48 h. In each case most of the injected radioactivity (over 60%) was recovered in the urine as ester sulphate. Similar results were obtained after oral administration of octadecyl [^{35}S]sulphate and after oral and intravenous administration of undecyl [^{35}S]sulphate.

After intravenous administration of decyl [^{35}S]sulphate (1 mg/200 g body wt.) to wholly anaesthetized rats with bile-duct and ureter cannulae 54–62% of the injected radioactivity was excreted in the urine as ester sulphate over 6 h; less than 2% appeared in the bile. In similar experiments with octadecyl [^{35}S]sulphate (0.1 mg/animal) approx. 73% of the radioactivity was recovered in the urine as ester sulphate; again less than 2% appeared in the bile.

Chromatography and electrophoresis of urine samples obtained from all animals receiving decyl [^{35}S]sulphate or octadecyl [^{35}S]sulphate showed one major radioactive component. This was identified as butyric acid 4-[^{35}S]sulphate.

When cannulated animals were injected with undecyl [^{35}S]sulphate (1 mg/200 g body wt.) 30% of the administered radioactivity appeared as ester sulphate in the urine, whereas at a lower dose (0.5 mg/200 g body wt.) 48% was excreted. At both dosages the amount of ^{35}S -labelled ester in bile obtained from male rats was low (approx. 2%), whereas bile from female rats contained between 6 and 14% of the injected radioactivity. The ester sulphate appearing in urine is associated with three metabolites, none of which can be identified with the parent ester. The chromatographic and electrophoretic properties of these metabolites suggest that they are short-chain sulphate esters structurally related to butyric acid 4-sulphate.

Whole-body radioautographic studies pinpoint the liver as the site of metabolism of all three parent esters.

Presumably the even-numbered alkyl sulphate esters studied are degraded by the mechanism proposed for the breakdown of dodecyl sulphate. The similar properties of butyric acid 4-sulphate and the metabolites derived from undecyl sulphate suggest that this odd-numbered alkyl sulphate ester might also be degraded by a similar mechanism.

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Denner, W. H. B., Olavesen, A. H., Powell, G. M. & Dodgson, K. S. (1969) *Biochem. J.* **111**, 43–51

Metabolism of [^{14}C]Phenacetin in Various Species

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The metabolic fate of phenacetin in various species is being investigated (a) to obtain information on interspecies variation in its metabolism and (b) to attempt to find an animal species that metabolizes the drug in a manner analogous to that reported to occur in certain human beings having a defective ability to de-ethylate phenacetin (Shahidi, 1968).

In this communication preliminary findings on the metabolism and excretion of [^{14}C]phenacetin in five species including man are reported. Animals were dosed orally with [^{14}C]phenacetin (100 mg/kg; 5 μCi) dissolved in aq. 70% (v/v) propane-1,2-diol, and the urine, faeces and expired air were collected for up to 4 days. Two male human subjects took a 500 mg oral dose of [^{14}C]phenacetin (5 μCi). The metabolites in the urine were identified and determined by quantitative radiochromatogram scanning and in some cases by reverse isotope dilution.

The species examined excreted 64–88% of an oral dose of [^{14}C]phenacetin in the urine in 3 days, the highest recoveries being obtained for the rabbit and guinea pig and the lowest for the ferret and rat. Faecal excretion varied from the low value of 0.2% found for the guinea pig to 4% found for the two human subjects and the rabbit. The excretion of radioactive CO_2 in the expired air, which could be regarded as a measure of the extent of deacetylation of the drug, accounted for 3–8% of the dose in the guinea pig, rabbit and two human subjects, but was higher in both the ferret (13%) and the rat (16%).

The major metabolite in the urine in all the species was conjugated *N*-acetyl-*p*-aminophenol, which accounted for 36–87% of the dose, the highest value being for the guinea pig and the lowest for the ferret. There were marked species variations in the pattern of conjugation of the *N*-acetyl-*p*-aminophenol, since the main conjugate in the urine in the rabbit, guinea pig and ferret was *N*-acetyl-*p*-aminophenyl glucuronide, whereas in the rat it was *N*-acetyl-*p*-aminophenyl sulphate. The two human subjects excreted slightly more of the glucuronide conjugate (38% of dose) than the sulphate (27%). In addition to these metabolites the urine contained small amounts of unchanged phenacetin (0.1–0.5% of dose), free 2-hydroxyphenacetin (0.1–0.3%) and free *N*-acetyl-*p*-aminophenol (0.9–3.2%), and also an unidentified metabolite that accounted for 0.4–4% of the dose in the rat, guinea pig and two human subjects and more (7–10%) in the rabbit and ferret.

