Glycoside content in plasma, liver, kidney, and skeletal muscle of anaesthetized rats (1.2 g/kg urethane) after 40 min single pass perfusion of the small intestine in vivo with Krebs-Ringer-solution containing 2×10^{-5} *M* ^{3}H-digitoxin in controls and under probenecid, ethacrynic acid, and mersalyl

Experiments	Glycoside concentration after 40 min perfusion with ^{3}H-digitoxin				
	Plasma (μg/ml)	Liver (μg/g)	Kidney (μg/g)	Muscle (μg/g)	*n*
Controls	1.08 ± 0.15	6.96 ± 1.50	1.85 ± 0.48	0.66 ± 0.17	5
Probenecid (1.0×10^{-2} *M*)	0.59 ± 0.12[a]	4.19 ± 1.40[a]	1.30 ± 0.31	0.45 ± 0.10[a]	5
Ethacrynic acid (0.5×10^{-3} *M*)	0.71 ± 0.19[a]	4.26 ± 1.55[a]	1.23 ± 0.47	0.40 ± 0.21	6
Mersalyl (8.0×10^{-3} *M*)	0.46 ± 0.14[a]	4.79 ± 0.80[a]	1.06 ± 0.33[a]	0.32 ± 0.14[a]	6

Results are given as the mean ± SD; *n* = number of experiments; [a] indicates significant difference from control values according to Student's *t*-test, $p < 0.01$.

amount of ^{3}H-digitoxin absorbed from the intestine as well as the glycoside concentration in plasma and tissues reached at the end of 40 min single pass perfusion. But, as shown in Figure 2, the inhibitory effects are significantly different from the controls not before a perfusion period of 30 min indicating a rather long latent period of the drug actions. The time course of the glycoside concentration in the plasma remained linear with ethacrynic acid and mersalyl, whereas in the inhibition with probenecid characteristics of saturation are obvious. In view of the mechanism of the drug actions, it should be mentioned that in a previous study the effects had been explained by inhibition of energy dependent processes which mediate in part the absorption of digitoxin[2]. In the case of ethacrynic acid and mersalyl specific effects on the Na^+, K^+ activated membrane, ATPase might be an appropriate explanation for the inhibition of digitoxin absorption[6,7]. It was shown by DAMM and WOERMANN[2] earlier that, with these drugs, the inhibition of digitoxin correlated strongly with the inhibition of the intestinal absorption of sodium, water and glucose. Probenecid is known as an inhibitor of organic anion transports[8,9] as well as for non-specific metabolic inhibitions[10–12]. The latter effect only seems to be involved in the inhibition of digitoxin absorption. In all, as demonstrated in vitro, digitoxin absorption in vivo can hardly be interpreted by simple diffusion; the present results indicate that active processes are at least partly involved in this transfer.

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Effect of Alcohol Ingestion on the Epithelial Cell Population in Rat Small Intestine

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Summary. Chronic administration of alcohol to well-nourished rats led to striking changes in the small intestinal cell population. The present experiments corroborate the view that alcohol is directly toxic to the small intestine.

Diarrhea, disturbances of digestion, and malabsorption of nutrients are frequently associated with chronic alcohol intake. Light and electron microscopic changes[2,3] and functional alterations[4–10] of the small intestine have been reported in alcoholic patients and experimental animals. The causes, however, are still unknown. Malnutrition and direct enterotoxic effect of alcohol have been assumed as pathogenic mechanisms of such abnormalities. The present investigation was undertaken to study the effect of a prolonged period of alcohol ingestion on the small intestinal cell population in otherwise well-nourished rats.

Materials and methods. 5 Wistar strain male rats (body weight 30–40 g) were allowed to drink only alcohol for 12 weeks. A 32% (v/v) ethyl alcohol solution in 25% (w/v) sucrose in tap water was used. 5 animals from the same stock were kept as controls and given no alcohol. All rats were fed on a solid semi-synthetic diet supplemented with large amounts of vitamins and lipotropes[11,12].

Tissues from both control and experimental groups were processed in exactly the same fashion. To determine the percentage of cells in division in the small intestine during a given time interval, the colchicine technique was applied[13,14]. The rats were injected s.c. with colchicine in a dose of 0.1 mg per 100 g of body weight 3 h prior to sacrifice. The animals were sacrificed by exsanguination under light ether anesthesia, and pieces of proximal jejunum and distal ileum were rapidly removed, flattened on cardboard, fixed in Bouin's solution for 72 h, and embedded in paraffin. Longitudinal sections were cut at 4 μm and stained with hematoxylin-eosin and PAS-hematoxylin. Areas of sections showing crypts cut through their length were selected for counting the total number of nuclei and the number of mitosis in metaphase and prophase. At least 10 crypts per section were counted in the particular tissue from each animal. The length of villi

Effect of alcohol feeding on villus and crypt length and crypt cell population

	Length (mm) [a]	No. of cells [b]	Nuclei in mitosis (%) [c]
Jejunum			
Controls	0.837 ± 0.056	83.72 ± 3.99	19.34 ± 2.51
Alcoholics	0.501 ± 0.049	66.22 ± 22.19	10.62 ± 3.26
Deviation from control (%)	− 40	− 21	− 55
p	< 0.005	< 0.1	< 0.005
Ileum			
Controls	0.699 ± 0.087	72.96 ± 6.15	14.20 ± 2.76
Alcoholics	0.407 ± 0.091	56.02 ± 14.21	5.91 ± 3.16
Deviation from control (%)	− 47	− 23	− 58
p	< 0.005	< 0.05	< 0.005

Values are means ± standard mean error. [a] Average length of villi plus crypts. [b] Average number of epithelial cells nuclei on longitudinal sections of crypts. [c] Metaphase plus prophase.

and crypts was measured in properly oriented sections by using a micrometric ocular. Student's t-test was used to check the significance of differences between control and alcoholic rats.

