crypt cells was significantly decreased in alcoholic animals, both in the jejunum (10.62 ± 3.26 mitosis against 19.34 ± 2.51 in the controls; $p < 0.005$) and in the ileum (5.91 ± 3.16 mitosis against 14.20 ± 2.76 in the control rats; $p < 0.005$).

It has been demonstrated that chronic administration of alcohol to human volunteers in the absence of nutritional deficiency produces ultrastructural changes in the small intestine[2]. Recent studies of BARAONA et al.[3] have shown that rats given alcohol in a liquid nutritionally adequate diet for 3 to 4 weeks have shorter jejunal villi with a reduced number of epithelial cells lining the villi. Furthermore, the jejunal and ileal crypts had a significantly increased number in epithelial cell count, while the number of mitosis per 100 crypt cells significantly increased in the ileum, but not in the jejunum. These findings are not in accordance with our experimental results. The different periods of time of alcohol ingestion, the age of the rats, and the type of diet used would seem to be the most likely causes of this discrepancy.

A continual dispute exists whether alcohol is directly toxic or indirectly injurious due to associated nutritional deficiency. In the present investigation, the alcohol-fed animals consumed a nutritionally adequate diet[12]. Thus our results support the view that alcohol is directly toxic to the small intestine, which may be one of the factors playing a role in the development of small intestinal morphological and functional changes.

The Biliary Excretion of [^{3}H] Lysergic Acid Diethylamide in Wistar and Gunn Rats

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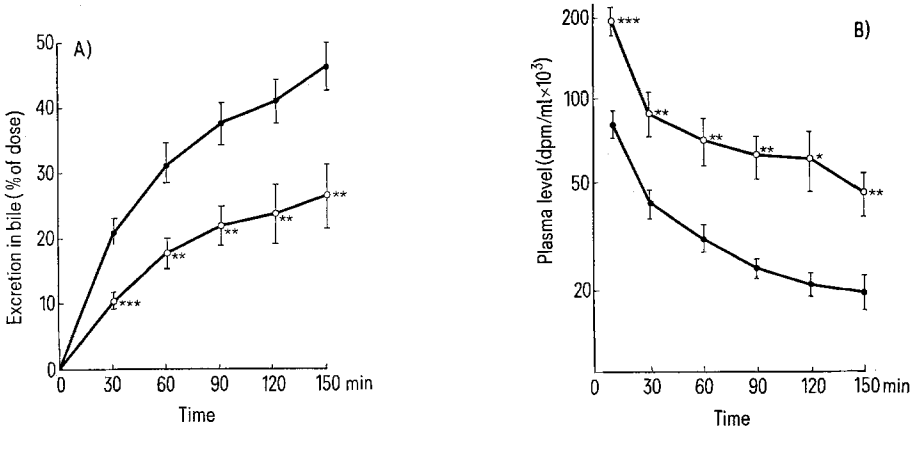
Summary. The biliary excretion of [^{3}H] LSD was studied in Wistar and homozygous Gunn rats. In Wistar rats approximately 46% of the given dose was recovered from bile in 2.5 h whilst in the homozygous Gunn rat 26% was recovered in the same time period. In both strains the main metabolites were glucuronides.

Glucuronide conjugation is an important mechanism in the biotransformation of many compounds. Previous work has shown that in normal rats LSD is extensively metabolized to glucuronides which are excreted mainly in the bile[1–3]. In the present study the biliary excretion of LSD was compared in normal Wistar rats and homozygous Gunn rats. In the homozygous Gunn rat, jaundice persists throughout life since the genetic lesion in the microsomal UDP-glucuronyltransferase prevents the synthesis of conjugated bilirubin. However, despite the lesion many exogenous compounds are excreted as glucuronides[4–9].

Materials and methods. Mature male Wistar rats and homozygous Gunn rats (250–350 g) were anaesthetised with urethane (14% w/v in 0.9% saline; 10.0 ml/kg, i.p.). Polyethylene catheters were inserted into a femoral vein, a carotid artery and the common bile duct. [2(n)-3H] lysergic acid diethylamide ([^{3}H] LSD; specific radioactivity 15.8 Ci/mmol; obtained from the Radiochemical Centre, Amersham) was dissolved in saline:methanol (19:1 v/v) after evaporation of the organic vehicle. Radiochemical purity was shown to be 97% by thin layer chromatography in solvent system chloroform:methanol:acetic acid (40:30:30).

[^{3}H] LSD (24 μCi/kg; 0.5 μg/kg) was injected i.v. and blood samples obtained at 5, 30, 60, 90, 120 and 150 min after administration of the drug. Bile was collected in preweighed vials at successive 30 min intervals for 150 min. The radioactive content of plasma samples (20–50 μl) and bile samples (50 μl) was determined by liquid scintillation spectrometry[10].

