Mechanisms of Reaction between Ultimate Chemical Carcinogens and Nucleic acid

By D. E. Hathway
IMPERIAL CHEMICAL INDUSTRIES CENTRAL TOXICOLOGY LABORATORY, ALDERLEY PARK, CHESHIRE SK10 4TJ

G. F. Kolar
INSTITUT FÜR TOXIKOLOGIE UND CHEMOTHERAPIE AM DEUTSCHEN KREBSFORSCHUNGSZENTRUM, D-6900 HEIDELBERG 1

1 Introduction

Ten years ago the Millers,[1,2] whose ideas arose from their discovery of positively charged intermediates in *N*-(2-fluorenyl)acetamide and amino-azo dye metabolism (*vide infra*), attempted to account for the interactions of chemical carcinogens by the electrophilicity of the ultimate, reactive forms. Their[2] argument, based on the work of Price *et al.*,[3,4] that 'S_N2 reactions or reactions of a type intermediate between S_N1 and S_N2 appear to predominate over pure S_N1 or free radical reactions with the reactive forms of chemical carcinogens' would make available a range of electrophilic reactivities, which would be expected to span a range of probable (nucleophilic sites in genetic material) receptors *in vivo*. As the electrophilic properties of chemical carcinogens or their reactive forms were held to be important to their carcinogenic mode of action, it followed by corollary that nucleophilic mutagens such as hydroxylamine would not be expected to be carcinogenic, and this supposition proved to be correct.[5] Moreover, nucleic acid-base analogues, which were mutagenic in micro-organisms, did not produce tumours in male and female rats.[6] However, it is doubtful whether the possible contribution of free radical mechanisms to chemical carcinogenesis has ever been assessed properly. Conceptually, it ought to be possible to increase or diminish the capacity of a potential mutagen or carcinogen to damage DNA by environmentally inhibiting or stimulating the drug-metabolizing enzymes or by adding agents which compete selectively with DNA as a substrate for these mutagens and carcinogens. This supposition seems in fact to be feasible, as hindered phenolic antioxidants like BHA (a mixture of 2- and 3-t-butyl-4-methoxyphenol) and BHT (3,5-di-t-butyl-4-hydroxytoluene) can reduce the potential of a chemical

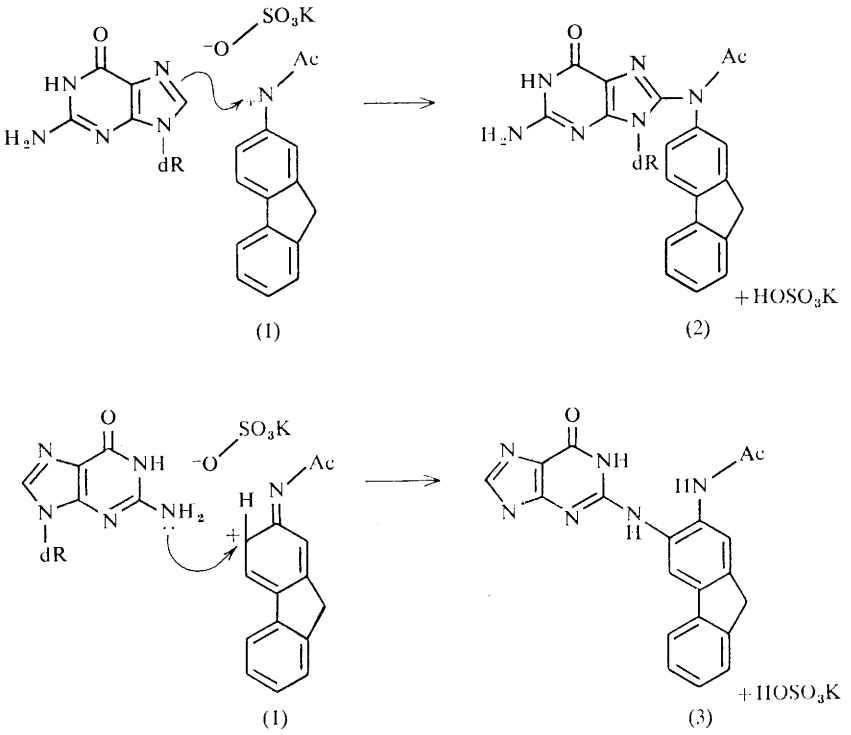
[1] J. A. Miller, *Cancer Res.*, 1970, **30**, 559.
[2] J. A. Miller and E. C. Miller, *J. Nat. Cancer Inst.*, 1971, **47**, V.
[3] C. C. Price, *Ann. New York Acad. Sci.*, 1958, **68**, 663.
[4] C. C. Price, G. M. Gaucher, P. Koneru, R. Shibakawa, J. R. Sowa, and M. Yomaguchi, *Ann. New York Acad. Sci.*, 1969, **163**, 593.
[5] L. A. Poirier, Thesis, University of Wisconsin-Madison.
[6] Z. Hadidian, T. N. Fredrickson, E. K. Weisburger, J. H. Weisburger, R. M. Glass, and N. Mantel, *J. Nat. Cancer Inst.*, 1968, **41**, 985.

carcinogen to induce chromosome breaks[7] and to initiate tumour formation.[8] Historically, the idea of the inhibition of tumour induction derives from Berenblum's discovery[9,10] 'that 2,2′-dichlorodiethyl sulphide was a potent anti-carcinogen for mouse skin.' These results [7,8] might suggest that the antioxidants may act as scavengers for carcinogen-derived free radicals *in vivo*. However, the fact that *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and some other carcinogens stimulate the guanylate cyclase system and cause cGMP to accumulate in rat-liver and colonic mucosa,[11] and that these findings are suppressed and reversed by antioxidants,[11] would suggest that the antioxidants participate in an alternative mechanism for the inhibition of chemical carcinogenesis.[11] But, a relationship between rapid stimulation of the guanylate cyclase system and subsequent induction of tumours has yet to be firmly established.[12]

The Millers'[1,2] great principle for unifying the reactivities of chemical carcinogens has been accepted in the years following its enunciation, and the present discussion paper shows that it applies as well to other interactions with DNA (*q.v.*), which have been elucidated during the ensuing decade. For example, in line with the ideas of the Millers, deoxyguanosine reacted both *in vitro* and *in vivo* with those centres of the *N*-(2-fluorenyl)acetamide derivative, *viz.* at the amido nitrogen and at carbon-3, which were activated through esterification of the *N*-hydroxylation product.[13] Thus, gentle chemical reaction between deoxyguanosine and the ultimate carcinogen of *N*-(2-fluorenyl)acetamide, *viz.* *N*-sulphonoxy-*N*-(2-fluorenyl)acetamide (1) (Scheme 1), gave (i) *N*-(deoxyguanosin-8-yl)-*N*-(2-fluorenyl)acetamide (2),[13] which had been identified previously as a reaction product,[14,15] and (ii) *N*-[3-(deoxyguanosin-N^2-yl)2-fluorenyl]acetamide (3), identical with the product obtained from hydrolysates of the modified DNA, extracted from the liver of rats injected with *N*-hydroxy-*N*-(2-fluorenyl)acetamide.[2,13,15] *N*-Sulphonoxy-*N*-(2-fluorenyl)acetamide (1), when caused to react chemically with methionyl peptides,[16] gave the same products as those obtained from the liver protein of rats, fed *N*-hydroxy-*N*-(2-fluorenyl)acetamide.

The mechanism (Scheme 1) that has been established for interaction of *N*-(2-fluorenyl)acetamide with DNA probably applies in the case of three industrial aromatic amines that have been associated for a long time with human bladder cancer (Table 1), since much the same distribution of tumours arises in various species of mammal for these substances as arose for the model substance, *N*-2-fluorenylacetamide. Although in these cases, the interactions between DNA and

[7] R. J. Shamberger, F. F. Baughman, S. L. Kalchert, C. E. Willis, and G. C. Hoffman, *Proc. Nat. Acad. Sci.*, 1973, **70**, 1461.
[8] L. W. Wattenberg, *J. Nat. Cancer Inst.*, 1973, **50**, 1541.
[9] I. Berenblum, 'Carcinogenesis as a Biological Problem', North-Holland, Amsterdam–Oxford, 1974, pp. 41, 148, and 201.
[10] I. Berenblum, *J. Pathol. Bacteriol.*, 1929, **32**, 425.
[11] P. A. Craven and F. R. De Rubertis, *Cancer Res.*, 1977, **37**, 4088.
[12] D. E. Hathway, in 'Foreign Compound Metabolism in Mammals' ed. D. E. Hathway, The Chemical Society, London, 1979, Vol. 5 pp. 190—243.
[13] J. G. Westra, E. Kriek, and H. Hittenhausen, *Chem.-Biol. Interactions*, 1976, **15**, 149.
[14] E. Kriek, J. A. Miller, U. Juhl, and E. C. Miller, *Biochemistry*, 1967, **6**, 177.
[15] E. Kriek, *Chem.-Biol. Interactions*, 1969, **1**, 1.
[16] P. D. Lotikar, J. D. Scribner, J. A. Miller, and E. C. Miller, *Life Sci.*, 1966, **5**, 1263.