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Shahidi, N. T. (1968) *Ann. N.Y. Acad. Sci.* **151**, 822

Increased Concentrations of Malonaldehyde in the Livers of Rats Treated with Carbon Tetrachloride

By P. J. JOSE and T. F. SLATER (*Department of Biochemistry, Brunel University, Kingston Lane, Uxbridge, Middlesex, U.K.*)

The administration of CCl_4 to rats results in numerous biochemical changes in the liver within the first hour after dosing. These changes are particularly evident in the endoplasmic reticulum and have been suggested to be, at least partially, dependent on lipid peroxidation, which, once initiated by free-radical metabolites of the CCl_4 , is an autocatalytic process (Recknagel & Ghoshal, 1966; Slater, 1966).

CCl_4 has been shown to stimulate the NADPH-dependent peroxidation of rat liver microsomal suspensions (Comporti *et al.*, 1965; Ghoshal & Recknagel, 1965; Slater & Sawyer, 1971). CCl_4 has also been shown to initiate the peroxidation of 'microsomal' lipids *in vivo* within 5 min of its administration, as evidenced by diene conjugation absorption (Rao & Recknagel, 1968). However, attempts to detect malonaldehyde (which is an extensively used index of lipid peroxidation) in the livers of rats treated with CCl_4 have failed (Priest *et al.*, 1962; Recknagel & Ghoshal, 1965). In retrospect this is not surprising, since malonaldehyde has been found to be metabolized *in vivo* (Placer *et al.*, 1965). We have overcome this problem by homogenizing a small piece of liver in cold 20% (w/v) trichloroacetic acid in a top-drive Waring Blendor as soon as possible after the animal was killed. The supernatant from the centrifuged homogenate was incubated (at 100°C for 10 min) with thiobarbituric acid. The pink chromogen, which is indicative of malonaldehyde, was extracted into butanol and its E_{535} determined.

The concentrations of malonaldehyde found in rat livers 2 h after the administration of liquid paraffin or a mixture of CCl_4 and liquid paraffin (1:1, v/v) were 1.39 ± 0.11 and 2.97 ± 0.31 nmol/g of liver respectively ($P < 0.001$). The administration of promethazine, an antioxidant that protects CCl_4 -treated rats against the onset of necrosis, decreased the concentrations of malonaldehyde in the livers of untreated rats or of rats that had been dosed with liquid paraffin or with the mixture of CCl_4 and liquid paraffin. However, promethazine did not completely inhibit the stimulation of lipid peroxidation caused

by CCl_4 , as assessed 2 h after dosage with the hepatotoxic agent.

The finding that CCl_4 stimulates lipid peroxidation *in vivo* is consistent with, and lends further support to, the suggestions that CCl_4 stimulates free-radical-motivated reactions in rat liver shortly after administration (Recknagel, 1967; Slater, 1972).

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Comporti, M., Saccocci, C. & Dianzani, M. U. (1965) *Enzymologia* **29**, 185

Ghoshal, A. K. & Recknagel, R. O. (1965) *Life Sci.* **4**, 1521

Placer, Z., Veselkova, A. & Rath, R. (1965) *Experientia* **21**, 19

Priest, R. E., Smuckler, E. A., Iseri, O. A. & Benditt, E. P. (1962) *Proc. Soc. Exp. Biol. Med.* **111**, 50

Rao, K. S. & Recknagel, R. O. (1968) *Exp. Mol. Pathol.* **9**, 271

Recknagel, R. O. (1967) *Pharmacol. Rev.* **19**, 145

Recknagel, R. O. & Ghoshal, A. K. (1965) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **24**, 299

Recknagel, R. O. & Ghoshal, A. K. (1966) *Lab. Invest.* **15**, 132

Slater, T. F. (1966) *Nature (London)* **209**, 36

Slater, T. F. (1972) *Free Radical Mechanisms in Tissue Injury*, Pion Ltd., London

Slater, T. F. & Sawyer, B. C. (1971) *Biochem. J.* **123**, 805

The Absorption of Aliphatic Carbamates by Everted Gut Sacs of Rat

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The everted-gut-sac technique (Wilson & Wiseman, 1954) has been used to study the absorption of a series of carbamates of general structure R-O-CO-NH_2 , where R is an aliphatic group ranging from methyl to *n*-heptyl. These compounds show a wide range of lipophilicity and are not ionized nor hydrolysed in aqueous solution.

Cannulated everted sacs of rat were incubated at 37°C in an oxygenated Krebs-Henseleit medium containing Tween 80 (5%, w/v) and one of the carbamates (10–50 mM). Samples (20 μl) were removed from the interior of the sac every 10 min for 1 h and assayed by g.l.c. on a Porapac P column. No metabolism was evident, recoveries averaging 97%. The results obtained, expressed as % of carbamate of the initial mucosal fluid absorbed/h per cm^2 were as follows: methyl, 2.81; ethyl, 3.37; *n*-propyl, 5.07; *n*-butyl, 6.60; *n*-pentyl, 3.75; *n*-hexyl, 2.25; *n*-heptyl, 0.67. It was found that the presence of Tween 80 did