DEPOT FAT AS SOURCE OF INCREASED LIVER TRIGLYCERIDES AFTER ETHANOL

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A number of studies have shown that the administration of single large doses of ethanol to the rat causes a fatty liver and that the increase in lipid consists almost entirely of neutral fat (Mallov and Bloch, 1956; di Luzio, 1958; Maling et al, 1960). The accumulation of triglycerides has been attributed to a stimulatory effect of ethanol on the biosynthesis of fatty acids by the liver (Lieber et al, 1959). Another possibility is that the increase in liver triglyceride content results from the mobilization of depot fat.

As a new approach to this problem, we have applied the technique of gas chromatography to the assay of linoleic acid in liver and adipose tissue triglycerides. Advantage was taken of the presence in depot lipids of large amounts of linoleic acid. Since this acid is not synthesized by the rat, it can serve as a convenient non-radioactive tracer to determine if fatty acids are synthesized in liver or are transported from fat depots. The results indicate that adipose tissue is the main source of fatty acids in triglycerides deposited in liver after ingestion of a single dose of ethanol.

METHODS

Female, Sprague-Dawley rats (170 to 200 g), fasted 16 to 24 hours, were given 4.8 g/kg of ethanol in water (50% v/v, by stomach tube). Eighteen hours later, the rats were killed by intraperi-

toneal injection of pentobarbital. The livers were rapidly removed, homogenized with 2 volumes of water, and extracted with 20 volumes of ethanol-ether (3:1). The ethanol-ether extract was filtered, evaporated to dryness, and the residual lipid was dissolved in a small volume of benzene. Total lipids were determined by evaporating an aliquot of the benzene extract and weighing the residue. The triglyceride and phospholipid fractions in the remaining extract were separated by silicic acid chromatography and weighed (Horning et al, 1960). Ten to 20 mg of triglycerides were transesterified by the methanol-sulfuric procedure, and the methyl esters were analyzed by gas chromatography.

Samples of abdominal adipose tissue were extracted with ethanol-ether. The lipids were transesterified, and the methyl esters were analyzed by gas chromatography, without prior silicic acid chromatography, since adipose tissue lipid is almost entirely triglyceride (Miller and Cooper, 1958).

RESULTS AND DISCUSSION

The oral administration of ethanol increased the liver triglycerides from 6.9 mg/g to 24.1 mg/g in 18 hours. A considerable proportion of the fatty acids in the deposited triglycerides was found to be linoleic acid (Table I). Calculation of the percentage of linoleic acid in the newly laid down triglycerides gave a value almost identical with that in depot fat (Table II).

To ensure that ethanol had not induced the synthesis of linoleic acid in liver, fatty acids in tissue lipids were prelabeled by injecting 2-C^{14}-acetate 24 hours before administration of ethanol. The fatty acid esters derived from liver triglycerides were analyzed by gas chromatography, and the radioactivity of the effluent esters was measured according to the procedure of Karmen and Tritch (1960). From the mass and

Table I

Linoleic Acid Composition of Liver Triglycerides and Phospholipids in the Control and Ethanol-Treated Rats. Values Are Expressed as Means ± S.E.

LIVER ANALYSES	CONTROL	ETHANOL-TREATED
Liver weights (g)	6.4 ± 0.7	7.5 ± 0.3
TRIGLYCERIDES (6 rats each group)		
mg/g liver	6.9 ± 1.8	24.1 ± 5.7
% C-18-2 (linoleic acid)	29.2 ± 1.2	25.8 ± 0.5
mg/g liver of C-18-2 (linoleic acid)	2.0 ± 0.6	6.1 ± 1.4
PHOSPHOLIPIDS (4 rats each group)		
mg/g liver	35.2 ± 1.0	31.9 ± 1.7
% C-18-2 (linoleic acid) in PL fatty acids	11.6 ± 0.6	15.4 ± 0.8
mg/g liver of C-18-2 (linoleic acid)	2.8 ± 0.1	3.2 ± 0.3

radioactivity record it is evident that all the fatty acids except linoleic were labeled (Fig. 1).

We also examined the possibility that the linoleic acid in liver triglycerides had been transferred enzymatically from liver phospholipids. The gas chromatographic analyses in Table I show almost no difference in the proportion of linoleic acid in phospholipids in control and ethanol-treated rats. Indeed, there was not enough linoleic acid present in the total liver phospholipids to account for the increased amount in the liver triglycerides.

