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HEPATOTOXICITY AND LIPID METABOLISM—I
STRUCTURE OF LIVER TRIGLYCERIDE IN RATS DOSED WITH CARBON TETRACHLORIDE

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Abstract—The positional distribution of fatty acids in triglycerides of liver and adipose tissue of fasted female and male rats dosed intragastrically with carbon tetrachloride (CCl_4) 6 and 24 hr previously were compared with that of the paired controls.

Liver triglycerides from rats sacrificed 6 hr after CCl_4 treatment contained, in terms of percentage composition, significantly more palmitic acid and less linoleic acid. This effect was attributed to a change in the composition at the 1,3-positions of the glycerides; namely, more palmitate and less linoleate presented at these loci. By 24 hr after, however, the positional composition of the triglycerides resembled that of the controls, in comparison with 6-hr specimens. This was mainly due to the correction in the percentage of palmitic acid, but not linoleic acid. The fatty acid composition of the 2-position of hepatic triglycerides was also slightly modified at the early time after CCl_4; thus, the percentage of oleic acid was increased. The structure of adipose tissue triglycerides was not influenced by CCl_4 and was not necessarily similar to the accumulated liver triglycerides.

These observations are indicative of the operation of alternative, unknown pathway(s) of triglyceride synthesis in the liver of rats intoxicated with this chlorinated hydrocarbon.

THE ACTION of most hepatotoxic drugs which cause fatty liver is, in general, associated with inhibition of hepatic protein synthesis.[1] Carbon tetrachloride (CCl_4), ethionine, white phosphorus and puromycin are examples of this type of compound in that interference with lipoprotein synthesis is a manifestation of an early general inhibition of protein synthesis, even though the metabolic alterations created by each agent are probably different.[2, 3] Although many investigators have investigated the composition of accumulated liver triglycerides and the rate of triglyceride accumulation after CCl_4,[2] no report is available of the structure of liver triglycerides treated with the chlorinated hydrocarbon. In a study with ethionine intoxicated rats, we have demonstrated an alteration in the structure of liver triglycerides.[4] Thus, differences in the composition of liver triglyceride observed after injection of ethionine were attributed to changes in the 1,3-position of the glycerides. Horning *et al.*[5] have reported that the percentage of linoleate is slightly decreased in the triglyceride fraction of livers from rats dosed with CCl_4. In addition, the livers of rats treated with CCl_4 consistently appear to incorporate much more radioactivity from labeled fatty acid into triglyceride and much less into phospholipid fractions, as compared to the controls.[6] It was also demonstrated unequivocally that the biosynthetic pathway from plasma long-chain

fatty acids to liver triglycerides remains intact for at least the first 12 hr after CCl_4 poisoning.[2] The rapid onset of liver triglyceride accumulation caused by CCl_4 most probably results mainly from a cessation of the function of the auxiliary coupling phase of hepatic triglyceride secretion, and less significantly from a decrease in the rate of hepatic protein synthesis.[2]

These changes in the metabolism of liver triglycerides produced by CCl_4 suggest probable difference in the structure of the hepatic glycerides. The present paper describes comparisons of the structure and composition of liver triglyceride in CCl_4-treated and paired control rats. The data show that the structure of liver triglyceride accumulated at the initial stage of CCl_4-poisoned rats is substantially different not only from the controls, but also from the ethionine-treated rats.

EXPERIMENTAL

Animals used. Male and female Wistar-strain rats weighing 140–190 g and fed on a commercial pellet ration (Oriental Rat Chow NMF) were fasted for 15 hr. Administration of CCl_4 (0·5 ml/100 g body wt.), diluted with an equal volume of liquid paraffin, was accomplished by stomach tube under light-ether anesthesia. Paired control rats were given either liquid paraffin or isotonic saline in a similar manner.