Scheme 1

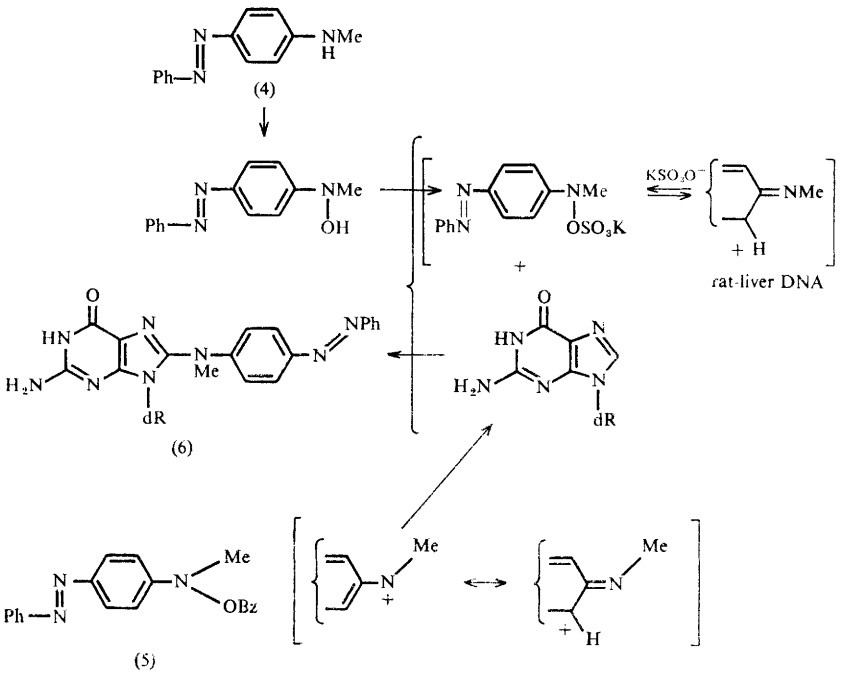
the reactive forms have never been investigated, such aromatic amines are known to be activated metabolically and, for example, *N*-hydroxy-2-naphthaleneamine is excreted in the urine of dogs and monkeys treated with 2-naphthylamine.[17] *cf.*[18] An *O*-glucuronic acid conjugate of *N*-hydroxy-4-biphenylamine is excreted in the urine of dogs treated with 4-biphenylamine.[19] Perhaps, it ought to be mentioned that guinea-pigs, which were unable to *N*-hydroxylate aromatic amines, developed tumours on administration of the *N*-hydroxy derivatives but not of the parent bases.[1]

In another connotation, degradation of the liver DNA and RNA from rats, which had been injected with 4-(*N*-methylamino)azobenzene (4) (Scheme 2) afforded respectively 4-[*N*-(deoxyguanosin-8-yl)-*N*-methylamino]azobenzene (6) and 4-[*N*-(guanosin-8-yl)-*N*-methylamino]azobenzene, identical with compounds, which were prepared by chemical reaction of synthetic 4-(*N*-benzoyloxy-*N*-

[17] J. L. Radomski and E. Brill, *Arch. Toxikol.*, 1971, **28**, 159.
[18] G. M. Bonser, D. B. Clayson, J. W. Jull, and L. N. Parah, *Brit. J. Cancer*, 1952, **6**, 412.
[19] J. L. Radomski, A. A. Rey, and E. Brill, *Cancer Res.*, 1973, **33**, 1284.

Table 1 *Tumour response of mammals to aromatic amines*

Mammal	*Model carcinogen* N-(2-*Fluorenyl*)*acetamide*	*Industrial carcinogens* 2-*Naphthaleneamine*	*Benzidine*	4-*Biphenylamine*
Man	not known	bladder tumours	bladder tumours	bladder tumours
Monkeys	bladder tumours	bladder tumours	not known	not known
Dogs	bladder tumours	bladder tumours	bladder tumours	bladder tumours
Rats	various, including hepatomata	none	acoustic, intestinal tumours, hepatomata	mammary, breast uterine tumours



Scheme 2

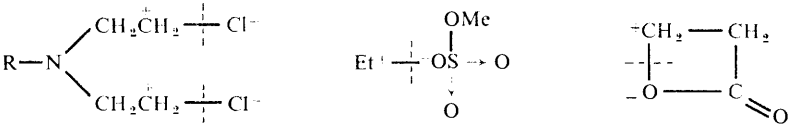
methylamino)azobenzene (5) with deoxyguanosine or guanosine.[20] 4(*N*-Methylamino)azobenzene (4) was also shown to react with the liver protein of rats *in vivo* to afford products with the constituent amino-acids.[21–25]

Although the Millers[2] made the cursory reference to S_N2 mechanisms, which has been quoted before (*vide supra*), they and their co-workers[1,2,13–16,20–25] used widely S_N1 mechanisms (Schemes 1, 2) involving carbenium and nitrenium ions, and this usage was entirely satisfactory, as the aromatic rings concerned permitted a distribution of charge, which conferred cation stability. However, their consideration[1,2] of the directly acting carcinogens, including the nitrogen mustards, the alkyl alkane sulphonates and β-propiolactone, as well as the strong carcinogens such as the *N*,*N*-dialkylnitrosamines, the *N*-alkylnitrosamides, methylazoxymethanol (the proximate carcinogen of the naturally-occurring carcinogen, cycasin) and the 3,3-dialkyl-1-aryltriazenes, which are all activated *in vivo* into reactive forms (see the Millers'[2] text-figure 4, which is reproduced here

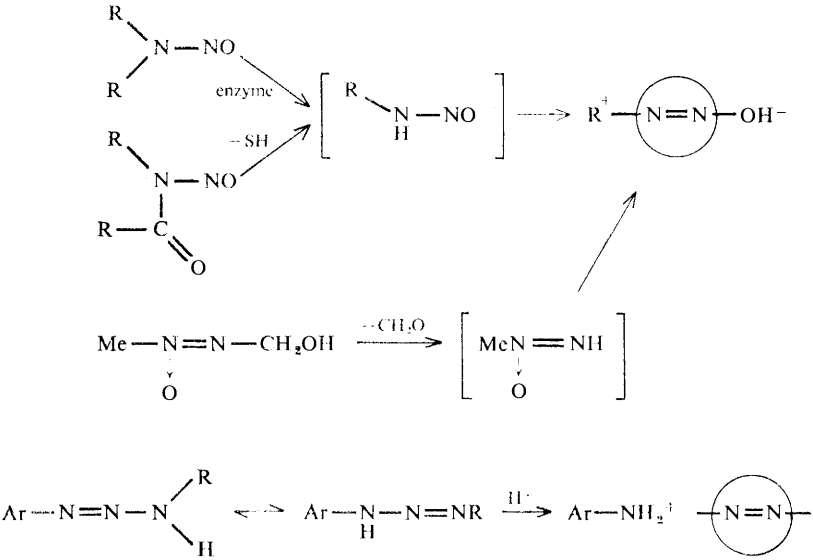
[20] J.-K. Lin, J. A. Miller, and E. C. Miller, *Cancer Res.*, 1975, **35**, 844.
[21] J. A. Miller and E. C. Miller, *Prog. Exp. Tumor Res.*, 1969, **11**, 273.
[22] J. D. Scribner, J. A. Miller, and E. C. Miller, *Biochem. Biophys. Res. Comm.*, 1965, **20**, 560.
[23] L. A. Poirier, J. A. Miller, E. C. Miller, and K. Sato, *Cancer Res.*, 1967, **27**, 1600.
[24] J.-K. Lin, J. A. Miller, and E. C. Miller, *Biochemistry*, 1968, **7**, 1889.
[25] J.-K. Lin, J. A. Miller, and E. C. Miller, *Biochemistry*, 1969, **8**, 1573.

as Scheme 3) was an uneasy extension of the foregoing concept. In particular, the liberation of the highly reactive species Me^+ that they showed[2] seemed to the present authors to be unlikely (see Section 3). This article results from our interest in occupational carcinogens (D.E.H.) and in chemical compounds as model carcinogens and therapeutic agents (G.F.K.).

The Millers' alkylating agents



Potential alkylating and arylating agents



Scheme 3

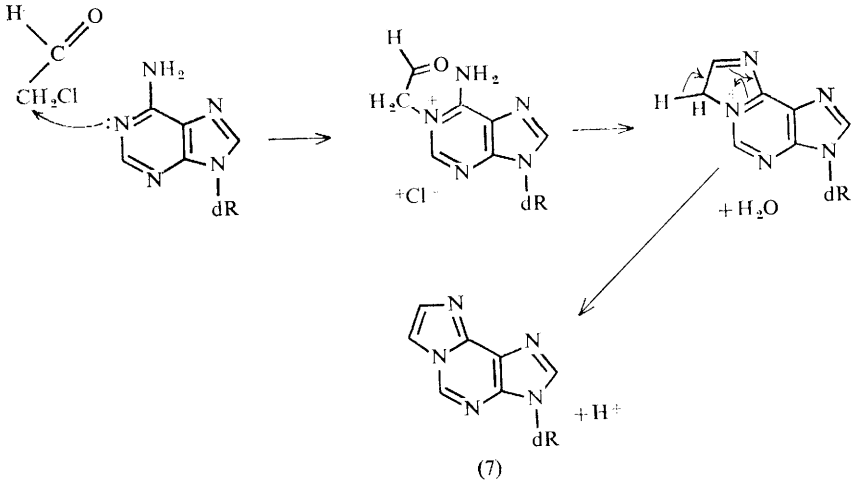
An attempt will be made in Sections 2—5 to analyse the reactivities of various carcinogens in respect of DNA in order to determine whether any feasible relationship therefrom is forthcoming. The penultimate section will deal with the distortion to DNA brought about by reaction with chemical carcinogens.

2 Some Occupational and Some Environmental Carcinogens

In line with the broad objectives of this article, the reactivities of a few examples

of industrial carcinogens and of naturally occurring ones (*q.v.*), for which the various interactions with DNA have been elucidated since the time when the Millers made their proposals, are considered in this section. The discussion (*vide infra*) does not reveal any clear relationship between the reactivity and carcinogenic potency for human carcinogens in general.

