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BILIARY EXCRETION OF GAMMA-GLUTAMYLTRANSFERASE

SELECTIVE ENHANCEMENT BY ACUTE ETHANOL ADMINISTRATION

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Abstract—To study the acute effect of ethanol on various constituents of the bile, female Wistar rats received by intravenous administration 0.9% NaCl solution either alone or containing in addition ethanol (0.1 ml ethanol 96% hr^{-1} 100 g body $weight^{-1}$). Compared to saline-treated controls there was a significant enhancement of biliary gamma-glutamyltransferase excretion after ethanol infusion for 5 hr by 166% (22.1 ± 2.8 μU/min/100 g body weight vs. 58.2 ± 13.7; $P < 0.0125$), whereas no changes or only marginal alterations have been observed for bile flow and the biliary excretion of total bile acids and alkaline phosphatase. The selective enhancement of biliary gamma-glutamyltransferase excretion by ethanol can be ascribed to an increased solubilization of the membrane-bound enzyme originating from the bile canaliculi of the hepatocytes and/or the epithelial cells of the bile ducts. Since the biliary excretion of total bile acids remained unchanged by ethanol, the observed selective solubilization of gamma-glutamyltransferase may occur by a mechanism primarily not involving total bile acids and could be linked to a direct effect of ethanol on physico-chemical properties such as an increased fluidity of liver plasma membranes.

A variety of studies have suggested that an acute administration of ethanol may impair the secretion of some secretory glycoproteins [1–5]. In these studies the glycoprotein content has been measured either in the medium after addition of ethanol to liver slices [1–4] or in the blood after *in vivo* application of ethanol [5]. However, hepatic glycoproteins are excreted under physiological conditions not only into the blood but also into bile [6, 7], and this is especially the case for the clinically important enzymes gamma-glutamyltransferase [8–10] and alkaline phosphatase [9–11] which are both glycoproteins [6–8].

In the present study the effect of an acute load of ethanol by intravenous application during a short period of time on the biliary excretion of gamma-glutamyltransferase and alkaline phosphatase was examined.

MATERIALS AND METHODS

Animals. Female Wistar rats (N = 20) with a body weight of 290–310 g were obtained from the Zentralinstitut für Versuchstierzucht, Hannover (F.R.G.). They were kept on laboratory chow (Altromin®) and tap water *ad libitum* until the start of the experiment.

Operative procedures. All animals (N = 20) were operated under intraperitoneal pentobarbital anesthesia (50 mg/kg body weight). First a Teflon catheter (outer diameter 1.1 mm, inner diameter 0.6 mm; H. C. Ulrich Corp., Ulm, F.R.G.) was introduced via the right jugular vein. By this route the rats received physiological saline (0.2 ml/hr/100 g body weight). The common bile duct was subsequently cannulated with the catheter described above approximately 1 cm distal of the hepatic duct bifurcation. To avoid contribution by pancreatic secretion, the distal common bile duct was ligated. Bile collection was started from the moment of cannulation. After the operation the animals were placed in Bollman-type restraining cages and kept at 37° using a heating lamp. After 1 hr of bile drainage under infusion of physiological saline solution, half of the animals (N = 10) received alcohol (0.1 ml ethanol 96%/hr/100 g body weight) for a total of 5 hrs intravenously which was incorporated into the volume of the physiological saline solution. The other half of the animals (N = 10) continued to receive physiological saline solution intravenously for 5 hrs. Bile was drained for a total of 6 hrs, and every hour bile sample was collected in pre-weighed tubes, weighed and stored at −20° until analysed. The enzymes and total bile acids were stable under the conditions of storage.

Analytical assays. Biliary gamma-glutamyltransferase activity was measured by spectrophotometric assay at 25° according to the method of Szasz [12], and the biliary activity of alkaline phosphatase was assayed at 25° by the method of Hausamen *et al.* [13]. The determination of protein was performed according to the method of Lowry *et al.* [14] using crystalline human albumin as standard. Total bile acids were analysed with the 3α-hydroxysteroid dehydrogenase enzyme assay [15].