Lipid analyses. At a selected time after the ingestion of CCl_4, rats were sacrificed by decapitation. The livers and the abdominal adipose tissues were quickly excised and total lipids were extracted by the procedure of Folch *et al.*[7] Solvent was removed from the extracts under reduced pressure in an atmosphere of nitrogen, and the residue was taken up in light petroleum ether (b.p., 30–60°). Total lipid was determined by weighing the residue after evaporation of an aliquot *in vacuo*. Separation of triglyceride was carried out with thin-layer chromatography using a 1mm-thick layer of silica gel G and a solvent mixture composed of light petroleum:ether–diethyl:ether–acetic acid (82:18:1, v/v/v).[8] Triglyceride visualized with 2,7-dichlorofluoresein (0·02% in 95% ethyl alcohol) and u.v. light was extracted with a chloroform:methanol mixture (4:1, v/v). Samples of triglycerides were digested with pancreatic lipase (EC 3.1.1.3),[9] and the resulting 2-monoglycerides were separated by thin-layer chromatography, using a light-petroleum:ether–diethyl:ether–acetic acid (30:70:1, v/v/v) mixture as the developing solvent.[10] Fatty acids liberated from triglycerides and 2-monoglycerides were methylated in a BF_3:methanol mixture.[11] Methyl esters were analyzed by gas-liquid chromatography on a Yanagimoto gas chromatograph model 550F apparatus with a hydrogen flame ionization detector. The samples were separated on a 4 mm $\times$ 2 m stainless steel column packed with 10% diethyleneglycol succinate polyester on 60–70 mesh Anakrom A or 80–100 mesh Diasolid S (Nihon Chromato Kogyo).[4] The accuracy of recovery of fatty acids from a mixture was checked frequently by means of standard fatty-acid mixtures provided by Applied Science Labs. Quantitative results with known standards agreed with the stated composition, the relative error being less than 5 per cent for major components and less than 8 per cent for minor components.

Plasma lipids were extracted according to Sperry and Brand.[12] Gas chromatographic analyses were carried out with the free-fatty acid and triglyceride fractions separated by thin-layer chromatography.[8]

RESULTS

Accumulation of lipids in the liver

Administration of CCl_4 resulted in a considerable increase in liver lipids (Table 1), i.e. 1·3 to 1·4 times more lipids infiltrated 6 hr after CCl_4 ingestion, and 2·3 to 3 times more at 24 hr after CCl_4, in comparison to paired control rats. Ingestion of paraffin alone did not influence the liver-lipid level. Female rats responded to CCl_4 by accumulation of lipid to a greater extent than the males.

TABLE 1. CONCENTRATION OF LIVER LIPIDS*

Treatments	Female		Male	
	6 hr	24 hr	6 hr	24 hr
Saline	57·0±0·9 (5)		62·5±1·4 (5)	
Paraffin	56·0±2·6 (5)	60·3±1·7 (6)	66·4±0·7 (5)	58·4±2·3 (4)
CCl_4	82·2±3·4 (5)	182·6±8·7 (4)	81·9±2·0 (5)	146·7±5·1 (4)

* Rats were treated as described in Experimental section and sacrificed at the indicated times. Body weights of rats were 140–190 g for female and 157–174 g for male. Values are expressed as mg total lipids per g of liver. Means (numbers of rats in parentheses) and S. E.

Fatty acid composition and structure of liver triglycerides

Experiments with female rats. The data are summarized in Table 2. Since there were no demonstrable differences in the composition of liver triglycerides of the two paired controls, comparisons were made between CCl_4-treated and paraffin-fed control rats. Liver triglycerides from female rats sacrificed at 6 hr after the administration of CCl_4 contained, in terms of percentage composition, significantly more palmitic acid and less linoleic acid in comparison with those of the controls. The fatty-acid composition of the 2-position of the triglycerides was practically similar between untreated rats and those treated with CCl_4, except for a decrease in palmitic acid in rats dosed with CCl_4. However, when the comparison was made with the saline-treated control, oleic acid was increased and arachidonic acid was decreased by this haloalkane. From these positional analyses it was evident that the composition of the 1,3-positions was modified by CCl_4. Thus, CCl_4 treatment resulted in a significant increase in palmitic and arachidonic acids and a decrease in linoleic acid at these loci.

Rather complicated results were obtained when rats were killed 24 hr after the CCl_4. With regard to total triglycerides, the percentage composition of linoleic acid was markedly decreased, while myristic, palmitoleic and linolenic acids increased with the administration of CCl_4. The percentage composition of palmitic acid did not change. Fatty acids at the 2-position of the triglyceride from CCl_4-poisoned rats were unchanged, except for linolenic acid. Calculation of the composition at the 1,3-positions demonstrated changes in myristic, palmitoleic and linoleic acids. The percentage of palmitic acid at these loci was not influenced by CCl_4.