Formation of 9-(β-D-2′-deoxyribofuranosyl)imidazo[2,1-*i*]purine (7) (Scheme 4) and 1-(β-D-2′deoxyribofuranosyl)imidazo[1,2-*c*]pyrimidin-2(1*H*)-one by reaction of vinyl chloride-derived chloroethylene oxide or its rearrangement product, chloroacetaldehyde with calf-thymus DNA, and from liver DNA prepared from rats that had been exposed chronically to vinyl chloride[26] implied a common reaction mechanism. In both cases (Scheme 4), initial alkylation



(7)

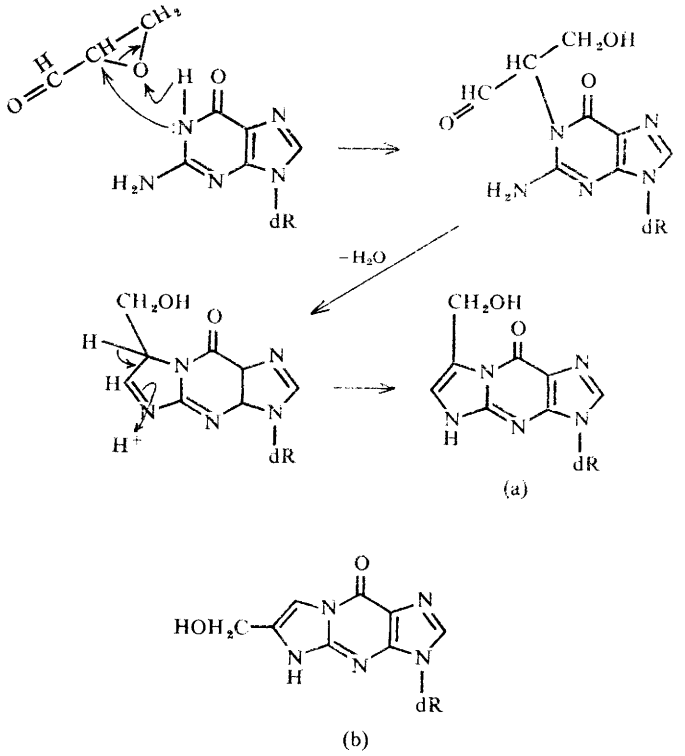
Scheme 4

occurred at the most nucleophilic ring-nitrogen (N-1 of the deoxyadenosine residues and N-3 of the deoxycytidine ones), followed successively by loss of the elements of water with ring-closure between the oxo-group and the amino-group belonging to C-6 of the deoxyadenosine residues (and to C-4 of the deoxycytidine ones) and by proton loss. There is a strong supposition that in the reaction of chloroethylene oxide or chloroacetaldehyde with DNA an S_N2 reaction is involved, and in this respect vinyl chloride differs from the other carcinogens that have been mentioned hitherto (Section 1). Nevertheless, vinyl chloride, which has been reported to induce human haemangiosarcoma of the liver with the shortest latent period recorded for any chemical carcinogen, must be regarded as one of the most powerful of carcinogens.

Imidazo-cyclization of purine and pyrimidine residues in DNA that has been

[26] T. Green and D. E. Hathway, *Chem.-Biol. Interactions*, 1978, **22**, 211.

found for vinyl chloride *in vivo*[26] appears to be an important biochemical lesion. Thus, glycidaldehyde which contains both an aldehyde and an alkylating function reacts with deoxyguanosine at weakly alkaline pH to effect imidazo-cyclization between the N-1 position and the C-2 amino group.[27,28] Van Duuren *et al.*[27] did not identify their product and suggested the alternative formulae (a) and (b) (Scheme 5) but, regardless of the structure, bimolecular alkylation at the



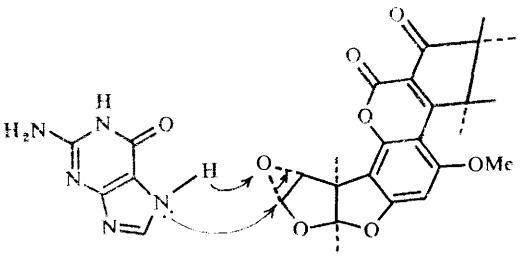
Scheme 5

ring-nitrogen would seem to occur initially (Scheme 5). Imidazo-cyclization would appear to provide a basis for glycidaldehyde mutagenicity[29–31] and carcino-

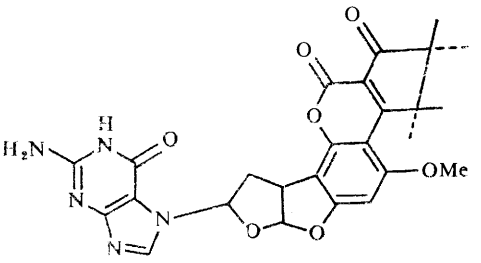
[27] B. M. Goldschmidt, T. P. Balzej, and B. L. Van Duuren, *Tetrahedron Letters*, 1968, 1583.
[28] B. L. Van Duuren, *Ann. New York Acad. Sci.*, 1969, **163**, 633.
[29] J. McCann, E. Choi, E. Yamasaki, and B. N. Ames, *Proc. Nat. Acad. Sci., U.S.A.*, 1975, **72**, 5135.
[30] M. C. Izard, *Compt. rend.*, 1973, **276**, 3037.
[31] T. H. Corbett, C. Heidelberger, and W. F. Dove, *Mol. Pharmacol.*, 1970, **6**, 667.

genicity[32–35] and possibly for the carcinogenicity in rodents of glycidyl esters.[36] The imidazo-cyclization of the purine and pyrimidine residues in DNA which has been found for chloroacetaldehyde,[26] would account for the covalent binding to DNA attributable to the reactive bromoacetaldehyde metabolite of 1,2-dibromoethane[37] and for the mutagenicity[38–42] and carcinogenicity in rodents[43] observed for 1,2-dibromoethane.

A bimolecular mechanism would also appear to apply to the reaction of the powerful mould carcinogen, aflatoxin B_1 (8) (Scheme 6) with DNA (and RNA). The structure of the DNA- and ribosomal RNA-bound (8) adducts obtained[44]



(8)



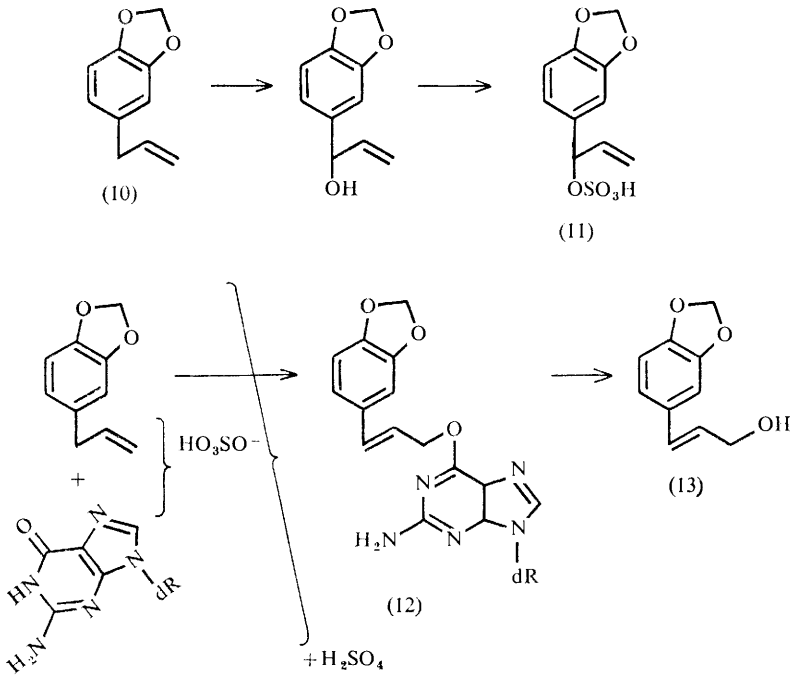
(9)

Scheme 6

[32] B. L. Van Duuren, L. Orris, and N. Nelson, *J. Nat. Cancer Inst.*, 1965, **35**, 707.
[33] B. L. Van Duuren, L. Langseth, L. Orris, G. Teebor, N. Nelson, and M. Kuschner, *J. Nat. Cancer Inst.*, 1966, **37**, 825.
[34] B. L. Van Duuren, L. Langseth, L. Orris, M. Baden, and M. Kuschner, *J. Nat. Cancer Inst.*, 1966, **39**, 1213.
[35] B. L. Van Duuren, L. Langseth, B. M. Goldschmidt, and L. Orris, *J. Nat. Cancer Inst.*, 1967, **39**, 1217.
[36] D. Swern, R. Wieder, M. McDonogh, D. R. Meranze, and M. B. Shimkin, *Cancer Res.*, 1970, **30**, 1037.
[37] D. L. Hill, T.-W. Shih, T. P. Johnston, and R. F. Struck, *Cancer Res.*, 1978, **38**, 2438.
[38] B. N. Ames, *Chem. Mutagens*, 1971, **1**, 267.
[39] H. Brem, A. B. Stein, and H. S. Rosenkranz, *Cancer Res.*, 1974, **34**, 2576.
[40] W. Buselmaier, G. Röhrborn, and P. Propping, *Mutat. Res.*, 1973, **21**, 25.
[41] R. B. Cumming and M. F. Walton, *Food Cosmetics Toxicol.*, 1973, **11**, 547.
[42] H. V. Malling and H. V. De Serres, *Genetics*, 1969, **61**, 39.
[43] W. A. Olson, R. T. Habermann, E. K. Weisburger, J. M. Ward, and J. H. Weisburger, *J. Nat. Cancer Inst.*, 1973, **51**, 1973.
[44] J.-K. Lin, J. A. Miller, and E. C. Miller, *Cancer Res.*, 1977, **37**, 4430.

from salmon-sperm DNA and rat-liver ribosomal RNA with fortified rat- and hamster-liver microsomes was inferred by hydrolysis, which yielded 2,3-dihydro-2-(guan-7-yl)-3-hydroxyaflatoxin B_1 (9) as the major product and 2,3-dihydro-2-[2,5,6-triamino-4-oxopyrimidin-N^5-yl)-*N*-formyl]-3-hydroxyaflatoxin B_1 as a minor one. Chemical reaction between the 2-position of the aflatoxin B_1-epoxy metabolite and N-7 of DNA or RNA guanine residues thus occurred both *in vitro* and *in vivo*.

