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Reduced serum very low-density lipoprotein levels after acute ethanol administration

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CONSIDERABLE doubt persists as to the mechanism of the acute ethanol-induced fatty liver.[1] Though the action of a wide variety of agents producing fatty liver is now believed to be mediated via interference with the release of triglycerides from the liver into the circulation,[2] evidence against this concept in the acute ethanol-induced fatty liver has been furnished by several workers,[3–5] while others have presented results which favor the concept.[6, 7] The work to be reported here was therefore undertaken to clarify current discrepancies. Part of the evidence showing that the hepatotoxins, carbon tetrachloride and ethionine, inhibit hepatic secretion of triglycerides was the demonstration of a lowered level in the serum of very low-density (VLD) lipoproteins (d < 1·019), the form in which triglycerides are transported out of the liver.[8, 9] Determinations of serum VLD lipoprotein levels after acute ethanol administration have similarly been performed as an index of the effect of ethanol on hepatic triglyceride transport.

Adult, male Sprague–Dawley rats (250–350 g) were used. The animals were maintained prior to the experiments on an adequate rat pellet diet. Four hr before dosage, food was removed from all cages. After dosage, the animals were deprived of drinking water to avoid the possibility of a differential water intake between groups. The 12 ethanol-treated animals received by stomach tube 6 g/kg of ethanol as a 50% (v/v) aqueous solution, which also contained 0·9% (w/v) NaCl. The 12 controls received a corresponding volume of 0·9% NaCl (1·5 ml/100 g body weight). All doses were given under light ether anaesthesia. Four hr later, the animals were killed by severing neck vessels under light ether anaesthesia and blood was collected. After standing at room temperature, the samples were centrifuged to obtain serum. For separation of VLD lipoproteins, 1·5-ml aliquots were pooled from the serum samples of 2 control or 2 treated rats. The density of the pooled samples was raised to 1·019 as described by Havel *et al.*[10] Isolation of the serum fractions containing the VLD lipoproteins was achieved by ultracentrifugation.[8] Determinations of protein[11] and total lipid[12] content were made on aliquots from each fraction. For the protein analysis, the lipoproteins were first precipitated by the method of Burstein[13] followed by delipidation with $CHCl_3$: CH_3OH (2:1, v/v) and ether. Total lipid was extracted and purified according to Folch *et al.*[14] Lipoprotein levels were calculated from the sum of the levels of the protein and lipid moieties.

In Table 1 it is shown that a significant fall in the level of serum VLD lipoproteins occurred 4 hr after the administration of an acute dose of ethanol. The decrease was observed in both the protein

TABLE 1. SERUM VERY LOW-DENSITY LIPOPROTEIN LEVELS 4-HR AFTER ACUTE ETHANOL ADMINISTRATION*

Parameter	Control animals	Ethanol-treated	Significance† (P value)	Per cent change from controls
Protein	2·41 ± 0·20	1·47 ± 0·09	<0·01	−39
Lipid	34·0 ± 2·5	25·9 ± 2·1	<0·05	−24
Lipoprotein	36·41 ± 2·64	27·37 ± 2·18	<0·05	−25

* Levels are expressed as mg/100 ml serum. The data shown in each case are means ± S.E.M. for 6 determinations.

† P values were calculated by using Student's *t*-test.

and lipid moieties of the lipoproteins, though to a greater extent in the former. It is noteworthy that a similar more pronounced reduction in the level of the protein moiety of the serum VLD lipoproteins has been demonstrated after carbon tetrachloride treatment.[8] The results are compatible with those of Dajani and Kouyoumjian,[7] who found that 3·5 hr after acute ethanol treatment serum triglyceride levels were decreased about 33 per cent. Other workers[15] failed to show an effect of acute ethanol administration on incorporation of labeled amino acid into serum low-density (< 1·063) lipoprotein.

Nevertheless, a specific effect on the critical VLD fraction may have been overlooked. On the other hand, it was recently reported that the ethanol-induced increase in liver lipid was associated with a decrease in the level of serum high-density lipoproteins.[16] These results, together with those presented in Table 1, are indicative of a nonspecific disturbance of hepatic lipoprotein secretion by ethanol.

If a causal role in the ethanol-induced accumulation of liver triglycerides is to be attributed to decreased formation or release (or both) of hepatic lipoproteins, the reduction in the level of serum VLD lipropoteins must be present prior to the rise in liver triglycerides, as in the case of carbon tetrachloride.[8] Such a role may now be postulated because experiments in this laboratory and elsewhere[3, 5] have shown that an increase in liver triglyceride levels does not begin until 6–8 hr after administration of the standard dose of ethanol.

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Determination of rat liver hexobarbital oxidase activity with a gaschromatographic method

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IN TOXICITY experiments the determination of the so called "drug metabolizing enzymes" or "processing enzymes"[1] becomes more and more of importance since it was demonstrated that many drugs and insecticides have the capacity to stimulate this type of microsomal enzymes.[2,3] One of the determinations, which are frequently used for this purpose, is the hexobarbital oxidase activity of liver microsomal fraction. In most experiments the non-metabolized hexobarbital after incubation is determined according to Cooper and Brodie.[4] This requires a time consuming extraction procedure after which the hexobarbital is measured by u.v. spectrophotometry.