Experiments with male rats. In general, results similar to those obtained with female rats were obtained in experiments with male rats (Table 3). At 6 hr after a dose of CCl_4, triglycerides from treated rats contained less linoleate and more palmitate than the controls. In addition, myristate, palmitoleate and linolenate were increased by CCl_4. Fatty acids at the 2-position of the triglycerides from the treated rats consisted

TABLE 2. FATTY-ACID COMPOSITION AND THE STRUCTURE OF LIVER TRIGLYCERIDES (FEMALE RATS)*

Fatty acids	$C_{1,2,3}$			C_2			$C_{1,3}$		
	Saline	Paraffin	CCl_4	Saline	Paraffin	CCl_4	Saline	Paraffin	CCl_4
6 hr									
14:0	0·4±0·0	0·3±0·0	0·4±0·0	0·2±0·0	0·1±0·0	0·1±0·0	0·5±0·0	0·5±0·1	0·5±0·1
16:0	25·1±0·3†	26·7±0·6†	29·9±0·7	3·6±0·2†	3·3±0·3†	1·8±0·1	35·8±0·3†	38·2±0·9†	43·8±1·1
16:1	1·8±0·2	2·0±0·2	2·5±0·2	1·1±0·2	1·3±0·2	1·4±0·2	2·1±0·2	2·4±0·2	3·1±0·3
18:0	3·6±0·4	3·1±0·5	3·3±0·2	0·7±0·1	0·6±0·1	0·3±0·1	5·1±0·5	4·4±0·7	4·8±0·1
18:1	19·8±0·9	19·9±0·6	19·0±0·9	22·4±1·0‡	23·6±0·8	26·0±0·6	18·5±1·2	18·1±0·5	15·5±1·0
18:2	40·8±1·0†	39·8±0·5†	34·7±0·8	62·8±4·8	63·0±0·9	62·7±0·8	29·7±1·1†	28·0±0·6†	20·9±1·1
18:3§	3·9±0·4	4·1±0·5	4·3±0·1	2·9±0·3	3·2±0·3	3·8±0·2	4·4±0·4	4·6±0·5	4·6±0·1
20:4	4·6±0·4	4·1±0·2	5·8±0·4	6·3±0·9‡	4·9±0·9	3·9±0·5	3·9±0·5†	3·8±0·6‡	6·8±0·7
24 hr									
14:0		0·4±0·0†	0·9±0·1		0·2±0·1	0·2±0·1		0·5±0·0†	1·3±0·1
16:0		25·2±0·5	26·0±0·7		3·0±0·3	2·3±0·3		36·1±0·8	37·8±1·1
16:1		2·2±0·1†	4·2±0·3		1·5±0·1	2·3±0·3		2·5±0·1†	5·2±0·4
18:0		2·6±0·2	1·9±0·2		0·4±0·1	0·4±0·1		3·7±0·2	2·6±0·4
18:1		20·1±0·4	19·6±0·8		23·8±0·5	25·1±0·1		18·8±0·6	16·9±1·2
18:2		42·0±0·9†	37·2±0·4		65·7±0·7	62·6±0·3		30·6±1·0†	24·4±0·8
18:3§		3·8±0·3†	6·0±0·5		2·8±0·3†	5·4±0·7		4·4±0·4	6·3±1·1
20:4		3·7±0·3	4·2±0·3		2·6±0·3	1·6±0·3		4·3±0·4	5·5±0·6

* Fatty-acid compositions of the 1,3-positions of triglycerides are calculated according to the following equation: $C_{1,3} = [3C_{1,2,3} - C_2]\frac{1}{2}$. Values are means of the per cent of total fatty acids and S. E. Five rats per group. The stereospecific numbering system recommended by Hirschmann[13] is used.

† Difference from CCl_4-treated rats is significant at $P < 0{\cdot}01$.

‡ Difference from CCl_4-treated rats is significant at $P < 0{\cdot}05$.

§ This also contains 20:1 acid.