In contrast, however, the reactive form of the naturally occurring hepatocarcinogen safrole (10) (Scheme 7), *viz.* safrole-1′-sulphate (11) reacts both *in*



Scheme 7

vitro and *in vivo* with deoxyguanosine residues of target-organ DNA to afford O^6-(isosafrol-3′-yl)deoxyguanosine (12), which was characterized both by n.m.r. spectroscopy[45] and by mild acid degradation which gave 3′-hydroxyisosafrole.[46] The reaction between the reactive safrole (10) metabolite, (11), which is a benzylic ester, and the deoxyguanosine residues of DNA is described almost certainly by an S_N1 mechanism, and there is a similarity between the alkylating

[45] P. Borchert, P. G. Wislocki, J. A. Miller, and E. C. Miller, *Cancer Res.*, 1973, **33**, 575.
[46] P. G. Wislocki, P. Borchert, J. A. Miller, and E. C. Miller, *Cancer Res.*, 1976, **36**, 1686.

action of safrole (*q.v.*) and that of *N*-(2-fluorenyl)acetamide and 4-(*N*-methylamino)azobenzene (Schemes 1, 2), discussed in Section 1.*

3 Methylating Carcinogens

Although there does not appear to be a simple relationship between reactivity and carcinogenic potency for chemical carcinogens in general (Section 2), within a field that is limited strictly to alkylation of purine and pyrimidine bases of DNA (and RNA) by simple aliphatic agents, there is a fair correlation. Such a finding is clearly important, and over the years, a big conspectus of fact has been assembled with regard to this subject area. These data are described here in some detail, particularly as the present authors believe that they may have some ideas to contribute to the interpretation of some of the mechanisms concerned.

In this section, attention is paid to reactions occurring at the different nucleophilic sites in DNA, and their significance is discussed. The principal sites are: (i) the N-7 position of guanine,[47,48] which is the major nucleophilic site in DNA; (ii) the N-3 position of adenine, which might be described as a principal minor (nucleophilic) site, and at which methylation causes facile depurination (of 3-methyladenine) from DNA; (iii) the exocyclic O-6 position of guanine at which methylation affords a miscoding base (which is also detachable from DNA *in vivo*, but much less readily than 3-methyladenine) in the Watson–Crick sense.[49–51]

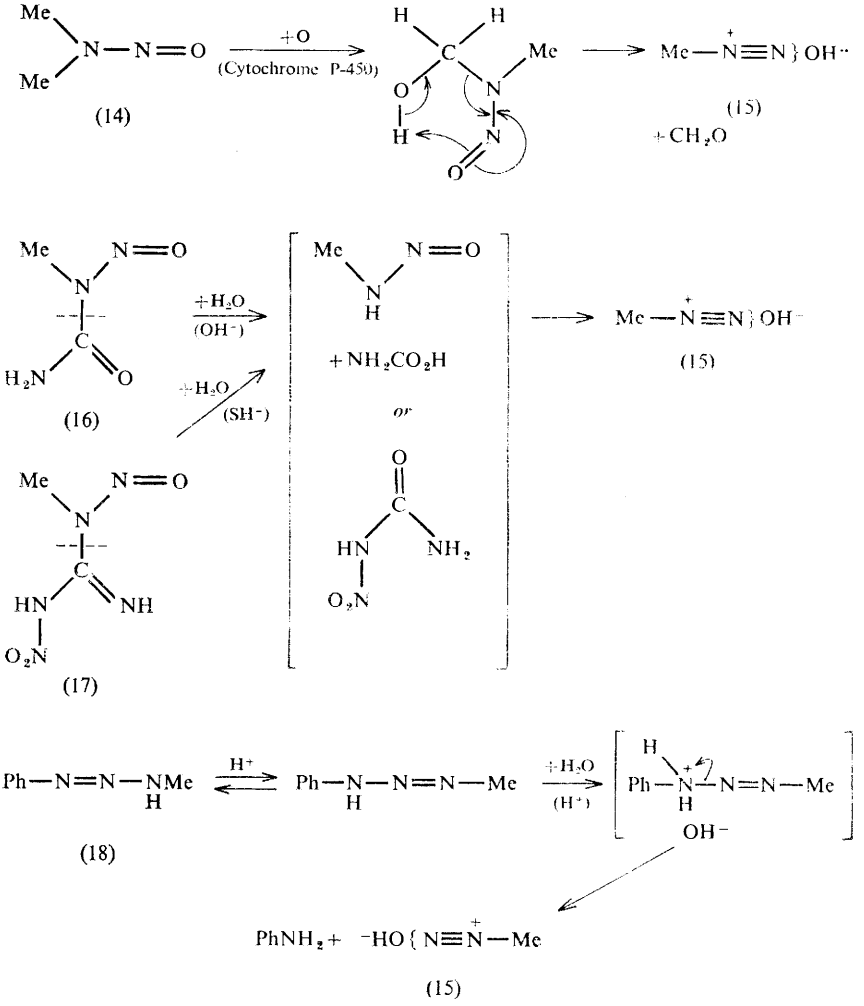
Differences in methylating reactivity can be rationalized on chemical grounds. Thus, amongst the directly acting methylating carcinogens, the non-nitrogen containing dimethyl sulphate (DMS), methyl methanesulphonate (MMS) and the methyl halides show low eletrophilic reactivity. They are typical Ingold S_N2 reagents, and the transferable alkyl group is thus bonded through oxygen, sulphur, *etc*: the values for the Swain–Scott[52] substrate constant s are relatively high (for MMS, s = 0.83).[53]

There is no evidence for chemical or bio-transformation of these substances into reactive forms. On the other hand, other direct-acting methylating carcinogens like *N*-methyl-*N*-nitrosourea (MNU), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and 3-methyl-l-phenyltriazene (MPT) contain either R—N—N—O or R—N—N—N systems, and generate diazomethane on treatment with alkali. These reagents show a co-ordination between the intermediate produced and the nucleophile, which lies between that of Ingold's S_N1 and S_N2

*The possibility of an S_N2 mechanism for the reaction of (11) with deoxyguanosine residues of target-organ DNA ought to be considered. In which case, there would be an attack at the 3'-carbon atom of the safrole residue by the nucleophile (see ref. 45), accompanied by a shift in double-bond position and loss of the sulphate to yield (12). However, the authors of this discussion paper stress the electrolytic properties of the benzylic ester (11) and consider the S_N1 reaction (*vide supra*) to be mechanistically favoured.

[47] B. Pullman, *Biopolymer Symp.*, 1964, **1**, 141.
[48] B. Pullman, *J. Chem. Phys.*, 1965, **43**, S233.
[49] A. Loveless, *Nature*, 1969, **223**, 206.
[50] L. L. Gerchman and D. B. Ludlam, *Biochim. Biophys. Acta*, 1973, **308**, 310.
[51] P. J. Abbott and R. Saffhill, *Brit. J. Cancer*, 1977, **36**, 404.
[52] C. J. Swain and C. B. Scott, *J. Amer. Chem. Soc.*, 1953, **75**, 411.
[53] S. Osterman-Golkar, L. Ehrenberg, and C. A. Wachtmeister, *Radiation Bot.*, 1970, **10**, 303.

categories[54] with a Swain–Scott s-value of 0.42.[55,56] *In vivo*, hydrolytic formation of methyl-diazonium ($Me—N_2^+$) ions (15) (Scheme 8) from MNU (16) is



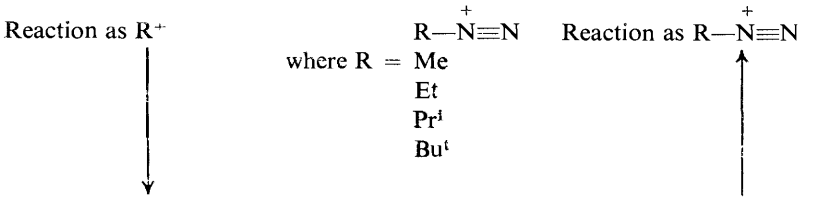
Scheme 8

catalysed by OH^-, from MNNG (17) by thiol-containing cellular constituents

[54] E. R. Garrett and S. Goto, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 1811.
[55] J. Veleminsky, S. Osterman-Golkar and L. Ehrenberg, *Mutat. Res.*, 1970, **10**, 169.
[56] S. Osterman-Golkar, *Mutat. Res.*, 1974, **24**, 219.

such as glutathione and cysteine,[57] and from MPT (18) by H^+ [58] and OH^-;[58a] metabolic activation is not involved in any one of these cases. Theoretically, alkylation by agents that generate alkyldiazonium ions might occur either through the reactive alkyldiazonium cations *per se* or through the separate carbenium ions (Table 2), but methylation is most likely to occur by a mechanism

