Effect of Ethionine and Ethanol on the Function of the Perfused Rat Liver[1] (35680)

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Previous studies have shown that perfusion of the normal rat liver with ethanol results in impairment of biliary excretion of test dyes and reduction of bile production (1). As these changes were not accompanied by an interference with removal of the dye from the perfusing medium it was inferred that, under these conditions, ethanol may affect the excretory function of the isolated liver.

Since alcoholic patients often have a fatty metamorphosis of the liver due to the effects of alcohol, malnutrition or both (2–4) the effect of fatty liver on the susceptibility to acute adverse effects of ethanol treatment seemed to warrant consideration. Ethionine was chosen to produce an acute fatty metamorphosis of the rat liver. While the apparent mechanism for the production of hepatic steatosis by ethionine differs from that assumed to be responsible for the ethanol-induced fatty liver, the hepatic lesion is that of fat accumulation accompanied by little or no necrosis (5). This report deals with the adverse effects of ethionine-induced fatty metamorphosis on hepatic function, measured by *ex vivo* perfusion and with the effects of perfusion of this fatty liver with ethanol.

Materials and Methods. Animals. Female, Sprague-Dawley rats (220–280 g) were kept under standard dietary and environmental conditions and used as liver donors. The animals were not allowed access to food following administration of ethionine, but were permitted to drink *ad lib.*

Perfusion. The method of liver perfusion and accompanying procedures and equipment have been described (1). The perfusion medium was wholly synthetic and consisted of Krebs–Henseleit buffer, pH: 7.4. It contained, per 100 ml, 2.5 g of bovine albumin (35% solution, Pentex Biochemicals), 240 mg of glucose, and 3,000 USP units of heparin (Liquaemin, Organon). Total perfusion volume was maintained at 70–80 ml.

Drugs. DL-Ethionine (99%, Pierce Chemical Co., Illinois) was dissolved in hot saline (25 mg/ml) and injected intraperitoneally in a concentration of 80 mg/100 g rat, body weight. The injections were given without anesthesia. Excision of livers and subsequent perfusions were carried out 17 hr after the administration of the drug.

Ethanol (absolute, USP) was perfused through livers in a concentration of 250 m*M*/liter. The ethanol was added after the first 30 min of equilibration, allowed in every experiment. The experimental design of the experiments is depicted in the figures that indicate the results.

The test dyes, sulphobromophthalein (BSP)[4] or indocyanine green (ICG)[4] were added to the medium 60 min after perfusion had been started, and the perfusion was then continued for an additional 45 min. BSP was added in a concentration of 10 mg/100 ml and ICG in a concentration of 2 mg/100 ml of perfusate. At intervals, samples of perfusate were drawn (0.2 ml) for determination of BSP concentration in the perfusate, while ICG removal rate by the liver was continuously recorded by a MD-40 densitometer adapted to an "ear piece" with a flow-thru

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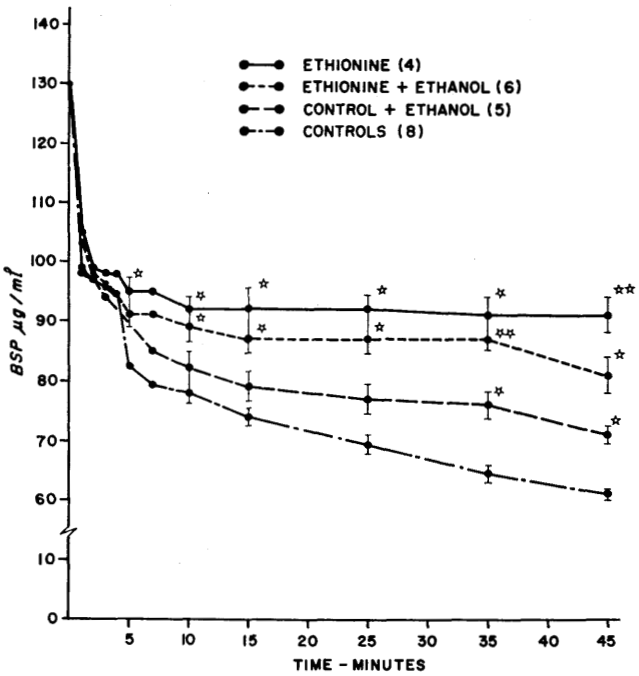

FIG. 1. BSP disappearance from the perfusate in control and ethionine pretreated preparations. Bars shown are SEM * significantly different from controls ($p<0.05$). Number of experiments is shown in brackets.

cuvette in the circuit.[5]

Bile was collected into 20-μl pipettes at intervals that were carefully timed.