TABLE 3. FATTY-ACID COMPOSITION AND THE STRUCTURE OF LIVER TRIGLYCERIDES (MALE RATS)*

Fatty acids	$C_{1,2,3}$			C_2			$C_{1,3}$		
	Saline	Paraffin	CCl_4	Saline	Paraffin	CCl_4	Saline	Paraffin	CCl_4
6 hr									
14:0	0·3±0·0†	0·3±0·0†	0·6±0·1	0·1±0·0	0·0±0·0	0·1±0·0	0·4±0·0†	0·4±0·1†	0·8±0·1
16:0	26·4±0·6†	25·5±0·6†	29·7±0·7	3·9±0·5‡	3·1±0·2	2·5±0·2	37·5±0·9†	36·7±1·0†	43·2±1·0
16:1	1·6±0·3‡	1·7±0·1‡	2·7±0·3	1·1±0·0‡	1·1±0·1	1·8±0·3	1·8±0·1†	2·0±0·2‡	3·2±0·3
18:0	2·6±0·3	2·2±0·2	1·9±0·1	0·6±0·1	0·5±0·1	0·3±0·1	3·7±0·5	3·0±0·2	2·6±0·2
18:1	19·0±0·6	18·9±0·7	19·4±0·5	22·3±0·6†	24·3±0·4‡	27·5±1·2	17·3±0·6	16·3±1·2	15·4±0·4
18:2	43·8±0·8†	44·7±0·5†	36·6±1·1	66·3±1·4	65·8±0·7	62·8±1·3	32·4±1·1†	34·1±1·0†	23·6±1·2
18:3§	3·6±0·4‡	4·2±0·3	4·9±0·3	2·6±0·3	2·8±0·4	3·6±0·0	4·4±0·4	4·9±0·5	5·4±0·3
20:4	2·6±0·2	2·5±0·2	4·2±0·7	3·0±0·5	2·2±0·4	1·3±0·6	2·5±0·3‡	2·6±0·1‡	5·7±1·0
24 hr									
14:0		0·4±0·0†	1·1±0·1		0·4±0·2	0·2±0·0		0·4±0·1†	1·4±0·2
16:0		29·9±0·6	29·1±0·2		5·2±1·2‡	2·2±0·3		42·4±1·0	42·5±0·5
16:1		3·2±0·1†	5·8±0·6		2·5±0·7	3·7±0·6		3·6±0·4†	6·8±0·6
18:0		2·8±0·5†	1·5±0·0		0·9±0·2	0·3±0·1		3·6±0·6	2·1±0·0
18:1		19·1±0·5	18·0±1·0		26·0±1·2	25·3±1·2		15·5±0·8	14·3±1·1
18:2		39·0±1·0‡	34·5±1·5		60·0±2·9	60·0±2·8		28·6±0·6†	21·9±1·4
18:3§		3·5±0·3†	7·9±0·5		3·1±0·3†	7·3±0·6		3·7±0·5†	8·2±0·5
20:4		2·1±0·3	2·1±0·3		1·9±0·1	1·0±0·2		2·2±0·5	2·7±0·5

* See Table 2 for details. Five rats per group.
† Difference from CCl_4-treated rats is significant at $P \leqslant 0{\cdot}01$.
‡ Difference from CCl_4-treated rats is significant at $P \leqslant 0{\cdot}05$.
§ This also contains 20:1 acid.

