LA-fed animals. Biliary cholesterol concentrations rapidly increased while total B.A. first increased and then decreased, thus causing a dramatic decrease in the total B.A./cholesterol ratio. Between 1.5 and 2.5 days there was a large decrease in cholic acid concentration: LA and its major metabolite 3α,6β-dihydroxy-5β-cholanoic acid, not detectable in bile of control animals, appeared in increasing amounts. These marked changes in bile acids preceded the appearance of liver damage which was well established only after 2.5 days. The livers of LA-treated animals showed areas of necrosis of irregular distribution with some dilatation of centro-lobular venules with inflammatory and neutrophil infiltration. LA may exert its hepatotoxic effect, in mice by alteration in cholesterol and bile acid biosynthesis in the liver, but the sulphating mechanism may reduce potential toxicity in man.

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Haem biosynthesis and hepatic drug metabolism in lead poisoned rats

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Previous animal studies (Scoppa, Roumengous & Penning, 1973; Alvares, Leigh, Cohn & Kappas, 1972) have produced indirect evidence that the depression in hepatic microsomal cytochrome P-450 content and the mixed function oxidase system in lead intoxicated rats, is associated with depressed haem synthesis. This, however, remains to be proven. The present study therefore investigated the inter-relationship of haem biosynthesis and cytochrome P-450 in lead intoxicated rats.

With increasing pretreatment of rats with intra-peritoneal injections of lead, there was a progressive decrease in hepatic microsomal cytochrome P-450 and b_5 content and decreased activity of the enzymes aniline hydroxylase and aminopyrene demethylase. Associated with this impairment of the microsomal mixed function oxidase system there was a depression in haem synthesis, as assessed by decreased activity of the enzymes δ-aminolaevulinic acid (ALA) dehydratase, coproporphyrin oxidase and ferrochelatase whilst the activity of the rate-limiting enzyme of haem biosynthesis ALA synthase was increased. The activity of ALA synthase has been shown to be regulated by free haem levels; thus when free haem levels are depressed the activity of the

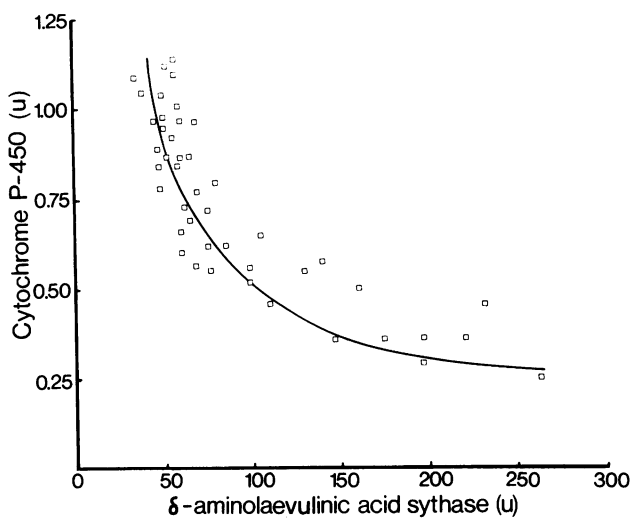

Figure 1 The correlation of hepatic γ-aminolaevulinic acid (ALA) synthase activity with microsomal cytochrome P-450 content ($r=0.76$; $P<0.001$), where cytochrome P-450 = $(41.4 \pm 3.4/\text{ALA synthase}) + (0.098 \pm 0.034)$.

enzyme is increased by a negative feedback mechanism. A highly significant ($P<0.001$) inverse relationship ($r=0.76$) was found between hepatic ALA synthase activity and microsomal cytochrome P-450 content (Figure 1). This indicates that the depression in the levels of the haemoprotein cytochrome P-450 in animals treated with lead is due to impaired haem synthesis resulting in decreased

availability of haem for synthesis of the haemoprotein and a decrease in the regulatory 'pool' of haem.

The activity of microsomal haem oxygenase was also increased by lead pretreatment. It is therefore concluded that the depression in the microsomal mixed function oxidase system in lead intoxication is the result of depressed haem synthesis and also possibly by an increase in haem degradation.

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The metabolism of bupivacaine in the rat

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Bupivacaine (Marcain, *N*-*n*-butylpipecolylxylidine) is a long-acting local anaesthetic widely used for lumbar epidural anaesthesia in childbirth. It is almost completely metabolized in human volunteers, mothers in childbirth and by their babies (Caldwell, Moffatt, Smith, Lieberman, Cawston & Beard, 1976) but little is known of the pathways of metabolism. Accordingly we have investigated the fate of this drug in the rat as a preliminary to human studies.

[^{14}C]-Bupivacaine hydrochloride, labelled in the carbonyl group, was synthesized, administered to female Wistar albino rats (30 mg/kg; 2.5 μCi/animal) by intraperitoneal injection, and their urine and faeces collected for four days. The excretion of [^{14}C] was monitored by liquid scintillation counting, and 74% of the dose was recovered (33% urine: 41% faeces). The bulk of the [^{14}C] excreted (urine 27%, faeces 29%) appeared on the first day.

Urinary metabolites were examined by thin-layer chromatography followed by radiochromatogram scanning, reverse isotope dilution, gas liquid chromatography, gas chromatography-mass spectrometry (g.c.-m.s.) and u.v. spectrometry. Five urinary metabolites were identified, together with two unknowns, and their identities and quantities are given in Table 1. The major routes of metabolism of bupivacaine in the rat involve hydroxylation of the aromatic ring to yield two isomeric phenolic metabolites, 3′- and 4′-hydroxybupivacaine, excreted free and as glucuronic acid conjugates, the ratio of free to conjugated material being about 0.25 in both cases. Standard compounds were not available, and the g.c.-m.s. properties of these metabolites do not permit unequivocal assignment of the position of the hydroxyl group and the assignment of these metabolites is by analogy with those of local anaesthetics of similar structure (see Dring, 1976). *N*-dealkylation to yield desbutylbupivacaine was a minor pathway, as was hydrolysis of the amide bond giving pipecolic acid. Two unknown minor metabolites were also separated but not characterized, and a small amount of bupivacaine was excreted unchanged.

The fate of bupivacaine in the rat is similar to that of other xylidide local anaesthetics, such as lignocaine and mepivacaine, especially in the predominance of aromatic hydroxylation (see Dring, 1976). However, the extent of *N*-dealkylation and amide hydrolysis is much lower than in the case of lignocaine, probably due to the size of the *N*-*n*-butyl substituent and to steric factors associated with the pipecolic acid moiety.

Table 1 Urinary metabolites of bupivacaine in the rat

Compound	*% Urinary ^{14}C as that compound*
Bupivacaine	3.4
Desbutylbupivacaine	1.1
3′-Hydroxybupivacaine	
free	8.9
glucuronide	36.4
4′-Hydroxybupivacaine	
free	5.6
glucuronide	22.7
Pipecolic acid	6.0
Unknown 1 (neutral)	2.2
Unknown 2 (acidic)	13.1

0–24 h urine of rats dosed with [^{14}C]-bupivacaine was analysed as described. Figures are the means of 3 animals.

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