Table 2 *Reactivity of alkyl diazonium ions*



involving Me—N_2^+ cations rather than the well-known highly reactive species Me^+. This supposition agrees with the conclusion of Friedman[59] who, after lengthy consideration of the intermediates and pathways of diazonium ion decomposition, says that, 'it appears that the products formed from primary alkyldiazonium ions can be accounted for without invoking a free primary carbonium ion'. Very recent experiments in rats given *N,N*-di-n-propylnitrosamine produced 7-n-propylguanine, but not 7-isopropylguanine, in liver DNA and thereby excluded carbocation participation.[59a]

In cytosol (pH 7.4), the energy-rich and very unstable Me—N_2^+ ions are in equilibrium with methanediazohydroxide[60] (Equation 1),

$$Me—\overset{+}{N}\equiv N + H_2O \rightleftharpoons H^+ + Me—N=N—OH \qquad (1)$$

which could provide a source of carbocations only in strongly acidic, unphysiological medium.[60] The ultimate carcinogenic form of the nitrosamide and nitrosamine agents and of MPT and DMPT is one of the electrophilic intermediates (Equation 1), *viz.* Me—N_2^+ or methanediazohydroxide. Competitive with the faster loss of molecular nitrogen from Me—N_2^+ ions through collapse of methanediazotate to generate methanol (Equation 1), the alkylation of nucleophilic sites in nucleic acid takes place by a reaction, which is described more correctly as S_N2 than S_N1.[2]

By analogy with the foregoing, it is feasible that generation of Me—N_2^+ ions in close proximity to DNA may result in the specific methylation of purine and

[57] P. D. Lawley and C. J. Thatcher, *Biochem. J.*, 1970, **116**, 693.
[58a] R. J. LeBlanc and K. Vaughan, *Canad. J. Chem.*, 1972, **50**, 2544.
[58] H. Bartsch, G. P. Margison, C. Malaveille, A. M. Camus, G. Brun, J. M. Margison, G. F. Kolar, and M. Wiessler, *Arch. Toxikol.*, 1977, **39**, 51.
[59] L. Friedman, Carbonium Ion Formation from Diazonium Ions, in '*Carbonium Ions*', ed. G. A. Olah and P. von R. Schleyer, John Wiley & Sons, New York, 1970, Vol. 2, pp. 655—711.
[59a] K. K. Park, M. C. Archer, and J. S. Wishnok, *Chem.-Biol. Interactions*, 1980, **29**, 139.
[60] R. A. Moss, *Acc. Chem. Res.*, 1974, **7**, 421.

pyrimidine nucleophilic sites *via* formation of transient intermediates (Equations 2 and 3).

$$Me—\overset{+}{N}\equiv N + ROH \rightarrow H^+ + Me—N=N—OR \quad (2)$$

$$Me—\overset{+}{N}\equiv N + NHRR' \rightarrow H^+ + Me—N=N—NRR' \quad (3)$$

Whence, concomitant loss of molecular nitrogen and collapse of the fugitive intermediates would transfer the methyl groups to the oxygen and nitrogen nucleophiles (Equations 4 and 5).

$$Me—N=N—OR \rightarrow MeOR + N_2 \quad (4)$$

$$Me—N=N—NRR' \rightarrow MeNRR' + N_2 \quad (5)$$

In fact, diazoethers have been isolated from other coupling reactions,[61–63] the coupling to nitrogen is well-known, and MPT decomposes to give *N*-methylaniline (G.F.K., unpublished work). There is thus chemical analogy for the various reaction processes concerned, but crucial experimental evidence is missing, and the concept in this paragraph must be regarded as a speculative or reasonable chemical possibility.

Product analysis is relevant to reactivity considerations. Thus, whilst the reaction of DMS or MMS with DNA yields a relatively small proportion of *O*-alkylation products, the ratio of O^6-methylguanine to 7-methylguanine being about 0.004[64] (and the proportion of phosphotriesters in the methylation product being approximately 0.01[65]), MNU and MNNG give relatively high yields of *O*-alkylation products, the ratio of O^6-methylguanine to 7-methylguanine being about 0.11 (and the proportion of phosphotriesters being approximately 0.2[66,67]). In the case of MPT, the ratio of O^6-methylguanine to 7-methylguanine ranges from 0.08 for liver DNA through 0.12 for DNA from the kidneys and lungs to 0.15 for that from the brain (one of the target organs for tumour induction).[58] Thus, the (S_N2) DMS and MMS reagents react more exclusively at N-7 of guanine than the $Me—N_2^+$ generating MNU, MNNG, and MPT reagents. Reaction of DMS or MMS with DNA seems to give relatively more of the minor products, 1- and 3-methyladenine (so that the ratio of 3-methyladenine to 7-methylguanine is approximately 0.2) and 3-methylpyrimidines, and a relatively smaller proportion of 7-methyladenine and 3-methylguanine than MNU and MNNG (which give a ratio of 3-methyladenine to 7-methylguanine of approximately 0.13).[68–70] Reaction of MPT with DNA *in vivo* also affords 3-methyladenine and 3-methylpyrimidines.[71]

[61] A. Ginsberg and J. Goerdeler, *Chem. Ber.*, 1961, **94**, 2043.
[62] H. T. Bucherer, *Ber.*, 1909, **42**, 47.
[63] E. Müller and H. Haiss, *Chem. Ber.*, 1962, **95**, 1255.
[64] P. D. Lawley and S. A. Shah, *Chem.-Biol. Interactions*, 1972, **5**, 286.
[65] P. Bannon and W. G. Verly, *European J. Biochem.*, 1972, **31**, 103.
[66] J. V. Frei and P. D. Lawley, *Chem.-Biol. Interactions*, 1976, **13**, 215.
[67] D. H. Swenson, P. B. Farmer, and P. D. Lawley, *Chem.-Biol. Interactions*, 1976, **15**, 91.
[68] P. D. Lawley, *Mutat. Res.*, 1974, **23**, 283.
[69] P. D. Lawley, in 'Screening Tests in Chemical Carcinogenesis,' I.A.R.C. Scientific Publications no. 12, ed. R. Montesano, H. Bartsch, and L. Tomatis, Lyon, France, 1976, pp. 181—208.
[70] A. E. Pegg, *Adv. Cancer Res.*, 1977, **25**, 195.
[71] G. P. Margison, A. J. Likhachev, and G. F. Kolar, *Chem.-Biol. Interactions*, 1979, **25**, 345.

In the case of the indirectly acting *N,N*-dimethylnitrosamine (DMN) (14) (Scheme 8), generation of the reactive form (15) involves an oxidative *N*-demethylation step.[58] The pattern of DNA methylation products derived from DMN *in vivo*[72-74] parallels the one given by chemically activated MNU (*vide supra*) and thus reflects the common methylation mechanism *via* Me—N_2^+ ions (15). Furthermore, the relative amounts of methylated bases formed by MPT[71] were essentially similar to those afforded by the indirectly acting 3,3-dimethyl-1-phenyltriazene (DMPT).[75] This observation implies that both DMPT and MPT react with DNA through the Me—N_2^+ species, and that oxidative *N*-demethylation of DMPT *in vivo* affords MPT as a relatively stable transport form.

The feasible correlation of carcinogenic potency with chemical reactivity is at first sight very confusing, as there is no relationship whatever between the capacity to form 7-methylguanine in DNA and carcinogenicity,[76] and considerable clarification is necessary. Thus, whilst the broad categories of nitroso-compounds and alkyl aryltriazenes are powerful carcinogens, the non-nitrogen containing alkylating agents are poor ones.[76] Direct-acting nitrosamides, which are transformed into reactive forms without metabolic activation (*vide supra*), produce tumours in numerous organs and at the site of administration, but the action of the *N,N*-dialkylnitrosamines is at any rate modulated by those organs that metabolize them into reactive forms.[77-80] *sym-N,N*-Dialkylnitrosamines afford hepatomas at the main site of metabolism, but *asym-N,N*-dialkylnitrosamines (like *N*-methyl-*N*-pentylnitrosamine and *N*-methyl-*N*-phenylnitrosamine), which are metabolized through relatively stable intermediates that can be transported from the site of metabolism to another organ, where they are metabolized further into R—N_2^+, are oesophageal carcinogens.[78] DMPT and its MPT transport form are non-hepatic carcinogens.

