Carcinogens and DNA

from Stephen Neidle

THE physical and chemical properties of the potent diolepoxide carcinogens have been much studied in the hope of determining the molecular basis of their carcinogenic and mutagenic effects. They are known to bind directly to DNA, and this reaction has formed the focus of both experimental and theoretical investigations into the activity of diolepoxides such as benzo(*a*)pyrene 7,8 dihydrodiol 9,10 oxide, the most important active carcinogenic metabolite of benzo(*a*)pyrene.

Although theoretical approaches to the likely conformation and chemical reactivity of the four possible stereoisomers of benzo(*a*)pyrene diolepoxide, using quantum-mechanical methodology, have had great influence on thinking in this field, in many cases the theoretical findings conflict with the experimental evidence and it is fair to say that only some of the recent theoretical treatments are successful or other than trivial in their predictions. The deficiencies and limitations of some approaches are highlighted for example, by their inability to explain satisfactorily the considerably greater tumorigenicity and DNA binding ability of the *anti* compared with the *syn* isomer (Brookes *Cancer Lett.* **6**, 285; 1979), even though the theoretically-derived data indicate that the *syn* isomer is the more stable (Klopman, Grinberg & Hopfinger *J. theor. Biol.* **79**, 355; 1979) and until relatively recently was believed to be the biologically significant one.

Application of the ϕX174 DNA assay system developed by Harvey and coworkers to a series of diolepoxide carcinogens, has led to the revelation of further deficiencies in some quantum mechanical analyses (Hsu *et al. Biochem. biophys. Res. Commun.* **87**, 416; **88**, 1; 1979). This assay system is remarkably effective in correlating inhibition of viral infectivity with carcinogenic potency of the various diolepoxides, yet this correlation was not followed either by predictions of reactivities from molecular orbital theory or by the relative structure positions of the diolepoxide groupings on the hydrocarbon skeletons.

Clearly, studies of the chemicals themselves are not enough to explain their different carcinogenic potencies and other factors such as the geometry and kinetics of their binding to the DNA receptor sites will have to be taken into account.

Benzo(*a*)pyrene diolepoxide carcinogenesis is generally considered to involve direct binding to DNA, with guanine residues as the major sites of attachment at least in terms of stoichiometry. About 10% of the total binding is to adenine however, and although its significance is unclear, some recent evidence correlates binding to adenines and not to guanines with mouse skin tumorigenicity (Di Giovanni *et al. Cancer Lett.* **7**, 39; 1979), although in this report no adducts have actually been characterised as diolepoxide ones. Adenine-modified regions of DNA are known to have rather different conformational properties in terms of greater local denaturation than their guanine counterparts. The *anti* form of the diol epoxide can be resolved to two stereoisomers. Their interactions with DNA are, not surprisingly, stereospecific (Meehan & Straub *Nature* **277**, 410; 1979), with essentially only the (+) isomer binding to double-stranded DNA (to guanines) whereas denatured, single-stranded DNA was equally susceptible to both isomers. These differences in binding ability correlate with biological activity in several cell lines — the (+) isomer being more carcinogenic — and Meehan and Straub suggest that DNA secondary structure is an important determinant in carcinogenicity.

One question of interest in looking at the binding of carcinogens to DNA is whether any specific base sequence is involved. Weinstein, Grunberger and coworkers (*Biochemistry*, in the press) do to a large extent confirm Meehan & Straub's findings, with the additional important observation from restriction enzyme analysis of modified SV40 and lambda DNA, that no appreciable specificity of binding in respect of base sequence was apparent. However, subtle and possibly important effects of binding specificity may occur. Probably more crucial to carcinogenicity is the geometry of the binding in relation to subsequent excision repair by endonucleases. Binding to different residues and different chemical groups within a residue dramatically affects excisability. Increased excisability implies attachment on the outside rather than to deeply buried sites within the molecule.

