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.

I thank Professor Sir Ernst Chain, F.R.S., for enthusiastic support and for facilities, and Dr. Anne Beloff-Chain for her encouragement. The skilled technical assistance of Miss Helga Wille is gratefully acknowledged.

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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.

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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

(II) as pheromones (Melrose *et al.*, 1968), the present study was undertaken to determine the role of compound (I) in the testicular biosynthesis of compound (II) and of 5 α -androst-16-en-3 β -ol (III).

The metabolism of [5 α -³H]androst-16-en-3-one (obtained through the courtesy of Dr. W. Hafferl, Syntex Research, Palo Alto, Calif., U.S.A.) has been studied in boar testis homogenate for 5, 10, 20, 30 and 60 min at 37°C. Each flask contained tritiated compound (I) (1.75×10^6 d.p.m.; 14 ng), 1 ml of homogenate (10%, w/v) in tris-HCl buffer, pH 7.4 (50 mM), NAD⁺, NADPH and ATP (each 0.3 mM) in a final volume of 4 ml. After extraction, steroids were isolated by column chromatography on alumina (Gower, 1971). Unmetabolized compound (I) was further purified by t.l.c. in benzene-ether (9:1, v/v), and compound (II) was run in benzene-methanol (9:1, v/v). Compound (III) was subjected to t.l.c. on AgNO₃-impregnated silicic acid in benzene-ethyl acetate (1:2, v/v) (Katkov & Gower, 1970). Further column chromatography, on alumina for compounds (I) and (II) or on AgNO₃-impregnated silicic acid for compound (III) (Katkov & Gower, 1970), was performed and constancy of specific radioactivity was observed over each peak eluted.

Compound (III) was formed from tritiated compound (I) in high yields (up to 49% in 5 min), whereas compound (II) was obtained in smaller yield (3% in 5 min, increasing progressively to 14% in 60 min). These results are essentially in accord with those of Ahmad & Gower (1968), who showed that, in boar testis preparations, the yield of compound (III) from progesterone was some five times that of compound (II); moreover, similar relative amounts have been isolated from mature boar testis (Booth, 1970).

Cofactor requirement studies have shown that NADH is the most active cofactor for the conversion of compound (I) into the 3 β -alcohol (III), whereas NADPH is the most active cofactor for the production of the 3 α -alcohol (II). Preliminary results with [7 α -³H]androst-4,16-dien-3-one (IV) as substrate have shown that it also can be reduced to a mixture of compounds (II) and (III) in boar testis preparations. These results clearly establish the ketones (I) and (IV) as intermediates in 16-unsaturated C₁₉ steroid biosynthesis.

The fact that compound (I) can be metabolized to compound (II) in boar parotid and submaxillary salivary-gland preparations (T. Katkov, personal communication) explains the occurrence of these compounds in boar saliva (see references cited in Gower, 1972).

The financial support of P. J. B. by the Agricultural Research Council (Grant no. AG35/19) is gratefully acknowledged. We are indebted to Dr. W. Hafferl for the gift of [5 α -³H]androst-16-en-3-one.

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Benzene as a Metabolite of Triphenyl-lead Acetate in the Rat

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Triphenyl-lead acetate has been proposed as an anti-fouling agent in paint (see, e.g., Guillen *et al.*, 1969) and as a molluscicide for the control of bilharzia (Hopf *et al.*, 1967).

This compound is quite toxic to rats when injected intraperitoneally in dimethyl sulphoxide (approx. LD₅₀ 6 mg/kg), but is considerably less toxic by mouth (approx. LD₅₀ 200–300 mg/kg). Tri[U-¹⁴C]-phenyl-lead acetate was synthesized from bromo-[U-¹⁴C]benzene and lead dichloride and administered orally dissolved in dimethyl sulphoxide to rats (dose 25 mg/kg). The urine, faeces and expired air of the animals were collected daily for 7 days. During this period 25% of the ¹⁴C was recovered in the urine, 29% in the faeces and 20% in the expired air, these being average values for three rats. With an oral dose (200 mg/kg) of unlabelled triphenyl-lead acetate, no lead could be detected in the urine, but 75% was found in the faeces in 7 days.

The ¹⁴C of the expired air was found to be present almost entirely as benzene, with a little ¹⁴CO₂ (1%). The benzene was trapped in methanol at -70°C and isolated as the nickel cyanide ammonia clathrate (Parke & Williams, 1953a) after addition of non-radioactive benzene. The benzene was released from the clathrate by heating and sulphonated in fuming H₂SO₄. The sodium benzenesulphonate was recrystallized to constant specific radioactivity, and the benzenesulphonic acid was identified as its *p*-toluidine salt, m.p. 195–198°C, and by the i.r. spectrum of the sodium salt. By radiochromatogram scanning, the ¹⁴C of the urine was found to be present as mainly phenyl sulphate, with a smaller amount of quinol sulphate. The faecal ¹⁴C has not yet been identified.

These experiments suggest that triphenyl-lead acetate as such is not absorbed from the intestine. It appears that the compound is at least partly decomposed in the gastrointestinal tract, possibly by the gut flora, to give benzene, which is then absorbed. Some, possibly nearly a half, of the absorbed benzene is eliminated as such in the expired air and the rest is