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COMPARISON OF HEPATIC TRIGLYCERIDE CONTENT AND HEPATIC LIPID SECRETION AFTER VARIOUS DOSES OF ETHANOL

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Abstract—The effect of ethanol (1, 2 and 4 mg/g i.v.) on hepatic triglyceride content, hepatic lipid release and plasma esterified fatty acid levels was investigated in mice. Within 4–8 hr all doses of ethanol increased the triglyceride content of the liver to about the same degree. Lipid secretion from the liver was inhibited 2 hr after the administration of 4 mg/g ethanol and increased 8 hr after the injection of 2 and 4 mg/g ethanol. Plasma esterified fatty acids showed an initial increase after 1 mg/g and an initial decrease after 4 mg/g ethanol. Eight hr after the administration of 4 mg/g ethanol the level of esterified fatty acids in the plasma was increased. Two mg/g ethanol did not have any effect on plasma esterified fatty acid levels. From the absence of a clear correlation of the three parameters tested it is concluded that inhibition of lipid secretion from the liver is not a major reason for the development of acute alcoholic fatty liver in mice.

In order to explain the deposition of triglycerides in the liver after ingestion of ethanol three main mechanisms have been discussed for several years: (1) increased uptake of fatty acids into the liver as result of increased adipose tissue lipolysis, (2) enhanced synthesis of fatty acids within the liver as a consequence of a shift in the redox state of the liver cell, and (3) a reduced rate of lipoprotein secretion from the liver into the blood [1]. However, agreement about the relative importance of each machanism could not be reached.

There was good reason to assume that ethanol might act by impairing the release of lipids from the liver, since a number of other hepatotoxic agents are known to raise the fat content of the liver by interfering with hepatic protein and lipoprotein synthesis [2–4]. Though measurements of plasma triglycerides and lipoproteins and of lipid secretion from the liver have already been made by several investigators the results were equivocal depending on the experimental conditions [4–13]. Therefore, having tried previously to evaluate the role of ethanol-induced adipose tissue lipolysis [14] and changes in the hepatic NAD/NADH-ratio [15] in the development of acute alcoholic fatty liver in mice, we felt justified in making a further attempt to assess the importance of hepatic lipid release in the development of acute alcoholic fatty liver. Our special aim was to compare the changes in the hepatic triglyceride content with changes in the levels of esterified fatty acids in the blood and in the rate of lipid secretion from the liver brought about in mice by various doses of ethanol. The experiments were designed to be comparable with our earlier experiments.

METHODS

Female NMRI-mice were kept at 25° and, unless stated otherwise, fed with standard diet (Altromin®) and tap water *ad lib.* before and during the experiment. The animals were treated acutely with ethanol (1, 2, and 4 mg/g i.v.) and sacrificed 2, 4, or 8 hr later by decapitation. Their livers were removed quickly and prepared for the determination of triglycerides according to the method of Eggstein and Kreutz [16]. Blood was collected for the estimation of esterified fatty acids by the method of Fried and Hoeflmayr [17]. Estimation of esterified fatty acids was preferred to the determination of plasma triglycerides since the former reflect better changes in plasma lipids. Earlier experiments [18] indicated that not only triglycerides but also cholesterol esters in the blood were affected by ethanol.

Lipid secretion from the liver to the blood was measured by the method of Byers and Friedman [19]. All mice were deprived of food for 8 hr before sacrifice. Six hr before sacrifice 0·5 mg/g Triton WR 1339 (oxyethylated tertiary octylphenol-formaldehyde polymer), as a 10% solution in saline, was given intravenously to inhibit the plasma lipid clearing system. According to Byers and Friedman, under these conditions the increase in the plasma level of esterified fatty acids which subsequently occurs is proportional to the rate of fatty acid release from the liver. Ethanol was given to Triton-treated animals at doses of 1, 2 and 4 mg/g, 2 or 8 hr before sacrifice.

The number of animals in each treatment group was 10 for liver triglycerides, 8–18 for plasma esterified fatty acids, and 15–20 for the lipid secretion experiments. Mean values ± S.E.M. are given in the figure and table. The significance of the changes was evaluated by the Student's *t*-test; $P \leqslant 0{\cdot}05$ was regarded as significant.