However, intravenous injection of MNU, which causes an high incidence of brain tumours in animals, also methylates rat-brain DNA to give a relatively large yield of *O*-alkylation products,[81] the ratio of O^6-methylguanine to 7-methylguanine being approximately 0.12. When MMS, which these workers[81] claimed did not induce brain tumours (*vide infra*) was used instead of MNU, no O^6-methylguanine resulted.[81] Separate investigation[73] of the liver DNA of rats that were given either the hepatocarcinogen DMN or MMS, which does not induce liver cancer, afforded a ratio of O^6-methylguanine to 7-methylguanine of about 0.12 in the case of DMN. In the two sets of experiments,[73,81] the two types

[72] P. J. O'Connor, M. J. Capps, and A. W. Craig, *Brit. J. Cancer*, 1973, **27**, 153.
[73] V. M. Craddock, *Biochim. Biophys. Acta*, 1973, **312**, 202.
[74] J. W. Nicholl, P. F. Swann, and A. E. Pegg, *Chem.-Biol. Interactions*, 1977, **16**, 301.
[75] P. Kleihues, G. F. Kolar, and G. P. Margison, *Cancer Res.*, 1976, **36**, 2189.
[76] P. F. Swann and P. N. Magee, *Biochem. J.*, 1968, **110**, 39.
[77] P. N. Magee and J. M. Barnes, *Adv. Cancer Res.*, 1967, **10**, 163.
[78] H. Druckrey, R. Preussmann, S. Ivankovic, and D. Schmähl, *Z. Krebsforsch.*, 1967, **69**, 103.
[79] P. N. Magee, R. Montesano, and R. Preussmann in 'Chemical Carcinogens' A.C.S. Monograph no. 173, ed. C. E. Searle, American Chemical Society, Washington, D.C., 1976, pp. 491—625.
[80] R. Montesano and H. Bartsch, *Mutat. Res.*, 1976, **32**, 179.
[81] P. Kleihues and P. N. Magee, *J. Neurochem.*, 1973, **20**, 595.

of alkylating agent gave the same minor products of DNA alkylation, *viz.* 1- and 3-methyladenine and 3-methylcytosine. Thus, there is a strong supposition that formation of the miscoding O^6-methylguanine base[49,51] in DNA provides a possible relationship between the carcinogenic potency and reactivity of these methylating agents. Other experiments[72,81–91] with nitrosamides and nitrosamines in hamsters, mice, and rats strongly support this correlation, but reinvestigation (*vide supra*) showed that in fact MMS caused some tumours of the nervous system.[79,80] However, this result can be reconciled with the very small amounts of O^6-methylguanine, formed in the brain by MMS,[85] which may be sufficient, as the nervous system of the developing rat is particularly sensitive to carcinogenesis by alkylating agents.[77–80]

The concept that the formation of O^6-methylguanine in DNA constitutes an important biochemical lesion in chemical carcinogenesis is strongly supported by the fact that it is only this modified base, out of all of the methylated products, which persists in the DNA of target tissues, which are particularly susceptible to tumours, for a much longer time than in other tissues, which are resistant to tumour induction.

4 Ethylating Carcinogens

Nucleic acid is alkylated to a smaller extent by ethylating agents than by methylating agents, and phosphotriesters account for up to 70% of the ethylation products.[82,83,93] The difference in reactivity between the two types of alkylating carcinogen that was discussed in the case of methylating agents (Section 3) applies also to ethylating agents. Thus, typical Ingold S_N2 ethylating carcinogens have relatively high s-values: s = 0.64 for diethyl sulphate (DES) and s = 0.67 for ethyl methanesulphonate (EMS).[53] On the other hand, *N*-ethyl-*N*-nitrosourea (ENU), *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG) and *N*,*N*-diethylnitrosamine (DEN), which have low s-values of approximately 0.26,[55,56,94] afford alkyl—N_2^+ ions (Section 3), and would be expected to react through a mechanism involving Et—N_2^+ ions *per se*.[59] Whilst EMS produces a significant amount of

Whilst the Me—N_2^+ generating alkylating agents, which are considered in this section, might be regarded as model carcinogens, there is a single report of an accidental exposure of scientific staff to DMN, (ref. 92), which used to be employed as an intermediate in the manufacture of 1,1-dimethylhydrazine for rocket fuel.

[82] R. Goth and M. F. Rajewsky, *Proc. Nat. Acad. Sci., U.S.A.*, 1974, **71**, 639.
[83] R. Goth and M. F. Rajewsky, *Z. Krebsforsch.*, 1974, **82**, 37.
[84] P. Kleihues and G. P. Margison, *J. Nat. Cancer Inst.*, 1974, **53**, 1839.
[85] P. Kleihues, K. Patzschke, G. P. Margison, L. A. Wegner, and C. Mende, *Z. Krebsforsch.*, 1974, **81**, 273.
[86] L. Den Engelse, *Chem.-Biol. Interactions*, 1974, **7**, 329.
[87] J. V. Frei and P. D. Lawley, *Chem.-Biol. Interactions*, 1975, **10**, 413.
[88] V. M. Craddock, *Chem.-Biol. Interactions*, 1975, **10**, 323.
[89] G. P. Margison, J. M. Margison, and R. Montesano, *Biochem. J.*, 1976, **157**, 627.
[90] J. W. Nicholl, P. F. Swann, and A. E. Pegg, *Nature*, 1975, **254**, 261.
[91] A. E. Pegg, J. W. Nicholl, P. N. Magre, and P. F. Swann, *Excerpta Med. Internat. Congress Series*, 1976, **376**, 39.
[92] H. A. Freund, *Ann. intern. Med.*, 1937, **10**, 1144.
[93] L. Sun and B. Singer, *Biochemistry*, 1975, **14**, 1795.
[94] A. E. Pegg and J. W. Nicholl, Ref. 69, pp. 571—590.

O^6-ethylguanine in DNA, the ratio of O^6-ethylguanine to 7-ethylguanine being 0.03,[93] ENU and DEN yield ethylated DNA in which the ratio of O^6-ethylguanine to 7-ethylguanine ranges from 0.48 to at least 0.70.[93] After 7-ethylguanine and O^6-ethylguanine, 3-ethyladenine is the third most abundant product of DNA ethylation.[82,83,93]

The capacity of alkylating agents to form the miscoding O^6-alkylguanine bases in the DNA of target tissues correlates with their carcinogenic potency (Section 3), and formation of O^6-ethylguanine agrees with this concept. Thus, doses of ENU and DEN, which gave the same incidence of kidney tumours as that given by MNU or DMN, yielded a smaller amount of 7-alkylguanine in kidney nucleic acids than the analogous methyl reagents,[95] but the relative proportion of O^6-alkylguanine to 7-alkylguanine formed by ENU and DEN exceeds that given by MNU and DMN, which in turn exceeds by far that produced by DES and DMS. The apparent anomaly of rats producing kidney tumours after three closely spaced intraperitoneal injections of EMS[95] can be explained by the small amounts of O^6-ethylguanine that are formed through the reaction of EMS with kidney DNA in those animals. In fact, the s-value for EMS (0.67) lies between the one (0.42) for MNU and that (0.83) for MMS.

5 Metal Carcinogens and Cancer Chemotherapeutic Agents

Chromium, nickel, and cadmium are suspected of being associated with industrial cancer, but the reactive forms are largely unknown, and nothing at all is known about the nature of interactions with nucleic acid. However, it has been established recently in the case of chromium that Cr^{VI} is a mutagen in standard test systems,[96–99] but Cr^{3+} is non-mutagenic.[96,97] These biological findings provide strong evidence for the chemical reaction of Cr^{VI} with DNA, but the basis for nickel carcinogenicity is unknown.

In standard test systems, *cis*-diamminedichloroplatinum(II) is a mutagen[100–102] and it is the parent compound of a number of cancer chemotherapeutic agents,[103] whereas the *trans*-isomers are inactive. $Pt(en)Cl_2$ reacts with nucleosides in the order of guanosine > adenosine ≃ deoxycytidine.[104] These platinum S_N2 electrophiles show approximately the same specificity[105,106] towards DNA nucleophilic sites, *i.e.* guanine > adenine > cytosine as the DES, DMS, and

[95] P. F. Swann and P. N. Magee, *Biochem. J.*, 1971, **125**, 841.
[96] S. Venitt, *Nature*, 1974, **250**, 493.
[97] F. L. Petrilli and S. de Flora, *Appl. Environ. Microbiol.*, 1977, **33**, 805.
[98] H. Nishioka, *Mutat. Res.*, 1975, **31**, 185.
[99] H. Nishioka, *Japanese J. Genetics*, 1975, **50**, 485.
[100] D. J. Beck and R. R. Brubaker, *Mutat. Res.*, 1975, **27**, 181.
[101] C. Monti-Bragadini, M. Tanaro, and E. Banfi, *Chem.-Biol. Interactions*, 1975, **11**, 469.
[102] P. Lecointe, J.-P. Macquet, J.-L. Butour, and C. Paoletti, *Mutat. Res.*, 1977, **48**, 139.
[103] J. J. Roberts and A. J. Thomson, *Progr. Nucleic Acid Res. Mol. Biol.*, 1979, **22**, 71.
[104] A. B. Robins, *Chem.-Biol. Interactions*, 1973, **6**, 35.
[105] S. Mansy, G. Y. H. Chu, R. E. Duncan, and R. S. Tobias, *J. Amer. Chem. Soc.*, 1978, **100**, 607.
[106] D. M. L. Goodgame, I. Jeeves, F. L. Phillips, and A. C. Skapski, *Biochim. Biophys. Acta*, 1975, **378**, 153,

MMS agents considered in Sections 3, 4.[107] Under neutral conditions, these reagents alkylate preferentially N-7 of guanine, N-1 of adenine, and N-3 of cytosine. Rosenberg's[108] idea that *cis*-$[PtCl_2(NH_3)_2]$ would undergo aquation *in vivo* before reaction with nuclear DNA now appears to be unlikely, as *cis*- and *trans*-aquo species show poor selectivity, whereas the bimolecular displacement of Cl would be consistent with the degree of selectivity that has been observed. There is no crystallographic evidence for bidentate binding of these Pt electrophiles to guanine residues of DNA.[106*cf.*103]

6 Pearson's Hard and Soft Acids and Bases with Reference to Sections 3—5

Some of the conclusions (*q.v.*) about the reactivities of electrophilic agents for various nucleophilic sites in DNA might have been reached had consideration been given alternatively to Pearson's principle of hard and soft acids and bases.[109–111] But, the impact of this work[109–111] might not have registered fully at the time when the Millers, who used[2] S_N1 and S_N2 arguments, made their proposals. In the present discussion paper, first consideration was given (Sections 1—5) to that approach to the alkylation of nucleic acid which the Millers[2] adopted.