Statistical analysis. Each determination was carried out in duplicate. The results obtained were expressed

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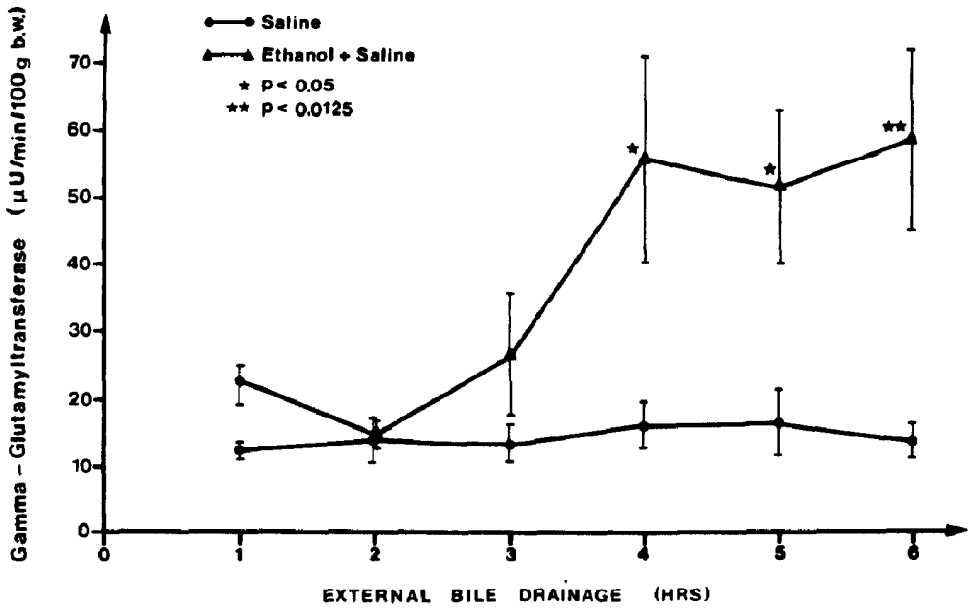

Fig. 1. Effect of ethanol infusion on biliary excretion of gamma-glutamyltransferase in comparison to saline-treated controls. Values represent means ± S.E.M. out of 10 rats each.

as means (± S.E.M.), and the significance of the differences was assessed by the Student's *t*-test.

RESULTS

Gamma-glutamyltransferase

Biliary excretion of gamma-glutamyltransferase remained unchanged in animals receiving physiological saline intravenously for a period of 6 hrs (Fig. 1). In the experimental group receiving physiological saline solution alone for 1 hr and subsequently ethanol in physiological saline solution for 5 hrs, biliary gamma-glutamyltransferase excretion remained unchanged during the first 2 hrs and gradually increased during the subsequent 4 hrs under the influence of alcohol infusion when compared to animals receiving physiological saline alone. After 5 hrs, the alcohol mediated increase of biliary gamma-glutamyltransferase excretion was 166% ($P < 0.0125$) compared to equally treated animals receiving physiological saline alone.

Alkaline phosphatase

In animals treated with physiological saline for 6 hrs, biliary excretion of alkaline phosphatase remained virtually unchanged (Fig. 2). In the exper-

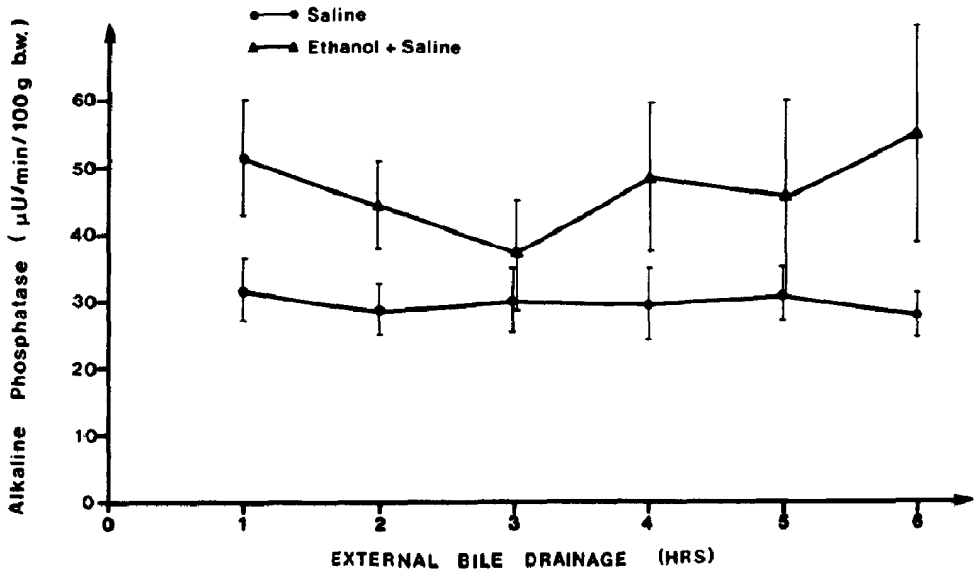

Fig. 2. Effect of ethanol infusion on biliary excretion of alkaline phosphatase in comparison to saline-treated controls. Values represent means ± S.E.M. out of 10 rats each.