TABLE 4. FATTY-ACID COMPOSITION AND STRUCTURE OF ADIPOSE TRIGLYCERIDES*

Fatty acids	Female rats						Male rats					
	$C_{1,2,3}$		C_2		$C_{1,3}$		$C_{1,2,3}$		C_2		$C_{1,3}$	
	Paraffin	CCl_4	Paraffin	CCl_4	Paraffin	CCl_4	Paraffin	CCl_4	Paraffin	CCl_4	Paraffin	CCl_4
6 hr												
14:0	1·1±0·1	1·0±0·0	0·5±0·0	0·5±0·0	1·4±0·1	1·3±0·0	1·2±0·1	1·3±0·0	0·5±0·1	0·4±0·1	1·6±0·1	1·8±0·1
16:0	22·8±0·6	22·4±1·1	7·7±0·2	8·2±0·3	30·2±0·8	29·4±1·6	23·4±0·7	22·7±0·6	6·0±1·2	5·9±0·1	32·0±1·2	31·2±1·1
16:1	5·1±0·4	4·5±0·2	3·8±0·3	3·4±0·3	5·8±0·5	5·1±0·3	6·5±0·1	6·9±0·4	4·0±0·5	4·4±0·5	7·8±0·1	8·3±0·6
18:0	2·9±0·2	3·1±0·2	0·6±0·1	0·8±0·2	4·1±0·3	4·2±0·3	3·1±0·2	3·0±0·1	0·4±0·1	0·4±0·1	4·5±0·3	4·2±0·2
18:1	27·5±0·7	26·7±0·5	28·8±1·0	28·4±0·9	26·9±0·7	25·8±0·4	25·5±0·6	27·2±0·5	23·4±1·1	23·8±1·6	26·8±0·8	29·0±0·4
18:2	34·9±1·2	36·3±1·5	54·2±1·2	55·0±1·0	25·2±1·1	27·0±1·8	35·0±0·9	33·6±0·9	60·6±1·6	60·5±1·4	21·8±2·1	19·8±0·8
18:3†	4·1±0·3	4·4±0·2	4·1±0·3	3·3±0·4	4·1±0·3	4·9±0·2	4·2±0·1	4·2±0·1	5·0±0·3	4·5±0·5	3·8±0·3	3·9±0·2
Unknown	1·0±0·1	1·0±0·0	trace	trace	1·5±0·1	1·5±0·1	0·9±0·0	1·0±0·1	trace	trace	1·3±0·1	1·6±0·1
20:4	0·6±0·1	0·6±0·1	0·3±0·0	0·4±0·1	0·8±0·2	0·8±0·1	0·2±0·1	0·1±0·1	0·1±0·1	0·1±0·1	0·4±0·2	trace
24 hr												
14:0	1·0±0·0	1·0±0·0	0·6±0·0	0·6±0·0	1·2±0·1	1·4±0·0	1·2±0·1	1·4±0·2	0·6±0·0	0·7±0·1	1·6±0·2	1·8±0·3
16:0	21·6±0·4	21·6±0·8	9·3±0·1	9·3±0·3	27·7±0·7	27·7±1·3	23·3±0·5	24·0±1·4	7·2±0·2	7·6±0·2	31·2±0·6	31·9±2·1
16:1	5·1±0·1	5·3±0·1	4·1±0·1	4·1±0·2	5·7±0·1	5·9±0·2	6·3±0·2	6·8±0·6	4·6±0·3	5·8±0·4	7·2±0·5	7·3±0·7
18:0	2·7±0·1	2·8±0·1	0·6±0·1	0·7±0·1	3·8±0·1	4·0±0·2	3·1±0·1	3·0±0·1	0·7±0·1	0·6±0·0	4·4±0·1	4·3±0·2
18:1	28·8±0·2	29·2±0·3	28·4±0·7	29·6±0·4	28·9±0·3	28·9±0·1	26·4±0·6	27·0±0·8	27·5±1·1	29·1±1·5	25·7±0·1	25·8±0·8
18:2	35·4±0·4	35·0±0·9	53·0±0·7	52·4±0·9	26·6±0·6	26·0±1·0	34·6±0·8	32·4±2·3	54·7±1·3	52·1±2·2	24·4±0·8	22·8±2·6
18:3†	4·1±0·1	3·8±0·2	3·8±0·3	3·1±0·4	4·2±0·2	4·1±0·1	4·1±0·6	4·2±0·4	4·6±0·4	4·0±0·3	3·9±0·7	4·3±0·8
Unknown	0·9±0·1	0·9±0·1	trace	trace	1·4±0·1	1·4±0·1	0·7±0·1	0·9±0·1	trace	trace	1·1±0·2	1·3±0·1
20:4	0·4±0·1	0·4±0·1	0·2±0·0	0·2±0·1	0·5±0·1	0·6±0·3	0·3±0·1	0·3±0·0	0·1±0·0	0·1±0·1	0·5±0·1	0·5±0·1

* Values are means of per cent of total fatty acids and S. E. See Table 2 for experimental details. Five rats per group.
† This also contains 20:1 acid.

of more monoenoate (especially oleate) and less palmitate (significant only against saline control rats). Fatty acids at the 1,3-positions of the triglycerides from treated rats consisted of more myristic, palmitic, palmitoleic and arachidonic acids and less linoleic acid.

At 24 hr after the dose of CCl_4, triglycerides from the treated rats contained more myristate, palmitoleate and linolenate and less stearate and linoleate. Again, as in the case with female rats, no difference could be seen in palmitic acid. Changes due to CCl_4 in the composition of the 2-position were only seen in the minor components, i.e. palmitate was decreased and linolenate increased. These changes in the total and the 2-position of triglyceride after CCl_4 caused an increase in the percentage of myristic, palmitoleic and linolenic acids and decrease in linoleic acid at the 1,3-positions.