There is no lack of molecular models for the structure of benzo(*a*)pyrene diol epoxide-modified DNA, some of which owe more to imagination than to reality. The recent suggestions of Beland (*Chem.-Biol. Interactions* **22**, 329; 1978) are among the more plausible and detailed with the benzo(*a*)pyrene chromophore being situated in the DNA minor groove and causing some local denaturation of the double helix. This general type of non-intercalative model is strongly supported by several recent physical measurements on the diol epoxide-DNA complex. Fluorescence probe techniques (Prusik *et al. Photochem. Photobiol.* **29**, 223; 1979; Prusik & Geacintov *Biochem. biophys. Res. Commun.* **88**, 782; 1979) give data consistent with the benzo(*a*)pyrene chromophore attached (by N2 of guanine) externally to the DNA double helix, and oriented with at most a 35° angle to the helix axis. In particular, intercalation (at least in the sense of the classical Lerman model) is ruled out by these workers on account of the accessibility of the chromophore to external probings. Additional evidence for a non-intercalative model is also provided by the new technique of optically-detected magnetic resonance (Lefkowitz *et al. FEBS Lett.* **105**, 77; 1979)

Unfortunately such techniques cannot provide detailed information on the molecular geometry of the modified residues of DNA. The situation is made yet more complex by the growing realisation that DNA structure is not always necessarily of the classical forms. There is now powerful evidence for example, that tracts of alternating pyrimidine-purine residues have highly unusual structures (see for example *Nature* **282**, 680; 1979; *News and Views*, **283**, 11; 1980); it would not be surprising if binding of carcinogens did not sometimes exploit such peculiarities. Molecular model-building of nucleic acid-carcinogen interaction urgently requires input of firm data from the fine structure techniques of X-ray crystallography and NMR in order to achieve credibility. Questions of the relevance of the structural peculiarities mentioned above to differences in excisability and repair, and to carcinogenic and mutagenic processes in general must await future investigations. □

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The specificity of plant defences

from R.A. Dixon & C.J. Lamb

WHEN plants are attacked by bacterial or fungal pathogens they synthesise and accumulate nonspecific antimicrobial compounds known as phytoalexins. A few years ago it was established that phytoalexins could be induced to accumulate by glucan and glycoprotein 'elicitor' molecules associated with the cell wall of the invading pathogen (see *News & Views* **273**, 266; 1978) and this has started a search for the molecular basis of the exquisite specificity seen in the classical 'gene-for-gene' relationship between plant cultivars and physiological races of a pathogen, looked at in terms of elicitors and phytoalexin production.

In the simplest terms the gene-for-gene relationship consists of a gene for 'avirulence' in the pathogen being complemented by a specific one for resistance in the plant — the plant and pathogen are in this case incompatible.

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Disease results when a mutation in the pathogen produces a new avirulence gene (now a 'virulence' gene) that is not matched by the available resistance genes carried by the host cultivar — the plant and pathogen are now compatible. Plant breeders have long been familiar with the ability of plant pathogens to keep one jump ahead by continually throwing up new races to which hitherto resistant varieties of crops are completely susceptible.

The simplest model for the gene-for-gene phenomenon in terms of elicitors requires that a race-specific elicitor (presumably the product of the pathogen's avirulence gene) is recognised by a specific receptor on the plant cell (presumed to be the product of the plant's resistance gene); phytoalexin production is induced and the pathogen is limited. If a mutation either in the pathogen or plant means that the elicitor is no longer recognised by the plant, then the infection is not limited and disease follows. The key predictions of this model are first, the existence of race-specific elicitors, and second, that the differential accumulation of phytoalexin is the most important determinant of disease resistance.

Recent work suggests that this model is indeed an oversimplification and that the real world is considerably more complex. On the question of race-specific elicitors Keen *et al.* (*Physiol. Plant Pathol.* **15**, 43; 1979) recently claimed to show race-specific elicitors in the cell envelopes of physiological races of the bacterium *Pseudomonas glycinea*, which causes bacterial blight in soybeans. High molecular weight glycoproteins solubilised by detergent treatment showed the same specificity, with one exception, in eliciting accumulation of the phytoalexin glyceollin in cotyledons of two varieties of soybean as did the bacterial races from which the elicitors were isolated.

In contrast, however, Albersheim and coworkers (*Plant Physiol.* **57**, 760; 1976) have shown that glucan elicitors from four different races of the fungal pathogen *Phytophthora megasperma* var. *sojae* (Pms) are qualitatively and quantitatively identical suggesting that further 'specificity factors' are needed to account for race-specific resistance. A recent report by Wade and Albersheim (*Proc. natn. Acad. Sci. U.S.A.* **76**, 4433; 1979) now identifies glycopeptides secreted into the culture medium by Pms as the proposed factors. Glycopeptides from three races of Pms were tested for their ability to prevent the expression of serious symptoms in four soybean cultivars. Glycopeptides from incompatible races of the pathogen but not from compatible ones protected seedlings from attack by compatible races. Glycopeptides from compatible races did not cause cultivars resistant to a given Pms race to become susceptible to that race. The glycopeptides themselves are only weak elicitors and their effect on glucan elicitor action remains to be established. Wade and Albersheim postulate that synthesis of the glycopeptides is controlled by the pathogen's avirulence genes and that in incompatible but not compatible interactions they trigger a defence reaction which decreases the rate of pathogen development and enables inhibitory levels of phytoalexin to accumulate in the area of initial fungal penetration.