Further evidence that the fatty acids in the deposited triglycerides came from adipose tissue was the finding that the proportion of oleic acid was higher than in control liver triglycerides, but the same as in adipose tissue (Table II).

Table II

The Fatty Acid Composition of Liver and Adipose Tissue Triglycerides in the Control and Ethanol-Treated Rats. Values Are Expressed as Means ± S.E.

Fatty Acid	Fatty Acid in Triglycerides			
	Controls (6 rats)	Ethanol-treated (6 rats)	Calculated* in deposited triglycerides	Adipose Tissue (12 rats)
	% ± S.E.	% ± S.E.	%	% ± S.E.
Palmitic	29.1 ± 0.8	28.1 ± 1.4	27.2	25.3 ± 0.5
Oleic	29.3 ± 1.3	34.6 ± 0.9	38.2	38.1 ± 0.5
Linoleic	29.2 ± 1.2	25.8 ± 0.5	23.3	22.5 ± 0.3

* From the following equation, the percent fatty acid in the triglycerides deposited in the liver was calculated for each treated rat and averaged for the 6 rats:

$$y = \frac{AB - 6.9\,C}{A - 6.9} = \text{percent fatty acid in deposited triglycerides,}$$

where A = total liver triglycerides in mg/g after ethanol,

B = percent fatty acid in total liver triglycerides after ethanol,

C = average percent fatty acid in 6 control rats (Table II), and the value of 6.9 (mg/g) is the mean liver triglycerides in 6 control rats (Table I).

In separate experiments it was observed that a marked elevation of free fatty acids in the blood occurred after a single large dose of ethanol (Brodie et al, 1960). This is additional support for the view that the depot fat is the origin of the deposited triglycerides.

It may be concluded that depot fat is the primary source of the fatty acids in liver triglycerides deposited after ethanol.

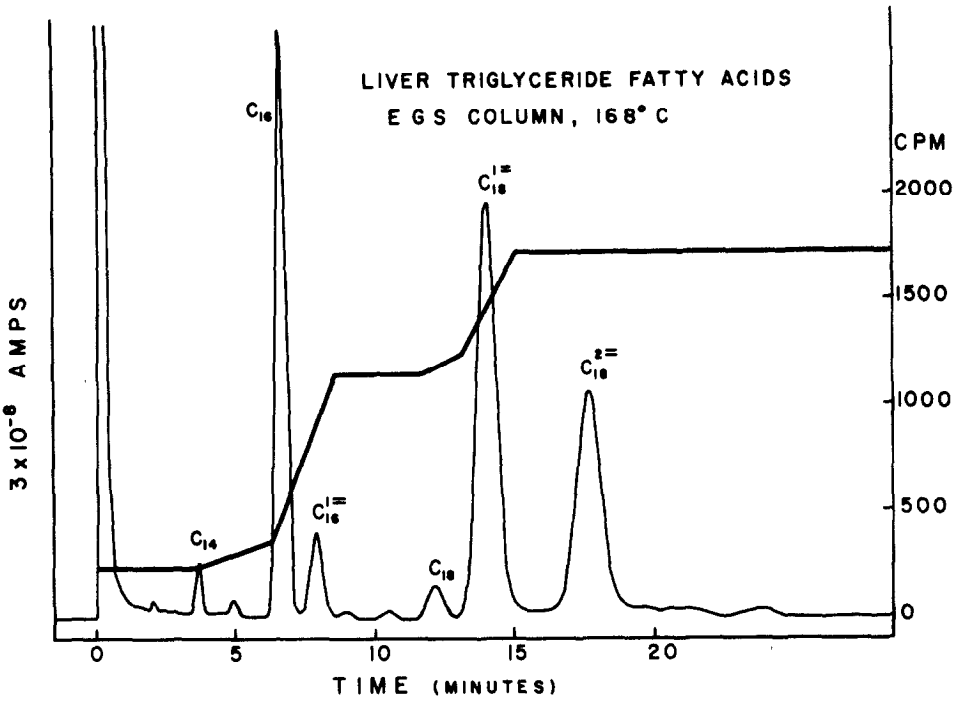

Fig. 1. Gas chromatographic analysis of rat liver triglycerides. The heavy line is the integral record of the radioactivity of the effluent peaks. An ethylene glycol-succinate column (6 ft., 4 mm. I.D., U-tube) was used at 198° C. with an argon detector.

However, these observations do not rule out the possibility that ethanol also causes enhanced biosynthesis of fatty acids in liver.

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