Fatty acid composition and structure of adipose triglycerides

There were no significant differences in the composition, as well as the positional distribution of fatty acids, of adipose triglycerides, regardless of the treatment, duration of the treatment, or sex of the animals, when compared with each paired control (Table 4).

Molecular families of liver and adipose triglycerides

Analyses of molecular families of liver and adipose triglycerides were based on the 2-random-1,3-random hypothesis.[14] As can be seen in Table 5, both in the liver triglycerides from the females and males, CCl_4 treatment resulted in an increase in the

TABLE 5. MOLECULAR FAMILIES OF LIVER AND ADIPOSE TRIGLYCERIDES*

Tissues	Treatment	Sex	Hours after CCl_4	Molecular families					
				SSS	SUS	SSU	USU	UUS	UUU
Liver	Saline	Female	6	1	16	2	1	47	33
	Paraffin	Female	6	1	18	2	1	47	31
	CCl_4	Female	6	1	23	1	1	49	25
	Paraffin	Female	24	1	15	3	1	46	34
	CCl_4	Female	24	1	17	1	1	47	33
	Saline	Male	6	1	16	2	2	46	33
	Paraffin	Male	6	1	15	2	1	47	34
	CCl_4	Male	6	1	21	1	1	48	28
	Paraffin	Male	24	1	20	3	2	47	27
	CCl_4	Male	24	1	21	1	1	48	28
Adipose	Saline, paraffin	Female	6	1	12	4	4	42	37
	CCl_4	Female	6	1	12	4	4	41	38
	Paraffin	Female	24	1	10	5	5	39	40
	CCl_4	Female	24	1	11	5	5	39	39
	Saline, paraffin	Male	6	1	14	3	2	44	36
	CCl_4	Male	6	1	14	3	2	44	36
	Paraffin	Male	24	1	13	4	3	43	35
	CCl_4	Male	24	1	14	4	3	43	34

* Values are calculated based on a 1,3-random-2-random distribution,[14] and are expressed as a per cent of the total triglycerides. Means of five rats per group. Abbreviations used are: S, saturated fatty acids; U, unsaturated.

SUS type of triglycerides and a decrease in UUU, at 6 hr after the dose of this agent. The modification of molecular families of hepatic triglycerides caused by CCl_4 was also noted in the minor components, but this seemed to be of little importance with respects to the effectiveness of CCl_4 because of their minority. The effects of CCl_4 on the molecular families of liver triglycerides were no longer seen in the rats killed at 24 hr after the dose of the hydrocarbon.

Fatty acid composition of plasma triglycerides and free-fatty acids

Table 6 shows the fatty acid compositions of plasma free-fatty acids and triglycerides from female rats that were sacrificed 6 and 24 hr after intoxication with CCl_4. There were no demonstrable changes in the composition of free-fatty acids between untreated rats and those that received CCl_4. CCl_4 intoxication, however, caused alteration in the composition of plasma triglycerides; namely, the percentage of palmitoleic and stearic acids was increased and that of linoleic acid was decreased by the treatment.

TABLE 6. FATTY-ACID COMPOSITION OF PLASMA FREE-FATTY ACIDS AND TRIGLYCERIDES IN FEMALE RATS*

Fatty acids	6 hr after treatment						24 hr after treatment					
	Free-fatty acids			Triglycerides			Free-fatty acids			Triglycerides		
	S	P	C	S	P	C	S	P	C	S	P	C
14:0	1·5	1·4	1·4	0·5	0·4	1·8	1·3	1·1	1·2	0·4	0·4	1·6
Unknown	0·4	0·4	0·3	0·3	0·3	0·4	0·4	0·2	0·3	0·3	0·4	0·5
16:0	28·9	32·3	29·4	26·8	25·4	25·1	28·1	28·0	24·2	24·3	25·6	20·5
16:1	6·1	5·7	6·5	2·1	2·2	5·3	4·2	4·8	4·6	1·9	2·3	4·5
18:0	8·0	8·7	7·5	3·1	3·4	9·1	7·3	8·6	8·0	3·4	3·9	8·3
18:1	23·5	23·7	25·3	22·6	20·1	23·5	24·5	23·9	27·1	20·2	20·2	25·9
18:2	25·4	23·3	24·7	31·0	35·7	26·0	28·1	26·3	28·7	34·8	37·0	27·9
18:3†	3·5	2·7	3·1	2·2	2·8	1·6	3·6	2·6	3·7	2·8	3·2	3·9
Unknown	1·1	0·8	0·7	1·5	1·3	1·0	0·8	1·0	1·1	1·2	0·7	2·0
20:4	1·6	1·0	1·1	4·3	5·7	4·9	1·7	3·5	1·1	5·7	6·3	4·9
Unknown	tr	tr	tr	5·6	2·7	1·3	tr	tr	tr	5·0	tr	tr