Pooled samples of Wistar and Gunn rat bile (0–2.5 h) were spotted on strips of Whatman No. 1 paper (6 × 50 cm) and resolved by descending chromatography in sol-



A) Excretion of radioactivity in bile after i.v. injection of [^{3}H] LSD (24 μCi/kg body wt.). Results were summated at 30 min intervals. B) The plasma disappearance of radioactivity after i.v. injection of [^{3}H] LSD.
●, Normal Wistar rats; ○, homozygous Gunn rats. Each value represents the mean ± SE of at least 5 experiments. Significantly different from Wistar, $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*).

vent system, *n*-butanol:acetic acid:water (12:3:5). Zones (1 cm) were serially cut from the chromatograms, transferred to glass vials and radioactivity determined by scintillation counting. In some studies samples of bile (0.2 ml) were adjusted to pH 5.0 (0.1 *M* HCl) and added to β-glucuronidase (8000 Fishman units, Ketodase, Warner Chilcott). Control specimens were prepared by adding β-glucuronidase to acidified bile containing glucaro-(1→4)-lactone (5 m*M*; Calbiochem). After incubation at 37 °C for 40 h, the specimens were resolved, by paper chromatography.

Results and discussion. The excretion of radioactivity in the bile of normal Wistar and homozygous Gunn rats after i.v. administration is shown in Figure A. Marked differences were observed. Approximately 46% of the given dose was recovered from normal rat bile in 2.5 h. In contrast, only about 26% was recovered from Gunn rat bile in the same time period. The excretion of radioactivity in the bile of normal rats was therefore roughly twice the excretion in Gunn rat bile. The reduced biliary excretion in Gunn rats was reflected in elevated plasma levels of radioactivity (Figure B).

When Wistar rat bile was resolved by paper chromatography (Table) an essentially similar pattern of metabolites was found as previously reported[3,5], despite a considerable difference in the dose level of LSD given (present study 0.5 μg/kg, Siddik et al.[3] 1.33 mg/kg). The major metabolites, (M3 and M4) which on some chromatograms were difficult to separate and have therefore been considered together, were identified as glucuronides by complete hydrolysis with β-glucuronidase. Hydrolysis of M3 and M4 was inhibited by the specific inhibitor glucaro-(1→4)-lactone, and under these conditions the radiochromatograms closely resembled those obtained when untreated bile was resolved. A similar pattern of metabolites was seen in Gunn rat bile (Table) with M3 and M4 shown to be glucuronides by the procedure outlined above. Therefore, despite the genetic lesion in UDP-glucuronyltransferase, glucuronides (probably of 13- and 14-hydroxy-LSD[3]) form the major part of the LSD excreted in the bile of Gunn rats.

There is considerable evidence that several glucuronyltransferases are present in the liver endoplasmic reticulum[11–13] and that the homozygous Gunn rat is deficient in only some of the enzymes[14,15]. It has recently been shown[16] that the defect in conjugation with glucuronic acid of many phenols in the cat is not absolute and small amounts of conjugates are formed. Similar considerations may apply in the Gunn rat and glucuronides might be expected if glucuronyltransferase is at a low level.

It has previously been shown[17] that the metabolism of the quaternary amine edrophonium is qualitatively similar in both normal Wistar and homozygous Gunn rats, the main metabolite being a glucuronide. However, the rate of biliary excretion of edrophonium glucuronide is approximately 10 times greater in homozygous Gunn rats than in Wistar rats. Such an increase was shown not to be due to an increased rate of metabolism but an increased rate of transfer across the canalicular membrane[17].

Different considerations apply in the present study. If it is assumed that, due to the absence of endogenous bilirubin glucuronide in the Gunn rat, there is a marked increase in canalicular transfer of a drug-glucuronide conjugate, there would seem to be a significantly depressed rate of formation of glucuronides of LSD or metabolites in this strain. This suggests the direct involvement of bilirubin glucuronyltransferase in the formation of such conjugates in the normal rat. The higher plasma levels of radioactivity in Gunn rats adds support to the concept of reduced formation of the conjugates.

Resolution of metabolites of [^{3}H] LSD by paper chromatography (see text for details)

Metabolite	Rf	Proportion of total radioactivity (%)	
		Wistar bile	Gunn bile
M1	0.14	2.1	1.1
M2	0.22	1.1	ND
M3	0.35	76.3	72.4
M4	0.43		
M5	0.72	1.1	0.9
M6	0.80	19.4	25.6

Values represent the proportion of total radioactivity detected on paper chromatograms. ND, not detectable.

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