

The results of the benzoate experiments show that the variable amounts of the mercapturic acid excreted are not due to poor efficiency in the collection of urine from rat pups; they would also appear to rule out any effects on urine excretion owing to the different dietary treatments of animals of different ages.

The variations in propylmercapturic acid excretion in animals of different ages could nevertheless be associated with their dietary history up to the time of dosing. It is perhaps significant that milk intake increases steadily with age up to approx. 15 days and then falls rapidly as the pups begin to consume solid food. Weaning is usually complete by 22 days, by which time acetylation of propylcysteine has returned to a high value. The fate of the propylcysteine that is not acetylated remains to be established. Chromatography has failed to detect the excretion of propylcysteine or of cysteine itself. It might therefore be suggested that the cysteine moiety is either extensively degraded or is incorporated into protein during this period of rapid growth.

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The Metabolism and Toxicity of Phenols in Cats

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The cat is known to be highly susceptible to certain phenols. For example, Oettel (1936) reported quinol poisoning of cats with concomitant excretion of 'typically black phenol urine'. The present study was designed to investigate and explain phenol toxicity in cats.

The following compounds were selected for investigation: phenol, 2,6-dimethoxyphenol, quinol and *p*-chlorophenol. Each compound was usually investigated at two dosages, 20mg/kg body wt. and 40mg/kg body wt.

Preliminary qualitative experiments were carried out in which free-ranging animals received intraperitoneal injections of the compound under investigation. Animals were placed in metabolic cages and allowed food and water; urine was collected over 24h. Animals receiving phenol, 2,6-dimethoxyphenol and quinol excreted very dark urine at the higher dosage. At the lower dosage, some darkening of the urine was noted but the coloration was markedly less than at the higher dose. Urine with normal appearance was obtained from cats receiving four doses of *p*-chlorophenol (40mg/kg body wt.) at 3h intervals. These results suggested that the urine coloration was associated with the substitution pattern of the aromatic ring. This idea was further explored by experiments with wholly anaesthetized animals.

Anaesthesia was induced by intraperitoneal injection of Urethane (1250mg/kg body wt.). The trachea and external jugular vein were incised and cannulated. The compound under investigation ([U-¹⁴C]phenol, 2,6-dimethoxy[U-¹⁴C]phenol or [2,3,5,6-¹⁴C]-quinol) was administered via the jugular-vein cannula. Urine and bile samples were collected at 1 h intervals over 6h. The coloration of urine already recorded with free-ranging animals was also noted with anaesthetized animals. Urine and bile samples were analysed for radioactive content, and in all experiments most of the radioactivity appeared in the urine with only small amounts (less than 5%) appearing in the bile. The greatest proportion of the injected radioactivity was present in urine samples collected over the first 3h and these were subjected to paper chromatography in butan-1-ol-acetic acid-water (3:1:1, by vol.) and propan-2-ol-aq. NH₃-water (20:1:2, by vol.) in the presence of appropriate authentic marker compounds. Radioactive areas on paper chromatograms were located by scanning. Phenols, glucuronic acid conjugates and sulphuric acid esters were located on chromatograms by the methods of Randerath (1963), Edwards (1969) and Denner *et al.* (1969) respectively.

[U-¹⁴C]Phenol

Chromatograms of urines obtained from cats receiving [U-¹⁴C]phenol (20mg/kg body wt.) revealed the presence of two radioactive components; the major component (80% of radioactivity in urine) was identified as phenyl sulphate and the remaining radioactivity was associated with quinol sulphate. In contrast, four radioactive components were present after the administration of [U-¹⁴C]phenol at the higher dosage (40mg/kg body wt.). The major component (78% of radioactivity in urine) was again identified as phenyl sulphate with quinol sulphate representing 16%. Quinol (4%) and phenol (2%) were also detected.