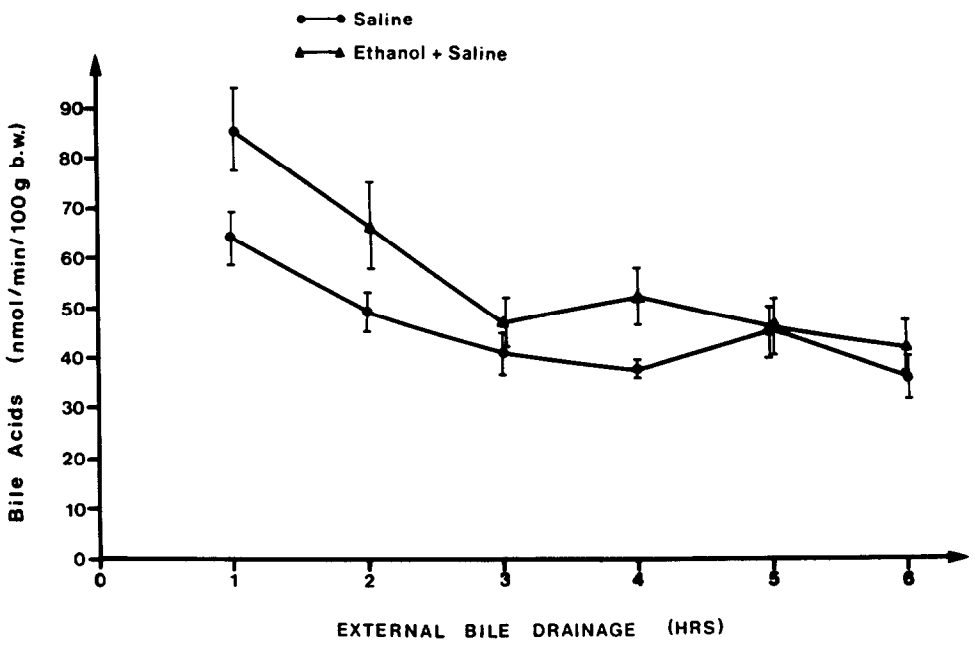

Fig. 3. Effect of ethanol infusion on biliary excretion of total bile acids in comparison to saline-treated controls. Values represent means ± S.E.M. out of 10 rats each.

imental group treated with ethanol intravenously there was also no change.

Total bile acids

Total bile acid excretion in the bile was slightly diminished in comparison to initial values in animals receiving physiological saline or ethanol (Fig. 3).

Protein

Biliary protein excretion was virtually unchanged under the infusion of physiological saline and showed only a slight enhancement during alcohol treatment (data not shown).

Bile flow

Bile flow remained unaltered in both experimental groups treated either with physiological saline alone or ethanol (Fig. 4).

Ratio gamma-glutamyltransferase: total bile acids

When the ratio of the biliary excretion of gamma-glutamyltransferase (expressed as μUmin/100 g b.w.): total bile acids (expressed as nmoles/min/100 g b.w.) was evaluated, the value was significantly higher after 5 hrs of ethanol infusion compared to the initial infusion period with physiological saline solution for 1 hr (1.54 ± 0.34 vs 0.28 ± 0.03; $P < 0.0025$). Moreover, a low value of only 0.36 ± 0.22 was obtained after 6 hrs of saline infusion.

DISCUSSION

The present study shows that an acute intravenous infusion of ethanol strikingly increases the biliary excretion of gamma-glutamyltransferase (Fig. 1), whereas there were either no changes or only marginal alterations of the biliary excretion of alkaline phosphatase (Fig. 2) and total bile acids (Fig. 3). Similarly, bile flow also remained unaffected (Fig. 4). Since enzymes such as gamma-glutamyltransferase and alkaline phosphatase present in the bile are commonly thought to be derived from the bile canaliculi of the hepatocytes and/or the epithelial cells of the bile ducts by a process involving solubilization of the membrane-bound enzymes and subsequent release of the enzymes into the bile [6–11], the results of the present study are suggestive of a selective solubilization of gamma-glutamyltransferase elicited by the acute administration of ethanol.

