SHORT COMMUNICATION

Accentuation of ethanol-induced fatty liver by phenobarbitone

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Summary
1. Four groups of rats received a liquid formula diet, given alone (control), or supplemented with either ethanol or phenobarbitone, or both together.
2. The ethanol-treated group showed a fall in plasma triglyceride and a rise in liver triglyceride, as compared with the control rats.
3. The phenobarbitone-treated group showed a marked rise in plasma triglyceride but not in liver triglyceride.
4. The group receiving both ethanol and phenobarbitone also showed a rise in plasma triglyceride but this was accompanied by a striking increase in liver triglyceride.
5. These results suggest that ethanol and phenobarbitone each stimulate triglyceride synthesis but that in addition ethanol has an inhibitory effect on hepatic triglyceride secretion.

Key words: cholesterol, ethanol, microsomes, liver, enzyme induction, phenobarbitone, triglycerides.

Introduction
Acute or chronic administration of ethanol to rats causes a marked increase in liver triglyceride content (DeCarli & Lieber, 1967; Johnson, Hernell & Olivecrona, 1975). Chronic administration of ethanol to humans has a similar effect, even in the presence of a high-protein, low-fat diet (Lieber & Rubin, 1968). It was of considerable interest, therefore, when it was reported that the development of acute, ethanol-induced fatty liver in rats could be prevented by pretreatment with phenobarbitone (Koff, Carter, Lui & Isselbacher, 1970). The object of the present study was to examine further this apparent protective action by investigating the effects of long-term, concomitant administration of phenobarbitone and ethanol to rats.

Materials and methods
Four groups each of eight male Wistar rats (Carworth Europe) were allocated as follows: group A, control group, basal diet; group B, ethanol-fed; group C, basal diet plus phenobarbitone; group D, ethanol plus phenobarbitone (this group was eventually reduced to six rats, owing to two deaths 10 days after commencing ethanol).

Measurement of sleeping time after intraperitoneal pentobarbitone (30 mg/kg body weight) was carried out to confirm that liver microsomal enzyme induction had occurred in phenobarbitone-fed rats. At the end of the study, the animals were guillotined and blood samples collected. The livers were removed immediately, weighed, and aliquots extracted
with chloroform/methanol (Folch, Lees & Sloane-Stanley, 1957). Plasma and liver triglycerides and cholesterol were estimated respectively by a semi-automated fluorimetric method (Technicon N.78; Cramp & Robertson, 1968) and by the ferric chloride/sulphuric acid colour reaction (Technicon N.24a; based on Levine & Zak, 1964).

Rats have an aversion to alcohol in drinking water, and we therefore used liquid formula diets, based on those described by DeCarli & Lieber (1967). The basal diet was made up in emulsion form to contain 4.184 kJ/ml, of which casein accounted for 18%, olive and sunflower oils for 35% and sucrose for 47% of the caloric content. Both ethanol-fed groups received an isocaloric diet with the same proportions of casein and vegetable oils, but ethanol was progressively substituted for sucrose until the final diet contained 11% of calories as sucrose and 36% as ethanol. Vitamins and minerals were added to both diets and phenobarbitone [0.5 mg/d (2.16 pmol/ml)], was added to the diet of the group C and group D rats. Feeding was continued for 22 days after the full dietary concentration of ethanol was reached. Feeding bottles were removed 5 h before the animals were killed.

Analysis of variance and Student's t-test were used for statistical comparison of the results.

Results

The lower body weight of both ethanol-fed groups (group B and group D) was presumably due to an overall reduction in their caloric intake, which occurred almost entirely during the first week of feeding (Table 1). Liver weight, expressed as a percentage of body weight, was markedly increased in both phenobarbitone-treated groups, especially the group that also received ethanol. Confirmation of enzyme-induction was obtained by the failure of phenobarbitone-fed rats to sleep after receiving intraperitoneal pentobarbitone.

Plasma triglyceride concentrations were significantly increased in rats given phenobarbitone alone (P<0.01) or phenobarbitone plus ethanol (P<0.02) but not in those given ethanol alone, in whom there was a significant fall as compared with control rats (P<0.005). Plasma cholesterol concentrations rose significantly in all three test groups (P<0.001).

Liver triglyceride concentrations rose in rats given ethanol alone (P<0.005) but not in those given phenobarbitone alone. A striking increase in liver triglyceride, however, occurred in rats given both ethanol and phenobarbitone (P<0.001). Parallel changes occurred in liver cholesterol.

