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INHIBITION OF RELEASE OF HEPATIC TRIGLYCERIDE BY ETHANOL--A REAPPRAISAL

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It has been demonstrated that administration of an adequate dose of ethanol to a rat leads to accumulation of triglycerides in the liver. Although ethanol can produce a variety of alterations, many of which may contribute to triglyceride accumulation, there is lack of agreement on the precise sequence of events leading to the hepatic steatosis[1]. One possible explanation of the ethanol-induced increase in hepatic triglycerides is a decrease in the movement of liver triglycerides into the serum lipoprotein pool. The proposition has been tested in the past, but the reported results are conflicting. Two approaches have been used to try to answer this question. The first involves examination of the effect of ethanol on concentration of serum triglyceride or of specific serum lipoproteins. The inherent weakness in such studies in vivo is that the measurements reflect a net balance between entrance and exit of triglycerides from the plasma compartment. The second approach avoids the complication of the exit of triglycerides from the plasma compartment by the use of a perfused liver, or by the intravenous injection of Triton WR 1339. This agent is presumed to prevent the action of clearing factor lipase on plasma lipoproteins[2]. As a result the plasma triglyceride level increases linearly for an appreciable period after Triton administration. The rate of increase has been used as a measure of the rate of release of triglycerides by the liver. This latter technique appears to be reasonably accurate in the case of normal starved rats as the removal rate of serum triglycerides in the

presence of Triton is only 5-10% of that in its absence[2,3]. Although some published data support the concept of an impairment of the release of liver triglycerides by ethanol[4-7], most of the available information does not support this thesis[8-14]. The problem in resolving such discrepant findings stems from variations in the parameters measured and the experimental conditions employed. One important omission from these studies has been an analysis of the temporal relationship between the concentration of plasma ethanol, the accumulation of liver triglyceride, and the release of liver triglyceride. The purpose of this paper is to provide such information and, in particular, to reexamine the key question of whether ethanol administration does affect the rate of release of triglyceride from the liver. Our data clearly indicate that one of the primary effects of ethanol may be on the release process.

Male Sprague-Dawley rats were acclimated for one week to a 12h light-dark cycle and daily handling. They were fed Purina rat pellets and starved 48h prior to use (200 gm final wt.). The dosage of Triton WR 1339 was 100 mg/100 g body weight administered intravenously as a solution (37°) containing 200 mg/ml 0.9% NaCl. Ethanol was injected intraperitoneally as a 20% solution (37°) in water. The dose of ethanol was 2 g/kg, and control animals received an equal volume of water. Identical results were obtained when the ethanol was diluted with 0.9% NaCl, and the control animals received an equal volume of 0.9% NaCl. All intravenous injections and the collection of blood samples were made with unanesthetized animals held in restraining cages to avoid complications introduced by ether anesthesia[3,15]. In order to facilitate intravenous injection into the tail vein, and the collection of blood samples into unheparinized capillary tubes from a cut in a tail vein, the animals were warmed for a few minutes with an infrared lamp. The blood was allowed to clot for 30 min and the serum separated in an International Model MB centrifuge. Serum triglyceride was measured by the method of Soloni (16). Liver triglyceride was determined in the following manner. At the designated time, the animal was killed by decapitation. Following cessation of bleeding, the liver was re-

moved in toto, washed in saline, blotted dry, and frozen in liquid nitrogen. The entire operation took about 45-60s. The livers were stored at -80° for a maximum of 24h. A sample was weighed and extracted by the method of Folch et al.[17], and an aliquot of the chloroform phase assayed by the method of Soloni[16].

The temporal relationship between the concentration of plasma ethanol and liver triglyceride is shown in Fig. 1. Each point represents the mean ± S.E.M. obtained with 20 rats. The use of intraperitoneal injection as the route of administration of ethanol yielded highly reproducible results in all of the parameters measured.

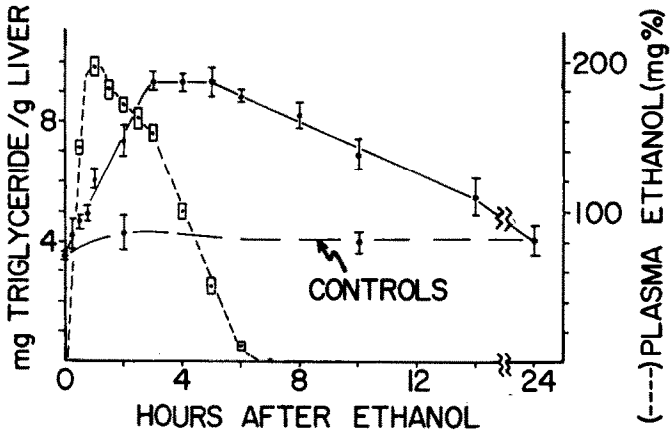

Fig. 1. Temporal relationship between plasma ethanol and liver triglyceride.