The methods for chemical determinations of BSP in perfusate and bile and of ICG in bile have been described previously (1).

A total of 46 perfusions are reported here. At the end of every perfusion, aliquots of the perfusate were taken for determination of the activity of the following enzymes: glutamic–oxaloacetic transaminase (GOT), glutamic–pyruvic transaminase (GPT), and alkaline phosphatase (7).

Analysis of variance has been calculated according to Snedecor (6). Probabilities of 0.05 or less have been considered significant.

Results. The production of fatty metamorphosis of the liver, 17 hr after the injection of ethionine, was confirmed by light microscopy.

BSP removal. Perfused livers from ethionine-treated rats showed a marked reduction in the rate of removal of BSP from the perfusate (Fig. 1). The decrease in rate of removal became statistically significant by 5 min after addition of BSP, when compared to normal, control livers.

Ethanol, in a concentration of 250 m*M*/liter decreased the rate of BSP removal by the normal livers. The impairment was apparent by 35 min after introduction of the dye. Prior to this time, no significant changes in the slope of BSP removal was noted in this group. Perfusion with 250 m*M*/liter of ethanol of the ethionine-pretreated livers did not significantly change the pattern of BSP removal from that of *their* controls (ethionine alone).

Excretion of BSP in bile. A "normal" rate of BSP excretion in the bile ranged, in these experiments, from 0.3 mg/ml at 5 min to 5.9 mg/ml at 35 min (Fig. 2). In the ethionine-treated livers, the BSP biliary concentration was significantly reduced to a maximum of 1.5 mg/ml of bile at 35 min. Perfusion of ethionine-treated livers with ethanol did not lead to any further decrease in biliary BSP concentrations from those found in ethionine-treated controls. Ethanol alone decreased the

[5] The Waters Co., Division of Flotronics, Inc.

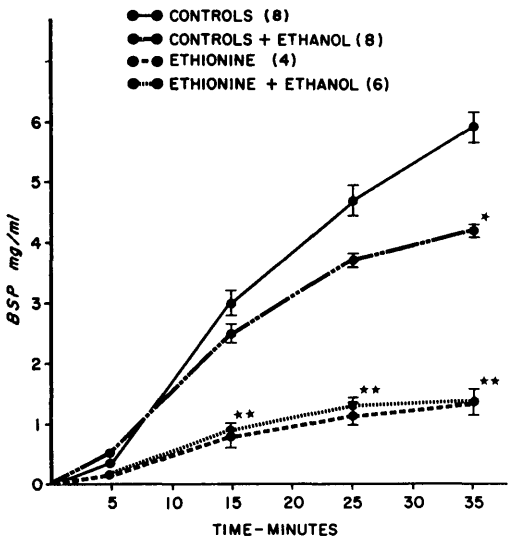

FIG. 2. Effect of ethionine and ethanol pretreatment on the biliary excretion of BSP. Bars represent SEM; *significantly different from controls ($p<$ 0.01). Number of experiments is shown in brackets.

concentration of BSP in the bile of normal livers, but the differences were not significant until 35 min after addition of the dye.

Bile excretion. Bile flow from control livers was maintained at 10.5 μl/min during the first hour of perfusion and decreased only after the addition of BSP (Fig. 3). Flow from the ethionine pretreated livers was significantly decreased by approximately 70% but it was not further depressed by perfusion with ethanol. Indeed, it was somewhat enhanced although still less than that of the control (no ethionine) livers perfused with ethanol. Perfusion of control livers with ethanol led to a moderate (30%), but significant ($p<0.01$) reduction in flow.

ICG removal and excretion in bile. The ICG removal rate from the perfusate was markedly decreased in ethionine-treated livers (Fig. 4). In control "normal" livers, removal of the dye was completed after 40 min of perfusion. A similar rate was found for normal livers to which ethanol was added, while in the ethionine-treated livers 35% of the dye still remained in the system at that time.

The excretion of ICG in the bile was reduced in the ethionine-treated livers (Table I). A maximal rate of excretion of 4.5 μg/ml was found in the normal livers at 35 min

