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ON THE INTERACTION OF CARCINOGENS WITH DNA

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Abstract—In considering the evidence for carcinogen–DNA interaction only the most recent data is discussed since excellent reviews of earlier work are available. While outlining the evidence relating to DNA binding of alkylating agent, aromatic amines and amides, alkylnitrosamines and hydrocarbons an attempt has been made to emphasise those aspects which seem most relevant or about which some doubt still remains. What emerges as the most significant feature of recent work in this area is the striking similarity in the nature of the metabolically produced ultimate carcinogen despite the widely different classes of compounds considered.

ZIMMERMAN[1] has presented evidence for a genetic hypothesis of cancer and has also dealt with the major objections to such a theory. He has indicated that carcinogenesis could result from:

(a) Mutation of nuclear or cytoplasmic DNA
(b) Mitotic recombination
(c) Mitotic gene conversion

The interaction of the carcinogen with DNA would seem essential for the first mechanism while for the latter processes such an interaction, or an effect on repair enzyme systems might be involved.

In the very limited space available I will discuss only the present evidence for carcinogen–DNA reaction. Since a number of excellent reviews on this general topic have appeared during the past 3 years I will draw attention to these, and confine myself to very recent results, and to points which seem of particular significance.

For convenience of presentation I will discuss the various broad classes of chemical carcinogens separately but as I hope will emerge, the most striking feature of the past 3 years has been the similarity in the nature of the metabolically produced ultimate carcinogens derived from very different classes of compounds.

Aromatic amines and amides

Much of the progress in this field is due to the work of the Millers who produced two reviews in 1966[2, 3] and a third[4] in 1969. A striking difference between these reviews is the much greater emphasis now placed on nucleic acid interactions. This follows from the implication of *N*-hydroxy derivatives of aromatic amines and amides as proximate carcinogenic[5] metabolites and the finding that esters of these compounds react non-enzymically with nucleic acids.[6] Most of the *in vitro* studies have been with esters of *N*-hydroxy-2-acetylaminofluorene, (*N*-hydroxy-AAF), in particular *N*-acetoxy-AAF,[7] but the *N*-benzyoyl, *N*-propionyl, *N*-butyryloxy, *N*-sulphate and the glucuronide of *N*-hydroxy-AAF have also been prepared.[4]

Studies with nucleosides and *N*-acetoxy-AAF showed significant reaction only with

guanosine and the product was identified[7] as *N*-(guanosin-8-yl)-AAF, while treatment of DNA and subsequent hydrolysis yielded *N*-(guanin-8-yl)-AF. Furthermore enzymic degradation of DNA and RNA isolated from the liver of rats injected with AAF-9-[^{14}C] also gave products identified as 8-substituted guanines.[4] The characterisation of these products seems beyond doubt but the mechanism of the reaction is obscure. Kriek,[8] when suggesting the possibility of such a reaction, reasoned that electrophilic substitution at C-8 might be expected, but as discussed by Robins,[9] such a reaction probably involves the purine anion and is inhibited by a substituent on N-7 or N-9 (see also Shapiro,[10] p. 104). Furthermore substitution at C-8 of purine nucleotides of anything larger than a fluorine atom requires the *syn*-configuration (I am indebted to Dr. A. M. Michelson for pointing this out) while in native DNA the nucleotides are in the *anti*-form. Therefore reaction of a bulky carcinogen with DNA *in vivo* to give C-8 guanine products must involve a denatured region of the DNA and must cause a permanent distortion of the helix configuration.

The C-8 reaction is certainly not a general one, even for closely related compounds, since it was reported[4] that *N*-acetoxy-4-acetylaminobiphenyl gave with guanosine mainly an acetylated guanine product while *N*-acetoxy-4-acetylaminostilbene gave a product which was not identified but was not substituted at C-8 and was not an acetylated derivative.