Such a hypothesis still leaves open the nature of the proposed defence reaction and the product of the host's resistance gene. This model assumes no determinative role for elicitor-induced phytoalexin accumulation in the soybean-Pms system and indeed Albersheim and coworkers (*Plant Physiol.* **57**, 760; 1976) have shown that initial rates of glyceollin accumulation are identical during infection of wounded hypocotyls by compatible and incompatible races of Pms. This contradicts earlier work of Keen (*Physiol. Plant Pathol.* **1**, 165; 1971) who observed differential rates of glyceollin accumulation in this system. However, in both studies no account is taken of the cellular and subcellular distribution of glyceollin and such considerations may be crucial for a full understanding of the role of phytoalexins in race-specific resistance.

A common feature of the models of Keen and Albersheim is that specificity is associated with the incompatible interaction. However, this may not always be the case. Two recent papers by Kuć and coworkers (*Physiol. Plant Pathol.* **15**, 117 and 127; 1979) suggest that in potato cultivars with R genes, specificity in resistance to physiological races of *Phytophthora infestans*, the causal agent of potato blight, is associated with compatibility, and a phytoalexin suppressor, or 'hypersensitivity inhibiting factor' is proposed as the determinant of specificity. They found that mycelia and zoospores of a compatible race of *P. infestans* contain β, 1-3, β, 1-6-linked low molecular weight glucans which could inhibit the rapid cell death, loss of electrolytes, tissue browning and accumulation of terpenoid phytoalexins associated with the hypersensitive reaction of potato tissues to treatment either with elicitor from the same compatible race or infection by an incompatible race. Although the incompatible race contained similar glucans they were much less active as suppressors of the hypersensitive reaction.

The glucans consisted of non-anionic and anionic fractions, the latter containing a small proportion of glucose residues as phosphoryl monoesters. In preliminary experiments, mixing elicitors from compatible or incompatible races with a membrane preparation from potato tubers led to decreased elicitor activity on subsequent application to tuber tissues, possibly as a result of sequestration of elicitors by binding sites on the membrane preparation. Only glucans from the compatible race were able to restore elicitor activity, suggesting that the small glucans may compete for the elicitor binding sites. These molecules may prove valuable tools in the search for elicitor receptor sites. Elicitors from *P. infestans* agglutinate potato protoplasts indicating cell surface binding sites (*Science* **201**, 364; 1978), but it is not clear whether these sites are receptors for elicitor induction of phytoalexin accumulation.

Although no unified hypothesis has yet emerged, the demonstration of molecules that determine specificity in plant-pathogen interactions is a major development in the study of the molecular basis of race-specific disease resistance. Clearly there is considerable scope for collaboration between biochemists and pathologists in this important field. □

Patterns of ungulate reproduction

from Robert M. May

ONE theme, around which much current ecological research is organised, concerns the relation between an animal's physical and biological environment (its habitat and the species it interacts with) and its foraging and reproductive behaviour. In this context, Robbins and Robbins (*Am. Nat.* **114**, 101; 1979) have recently drawn together a lot of data on ungulate and subungulate species, with a view to elucidating fetal and neonatal growth patterns, and trends in maternal reproductive effort. These authors believe ungulates to be especially interesting, because they inhabit a wide range of environments, and comprise species in many different stages of domestication.

By plotting the weight of the fetus (expressed as a fraction of the weight at parturition) as a function of time (expressed as a fraction of the gestation period) for several species, Robbins and Robbins show the fetal growth rate in ungulates is a power function that varies little among species, and is typically of the general mammalian pattern derived by Sacher and Staffeldt (*Am. Nat.* **108**, 593; 1974) and others. Using data for white tailed deer, domestic sheep and cattle, they also show the weight of the actual fetus, as a percentage of the gravid uterus, increases steadily throughout gestation, to around 60% at parturition. The pattern shows no significant differences between natural and domesticated ungulates; that is, selection for a larger neonate in domesticated animals entails a proportional enlargement of the uterus, fluids and membranes. As Robbins and Robbins point out, this fact

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