(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).

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

By BARBARA WILLIAMS, L. G. DRING and R. T. WILLIAMS (*Department of Biochemistry, St. Mary's Hospital Medical School, London W.2, U.K.*)

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

metabolized to known benzene metabolites (Parke & Williams, 1953b), mainly phenol, which is excreted in the urine as conjugates.

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The Effect of Testosterone on the Biliary Excretion of Tartrazine in Female Rats

By P. C. HIROM, P. MILLBURN, F. O. OSIYEMI, R. L. SMITH, H. B. TURBERT and R. T. WILLIAMS (*Department of Biochemistry, St. Mary's Hospital Medical School, London W.2, U.K.*)

It has been shown that there is a sex difference in the extent of the biliary excretion of tartrazine in rats, the females excreting 2-3 times as much of the unchanged dye in the bile as the males. Tartrazine is not metabolized and is excreted unchanged in the bile and urine (Gregson *et al.*, 1972).

It has now been found that such a sex difference does not occur in guinea pigs (Duncan Hartley albino), both sexes excreting about 30-40% of an intravenously injected dose of the dye (50 μ mol/kg) in the bile in 3 h.

The sex difference in rats (Wistar albino) has been further investigated. The renal pedicles of biliary cannulated male and female rats were ligated to prevent urinary excretion, and the extent of biliary elimination of intravenously injected tartrazine (50 μ mol/kg) was measured. Under these conditions the males excreted about 22% and the females 63% of the dose of tartrazine in the bile in 3 h. This suggested that the sex difference in biliary excretion was not due to more rapid urinary excretion of the dye by the males, since in rats with functioning kidneys the values were 18 and 33% for males and females respectively.

Female rats were injected subcutaneously each day for 28 days with testosterone propionate (2.5 mg/kg per day) in arachis oil (0.5 ml/kg) containing benzyl alcohol (10%, v/v). Control animals were injected daily with the same volume of the solvent only. On day 29 the rats were bile-duct-cannulated and the excretion of intravenously injected tartrazine (50 μ mol/kg) was measured. In the testosterone-treated females the biliary excretion in 3 h was 14%

(9-19%) and the urinary excretion 63% (55-70%) in eight animals. In eight control animals the biliary excretion was 31% (19-44%) and the urinary excretion 47% (41-53%). In normal male rats the corresponding values were 12 and 64% in 3 h. Pretreatment with testosterone thus altered the extent of the biliary excretion of tartrazine in females to that of males.

The pretreatment with testosterone also altered the hexobarbitone sleeping times, for in the control female rats the sleeping time was 102 min (91-138 min) and in the treated females 57 min (33-76 min), the intraperitoneal dose of hexobarbitone being 100 mg/kg. The hexobarbitone sleeping time for normal male rats was 23 min (16-36 min). Testosterone pretreatment of female rats therefore decreased the biliary excretion of tartrazine, which is not metabolized, and increased the rate of metabolism of hexobarbitone.

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Species Variations in the Metabolism of Phenol

By I. D. CAPEL, M. R. FRENCH, P. MILLBURN, R. L. SMITH and R. T. WILLIAMS (*Department of Biochemistry, St. Mary's Hospital Medical School, London W.2, U.K.*)

Marked species variations in the pattern of metabolism of a number of simple organic compounds, including benzoic acid (Bridges *et al.*, 1970), amphetamine (Dring *et al.*, 1970), phenylacetic acid (James *et al.*, 1971) and quinic acid (Adamson *et al.*, 1970), have been reported. These studies have now been extended to an examination of the species variation in the metabolism of [14 C]phenol in 19 species of animals. The animals were dosed orally or injected intraperitoneally with [14 C]phenol (25 mg/kg), and urine was collected for 24 h and then analysed for metabolites by paper chromatography followed by radiochromatogram scanning. For man (three male subjects) the oral dose was 0.01 mg/kg and for the rhesus monkey it was 50 mg/kg. The recovery of 14 C in the 24 h urine ranged from 30% for the squirrel monkey to 95% for the rat; for most species it was about 50-70%.

Eight of the species examined, namely, the rat, mouse, jerboa, gerbil, hamster, lemming, guinea pig and man, excreted four metabolites, these being the sulphate and glucuronic acid conjugates of phenol and its oxidation product, quinol.