Changes in fatty acid composition of liver lipids induced by carbon tetrachloride and ethionine

A number of studies have shown that the administration of single large doses of CCl_4, ethionine or ethanol to the rat causes a fatty liver, and that the increase in lipid consists almost entirely of triglycerides[1–3].

In previous studies in this laboratory[4] gas chromatography has been applied to the assay of linoleic acid in liver and adipose triglycerides. Since this acid is not synthesized by the rat, it serves as a tracer to determine if fatty acids in liver triglycerides are formed in liver or are transported from fat depots. From the results obtained[4], it was concluded that adipose tissue is the major source of fatty acids deposited in liver triglycerides after ethanol administration.

The present studies show that adipose tissue is also the main source of fatty acids in the triglycerides deposited in liver by single doses of CCl_4 and ethionine. In addition, CCl_4 and ethionine alter the fatty acid composition of the liver phospholipids: ethionine decreases the content of palmitic acid, and CCl_4 the content of arachidonic acid.

Experiments were carried out with female Sprague – Dawley rats (175 – 200 g), fasted overnight. CCl_4 (1.5 ml/kg or 2.5 ml/kg) dissolved in mineral oil was given by stomach tube. Ethionine (750 mg/kg) was adminstered in three divided doses, given 2.5 h apart. Animals were killed by intraperitoneal injection of pentobarbital 24 h after administration of CCl_4 or the first dose of ethionine.

Livers were rapidly removed, homogenized with 10 ml of chloroform – methanol (2:1), and extracted with 150 vol. of chloroform – methanol (2:1). The chloroform – methanol extract was filtered, the filtrate was shaken vigorously with 0.2 vol. of water, and the phases allowed to separate overnight in a separatory funnel at 4°. The organic (lower) layer was evaporated under reduced pressure and the residue immediately taken up in benzene. Total lipids were determined by weighing the residue after evaporation of an aliquot of the benzene solution. The triglyceride and phospholipid fractions in the remaining extract were weighed after separation by silicic acid chromatography[5]. 10–20 mg of triglyceride or phospholipid were transesterified by a methanol – H_2SO_4 procedure, and the methyl esters were analyzed by gas chromatography. Phospholipids were also determined colorimetrically by the method of BARTLETT[6].

Samples of abdominal adipose tissue were extracted with chloroform–methanol. The lipids were transesterified and the methyl esters analyzed by gas chromatography without prior silicic acid fractionation, since adipose tissue is almost entirely triglyceride[7].

The administration of CCl_4 increased the content in liver of triglycerides and of linoleic (C-18-2) acid more than 20-fold in 24 h (Table I). The linoleic acid must have been mobilized from the fat depots, since the amount of triglyceride linoleic acid was far greater than that originally present in liver. Moreover the linoleic acid in liver phospholipids did not decline.

The administration of ethionine increased the content in liver of triglycerides and of linoleic acid about 10-fold (Table I). This large increase in linoleic acid is also best explained by transport from adipose tissue.

However, there was a marked decrease in the percentage of palmitic (C-16)

TABLE I

ANALYSIS OF LIVER TRIGLYCERIDES AND PHOSPHOLIPIDS IN CONTROL, CCl_4- AND ETHIONINE-TREATED RATS

Values are expressed as means ± standard error of mean.

Liver analyses	*Control*	*CCl_4-treated*		*Ethionine-treated*
	9 rats	*1.5 ml/kg 4 rats*	*2.5 ml/kg 7 rats*	*750 mg/kg 8 rats*
Triglycerides				
mg/g liver	3.8 ± 0.4	77.7 ± 5.2	101.7 ± 5.7	37.9 ± 8.3
mg/g liver of linoleic (C-18-2) acid	1.0	21.0	25.0	10.7
% linoleic (C-18-2) acid	27.2 ± 1.0	27.1 ± 2.1	24.9 ± 1.3	28.2 ± 0.7
% palmitic (C-16) acid	28.7 ± 0.4	29.9 ± 1.4	30.1 ± 0.7	21.1 ± 0.7
Phospholipids				
mg/g liver*	32.2 ± 2.0	28.6 ± 0.4	27.1 ± 1.7	29.2 ± 2.3
mg/g liver of linoleic (C-18-2) acid	2.7	3.0	2.9	3.3
% linoleic (C-18-2) acid in phospholipid fatty acids	12.5 ± 0.8	16.1 ± 0.7	16.0 ± 0.5	17.5 ± 0.6

* mg of phospholipid × 0.65 = mg of phospholipid fatty acid.