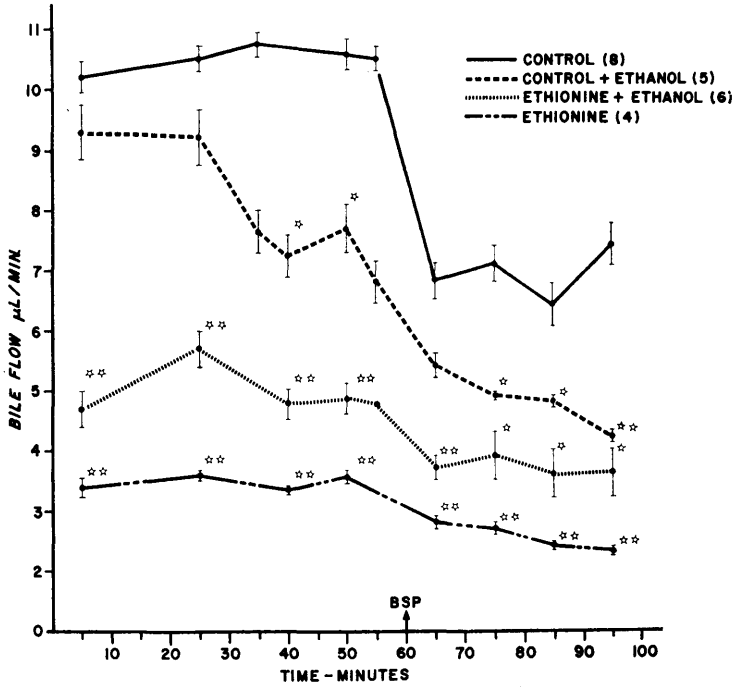

FIG. 3. Effect of ethionine and ethanol on the rate of bile flow excreted by perfused livers (μl $\pm$ SEM); significantly different from controls *($p<0.05$) and **($p<0.01$).

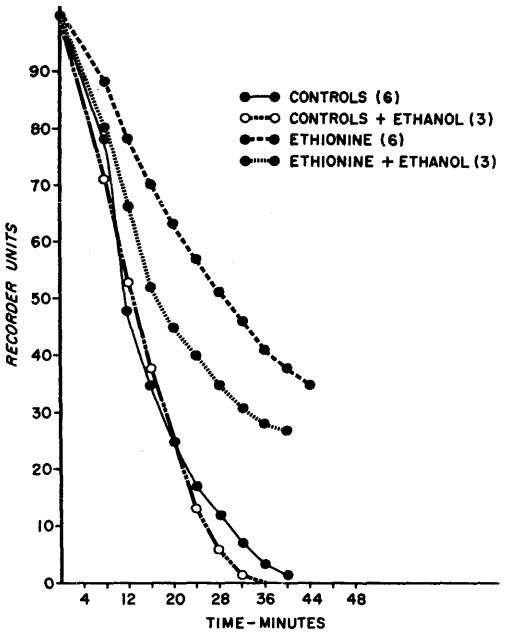

FIG. 4. Disappearance of ICG from the perfusate, expressed in recorder units. Number of experiments is shown in brackets.

after addition of the dye. In the ethionine group, however, ICG was found in a concentration of 1.4 μg/ml of bile. Perfusion of control livers with ethanol led to approximately the same degree of decrease in concentration of ICG in the bile. Perfusion of ethionine-pretreated livers with ethanol led to no greater decrease in ICG excretion than that induced by ethionine or ethanol alone.

Enzyme activity in perfusate. There were no measurable activities of the three enzymes studied in the buffer before perfusion. By the end of the perfusion period, the perfusate of the control livers contained measurable levels of GOT and GPT but virtually no alkaline phosphatase activity (Table II). Perfusion of the normal livers with ethanol led to an almost doubling of the levels of the transaminases. Ethionine-pretreated livers perfused without ethanol led to a threefold elevation of GOT and a fivefold elevation of GPT leakage. The perfusate of ethionine-pretreated livers in the presence of ethanol, had fourfold elevations of GOT values and fivefold elevations of GPT levels, *i.e.*, the perfusate content of GOT and GPT was approximately equal to the sum of the separate ethionine and ethanol effects. Alkaline phosphatase levels in the perfusates of the various preparations (ethionine, ethanol or both) were low (about 1.0 units) but significantly higher than those of the control group (0.2 units).

In order to ascertain more reliably the degree of enzyme leakage that could be attributed to the effect of ethanol, it seemed necessary to test the direct effect of ethanol on measurable enzyme activity. The results (Table III) demonstrate some degree of inhibition by ethanol of GOT and GPT but not of alkaline phosphatase activity.

Discussion. Perfusion *ex-vivo* of the rat liver, 17 hr after an *in vivo* dose of ethionine, demonstrated impairment of hepatic function. Removal of BSP and ICG from the perfusate was significantly reduced, especially during the "excretory" phase (8) as evident in the normal initial portion of the BSP removal curve but the distinctly abnormal second or "excretory" phase. Consistent with this interpretation are the data showing that the reduction in the flow of bile and the concentrations of BSP and ICG in the bile, was more striking than the reduction in removal of the dyes from the perfusate.