2,6-Dimethoxy[U-¹⁴C]phenol

At the lower dosage (20mg/kg body wt.), the major (93%) radioactive component in urine was identified as 2,6-dimethoxyquinol disulphate with small amounts of the parent phenol (5%) and a glucuronic acid conjugate (2%). Substantially the same results were obtained at the higher dosage (40mg/kg body wt.), but in this case small amounts (3% of radioactivity in urine) were identified as 2,6-dimethoxyquinol.

[2,3,5,6-¹⁴C]Quinol

The major radioactive metabolite in urine (87% of radioactivity in urine) was identified as quinol sulphate after administration of ¹⁴C-labelled quinol at a dosage of 20mg/kg body wt. Some unchanged quinol (10%) was present, together with small amounts (3%) of a glucuronic acid conjugate.

The results obtained with [U-¹⁴C]phenol are in agreement with those obtained by Capel *et al.* (1972), who showed that phenyl sulphate and quinol sulphate are the major metabolites of phenol. Sulphate conjugates are also the major metabolites of the other phenols when administered at the lower dosage. However, this detoxication process is apparently saturated when the higher doses of phenols are administered, since, in addition to sulphate conjugates, urines contained parent phenols together with their oxidation products in significant concentrations. It has been well established that quinols and quinones undergo oxidation to yield dark polymerization products (Erdtman & Hogberg, 1968), and such oxidations would be enhanced at the alkaline pH of cat urine. The dark appearance of cat urines after the administration of phenols is therefore explicable in these terms. No such darkening occurs after *p*-chlorophenol administration, since the blocked *para* position prevents the formation of quinol.

The formation of quinones from quinols provides an explanation for the toxic effects of phenols in cats. It has been shown that quinones inhibit mitochondrial respiration by acting as ubiquinone analogues (Redfearn & Whittaker, 1962; Smith & Lester, 1961).

The present investigation was therefore extended to examine the effects of *p*-benzoquinone and 2,6-dimethoxybenzoquinone on cat liver mitochondria (Chappell & Hansford, 1972). It was shown that both quinones are potent inhibitors of mitochondrial respiration. Mitochondria could be protected by an equimolar concentration of cysteine.

A predictable consequence of the presence of quinones is the formation of elevated amounts of methaemoglobin, and this prediction was verified: the value of methaemoglobin reached 12% in cats 1h after administration of [¹⁴C]phenol (20 mg/kg body wt.). Similar values were recorded with 2,6-dimethoxy[¹⁴C]phenol and [2,3,5,6-¹⁴C]-quinol.

In many other species, e.g. the rat, phenols are readily detoxicated by forming glucuronic acid conjugates (Dutton, 1966). However, it is known that cats do not readily form glucuronides (Robinson & Williams, 1958), and the present study shows that sulphate conjugation is a major detoxication mechanism. Further, this mechanism is apparently saturated readily and the toxic effects of phenols are explicable in terms of the oxidation products of unconjugated phenols.

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Studies on the Mechanism of Biliary Excretion of Aryl Sulphates in the Rat

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Previous studies with a series of structurally related aryl sulphate esters have shown that such compounds are excreted in the bile of rats after conjugation with glucuronic acid (Hearse *et al.*, 1969). It has been suggested that, although these sulphate esters satisfy anionic and lipophilic requirements for biliary excretion, the molecular-weight requirement is satisfied only after further conjugation (Millburn, 1970). Moreover, the mono- and di-sulphate esters of diethylstilboestrol, both of which satisfy the criteria for elimination in bile without metabolic modification, appear in trace amounts only in bile after their administration to rats. The major metabolite in bile in each case was diethylstilboestrol monosulphate monoglucuronide (Gregory *et al.*, 1971). These findings suggest that there is a mechanism for the elimination of glucuronic acid conjugates into bile in rats, whereas no such mechanism exists for sulphate esters. However, phenolphthalein disulphate is eliminated unchanged in rat bile (Hirom *et al.*, 1972), and the question arises whether phenolphthalein disulphate is eliminated via the same mechanism as the glucuronic acid conjugates of aryl sulphate esters. The present study was designed to