Discussion

Normally a significant proportion of the free fatty acids taken up by the liver undergo oxidation, the remainder being mainly incorporated into triglyceride (Scow & Chernick, 1970). Ethanol exerts a sparing effect on the former process and thus increases the amount of free fatty acids available for triglyceride synthesis (Baraona & Lieber, 1970; Baraona, Pirola & Lieber, 1973; Johnson et al., 1975). This leads to an increased concentration of very-low-density lipoprotein in plasma and thus to hypertriglyceridaemia (Wilson, Schreibman, Brewster & Arky, 1970; Chait, Mancini, February & Lewis, 1972; Avogaro & Cazzolato, 1975). However, should the rate of synthesis and secretion of very-low-density lipoprotein into plasma fail to keep pace with the increased

<table>
<thead>
<tr>
<th>Group</th>
<th>Food intake (kJ per rat/day)</th>
<th>Body wt. (g)</th>
<th>Liver wt. (% body wt.)</th>
<th>Plasma triglyceride (μmol/l)</th>
<th>Plasma cholesterol (μmol/l)</th>
<th>Liver triglyceride (mg/g)</th>
<th>Liver cholesterol (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 8)</td>
<td>210±4</td>
<td>169±2</td>
<td>4.5±0.01</td>
<td>1240±120</td>
<td>2000±100</td>
<td>17.8±0.6</td>
<td>3.5±0.1</td>
</tr>
<tr>
<td>B (n = 8)</td>
<td>182±4</td>
<td>137±8</td>
<td>4.7±0.1</td>
<td>730±50</td>
<td>3470±210</td>
<td>24.2±1.5</td>
<td>5.3±0.4</td>
</tr>
<tr>
<td>C (n = 8)</td>
<td>206±7</td>
<td>165±8</td>
<td>7.0±0.2</td>
<td>3240±290</td>
<td>3730±160</td>
<td>18.4±5.0</td>
<td>4.1±0.3</td>
</tr>
<tr>
<td>D (n = 6)</td>
<td>171±1</td>
<td>136±4</td>
<td>9.1±0.4</td>
<td>2880±770</td>
<td>3320±210</td>
<td>113±11.2</td>
<td>10.7±1.3</td>
</tr>
</tbody>
</table>
synthesis of hepatic triglycerides, these will tend to accumulate (Hawkins & Kalant, 1972). We found the expected increase in liver triglyceride in rats given ethanol, but this was accompanied by a fall rather than a rise in plasma triglyceride. It is possible that the lesser weight gain of ethanol-fed rats tended to counteract hypertriglyceridaemia by increasing the catabolism of circulating triglyceride, as occurs in obese human subjects during periods of negative caloric balance (Lewis, Mancini, Mattock, Chait & Fraser, 1972). Alternatively, the anomalous decrease in plasma triglyceride, which has also been reported in humans taking large amounts of ethanol (Schapiro, Drummey, Scheig, Mendelson & Isselbacher, 1963), could have been due to inhibition of secretion of very-low-density lipoprotein (Schapiro, Drummey, Shimizu & Isselbacher, 1964).

Elevation of plasma triglyceride, due to an increase in very-low-density lipoprotein, and of plasma cholesterol, due to an increase in low-density lipoprotein, have been described in healthy subjects given phenobarbitone (Miller & Nestel, 1973; Durrington, Roberts, Jackson, Branch & Hartog, 1976). We found that similar increases in plasma cholesterol and triglyceride occurred in our phenobarbitone-fed rats. The hypercholesterolaemia presumably reflected the increased hepatic synthesis of cholesterol which accompanies phenobarbitone-induced hypotrophy of the smooth endoplasmic reticulum (Jones & Armstrong, 1965). Unlike ethanol, administration of phenobarbitone alone did not cause any increase in liver triglyceride concentration, but when ethanol and phenobarbitone were given together, both the concentration and total amount of triglyceride in the liver increased fivefold. This observation does not support the concept that barbiturates are capable of counteracting the ability of ethanol to produce a fatty liver (Koff et al., 1970; Lieber, 1974), but suggests the opposite.

The synergistic effect of ethanol and phenobarbitone on the accumulation of hepatic triglyceride suggests that both drugs stimulate triglyceride synthesis. In addition, ethanol has been shown to decrease the elimination of phenobarbitone from the blood by impairing its rate of hydroxylation, thus maintaining an increased concentration of phenobarbitone in the liver (Coldwell, Paul & Thomas, 1973). However, the increase in liver triglyceride and absence of hypertriglyceridaemia in the rats receiving only ethanol contrasts with the hypertriglyceridaemia and absence of fatty liver in those receiving phenobarbitone alone. These findings support the concept that ethanol exerts an additional, inhibitory effect on secretion of very-low-density lipoprotein, as originally proposed by Schapiro et al. (1964). Thus the striking increase in liver triglyceride in rats given both ethanol and phenobarbitone was presumably due to a combination of increased synthesis and reduced secretion of triglyceride.

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