enzymes that are involved in the metabolism of substances foreign to the vertebrate body. Structure-activity relationships relative to induction have not been extensively studied. However, Abernathy *et al.* (1971) examined a number of compounds structurally related to DDT with respect to induction of the hepatic microsomal mixed-function oxidases in mice. Structural requirements for maximal induction were postulated in terms of interatomic distances and molecular dimensions. No correlations were found between toxicity of compounds and their inductive effects.

In the present work some 50 halogen derivatives of ethane, benzene, biphenyl and diphenylethane were examined for their effects on hepatic drug-metabolizing enzymes.

Adult male rats were treated daily for 7 days with the organohalogen compound; the substances were administered in arachis oil at a dosage of 100 μ mol/kg body wt. by intraperitoneal injection. In preliminary experiments the shortening or lengthening of hexobarbitone-induced sleeping time and the increase or decrease of microsomal cytochrome *P*-450 content were taken as indicative of induction of inhibition respectively. In later experiments certain compounds representative of each series were examined in greater detail for their ability to alter the rate of metabolism by microsomal enzymes.

An attempt has been made to correlate the results of these experiments with certain physicochemical properties (such as lipophilicity, electronic configuration and steric effects) by means of Hansch analysis. It is considered that this work may lead to a better understanding of the mechanism, which is at present unknown, of microsomal enzyme induction.

Abernathy, C. O., Hodgson, E. & Guthrie, F. E. (1971) *Biochem. Pharmacol.* **20**, 2385-2393

Biochemical and Pathological Differences in Hepatic Response to Chronic Feeding of Safrole and Butylated Hydroxytoluene to Rats

By TIMOTHY J. B. GRAY, DENNIS V. PARKE, PAUL GRASSO and REGINALD F. CRAMPTON (*Department of Biochemistry, University of Surrey, Guildford, Surrey, U.K., and British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey, U.K.*)

Safrole (4-allyl-1,2-methylenedioxybenzene) produces liver enlargement and induction of microsomal drug-metabolizing enzymes (Parke & Rahman, 1970). It is also known to be hepatotoxic, chronic administration producing nodular hyperplasia (Hagen *et al.*, 1965). Butylated hydroxytoluene (2,6-di-*t*-butyl-4-methylphenol) also produces liver enlargement and

enzyme induction (Creaven *et al.*, 1966), but no histopathological changes have been found even after 2 years at comparable dosages (Gilbert & Golberg, 1965). In view of the similar early biochemical effects but grossly different chronic pathological effects of these two compounds, the activities of certain microsomal enzymes were studied during chronic administration to determine whether changes in enzyme activity could be correlated with pathological changes.

Female rats were administered orally with butylated hydroxytoluene or safrole (approx. 250 and 150 mg/kg body wt. per day respectively). At four intervals between 1 and 32 weeks microsomal enzyme activities were measured and liver samples were taken for light and electron microscopy.

Rats given butylated hydroxytoluene at 1 week showed 50-100% increases in ethylmorphine *N*-demethylase, biphenyl 4-hydroxylase, aniline *p*-hydroxylase and NADPH-cytochrome *c* reductase activities, cytochrome *P*-450 and cytochrome *b*₅ contents, microsomal protein and liver weight. These increases were maintained at 32 weeks.

Rats given safrole at 1 week showed increases of 50-200% in the above parameters. After 8 weeks these values were markedly diminished, and by 16 weeks enzyme activities were inhibited by up to 50%, although the cytochrome *P*-450 content still showed a 30% increase. However, the cytochrome *b*₅ content remained greatly increased (250% of control). The loss of drug-metabolizing enzyme activities, the increase in cytochrome *P*-450 content and the apparent increase in cytochrome *b*₅ content are attributed to the interaction of safrole with the endoplasmic reticulum, as observed with a number of methylenedioxyphenol compounds (Franklin, 1971; Lake & Parke, 1972).

Light microscopy showed the presence of individual cell necrosis in the livers of safrole-treated animals after 8 weeks. No abnormalities were found with butylated hydroxytoluene.

Butylated hydroxytoluene therefore appears to produce maintained enzyme induction, whereas with safrole the induction is transient. The appearance of pathological changes with the latter coincided with the decrease in enzyme activity. Very similar enzymic changes were observed by Hutterer *et al.* (1969) with dieldrin (*endo-exo*-1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene), another compound known to produce nodular hyperplasia in the liver (Wright *et al.*, 1972).

T. J. B. G. thanks the Science Research Council for financial support.

Creaven, P. J., Davies, W. H. & Williams, R. T. (1966) *J. Pharm. Pharmacol.* **18**, 485

Franklin, M. R. (1971) *Xenobiotica* **1**, 581

- Gilbert, D. & Golberg, L. (1965) *Food Cosmet. Toxicol.* **3**, 417
- Hagen, E. C., Jenner, P. M., Jones, W. I., Fitzhugh, O. G., Brouwer, J. G. & Webb, W. K. (1965) *Toxicol. Appl. Pharmacol.* **7**, 18
- Hutterer, F., Klion, F. M., Wengraf, A., Schaffner, F. & Popper, H. (1969) *Lab. Invest.* **20**, 455
- Lake, B. G. & Parke, D. V. (1972) *Biochem. J.* **127**, 23 P
- Parke, D. V. & Rahman, H. (1970) *Biochem. J.* **119**, 53 P
- Wright, A. S., Potter, D., Wooder, M. F., Donniger, C. & Greenland, R. D. (1972) *Food Cosmet. Toxicol.* **10**, in the press