The work outlined above led the Millers[5] to suggest that reactive esters might be the ultimate reactive carcinogenic metabolites for aromatic amines and amides. The correlation found between the susceptibility of various species to *N*-hydroxy-AAF carcinogenicity, and the level of sulphotransferase enzyme activity in the liver led to the suggestion[11] of the *N*-sulphate as the likely *in vivo* reactive ester. Such a species generated in the cytoplasm would be highly charged and might be unable to penetrate the nuclear membrane and react with the genetic material. Failure to penetrate the cell membrane might account for the reported[11] failure of the *N*-sulphate of AAF to induce tumours at the sight of injection into rats although able to mutate *B. subtilis* transforming DNA.[12] The hypothesis of the reactive ester as ultimate carcinogen does not explain the need for an *N*-methyl group for carcinogenic activity in the azo-dye series of compounds. Like *N*-acetyl-4-aminoazobenzene (AAB) and 4-aminoazobenzene (AB) neither *N*-hydroxy-AAB, *N*-hydroxy-AB or *N*-acetoxy-AAB induced tumours in rats either on long term feeding or repeated i.p. or s.c. injections.[13]

Considerations of this sort led Dipple, Lawley and Brookes[14] to suggest the possibility of a reactive metabolite of the type $ArNR^+$ being generated directly by metabolism of an *N*-methyl group. This hypothesis would suggest that within a series of related compounds carcinogenic potency would depend on the stability of the reactive intermediate.

In summary it would seem that although *N*-hydroxylation and esterification almost certainly accounts for the protein binding of aromatic amine and amide carcinogens, there is still need for detailed studies of nucleic acid binding which might resolve the present uncertainty on the nature of the true ultimate carcinogen.

Alkylating agents

In this section I will consider the classical alkylating agents (e.g. sulphur and nitrogen mustards, ethyleneimines, epoxides, and alkane sulphonates), as well as the *N*-alkyl-*N*-nitroso amines, amides and amidines. In a comprehensive review of this

field Lawley[15] discussed the chemistry of DNA alkylation and the proposed[16] mechanism of alkylation mutagenesis based on the production of 7-alkylguanine moieties in alkylated DNA. Although these ideas seemed to accord with most mutation studies some anomalies remained, in particular the report by Loveless[17] that whereas ethyl methanesulphonate (EMS) was mutagenic towards T-even bacteriophage, methyl methanesulphonate (MMS) was toxic but not mutagenic. Both MMS and EMS were shown to alkylate the purines in the DNA of phage. Recently Loveless and Hampton[18] reported that *N*-methyl and *N*-ethyl-*N*-nitrosoureas (NMU and NEU) both caused mutation of T2 phage whereas only the *N*-methyl compound gave a significant yield of alkylated purines on treatment of DNA *in vitro* and subsequent acid hydrolysis. These results led to the suggestion[18] that EMS, NMU and NEU could give an alkylation product with DNA which was not given by MMS and which was acid labile and highly mutagenic. On the basis of studies with these agents and deoxyguanosine, Loveless[19] has suggested that *O*-6-alkylguanine could be this previously undetected product. Furthermore Lawley[20] has shown that treatment of DNA *in vitro* with *N*-methyl-*N*-nitroso-*N*-nitroguanidine (NMNG) gives in addition to the expected methylated purines, *O*-6-methylguanine, while MMS treated DNA does not yield this product.

If confirmed, this finding of *O*-6-alkylation presents an intriguing chemical problem. It might be expected that alkylation at *O*-6 would involve the guanine anion and if the intermediate alkylating species derived from the *N*-alkyl-*N*-nitroso derivatives (perhaps the diazoalkane) abstracted a proton from the guanine moiety, rather than from the solvent, then such a reaction would be favoured. This might explain the greater amount of *O*-6-alkylation with compounds which could yield ethyl-diazo intermediates compared to the corresponding methyl derivatives but does not explain the difference found between MMS and EMS. An alternative view namely that *O*-6-alkylation is favoured by agents reacting predominantly by an SNI mechanism might prove correct but is not an explanation of why this should be the case.