Results and discussion. The general appearance of the experimental animals was essentially similar to that of the controls. However, they gained 23–28% less weight than the controls. Clinical signs of inebriation were commonly seen among the alcohol-fed rats. The animals drinking alcohol consumed 27% of their calories as alcohol and the total amount of daily consumed calories was 10% less than the control group.

After 12 weeks of chronic alcohol ingestion, striking changes in small intestinal cell population could be observed. The jejunal and ileal mucosae from alcoholic rats were thinner than those of the control rats (Figure), and the intestinal crypts of the alcohol-fed animals had a decreased epithelial cell count, both in the jejunum (66.22 ± 22.19 cells against 83.72 ± 3.99 in the controls; $p < 0.1$) and in the ileum (56.02 ± 14.21 cells against 72.96 ± 6.15 in the controls; $p < 0.05$) (Table). The number of mitosis (prophase plus metaphase) per 100

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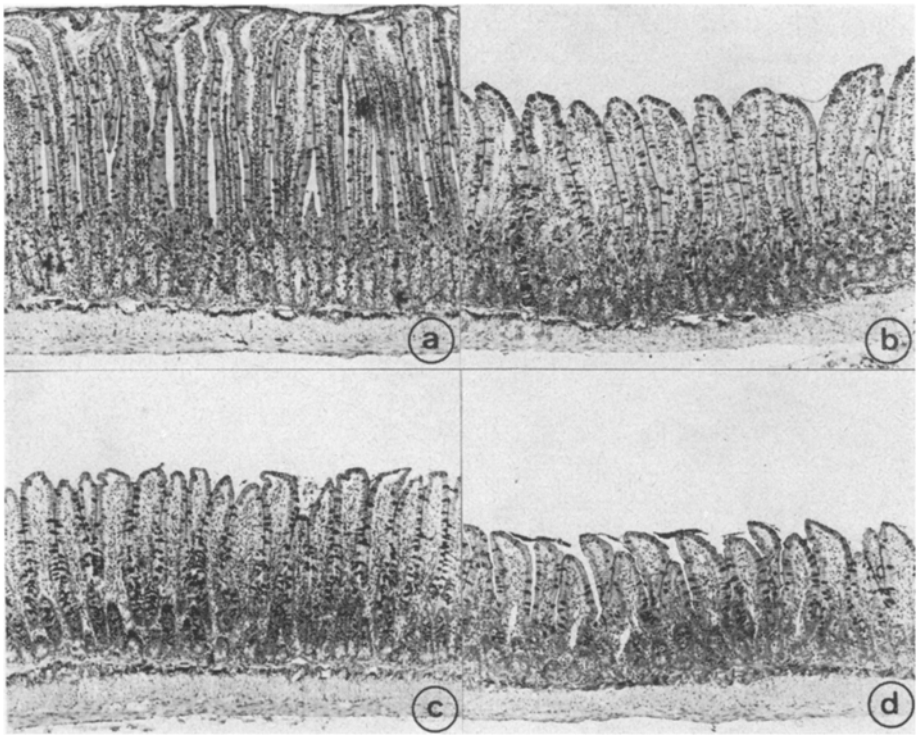

Representative longitudinal sections of jejunum and ileum stained with PAS-hematoxylin. a) Normal control jejunum; b) Jejunum from alcoholic rat; c) Normal control ileum; d) Ileum from alcoholic rat. × 50.

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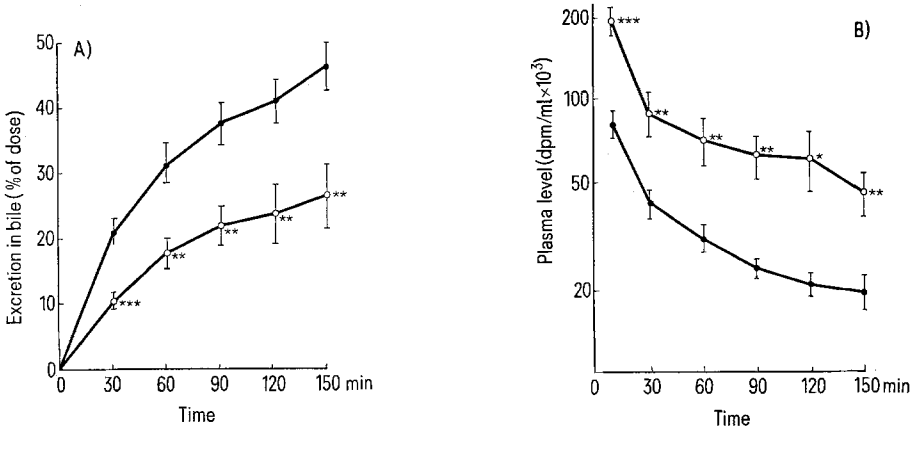
Summary. The biliary excretion of [^{3}H] LSD was studied in Wistar and homozygous Gunn rats. In Wistar rats approximately 46% of the given dose was recovered from bile in 2.5 h whilst in the homozygous Gunn rat 26% was recovered in the same time period. In both strains the main metabolites were glucuronides.

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, $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*).