was in a metabolite, as yet unidentified, the remainder being unchanged salbutamol. The urinary-excretion findings showed that the drug was well absorbed, 80% of the dose appearing in the urine (0-72h) and 6% in the faeces. Unchanged salbutamol accounted for 40% of urinary radioactivity, 60% consisting of the same metabolite as that found in plasma.

Administration of [³H]salbutamol by aerosol resulted in a similar metabolic pattern in the plasma and urine of patients receiving the drug. The metabolite is an acid-labile compound that is not hydrolysed by β -glucuronidase or arylsulphatase. Investigations so far have shown this metabolite to be unique to man.

This work was supported by a grant from the Medical Research Council.

Morgan, C. D., Sandler, M., Davies, D. S., Conolly, M. E., Paterson, J. W. & Dollery, C. T. (1969). *Biochem. J.* **114**, 8P.

Formation of Epoxides as Microsomal Metabolites of Polycyclic Hydrocarbons

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

Microsomal metabolism of polycyclic hydrocarbons *in vitro* leads to the formation of reactive intermediates (Grover & Sims, 1968; Gelboin, 1969) that could be epoxides. Evidence supporting the suggestion (Boyland, 1950) that epoxides are intermediates in the microsomal hydroxylation of double bonds has been obtained with benzene and naphthalene (Jerina, Daly, Witkop, Zaltzman-Nirenberg & Udenfriend, 1968, 1970) and with phenanthrene and dibenz[*a,h*]anthracene (Pandov & Sims, 1970). Synthetic 'K-region' epoxides of polycyclic hydrocarbons are active alkylating agents that react with nucleic acid either *in vitro* (Grover & Sims, 1970) or in cells in culture (Grover, Forrester & Sims, 1971a). In view of the activity of such epoxides in transforming rodent cells in culture (Grover *et al.* 1971b), it seemed important to establish the formation of epoxides as intermediates in the metabolism of polycyclic hydrocarbons.

In the present work ³H-labelled samples of phenanthrene, benz[*a*]anthracene and dibenz[*a,h*]anthracene have been incubated with rat liver microsomal fraction fortified with an NADPH-generating system. After incubation, non-polar products were extracted into ether, the appropriate carrier 'K-region' epoxide was added, the ether was removed by evaporation and the residue was chromatographed on an alumina column. Fractions

containing the radioactive peak that was eluted with the carrier epoxide were pooled and evaporated to dryness, and the epoxide, together with a further addition of unlabelled carrier epoxide, was crystallized from cyclohexane. Radioactively labelled 'K-region' epoxides obtained in this way have been crystallized to constant specific radioactivity and the associated radioactivity has been shown to be convertible (a) by treatment with methanolic hydrochloric acid into products that possess on thin-layer chromatograms the chromatographic properties of phenols, (b) by incubation with microsomal epoxide hydase, in the absence of cofactors, into products that possess on thin-layer chromatograms the chromatographic properties of dihydrodiols and (c) by heating with GSH in aqueous acetone at alkaline pH into water-soluble products that possess the paper-chromatographic properties of acid-labile glutathione conjugates. In addition, the only 'non-K-region' epoxide prepared to date, benz[*a*]anthracene 8,9-epoxide, has characteristics (P. Sims, unpublished work) that readily distinguish it from the 'K-region' derivative used in these studies. The role of epoxides in the biological effects produced by polycyclic hydrocarbons, which include carcinogenesis, is currently under investigation.

Boyland, E. (1950). *Biochem. Soc. Symp.* **5**, 40.

Gelboin, H. V. (1969). *Cancer Res.* **29**, 1272.

Grover, P. L., Forrester, J. A. & Sims, P. (1971a). *Biochem. Pharmac.* (in the Press).

Grover, P. L. & Sims, P. (1968). *Biochem. J.* **110**, 159.

Grover, P. L. & Sims, P. (1970). *Biochem. Pharmac.* **19**, 2251.

Grover, P. L., Sims, P., Huberman, E., Kuroki, T., Marquardt, H. & Heidelberger, C. (1971b). *Proc. natn. Acad. Sci. U.S.A.* (in the Press).

Jerina, D. M., Daly, J. W., Witkop, B., Zaltzman-Nirenberg, P. & Udenfriend, S. (1968). *Archs Biochem. Biophys.* **128**, 176.

Jerina, D. M., Daly, J. W., Witkop, B., Zaltzman-Nirenberg, P. & Udenfriend, S. (1970). *Biochemistry, Easton*, **9**, 147.

Pandov, H. & Sims, P. (1970). *Biochem. Pharmac.* **19**, 299.

Enzymic Formation of β -D-Monoglucuronide, β -D-Monoglucoside and Mixtures of β -D-Monoxylsides and β -D-Dixylsides of Bilirubin by Microsomal Preparations from Rat Liver

By K. P. M. HEIRWEGH, J. A. T. P. MEUWISSEN and J. FEVERY. (*Liver Physiopathology Laboratory, Rega Institute, University of Leuven, B-3000 Leuven, Belgium*)

Glucosyl transfer from UDP-glucose to several steroids by microsomal preparations from rabbit liver has been demonstrated by Collins, Williamson