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

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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

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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.

Six species, namely the squirrel monkey, capuchin, ferret, dog, hedgehog and rabbit, excreted only three metabolites. The two herbivorous New World monkeys excreted phenyl glucuronide, quinol glucuronide and phenyl sulphate, and, at the dose of phenol used, glucuronic acid conjugation appeared to be more effective than sulphate conjugation in these two species. The other four species, however, excreted phenyl sulphate, quinol sulphate and phenyl glucuronide, and these species varied according to the extent to which they utilized the two conjugation mechanisms and in the extent of oxidation of phenol to quinol.

Four species, the rhesus monkey, cat, fruit bat and chicken, excreted only two metabolites, and one species, the pig, only one. The rhesus monkey, fruit bat and hen excreted phenyl sulphate and phenyl glucuronide, but no quinol conjugates. The cat appeared to excrete only phenyl sulphate and quinol sulphate, whereas the pig appeared to excrete only phenyl glucuronide.

The apparent inability of the cat to utilize the glucuronic acid mechanism and the pig the sulphate mechanism for the conjugation of phenol was therefore investigated in greater detail. It was found that these mechanisms were not entirely absent as far as phenol is concerned, since they were found at a very low level. The pig given [^{14}C]phenol orally or intraperitoneally excreted more than 90% of the 24 h ^{14}C excretion as phenyl glucuronide, but small amounts of phenyl sulphate (0.2–8.3%) were also detected. Similarly, in the cat, although phenyl sulphate and quinol sulphate accounted for over 90% of an oral or injected dose of [^{14}C]phenol, small amounts (0.1–2.0% of the 24 h ^{14}C excretion) of phenyl glucuronide were also found in the urine.

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Dehydrogenation of DL-Methylphenylcarbinol in the Rat

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Although methylphenylcarbinol (α -hydroxyethylbenzene) gives rise to methylphenylcarbinol glucuronide, hippuric acid and mandelic acid after admin-

istration to rabbits (Smith *et al.*, 1954a), the dehydrogenation of the carbinol to acetophenone does not seem to have been reported. Reduction of acetophenone, however, does occur in the rabbit and the dog (Smith *et al.*, 1954b; Leibman, 1971; Sundvik, 1886). The present investigation was undertaken to determine whether methylphenylcarbinol is oxidized in a manner similar to other carbinols such as indan-1-ol (Billings *et al.*, 1971) and acenaphthen-1-ol (Simmons *et al.*, 1971), which gave rise to the corresponding ketones.

Ether extracts of the urine from rats injected subcutaneously with DL-methylphenylcarbinol were examined by t.l.c. and g.l.c. Acetophenone and the free carbinol were detected. The extent of the conversion into acetophenone was estimated from g.l.c. traces at 0.15% of the dose, which compares well with the conversion of indan-1-ol into indan-1-one (Billings *et al.*, 1970).

The oxidation of methylphenylcarbinol by rat liver preparations was also examined. The compound was incubated with a microsomal preparation and with a 100000g supernatant preparation in the presence of NAD^+ or NADP^+ at pH 7.4 and 37°C. Ether extracts of these systems were examined by t.l.c. and g.l.c. and were found to contain acetophenone in all cases. The rate of oxidation of methylphenylcarbinol was followed in similar systems by measuring the increase in E_{340} .

These experiments showed that both the microsomal system and the cytosol are active with NAD^+ and NADP^+ , and that NAD^+ is the preferred cofactor for both systems. These cofactor requirements for the microsomal oxidation of methylphenylcarbinol are similar to those for the oxidation of acenaphthen-1-ol and *cis*- and *trans*-acenaphthene-1,2-diol (Simmons *et al.*, 1971; Drummond, 1971). The oxidation of these latter compounds by the cytosol, however, has a specific requirement for NADP^+ . The results of this investigation also indicate that the activity of the rat liver cytosol differs from that of a similar bovine liver preparation that Billings *et al.* (1971) showed to have no activity towards methylphenylcarbinol.

A more detailed study of the rat liver supernatant preparation is being conducted.

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