The site of reaction in an ambident nucleophile with alternative hard and soft sites can be deduced usually through application[112] of the hardness–softness principle,[109–111] and considerable attention has been given to the problem of *C- versus O*-alkylation.[113–117] The same sort of argument would apply to the problem of *N- versus O*-alkylation. In the case, for example, of deoxyguanosine, the donor N-7 atom is a softer base than the donor (O-6) oxygen atom. Hence, the harder alkyldiazonium, alkyl—N_2^+, cations would be expected to react with deoxyguanosine to give a larger proportion of O^6-alkylation products, relative to that of N-7 ones, than the softer alkyl groups belonging to the DES, DMS, and MMS agents. This prediction agrees with the experimental evidence (Sections 3, 4). In contrast to the harder (acid) alkyl—N_2^+ cations, the softer $(H_3N)_2Pt^{2+}$ cations, for which the simple Me—Hg^+ is an archetype,[118] would be expected to react with deoxyguanosine residues of DNA to effect almost exclusive platination at N-7. This supposition agrees with the experimental findings (Section 5).

[107] B. Singer, *Progr. Nucleic Acid Res. Mol. Biol.*, 1975, **15**, 219.
[108] B. Rosenberg, *Naturwiss.*, 1973, **60**, 399.
[109] R. G. Pearson, *J. Amer. Chem. Soc.*, 1963, **85**, 3533.
[110] R. G. Pearson, *Science*, 1966, **151**, 172.
[111] R. G. Pearson and J. Sonstad, *J. Amer. Chem. Soc.*, 1967, **89**, 1827.
[112] R. F. Hudson, paper presented at a symposium on S.H.A.B., Cynamid European Research Institute, Geneva, May 1965: *Chem. Eng. News*, 1965, **43**, [no. 22], pp. 102, 103
[113] A. L. Kurts, N. K. Genkina, A. Macias, I. P. Beletskaya, and O. A Reutov, *Tetrahedron*, 1971, **27**, 4777.
[114] W. J. Le Noble and H. F. Morris, *J. Org. Chem.*, 1969, **34**, 1969.
[115] W. S. Murphy and D. J. Buckley, *Tetrahedron Letters*, 1969, 2975.
[116] J. A. Campbell and J. F. Wolfe, *Org. Prep. Proc. Internat.*, 1971, **3**, 303.
[117] J. P. Boisset, J. Boyer, and J. Rouzand, *Compt. rend., ser. C*, 1966, **263**, 1253.
[118] G. Schwarzenbach, paper presented at a symposium on S.H.A.B., Cynamid European Research Institute, Geneva, May 1965: *Chem. Eng. News*, 1965, **43** [no. 22], p. 92.

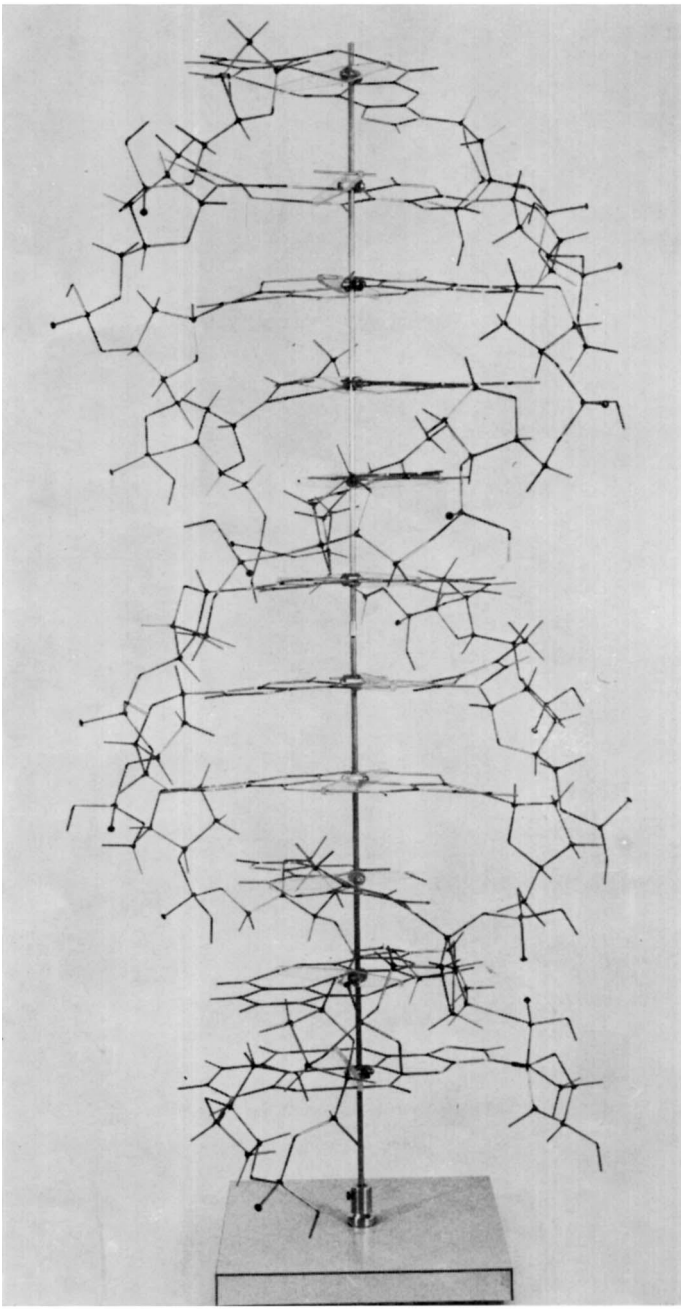
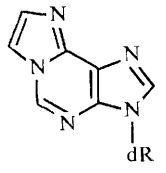


Plate 1 *A model of the Watson–Crick double helix for DNA*

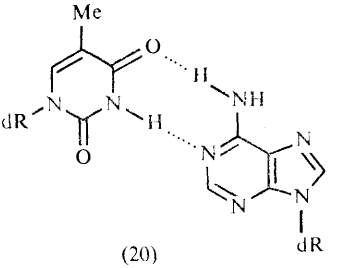
7 Nucleic Acid Modifications

Finally, an attempt is made in this section to consider changes (*q.v.*) to the structure of DNA that can be brought about through reaction with chemical carcinogens. It will be recalled that the Watson–Crick double helix for DNA (Plate 1) involves not only the maximum possible number of H-bonded base pairs,[119] but also those pairs which confer maximum stability. Two types of distortion to DNA that may cause miscoding and heritable change are envisaged. Firstly (i), the introduction of substituent groups at sites on the pairing faces of the bases nearly always breaks a number of H-bonds interconnecting the bases (*vide infra*), and diminishes stability in proportion to the incidence of H-bonds broken. Alternatively, (ii) the introduction of bulky substituent groups at sites on the backside of the purine bases tends to interfere with the stacking of the bases inside the double helix, by grossly distorting the sugar and phosphate residues at the periphery and, in the extreme case, causes 'unstacking' (*vide infra*).

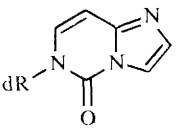
In the target-organ DNA of mammals exposed either to vinyl chloride or to 1,2-dibromoethane (Section 2), imidazo-cyclization of deoxyadenosine (19), coplanar with the purine and pyrimidine rings respectively, would break both of the H-bonds of the adenine–thymine pair (20).[26] Again, in the target-organ DNA of mammals exposed either to vinyl chloride or to 1,2-dibromoethane, imidazo-cyclization of deoxycytidine (21) would break two out of three H-bonds belonging to the cytosine–guanine pair (23),[26] and in the target-organ DNA of mammals exposed either to glycidaldehyde[27] or to glycidyl esters[36] (Section 2), the similar



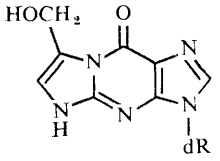
(19)



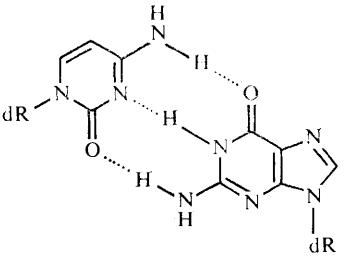
(20)



(21)



(22)

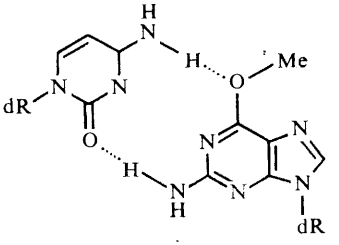


(23)

[119] L. Pauling and R. B. Corey, *Arch. Biochem. Biophys.*, 1956, **65**, 164.

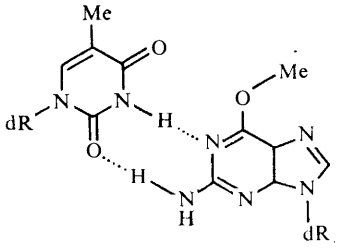
imidazo-cyclization of deoxyguanosine (22) would produce the same result on the base pair (23). The breaking of the pairs of H-bonds in the way that has been described would tend to decrease stability in the double helix in proportion to the incidence of that breaking in the macromolecule. Formation of such base analogues as the ones described and their persistence together with the likelihood of misincorporation occurring during DNA repair are consistent with the pro-mutagenic response concerned.