RESULTS AND DISCUSSION

Ethanol caused the well known long-lasting increase in hepatic triglycerides which was dose-dependent only in its initial stage. Later similar triglyceride levels were reached independent of the dose of ethanol (Fig. 1). The level of esterified fatty acids in the plasma was transiently elevated by 1 mg/g ethanol

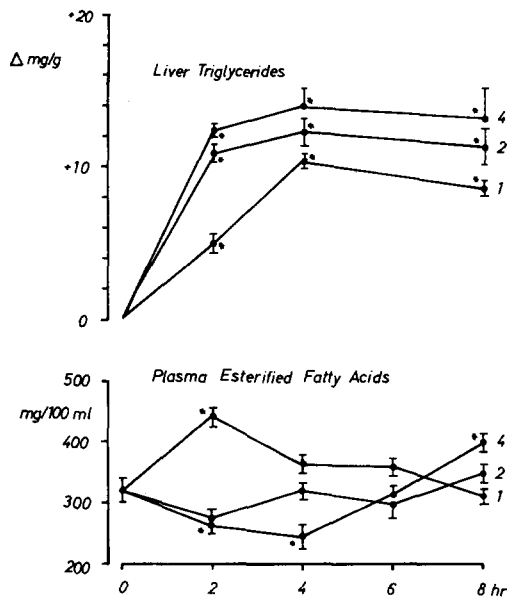

Fig. 1. Plasma esterified fatty acids and increase in liver triglyceride content of mice treated with various doses of ethanol (1, 2 and 4 mg/g, i.v.). Mean values ± S.E.M. are given. * Significantly different from the control value, $P \leq 0.05$.

and temporarily decreased by 4 mg/g ethanol. No significant changes occurred with the intermediate dose of ethanol (Fig. 1). These results closely resemble those obtained earlier in rats, except that the effects seem to be more short-lived [5, 18].

The increase in the plasma esterified fatty acids after 1 mg/g ethanol corresponds in time with the delay in the accumulation of hepatic triglycerides and one might assume that the higher plasma level of esterified fatty acids results from an increased secretion of lipids from the liver. The measurement of lipid secretion shows that with the lower alcohol doses the capacity of the liver to dispose of excess fat is indeed less seriously affected than with the higher dose, but no increase of lipid release could be demonstrated with this method (Table 1). Therefore, the possibility that ethanol interferes also with the lipid clearing capacity of the blood must be considered.

With 4 mg/g ethanol a slight but significant inhibition of the lipid secretion from the liver was observed initially, and this effect may well be the reason for the decline in the level of plasma esterified fatty acids (Fig. 1, Table 1). On the other hand, there is no strict correlation between the extent of the accumulation of triglycerides in the liver caused by the different ethanol doses and the rate of lipid release. The diminished secretion of esterified fatty acids seen after 4 mg/g ethanol cannot possibly account for the total amount of triglycerides accumulated in the livers of the animals. It may be responsible for the additional increase of the hepatic triglyceride content compared with that in mice treated with 2 mg/g ethanol.

The reduction of lipid secretion is only a temporary effect (Table 1). Eight hr after the administration of 2 or 4 mg/g ethanol the release of esterified fatty acids from the liver was significantly enhanced. Again there is no relationship between the rate of lipid secretion and the triglyceride content of the liver. A casual connection may, however, exist between the changes in lipid secretion and the esterified fatty acid level in the blood which in mice treated with 2 or 4 mg/g ethanol rose significantly between 2 and 8 hr. (Fig. 1, Table 1).

In summary the present results demonstrate that there are no close correlations between the rate of hepatic lipid secretion, plasma esterified fatty acid levels and triglyceride content of the liver. Hence, at least in mice, changes in the triglyceride release which may occur in the livers of ethanol-treated animals are not a major cause of ethanol-induced hepatic steatosis. This is in good agreement with other results from this and other laboratories which point out the effect of flooding the liver with extrahepatic fatty acids originating from adipose tissue lipolysis caused by ethanol [14, 15, 20, 21]. Furthermore, the experiments show that the incongruous results reported in the literature may well be explained by differences in the dose of ethanol used and the time point at which lipid release was measured.

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Table 1. Effect of ethanol on plasma esterified fatty acids in mice treated with Triton WR 1339

Treatment				Results	
Triton WR 1339 (0·5 mg/g i.v.) Time (hr)	Saline Time (hr)	Ethanol Time (hr)	Ethanol Dose (mg/g i.v.)	Esterified fatty acids in plasma (mg/100 ml)	Number of animals
—	—	—	—	337 ± 22	15
6	2	—	—	1286 ± 55	15
6	—	2	1	1160 ± 90	18
6	—	2	2	1228 ± 86	17
6	—	2	4	1064 ± 51*	15
6	8	—	—	1276 ± 62	20
6	—	8	1	1260 ± 65	20
6	—	8	2	1510 ± 78*	20
6	—	8	4	1605 ± 87*	19

Mean values ± S.E.M. are given.
* Significantly different from the saline-treated control, $P \leq 0.05$.

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