resembling human variegate porphyria in that protoporphyrin is the main pigment that accumulates in the liver (De Matteis, 1967). It is now found that rats given a relatively small dose of this drug exhibit a hepatic porphyria closely resembling variegate porphyria in its latent phase; they become very sensitive to barbiturates and other drugs that can exacerbate the metabolic disorder, changing its biochemical picture to that typical of the human acute attack.

Starved rats were given 3,5-diethoxycarbonyl-1,4-dihydrocollidine (100mg/kg) intraperitoneally and at the same time either phenobarbitone or another drug (50–100mg/kg) orally and killed 15h later. 3,5-Diethoxycarbonyl-1,4-dihydrocollidine given on its own increased the activity of liver 5-aminolaevulinate synthetase (threefold above normal) and the concentration of porphyrins (tenfold) without altering the concentration of either 5-aminolaevulinate or porphobilinogen. When phenobarbitone was given together with 3,5-diethoxycarbonyl-1,4-dihydrocollidine the following increases were observed in the liver: 5-aminolaevulinate synthetase (tenfold), porphyrins (60-fold), porphobilinogen (80-fold) and 5-aminolaevulinate (at least threefold). Like phenobarbitone, thiopentone, phenylbutazone, lindane, dicophane, 2-isopropyl-2-propylacetamide and ethynyloestradiol could all potentiate the porphyria caused by 3,5-diethoxycarbonyl-1,4-dihydrocollidine, but none of these when given alone increased the concentration of porphyrins in the liver. The mechanism by which this potentiation takes place will be discussed in the light of the rapid inhibition of liver ferrochelatase activity caused by 3,5-diethoxycarbonyl-1,4-dihydrocollidine in the rat (De Matteis & Gibbs, 1972) and of the suggestion that inhibition at some point in the pathway of liver haem synthesis may be the underlying defect common to all varieties of human genetic porphyria (Kaufman & Marver, 1970).

Dean, G. & Barnes, H. D. (1959) *S. Afr. Med. J.* **33**, 246–253

De Matteis, F. (1967) *Pharmacol. Rev.* **19**, 523–557

De Matteis, F. & Gibbs, A. (1972) *Biochem. J.* in the press

Eales, L. (1971) *S. Afr. J. Lab. Clin. Med.* **17**, 120–126

Kaufman, L. & Marver, H. S. (1970) *N. Engl. J. Med.* **283**, 954–958

Glycogen Synthetase and Hexokinase Activities in the Perfused Langendorff and Working Heart of Normal and Streptozotocin-Diabetic Rats

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It was shown in a previous study (Chain *et al.*, 1969) that, in the perfused working rat heart rendered insulin-deficient by injection of anti-insulin serum or the diabetogenic antibiotic streptozotocin into the intact animal before excision of the heart, glycogen synthesis from [U - ^{14}C]glucose was greatly decreased, to half or less than half the normal value, and could be restored to normality by addition of insulin (2 munits/ml) to the perfusion liquid, whereas glucose oxidation to $^{14}CO_2$ was not affected.

It was decided to ascertain whether the diminished glycogen synthesis in the insulin-deficient rat heart would reflect itself in decreased activities of some of the enzymes known to be involved in glycogen synthesis. The activities of two such enzymes have now been assayed in the perfused hearts in working and non-working conditions from normal and streptozotocin-diabetic rats in the presence and in the absence of insulin: the glucose 6-phosphate-dependent and -independent glycogen synthetases D and I, catalysing the final step in glycogen synthesis from glucose, and the hexokinases, bringing about the first step of glucose metabolism, which are known to occur in heart tissue as two main soluble isoenzymes (Katzen *et al.*, 1970). Glycogen synthetase was determined by the method of Villar-Palasi *et al.* (1966). Total and soluble hexokinase activities were determined spectrophotometrically, types I and II being distinguished by their different thermal stabilities (Grossbard & Schimke, 1966).

No decrease in total glycogen synthetase activity was found in the diabetic perfused heart, nor were changes in the I/D ratio observed, and the activities of these enzymes were not increased by addition of insulin (10 munits/ml) to the perfusion liquid.

The total hexokinase activity was found to be decreased in the diabetic heart, confirming the results of Katzen *et al.* (1970), and this decrease was shown to be due to a loss of activity of about 34% specifically of the type II isoenzyme; the activity of isoenzyme type I was not affected. However, the decreased activity of type II hexokinase could not be restored to normality by the addition of insulin (10 munits/ml) to the perfusion liquid.

Thus the marked stimulation of glycogen synthesis from glucose observed to occur in the perfused insulin-deficient heart after addition of insulin cannot be explained in terms of a stimulation of the

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

By P. J. BROPHY and D. B. GOWER (*Department of Biochemistry, Guy's Hospital Medical School, London SE1 9RT, U.K.*)

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