On the other hand, the alkylation of O-6 in deoxyguanosine obliterates only one out of three H-bonds of the cytosine–guanine pair. The nature of the miscoding of O^6-methylguanine may be considered in terms of its H-bonding with other bases. Thus, it is possible to construct an H-bonded base pair between O^6-methylguanine and cytosine (24a) as well as between O^6-methylguanine and thymine (24b). Experimental evidence shows that in fact the miscoding of O^6-



(a)

(24)



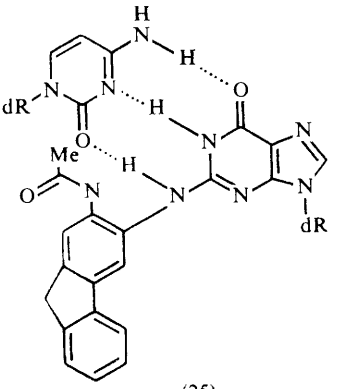
(b)

methylguanine[49–51] is competitive with normal base incorporation.[120] Whilst the formation and persistence of O^6-alkylguanine in target-cell DNA can be correlated with tumour induction (Sections 3, 4), O'Connor *et al.* suggest[120] that further investigation of this pro-mutagenic lesion and its repair may be desirable.

[120] P. J. O'Connor, R. Saffhill, and G. P. Margison, in 'Environmental Carcinogenesis', ed. P. Emmelot and E. Kriek, Elsevier–North Holland, Biomedical Press, Amsterdam, 1979, pp. 73–96.

Similar considerations to the foregoing would seem to apply to safrole (Section 2), which effects alkylation at O^6-position of deoxyguanosine residues.[45]

In contrast to previous examples, *N*-[3-deoxyguanosin-N^2-yl)-*N*-(2-fluorenyl)] acetamide[13] (Scheme 1) is readily accommodated by the Watson–Crick double helix without breaking any of the cytosine–guanine H-bonds (25) or interfering with the packing of the bases. Formation of this adduct is unlikely to be promutagenic.



(25)

Inspection of models shows the introduction of bulky substituents at C-8 of deoxyguanosine, as in the case of the formation of *N*-(deoxyguanosin-8-yl)-*N*-(2-fluorenyl)acetamide and 4-[*N*-(deoxyguanosin-8-yl)-*N*-methylamino]azobenzene (Section 1) to interfere with the stacking of the bases inside the double helix by tangling with and grossly distorting the sugar and phosphate residues at the periphery. It is suggested that similar distortion of the native DNA would break the H-bonds belonging to some of the base pairs, and would afford opportunity for misincorporation during subsequent DNA repair. In fact, some very important aromatic amines (Table 1) and amino-azo dyes are human carcinogens (Section 1).

That the foregoing arguments about the substitution of bulky groups at sterically hindered sites in the deoxyguanosine residues of DNA are reasonably correct is seen, for example, from the results of the study[105] of the reaction of *cis*-[$PtCl_2(NH_3)_2$] (Section 5) with four-membered polynucleotides and DNA by Raman difference spectroscopy. Highly selective binding to purine bases was observed in a four-membered polynucleotide model system,[105] and in particular, there were the characteristic shifts associated with substitution at the sterically hindered N-7 of the deoxyguanosine residues. In line with our foregoing arguments and expectations in this case, considerable scattering at 1240 cm^{-1} was shown[105] to be attributable to extensive 'unstacking' of the bases of DNA. This denaturation was further confirmed by the disappearance of the scattering at 835

cm^{-1}, which is diagnostic[105] for the biologically important B conformation.[121–123] In fact, these changes[105] were very similar to the ones[124] produced by heating calf-thymus DNA to 98 °C at pH7, which completely denatured the biopolymer.[124] Thus, in this case,[105] the presence of bulky Pt-containing groups, quaternizing the N-7 of the deoxyguanosine residues of DNA, has been shown to distort the stacking of the bases and to obliterate H-bonds interconnecting the base pairs.

Thus, there seems to be a reasonable correlation between the distortion to the Watson–Crick double helix for DNA, which is caused by chemical reaction, and carcinogenic potency. In the case of the simple alkylating agents (Section 3, 4), new biological testing has proved effective, and resulting information on the incorporation of base analogues (adducts) in model template systems for DNA polymerase and on DNA repair[120] agrees with the interpretation of the changes to DNA structure, which has been made (*vide supra*).

8 Concluding Remarks

(1) The Millers' concept (1970, 1971)[1,2] that reaction of chemical carcinogens with genetic material may be due to the electrophilicity of the ultimate reactive forms has been accepted subsequently. Nucleophiles are non-carcinogenic, and there is an alternative explanation to the obvious one for the intervention of free-radical scavengers with carcinogenicity. The Millers[2] attributed S_N1 mechanisms involving carbenium and nitrenium ions to the reactions between *N*-(2-fluorenyl) acetamide or *N*-methylamino-azobenzene and DNA *in vivo*: the aromatic rings of the reagents permit of a distribution of charge which confers cation stability.

(2) In general, there is no clear relation between the reactivity of the ultimate carcinogens and carcinogenic potency. It is considered that initial reaction between vinyl chloride-related chloroethylene oxide or chloroacetaldehyde and the deoxyadenosine or deoxycytidine residues of DNA is S_N2 and that the epoxy metabolite of aflatoxin B_1 reacts similarly with the deoxyguanosine residues, whereas the reactive form of safrole is a benzylic ester, which makes an S_N1 reaction with deoxyguanosine residues.

(3) Amongst directly-acting methylating carcinogens, DMS and MMS show low electrophilic reactivity and are typical S_N2 alkylating agents, whereas MNU, MNNG, and MPT show co-ordination between the intermediate produced and the nucleophile, and a reactivity somewhere between that of an S_N1 and an S_N2 mechanism. Methylation by the latter agents is likely to involve $Me—N_2^+$ ions or methanediazohydroxide rather than the highly reactive Me^+ species, and to be bimolecular. A mechanism is discussed. Whilst DMS and MMS yield a relatively low proportion of *O*-alkylation products, the ratio of O^6-methylguanine to N^7-methylguanine being 0.004, MNU, MNNG, and MPT give relatively high

[121] R. E. Franklin and R. G. Gosling, *Acta Cryst.*, 1953, **6**, 673.
[122] R. E. Franklin and R. G. Gosling, *Nature*, 1953, **171**, 742.
[123] A. Klug, *Nature*, 1968, **219**, 208.
[124] S. C. Erfurth and W. L. Peticolas, *Biopolymers*, 1975, **14**, 247.

yields of *O*-alkylation products, the ratio of O^6-methylguanine to N^7-methylguanine being *ca.* 0.12. Oxidative *N*-demethylation of DMN and DMPT occurs and alkylation involves Me—N_2^+ ions. Relatively stable transport forms of some *asym-N,N*-dialkylnitrosamines determine the site of tumour induction, where further metabolism into the powerful alkylating R—N_2^+ species takes place. The capacity of the agents to form O^6-methylguanine in target-tissue DNA correlates with their carcinogenic potency, and DES, DMS, and MMS fail to induce tumours in tissues *in vivo* where MNU, DMN, MPT, and DMPT are active carcinogens.

(4) Nucleic acid is alkylated to a lesser extent by ethylating than by methylating agents. The typical S_N2 agent EMS affords an O^6-ethylguanine to N^7-ethylguanine ratio of 0.03, whereas ENU and DEN, which react through Et—N_2^+, give a ratio of from 0.48 to at least 0.70. DEN and ENU are much more powerful carcinogens than EMS.

(5) Strong biological evidence is cited for the chemical reaction of Cr^{VI} with DNA *in vivo*. Like the DES, DMS, and MMS agents (*vide supra*), *cis*-$[PtCl_2(NH_3)_2]$ reacts with DNA by an S_N2 mechanism.

(6) Analysis (1)—(5) shows that very few reactions between ultimate chemical carcinogens and nucleic acid can be described as S_N1, and the argument about S_N2 reactions predominating over S_N1 applies to the subject-matter of this discussion.

(7) Qualitative application of Pearson's hardness–softness principle infers (i) that the harder alkyl—N_2^+ cations would be expected to react with deoxyguanosine residues to give a larger proportion of O^6-alkylation products than the softer alkyl groups of the DES, DMS, and MMS agents, and (ii) that the softer $(H_3N)_2Pt^{2+}$ cations would be expected to yield almost exclusive N^7-platination.

(8) There appears to be a reasonable correlation between the distortion to the Watson–Crick double helix for DNA, caused by chemical reaction, and carcinogenic potency. Two types of distortion to DNA that cause miscoding are envisaged: (i) introduction of substituent groups at sites on the pairing faces of the bases thereby breaking a number of H-bonds interconnecting the bases: (ii) introduction of bulky substituents at sites on the backside of the purine bases tending to interfere with the stacking of the bases inside the double helix by grossly distorting the sugars and phosphate groups at the periphery, and in extreme cases causing 'unstacking'. Imidazo-cyclization of purine bases by vinyl chloride, 1,2-dibromoethane, and glycidaldehyde exemplifies the first category, the special case of O^6-methylation (ethylation) is discussed, and respective formation of *N*-(deoxyguanosin-8-yl)-*N*-(2-fluorenyl)acetamide and 4-[*N*-(deoxyguanosin-8-yl)-*N*-methylamino]azobenzene and N^7-platination exemplifies the second category of distortion. By contrast, *N*-[3-deoxyguanosin-N^2-yl)-2-

fluorenyl]acetamide was readily accommodated by the double helix, and is unlikely to constitute a pro-mutagenic lesion.

(9) It ought to be stated clearly that no attempt has been made to treat the title subject exhaustively, but the authors have drawn attention to some important chemical carcinogens, where the interaction between the reactive form and nucleic acid has been explored.

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