A variety of studies has demonstrated a relationship between the biliary excretion of total bile acids and the biliary output of gamma-glutamyltransferase [10, 16] as well as of alkaline phosphatase [10, 11, 17, 18], suggesting that total bile acids are primarily responsible for the solubilization of these membrane-bound enzymes and for the subsequent release into the bile. In particular, infusion of bile acids has been shown to enhance the biliary output of gamma-glutamyltransferase [10] and of alkaline phosphatase [10, 11]. Moreover, *in vitro* addition of bile acids to subcellular fractions of liver cells leads to solubilization of gamma-glutamyltransferase [19–21] and of alkaline phosphatase [11], findings which are in line with a striking solubilizing potency of bile acids. In the present study, however, biliary output of total bile acids failed to show any increase during ethanol infusion (Fig. 3) under conditions of a strikingly enhanced biliary output of gamma-glutamyltransferase (Fig. 1), suggesting that this increase occurs primarily by a mechanism which may not involve the action of bile acids, at least in the initial stage. This is substantiated by the data of the present study showing a significantly increased ratio of biliary gamma-glutamyltransferase: total bile acids after

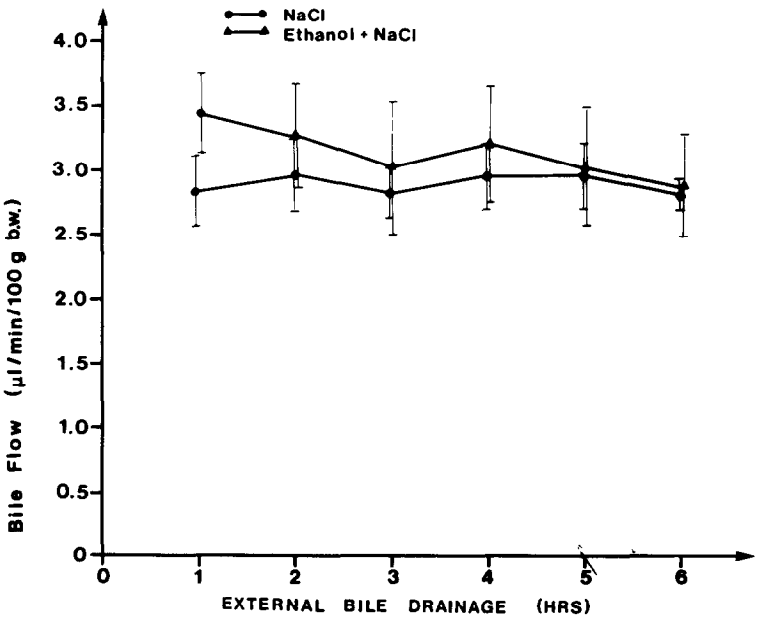

Fig. 4. Effect of ethanol infusion on bile flow in comparison to saline-treated controls. Values represent means ± S.E.M. out of 10 rats each.

ethanol infusion compared to saline treatment. It is obvious from these results that much smaller amounts of bile acids are required to solubilize the membrane-bound gamma-glutamyltransferase provided that ethanol has been administered.

Recent studies have shown that the acute administration of ethanol may alter a variety of characteristics and functions of liver plasma membranes [22–25]. These include the activities of various enzymes [23, 26], amino acid uptake [22], membrane glycoprotein assembly [25] and membrane fluidity [23]. Changes of liver plasma membrane fluidity are causally related to alterations of the composition and properties of the bilayer lipid environment, thereby affecting the functions of those membrane proteins which are embedded in or transverse the lipid core of the membrane [24]. Gamma-glutamyltransferase as well as alkaline phosphatase are constituents of the liver canalicular plasma membranes [7, 8, 27–29] where both gamma-glutamyltransferase [29] and alkaline phosphatase [30, 31] are located at the luminal side. Gamma-glutamyltransferase is known to be more easily solubilized than alkaline phosphatase by bile acids [10] or papain digestion [29], a finding which is also observed with ethanol in this study (Tables 1 and 2) and suggests that alkaline phosphatase may be more tidely bound to or embedded in liver plasma membranes. It is tempting to speculate that ethanol may selectively solubilize the membrane-bound gamma-glutamyltransferase after a time lag of two hours (Fig. 1) by a mechanism involving physico-chemical changes of the liver plasma membranes such as increased membrane fluidity, and the altered plasma membranes may then be more susceptible for the solubilizing property of the bile acids.

In conclusion, the present study shows a selective enhancement of biliary gamma-glutamyltransferase excretion by an acute load of ethanol. It appears that the primary event of this rise is not due to the action of total bile acids but rather to a direct effect of ethanol on physico-chemical properties such as an increased fluidity of liver plasma membranes and subsequent solubilization by bile acids.

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