Dialkylnitrosamines

The significance of the above discussion to carcinogenesis by both classical alkylating agents and dialkylnitrosamines is obvious. The recent review of carcinogenic nitroso compounds by Magee and Barnes[21] makes unnecessary any general discussion here. The attempt by Swan and Magee[22] to correlate methylation of nucleic acids of various tissues of the rat with the incidence of tumours emphasised the outstanding problems of this field. Although these authors point out the lack of a correlation this seems to be largely due to the failure of MMS to produce kidney tumours. In view of the extremely complex biological parameter being considered (namely production of tumours) the degree of correlation obtained seems remarkably good. Furthermore the methylation data suggested that NMU should yield tumours of the kidney and other organs in the rat, and that MMS would be expected to yield brain tumours. Both of these predictions were subsequently confirmed by experiment.[22] Studies of *in vivo* *O*-6-guanine alkylation by carcinogens of this type may add to knowledge on the significance of this site of reaction for tumour production.

Lactones

Carcinogenic lactones were reviewed by Dickens[23] and more recently a series of

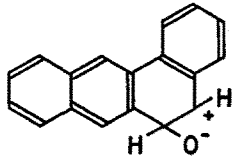
papers by Van Duuren *et al.*[24] have appeared dealing with these compounds. For the present discussion only β-propiolactone will be considered. This compound could have been considered under alkylating agents since it was shown[25] to yield 7-(2-carboxyethyl)guanine derivatives on reaction with guanosine, RNA and DNA. It has been used by Colburn and Boutwell[26, 27] in an elegant quantitative study relating tumour production with dose and extent of reaction with DNA, RNA and protein. These same authors also compared tumour initiation and binding to cellular constituents of β-propiolactone and a number of related alkylating agents.[28] As a result of this work they were able to conclude that DNA binding, but not RNA or protein binding correlates with tumour-initiating potency, a view also expressed by Dingman and Sporn[29] as a result of their work with a series of aminoazo-dyes and by Brookes and Lawley[30] for aromatic hydrocarbons.

Aromatic hydrocarbons

In their review *Molecular geometry and carcinogenic activity of aromatic compounds. New perspectives*, Arcos and Argus[31] cover most aspects of hydrocarbon carcinogenesis and any omissions are probably dealt with by the relevant papers presented at a recent symposium on "The physicochemical mechanism of carcinogenesis".[32]

It now seems generally agreed that carcinogenic hydrocarbons are metabolised to chemically reactive derivatives which bind to DNA, RNA and protein. Doubt remains on the nature of the ultimate carcinogen and which macromolecule represents the vital target for tumour initiation.

Dipple, Lawley and Brookes[14] proposed that the structure of the ultimate carcinogen, rather than that of the administered compound should be the determinant of carcinogenic potency for aromatic hydrocarbons. This concept, plus the assumption that the vital target for carcinogenesis was within the cell but distant from the site of hydrocarbon metabolism, led these authors to propose[14] that aralkyl carbonium ions of the general type I for unsubstituted hydrocarbons and type II for methyl substituted compounds, may be the ultimate carcinogen (Fig. 1).



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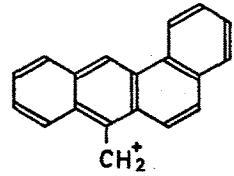

II

In an attempt to mimic the reactivity of such species *in vitro*, 7-bromomethylbenz[*a*]anthracene and 7-bromomethyl-12-methylbenz[*a*]anthracene were synthesized and shown to react very rapidly with nucleic acids in aqueous solution. The chemistry of the reaction of these derivatives is being investigated and some preliminary results have been published.[32] The bromomethyl derivatives were also tested as carcinogens by a single s.c. injection into rats, and compared with the parent hydrocarbons 7-methylbenz[*a*]anthracene and 7,12-dimethylbenz[*a*]anthracene (DMBA). Although these experiments are still not completed it can be said that 7-bromomethyl-12-methylbenz[*a*]anthracene is as active as DMBA itself in this system (Dipple, unpublished results).

The diverse nature of chemical carcinogens suggests that the structure which finally reacts with the initial cellular receptor is of lesser significance than the process which determines if such binding will occur. It seems possible therefore that the importance of structure in relation to activity results from the way in which structure influences the reactivity of the ultimate carcinogen.

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