The injection of Triton into control rats causes a linear increase in serum triglyceride over the 90 min test period[2,3]. When this same measurement was repeated with animals that received ethanol 2h prior to the Triton injection, the slope of the line displaying the Triton-induced hyperlipidemia was markedly decreased (Fig. 2). The data (mean ± S.E.M.) were obtained with 25 rats (control) and 10 rats (+ ethanol) respectively. The relationship between a series of rates of Triton-induced hyperlipidemias as a function of time after ethanol administration is shown in Fig. 3. The abscissa is the time after ethanol administration that Triton was injected. The rate of Triton-induced hyperlipidemia is plotted on the ordinate at the time corresponding

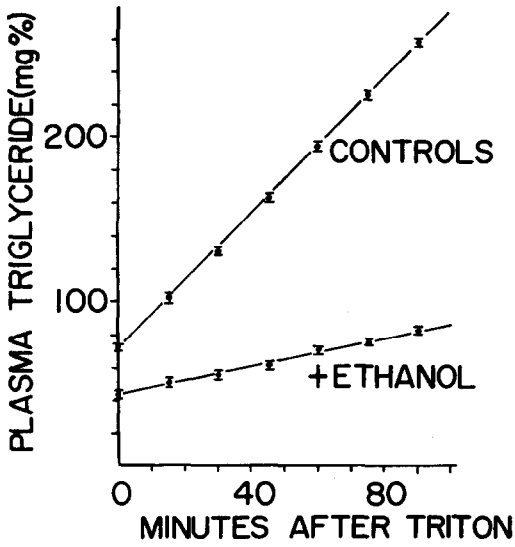

Fig. 2. Rate of Triton-induced hyperlipidemia. Triton was administered 2h after ethanol.

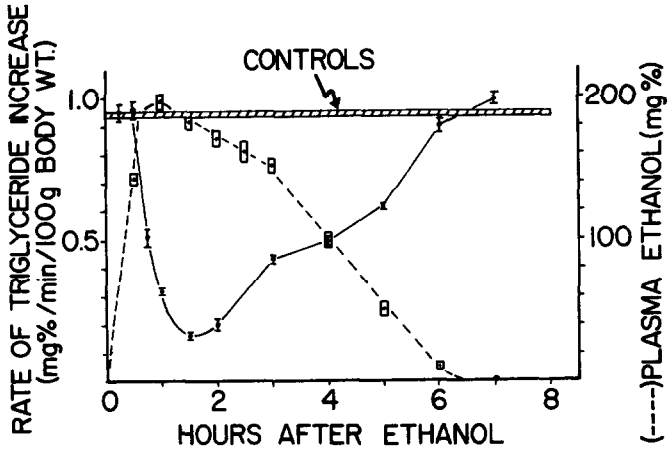

Fig. 3. Temporal relationship between plasma ethanol and Triton-induced hyperlipidemia. The horizontal dashed bar shows mean ± S.E.M. of post-Triton hyperlipidemia in 50 control rats.

to the Triton injection, although each rate was determined for the full 90 min time after Triton administration. Each point on the curve represents the mean ± S.E.M. obtained with 6 rats. It should be noted that there is a significant lag period (about 30 min) that occurs during the time the plasma ethanol concentration is markedly increasing. It is also of interest that the depression of the rate of Triton-induced hyperlipidemia persists until the

ethanol is removed from the blood; and except for the lag period, the degree of inhibition roughly parallels the change in concentration of plasma ethanol. To the best of our knowledge, the data shown in Fig. 3 is the first detailed analysis of this relationship. One obvious conclusion is that ethanol inhibits the movement of triglyceride out of the liver and that this inhibition may be related to plasma ethanol concentration. Although alternative explanations are possible and are being evaluated, the data are sufficient to warrant reappraisal of current views regarding ethanol induced hepatic steatosis.

Acknowledgment

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