activity of glycogen synthetase and the hexokinases. The findings that their activities are not affected by insulin are consistent with the previously expressed view (Chain *et al.*, 1969) that insulin acts by stimulating one of the enzyme components of the multi-enzyme system of oxidative phosphorylation concerned with ATP production specifically for the phosphorylation of glucose.

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- Chain, E. B., Mansford, K. R. L. & Opie, L. H. (1969) *Biochem. J.* **115**, 537
 Grossbard, L. & Schimke, R. T. (1966) *J. Biol. Chem.* **241**, 3546
 Katzen, H. M., Soderman, D. D. & Wiley, C. E. (1970) *J. Biol. Chem.* **245**, 408
 Villar-Palasi, C., Rosell-Perz, M., Hizukuri, S., Huijing, F. & Larner, J. (1966) *Methods Enzymol.* **8**, 374

Interaction of Saffrole and Isosaffrole with Hepatic Microsomal Haemoproteins

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Saffrole and isosaffrole are known inducers of the liver microsomal drug-metabolizing enzymes of the rat (Parke & Rahman, 1970) and have been shown to result in the formation of a new hepatic microsomal haemoprotein (Parke & Rahman, 1971). Male rats pretreated with saffrole or isosaffrole (125 mg/kg per day for 3 days intraperitoneally) gave rise to the appearance of a new redox difference absorption maximum at 455 nm, which was not identical with those of cytochrome *P*-450 and cytochrome *b*₅.

Similar spectral absorption curves with maxima at 455 nm were obtained when a liver microsomal preparation from mice was incubated *in vitro* with piperonyl butoxide plus NADPH (Philpot & Hodgson, 1971) or when a liver microsomal preparation from phenobarbital-pretreated rats was incubated *in vitro* with saffrole or isosaffrole plus an NADPH-generating system (M. R. Franklin, personal communication). The absorption maxima at 455 nm in these two experiments are the differences between the reduced spectra of liver microsomal fraction incubated with saffrole, isosaffrole or piperonyl butoxide and of liver microsomal fraction alone. The absorption maxima at 455 nm found by Parke & Rahman (1971) was on the other hand the difference between the reduced and oxidized forms of saffrole- and isosaffrole-pretreated liver microsomal fraction. It would therefore appear that these methylenedioxy-

phenyl compounds undergo metabolism by microsomal enzymes with subsequent binding of the metabolites to microsomal haemoprotein.

Addition of saffrole, isosaffrole, piperonyl butoxide, piperonal, piperonyl alcohol, piperonylic acid, piperonylacrylic acid or allylbenzene to rat microsomal preparations (washed 105 000g deposit) for 30 min but without added NADPH gave no absorption maximum in the reduced spectra at 455 nm. Further, pretreatment of male rats for 4 days with any of these compounds (100 mg/kg per day) resulted in the formation of the 455 nm redox difference absorption maximum only with saffrole and isosaffrole. This further suggests that the observation by Parke & Rahman (1971) was perhaps due to a combination of two effects, namely the production of a new modified haemoprotein and the modification of existing haemoprotein by binding of metabolites.

Pretreatment of rats with phenobarbital is known to result in an increased total binding (ΔE_{max}) of both type I (e.g. carbon tetrachloride) and type II (e.g. aniline, nicotinamide) substrates, but does not usually result in any decrease of the respective *K*_s values (Guarino *et al.*, 1969). In contrast, pretreatment with isosaffrole (150 mg/kg per day for 5 days intraperitoneally) results in an increase in the total binding of type I and type II substrates, but also gives rise to a decrease in *K*_s values for type II substrates (*K*_s values: for nicotinamide-untreated, 3.9 mM, and for isosaffrole-pretreated, 1.6 mM; for aniline-untreated, 0.15 mM, and for isosaffrole-pretreated, 0.07 mM), but not for type I substrates. This is taken to indicate that the increase of microsomal drug-metabolizing enzyme activity and of microsomal cytochromes after pretreatment with isosaffrole may be associated with the biosynthesis of a modified cytochrome *P*-450.

- Guarino, A. M., Gram, T. E., Gigon, P. L., Greene, F. E. & Gillette, J. R. (1969) *Mol. Pharmacol.* **5**, 131
 Parke, D. V. & Rahman, H. (1970) *Biochem. J.* **119**, 53 P
 Parke, D. V. & Rahman, H. (1971) *Biochem. J.* **123**, 9 P
 Philpot, R. M. & Hodgson, E. (1971) *Life Sci.* **10**, part II, 503

The Metabolism *in vitro* of 16-Unsaturated C₁₉ Keto Steroids in Boar Testis

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The urine-smelling steroid 5 α -androst-16-en-3-one (I) is formed in boar testis from pregnenolone and progesterone (Katkov & Gower, 1968; Gower *et al.*, 1972). In view of the possible physiological importance of this compound and of 5 α -androst-16-en-3 α -ol