

Enzyme-Catalysed Reactions of Polycyclic Hydrocarbons with Deoxyribonucleic Acid and Protein *in vitro*

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Although correlations have been found between the chemical structure and the carcinogenic activity of many polycyclic hydrocarbons (Pullman & Pullman, 1955), it is generally accepted that metabolic activation is a prerequisite for the covalent binding of these relatively inert compounds to cellular components (Wiest & Heidelberger, 1953; Brookes & Lawley, 1964; Goshman & Heidelberger, 1967), although the mechanism of such interactions is not understood. With other classes of chemical carcinogens such as the aromatic amines, much more is known about the type of reactive intermediate formed metabolically and, with *p*-dimethyl-aminoazobenzene and 2-acetylaminofluorene, derivatives have been found that are more carcinogenic than the parent amine (Poirier, Miller, Miller & Sato, 1967; Miller, Miller & Hartmann, 1961) and that also react chemically with constituents of nucleic acids (Poirier *et al.* 1967) and of proteins (Lotlikar, Scribner, Miller & Miller, 1966). The polycyclic hydrocarbons are mainly metabolized by ring hydroxylation and it has been suggested (Boyland, 1950) that epoxides may be formed as reactive intermediates in this process; with some hydrocarbons there is indirect evidence to support this view (Boyland & Sims, 1962, 1965). In animal tests, however, the 'K region' epoxides of some of these hydrocarbons have been found to be much less carcinogenic than the parent compounds (Miller & Miller, 1967; Boyland & Sims, 1967; Van Duuren, Langseth, Goldschmidt & Orris, 1967). Previous studies of the products formed by reaction of polycyclic hydrocarbons with RNA, DNA and protein *in vivo* have been hampered by the low level of reaction observed and by the difficulty in obtaining from animals sufficient pure material for further examination (Brookes, 1966). This communication presents some results obtained with a system *in vitro* devised to facilitate investigation of the chemical nature of the reactions between polycyclic hydrocarbons and cellular constituents. The system used involves incubation of polycyclic hydrocarbons with either DNA or protein in the presence of a microsomal hydroxylating system with the idea that active intermediates formed from the hydrocarbons may then react with the macro-

molecule *in situ*. It may be that the same principle can be applied to other biologically active compounds thought to require metabolic activation before interactions with cellular constituents.

Experimental. A 50mg. sample of DNA (ex salmon testes; Sigma Chemical Co., St Louis, Mo., U.S.A.) purified by a detergent/salt procedure (Kay, Simmons & Dounce, 1952) to a residual protein content of less than 0.2% or a 50mg. sample of protein (bovine plasma albumin; Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) was incubated in air in subdued light at 37° with 1mM-sodium citrate buffer, pH 7.4 (90ml.), containing resuspended washed rat liver microsomes (Boyland & Grover, 1961) (equivalent to 2.5g. of liver), NADP⁺ (8.5mg.), glucose 6-phosphate (63mg.), MgCl₂ (25mg.) and glucose 6-phosphate dehydrogenase [Boehringer Corporation (London) Ltd., London, W. 5] (0.7 unit) together with a tritiated polycyclic hydrocarbon (The Radiochemical Centre, Amersham, Bucks.) added in acetone (0.1ml.).

After incubation, the solution was cooled to 3°, the microsomes were removed by ultracentrifugation at 80000g for 1hr. and the unchanged hydrocarbon and non-polar metabolites were extracted from the supernatant fluid with ether (3 × 100ml.). Then NaCl (4g.) was added, and the DNA or protein was reprecipitated by ethanol (2vol.) or acetone (2vol.) respectively, washed in fresh solvent for at least 18hr. and dried. The fibrous DNA was deproteinized by a repetition of the detergent/salt procedure and reisolated. DNA (5mg.) was hydrolysed in deoxyribonuclease solution (0.5ml.) and protein (5mg.) was dissolved in tetraethylammonium hydroxide solution (1.0ml.) before the radioactivity was determined by liquid-scintillation counting.

Results and discussion. Preliminary experiments indicated that, when tritiated polycyclic hydrocarbons were incubated with rat liver microsomes under these conditions, reactions with protein or with DNA took place, but that detectable levels of radioactivity (namely at least 50% increase over the background level of the liquid-scintillation system) were not bound to protein or to DNA when

Table 1. *Enzyme-catalysed reaction of tritiated polycyclic hydrocarbons with protein and DNA in vitro*

A 50mg. sample of protein or DNA was incubated for 1 hr. with 5 μ g. of hydrocarbon under the conditions described in the text. When similar mixtures were incubated without any cofactors, binding of the hydrocarbons either to DNA or to protein could not be detected.

Hydrocarbon	Reaction with protein (μ -moles/mole*)	Reaction with DNA (μ moles/g.atom of DNA P†)
Benzo[a]pyrene	0.78	1.41
3-Methylcholanthrene	0.73	0.78
7,12-Dimethylbenz[a]anthracene	0.78	0.64
Dibenz[a,h]anthracene	0.95	0.44
Dibenz[a,c]anthracene	1.07	0.56
Benz[a]anthracene	0.76	0.70
Pyrene	1.15	0.31
Phenanthrene	0.72	0.05

*Mol.wt. of bovine plasma albumin taken as 64000.

†Calculated on a basis of 8% phosphorus.

all the cofactors were omitted from the reaction mixtures. Earlier work has shown that the activity of the microsomal enzymes metabolizing polycyclic hydrocarbons is markedly increased in rat liver in the post-weaning period (Sims & Grover, 1967); in the present experiments microsomal fractions were prepared from pooled livers from 25-day-old female rats. Although these preparations show greatly enhanced hydroxylating activity, they also vary from preparation to preparation in the amount of activity present. For this reason the values given in Table 1 for the reaction with protein were all obtained with one preparation of microsomes; a second was used to measure the binding to DNA.

Results obtained with the system described show that the binding of benzo[a]pyrene and pyrene to protein increases with the time of incubation up to 2 hr. Similarly, with dibenz[a,h]anthracene and phenanthrene, the extent of reaction with DNA increases linearly with increasing polycyclic hydrocarbon concentration for additions in the range 2–20 μ g. of hydrocarbon.

The values in Table 1 show that in the presence of liver microsomes the eight hydrocarbons tested react with both protein and DNA and that, whereas the reactions of each hydrocarbon with protein are similar in extent, those with DNA show a wider variation. The reactions presumably involve

the formation of metabolic intermediates produced by the microsomal system, and it is likely that similar intermediates are involved in the binding of polycyclic hydrocarbons to cell constituents *in vivo* that has been described previously (Wiest & Heidelberger, 1953; Brookes & Lawley, 1964; Goshman & Heidelberger, 1967). As yet there is little information about the site and nature of the metabolic activation of the hydrocarbon molecules that are important for reactions of the type described, but it is noteworthy that dibenz[a,c]anthracene, which does not possess a 'K region', reacts, when metabolized, to the same extent as the isomeric dibenz[a,h]anthracene, a finding that is in agreement with earlier studies *in vivo* (Goshman & Heidelberger, 1967).

Although the system *in vitro* described here may be of use in elucidating the chemical basis of the reaction between polycyclic hydrocarbons and biologically important macromolecules, it still remains to be seen what relationship reactions of this type bear to the obviously more complex process of carcinogenesis.

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