3-methylcholanthrene are in progress.

Acknowledgements

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