Capel et al. (1972) reported that in the cat [14C]phenol was excreted in the urine mainly as phenyl sulphate together with some quinol sulphate and just detectable amounts (<1% of dose) of phenylglucuronide. However, there was also reported an appreciable amount, up to 8% of the dose, of an unknown metabolite. This metabolite has now been identified as phenyl dihydrogen phosphate, C₆H₆OPO(OH)₂, which was characterized in a number of ways.

The urine obtained after administering [14C]phenol (5 and 25 mg/kg orally and intraperitoneally) to cats, was chromatographed on Whatman no. 1 paper in six different solvents. The unidentified ¹⁴C peak on radiochromatograms had RF values identical with those of authentic disodium phenylphosphate in these solvents. The area corresponding to the unidentified ¹⁴C material was eluted from twelve paper chromatograms with water (10 ml/chromatogram) and the combined eluates were concentrated at reduced pressure to a small volume. The metabolite present in this concentrated eluate was found to be hydrolysed at 37°C at pH 10.4 in 0.1 M-glycine buffer by alkaline phosphatase [Sigma (London) Chemical Co., London S.W.6, U.K.] to give [¹⁴C]phenol which was isolated as radioactive 2,4,6-tribromophenol, m.p. 94°C, after adding non-radioactive phenol carrier and then bromine water to the incubate. The compound, however, was not affected by β-glucuronidase (Ketodase; Warner-Chilcott Laboratories, Eastleigh, Hants., U.K.), arylsulphatase [from Helix pomatia; Sigma (London) Chemical Co.] or β-glucosidase [Emulsin; Sigma (London) Chemical Co.] preparations.

Two cats (2.5 and 3.5 kg) were each given 1 mg of Na₃H³²P0₄ (200 μCi) in 1 ml of water intraperitoneally on two consecutive days. On the second day they were also given 25 mg of phenol/kg in 1 ml of water neutralized by 11 M-NaOH. On the first day the excretion of ³²P in the urine was 85 and 97% of the dose respectively in the two cats and on the second day 79 and 105%. The output of the total dose of ³²P for the 2 days was thus 82 and 101%. Paper chromatography showed that most of this ³²P was present in the urine as P₃. The urines of each day were then concentrated at reduced pressure to a small volume. The viscous urine concentrate was cooled in ice and acidified by the slow addition of 11 M-HCl (5 ml). After being kept at 4°C for 1 h, the acidified concentrate was extracted by prolonged shaking with chloroform (3 x 50 ml). The chloroform extracts were evaporated to a brown oil which solidified on being kept in an evacuated desiccator at 4°C for 5 days. The product was extracted with chloroform to give colourless crystals which were repeatedly recrystallized from chloroform to a constant m.p. of 98°C [Jacobsen (1875) gives m.p. 97-98°C for monophenylphosphoric acid]. The mass spectra of the acid from urine, which was radioactive, and of its dimethyl ester prepared by methylation with diazomethane were examined in a Varian MAT CH5 mass spectrometer. The monophenylphosphoric acid isolated from cat urine showed a very small molecular ion at m/e 174 and its mass spectrum was almost identical with that of an authentic sample. The dimethyl ester showed a peak at m/e 202 corresponding to the molecular ion and its mass spectrum was almost identical with that of authentic dimethylphenyl phosphate.
1-[1-14C]Naphthol and 2-[8-14C]naphthol were also examined in the cat. In the case of 1-naphthol, 89% of the dose (25mg/kg injected intraperitoneally) was excreted as the sulphate and 1% as glucuronide in 24h and there was no evidence of a phosphate conjugate. In the case of 2-naphthol similarly administered, 57% of the dose was excreted in 24h as the sulphate of 2-naphthol, 15% as the sulphate of a dihydroxynaphthalene (probably 2,7-dihydroxynaphthalene) and 1% as 2-naphthylglucuronide, but there was no evidence of a phosphate conjugate.

The formation in vivo of the phosphates of foreign organic compounds is a reaction which has been rarely observed in the past. Di-(2-amino-1-naphthyl) hydrogen phosphate has been shown to be a metabolite of 2-naphthylamine in urine in dogs (Troll et al., 1959; Boyland et al., 1961) and in man (Troll et al., 1963). Troll & Belman (1967; cited by Irving, 1970) have also reported di-(2-hydroxylamino-1-naphthyl) hydrogen phosphate as a metabolite of 2-naphthylamine. 1-Naphthyl dihydrogen phosphate has been found to be a metabolite of 1-naphthol in houseflies, blowflies and New Zealand grass grubs (Binning et al., 1967). Monoethyl phosphate has been isolated from the liver of rats given large doses of ethanol intraperitoneally (Tomaszewski & Buckowicz, 1972). Heenan & Smith (1967) have found that p-nitrophenol and 1-naphthol form glucoside 6-phosphates in houseflies and blowflies, but in these conjugates the phosphate group is not attached directly to the phenol.

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Studies on the Conjugation of 4'-Hydroxyamphetamine in vitro

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One of the metabolites of amphetamine in a number of species is 4'-hydroxyamphetamine (Paredrine) (Dring et al., 1970) and this compound appears to be the immediate precursor of 4'-hydroxynorephedrine, the supposed false neurotransmitter produced during the metabolism of amphetamine. A study of the fate of 4'-hydroxyamphetamine (Capel, 1973; Sever et al., 1973) in a number of species has shown that it is metabolized mainly by conjugation of the phenolic hydroxyl group, but a species difference in the conjugation was found, the rat, guinea pig and pig conjugating it with glucuronic acid whereas man and four species of monkeys conjugated it with sulphate. The conjugation of 4'-hydroxyamphetamine has now been examined in vitro with tissue preparations.

By using 4'-hydroxy[14C]amphetamine free and conjugated 4'-hydroxyamphetamine could be readily estimated by solvent extraction. Incubation mixtures (3ml) were brought to pH9.5 with 0.5M-borate buffer, pH9.5 (3ml), and the free 4'-hydroxy-