TABLE II

FATTY ACID COMPOSITION* OF LIVER PHOSPHOLIPIDS IN CONTROL, CCl_4- AND ETHIONINE-TREATED RATS

Values are expressed as means ± standard error of mean.

Fatty acid	*Control*	*CCl_4-treated*		*Ethionine-treated*
	6 rats (%)	*1.5 ml/kg 4 rats (%)*	*2.5 ml/kg 7 rats (%)*	*750 mg/kg 8 rats (%)*
C-16 palmitic	21.4 ± 0.6	19.8 ± 1.2	20.9 ± 0.7	14.7 ± 0.2
C-18 stearic	34.0 ± 1.1	37.3 ± 1.8	33.6 ± 0.7	34.5 ± 1.5
C-18-1 oleic	5.1 ± 0.2	7.9 ± 0.3	11.5 ± 0.7	8.9 ± 0.8
C-18-2 linoleic	12.7 ± 0.8	16.1 ± 0.7	16.0 ± 0.5	17.5 ± 0.6
C-20-4 arachidonic	26.5 ± 1.2	18.4 ± 0.5	16.9 ± 0.9	24.0 ± 1.0

* A 15% ethylene glycol–succinate column, 6 ft, 4 mm internal dia. U-tube was used at 186° with an argon detector for the gas chromatography.

acid in liver triglycerides of ethionine-treated animals (Table I) but not in animals treated with CCl_4 (Table I) or ethanol[4].

Both CCl_4 and ethionine changed the fatty acid composition, but not the total amount, of phospholipids in liver. After CCl_4 the percentage of arachidonic (C-20-4) acid in liver phospholipids decreased by about one third (Table II). This was balanced by an increase in oleic (C-18-1) and linoleic (C-18-2) acids; the percentage of the saturated fatty acids, palmitic and stearic acids, was unchanged. Thus, despite the change in composition of fatty acids, the total amount of unsaturated fatty acids in the liver phospholipids did not change.

After ethionine treatment, the percentage of palmitic acid in liver phospholipids was decreased by one third (Table II); this was balanced by an increase in oleic and

linoleic acids rather than in stearic acid. Thus the amount of saturated fatty acids in the liver phospholipids decreased.

The liver phospholipids were fractionated by silicic acid chromatography. After CCl_4 treatment, the arachidonic acid content in the cephalin and lecithin fractions of liver decreased from 24.7 % to 17.6 % and from 25.5 to 15.2 %, respectively, while the saturated fatty acids were unaffected. After ethionine treatment, the palmitic acid content in the cephalin and lecithin fractions decreased from 17.1 % to 11.6 % and from 26.6 % to 17.8 %, respectively; however, the arachidonic acid level was not changed significantly.

The triglycerides which accumulate in the liver after CCl_4 and ethionine administration are synthesized primarily from fatty acids mobilized from the depots. In addition these two agents case qualitative changes in the composition of the liver lipids. CCl_4 lowers the arachidonic acid content and correspondingly raises the oleic and linoleic acid content of liver phospholipids; ethionine lowers the palmitic acid content of both liver triglycerides and phospholipids. Because arachidonic acid is an essential fatty acid, its decrease may be of particular significance in the action of CCl_4 on the liver.

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The mechanism of the reduction of mitochondrial DPN+ coupled with the oxidation of succinate

There is now considerable evidence[1–5] in support of CHANCE's original suggestion[6] that the reduction of mitochondrial DPN+ brought about by the addition of succinate to aerobic mitochondria in State 4 (*i.e.* rate of respiration limited by ADP concentration) is due to a reversal of the respiratory chain.

We have studied this reaction in rat-liver mitochondria by coupling it to the synthesis of glutamate in the presence of α-ketoglutarate and NH_3, with arsenite added to prevent the oxidation of α-ketoglutarate. Aspartate was found as well as glutamate; it must have been synthesised from malate (derived from succinate) according to Reactions 1 and 2.