Reactions of 'K'-Region Epoxides of Polycyclic Hydrocarbons with Nucleic Acids and Polyribonucleotides

By P. L. GROVER and P. SIMS (*Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London SW3 6JB, U.K.*)

'K'-region epoxides have been detected as microsomal metabolites of the polycyclic hydrocarbons phenanthrene and benz[a]anthracene (Grover *et al.*, 1971b), of pyrene and benzo[a]pyrene (Grover *et al.*, 1972) and of 7,12-dimethylbenz[a]anthracene (Keysell *et al.*, 1972). Synthetic 'K'-region epoxides have been found to possess biological activity as mutagens (Huberman *et al.*, 1971; Cookson *et al.*, 1971), to cause malignant transformation of rodent cells *in vitro* (Grover *et al.*, 1971c) and to be alkylating agents that react with the nucleic acids of cells in culture (Grover *et al.*, 1971a). In view of current interest in the role that 'K'-region epoxides may play in hydrocarbon carcinogenesis *in vivo*, we are examining in more detail the reactions of these compounds with nucleic acids.

In the present investigations, ³H-labelled 'K'-region epoxides (0.04 μmol) were incubated with nucleic acid (4.0 μmol of P) at 37°C for 2 h in neutral aqueous solution, and the water-soluble products were passed through a Sephadex G-25 column. The extents of reaction (mmol/mol of P) with DNA, RNA and apurinic acid respectively were: for phenanthrene 9,10-oxide, 0.015, <0.007 and <0.007; for benz[a]anthracene 5,6-oxide, 0.68, 0.51 and 0.021; for 7-methylbenz[a]anthracene 5,6-oxide, 1.63, 0.96 and 0.051; for dibenz[a,h]anthracene 5,6-oxide, 0.33, 0.42 and 0.020.

In similar experiments with synthetic polyribonucleotides, the 'K'-region epoxides did not appear to react with poly(U) or poly(C), reacted with poly(A) (0.02–0.57 mmol/mol of P) but reacted more extensively with poly(G) (0.29–1.86 mmol/mol of P). These results suggest that it is probably the purines in nucleic acids that are alkylated by polycyclic hydrocarbon epoxides; a study of the products formed is in progress.

This investigation was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital), from the Medical Research Council and the Cancer Campaign for Research.

Cookson, M. J., Sims, P. & Grover, P. L. (1971) *Nature New Biol. (London)* **234**, 186–187

Grover, P. L., Forrester, J. A. & Sims, P. (1971a) *Biochem. Pharmacol.* **20**, 1297–1302

Grover, P. L., Hewer, A. & Sims, P. (1971b) *FEBS Lett.* **18**, 76–80

Grover, P. L., Sims, P., Huberman, E., Marquardt, H., Kuroki, T. & Heidelberger, C. (1971c) *Proc. Nat. Acad. Sci. U.S.* **68**, 1098–1101

Grover, P. L., Hewer, A. & Sims, P. (1972) *Biochem. Pharmacol.* in the press

Huberman, E., Aspiras, L., Heidelberger, C., Grover, P. L. & Sims, P. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 3195–3199

Keysell, G. R., Booth, J., Sims, P., Grover, P. L. & Hewer, A. (1972) *Biochem. J.* **129**, 41 p

The Formation of an Epoxide in the Microsomal Metabolism of 7,12-Dimethylbenz[a]anthracene

By G. R. KEYSELL, J. BOOTH, P. SIMS, P. L. GROVER and A. HEWER (*Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London SW3 6JB, U.K.*)

The metabolism of 7,12-dimethylbenz[a]anthracene by rat liver microsomal fraction yields dihydrodiols and phenols (Sims, 1970), which probably arise from epoxide precursors: with phenanthrene and benz[a]anthracene (Sims *et al.*, 1971) and with pyrene and benzo[a]pyrene (Grover *et al.*, 1972) 'K'-region epoxides are formed. 'K'-region epoxides react with DNA, RNA and protein both chemically and in cells in culture (Grover *et al.*, 1971a), and possess biological activities (Grover *et al.*, 1971c) not shown either by the parent hydrocarbons or by the dihydrodiols and phenols into which they are converted. 7,12-Dimethylbenz[a]anthracene is a potent carcinogen that presumably also requires metabolic activation, and the possibility that this involves the formation of a 'K'-region epoxide was investigated.

[³H]7,12-Dimethylbenz[a]anthracene was incubated with rat liver microsomal fraction, an NADPH-generating system and an epoxide hydrazide inhibitor, and the metabolites were chromatographed on alumina (Grover *et al.*, 1971b). A radioactive metabolite eluted with benzene–light petroleum (b.p. 60–80°C) (2:1, v/v) was (a) incubated with the 'epoxide hydrazide' of rat liver microsomal fraction to yield a product that was chromatographically identical with *trans*-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene, (b) treated with HCl to yield a phenol that was identical with 5-hydroxy-7,12-dimethylbenz[a]anthracene, (c) allowed to react with GSH in aqueous acetone at pH 8.0 to form a water-soluble product that had the chromatographic characteristics of an acid-labile GSH conjugate, and (d) incubated with poly(G) in a neutral aqueous solution and the products passed through Sephadex G-25 to give a radioactive peak coincident with that of the u.v.-absorbance peak at 260 nm.

The conversion of the metabolite into a dihydrodiol