TABLE I. Effect of Ethionine and Ethanol on ICG Biliary Excretion. (μg/ml ± SEM).

Treatment	*n*	Minutes after dye addition			
		5	15	25	35
Control	6	1.5 ± 0.07	1.8 ± 0.08	2.5 ± 0.09	4.5 ± 0.1
Control and ethanol	3	0.6 ± 0.05[a]	0.7 ± 0.08[a]	1.0 ± 0.09[a]	1.5 ± 0.1 [a]
Ethionine	6	0.3 ± 0.1 [a]	0.6 ± 0.03[a]	1.3 ± 0.08[a]	1.4 ± 0.09[a]
Ethionine and ethanol	3	0.4 ± 0.1 [b]	0.5 ± 0.07[b]	0.9 ± 0.1 [b]	1.9 ± 0.1 [b]

[a] Significantly different from controls, $p < 0.005$.

[b] Significantly different from controls, but not from ethionine-treated "controls."

TABLE II. Activity of Enzymes in the Perfusate.

Treatment	*n*	GOT	GPT	Alkaline phosphatase
Controls, normals	8	26 ± 6	8 ± 1	0.2 ± 0.04
Ethanol, normals	5	41 ± 5	14 ± 3[a]	0.8 ± 0.1 [a]
Ethionine	4	78 ± 10[a]	42 ± 6[a]	1.2 ± 0.3 [a]
Ethanol, ethionine	6	110 ± 46[b]	52 ± 8[b]	1.0 ± 0.3 [b]

[a] SEM, significantly different from normal controls ($p < 0.01$).
[b] Significantly different from controls, but not from ethionine-treated "controls".

Presumably the initial portion of the curve of clearance of dye from the perfusate, reflects transport at the sinusoidal surface and the second main phase reflects excretion into the bile. Accordingly, it may be inferred that excretion into the canaliculus is impaired to a greater degree than transport into the hepatocyte, as the result of ethionine-produced injury. The mechanism by which parenchymal injury can lead to an excretory defect presumably relates to defective formation and/or excretion of bile salts (9, 10). Ethionine has been shown (11) to lead to impaired secretion of bile salts perhaps as the result of the rapid depletion of hepatic ATP (12, 13) and consequent defect in the active transport mechanisms.

Perfusion with ethanol led to no greater effect on the ethionine-produced fatty livers than on "normals". Apparently, this damaged liver shows no increased vulnerability to the previously demonstrated adverse effect of ethanol on bile excretion. Whether this lack of adverse effect of ethanol only reflects the fact that the ethionine impairment is already maximal, or whether the ethionine-damaged liver is unable to metabolize ethanol (assuming ethanol metabolites are responsible for its effect on bile secretion), is not deductible from the available data.

That ethanol can induce adverse effects in the liver already damaged by ethionine, however, is apparent in the observations on enzyme leakage into the perfusate. Perfusion of the "normal" livers with ethanol almost doubled leakage of GOT and GPT from the livers. The ethionine-pretreated livers showed a tripling of leakage values and the perfusion of the ethionine-pretreated livers with ethanol led to a fourfold (GOT) and fivefold (GPT) increases in enzyme leakage. This expression of hepatocyte injury suggests that the ethanol and ethionine effects were summated but not potentiated.

Summary. *In vivo* administration of ethionine to rats (17 hr before sacrifice) led to *in vitro* demonstrability of hepatic injury. The adverse effect was reflected in decreased flow of bile, impaired clearance of foreign dyes (BSP and ICG) by the perfused liver, and increased leakage of hepatic enzymes into the perfusate. Perfusion with ethanol led to no further worsening of bile flow or dye excretion but did enhance leakage of enzymes.

These observations indicate that, in the

TABLE III. Direct Effect of Ethanol on Enzyme Activity. (IU ± SEM).

Treatment	*n*	Perfusate[a]		Alkaline phosphatase
		GOT	GPT	
Control	4	49 ± 2	27 ± 9	0.2
Ethanol[b]	4	36 ± 1	20 ± .9	0.2
		Human Serum (# 368)		
Control	2	138	81	14.1
Ethanol[b]	2	106	42	15.8

[a] Obtained after 100 min of a "control" perfusion.
[b] Prior to incubation, 250 m*M*/liter of ethanol were added to the reaction vessel.

perfused liver, the adverse effect of ethionine on the hepatocyte can be reflected by impaired bile flow and dye excretion as well as by leakage of enzymes into the perfusate.

Perfusion with ethanol led to additional hepatic injury, evident in enhanced leakage of enzymes, but not demonstrable by additional impairment of bile flow, perhaps already maximally impaired by ethionine.

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