* Values are means expressed as a per cent of the total fatty acids. Analyses of pooled plasma are from five rats per group. Abbreviations used are: S, saline; P, paraffin; C, CCl_4; tr, trace.
† 20:1 acid is also included.

DISCUSSION

Although an explanation of the data presented in this paper appears difficult, they seem to open areas for speculation with respect to the effect of CCl_4 on the metabolism of hepatic lipids. Recknagel *et al.*[2] have reported that in perfusion experiments the biosynthetic pathway from plasma long-chain fatty acids to liver triglycerides remains intact for at least the first 12 hr after CCl_4. By 24 hr after the poisoning, however, when the liver mitochondria have undergone extensive functional degeneration, the capacity of the isolated perfused liver to incorporate plasma long-chain fatty acids into liver triglycerides was markedly reduced. This observation may provide a clue to explain the observed time difference in the liver triglyceride structure after CCl_4 poisoning. If it is assumed that at least two different mechanisms by which triglycerides are accumulated in the liver are operative after CCl_4 poisoning, the effect of the chlorinated hydrocarbon 6 hr after its dose could be attributed to enhancement of triglyceride

synthesis in the liver by an alternative or unknown pathway(s) in which more palmitic acid and less linoleic acid are incorporated into the 1,3-positions. There is the possibility that the decrease in the relative amounts of linoleic acid may be reflected by peroxidation of triglycerides.[15, 16] Peroxidative decomposition of liver lipids has been demonstrated to occur shortly after CCl_4 intoxication.

The delayed effect of CCl_4 on the positional distribution of fatty acids in hepatic triglycerides could in turn result simply from the known pathway of liver triglyceride synthesis, since the structure of the triglycerides 24 hr after the CCl_4 treatment resembled that of the paired controls, in comparison with 6-hr specimens. Several investigators[17, 18] have demonstrated the elevation of plasma free-fatty acid at a later stage of CCl_4 intoxication. Therefore, liver triglyceride from rats sacrificed at 24 hr after the poisoning is probably derived from free-fatty acid liberated from adipose tissue. Although triglyceride synthesis by the liver appears to decrease at this stage, oversupply of plasma free-fatty acids may be responsible for determining the composition and the structure of the triglycerides deposited in the liver of treated rats. Another possibility is that palmitic acid, incorporated initially and predominantly into the 1,3-positions of liver triglycerides, and linoleic acid vice versa, would shift to the 2-position to form triglycerides like those of normal fasting rats. This assumption may be reasonable, in part, since spontaneous shifting of partial glycerides is well known in the field of glyceride chemistry.[19] Also, this may in turn be the operation of a compensatory action in the treated rats in order to transport accumulated triglycerides, as much as possible, from liver to extrahepatic tissues.

The fatty-acid composition of the 2-position of liver triglycerides was also modified slightly by CCl_4. The most prominent change was noted in the liver of rats treated 6 hr previously with CCl_4. Except for changes in minor components at this locus, the increase in the percentage of oleic acid is also indicative of probable modifications of structure. This change was no longer seen in the liver of rats sacrificed 24 hr after the hydrocarbon. Although the physiological significance of the initial alteration in the composition of combined fatty acids at the 2-position of hepatic triglycerides is not apparent, the observation also suggests that the triglyceride biosynthetic pathway in the liver is substantially altered by CCl_4.

Analyses of plasma free-fatty acids revealed similarity in their composition in the treated and untreated rats. This finding implies that both groups of rats are supplying similar fatty acids for triglyceride synthesis, though there is a possibility of a difference in their concentration.[20] The composition of plasma triglyceride was changed after CCl_4 intoxication. Since the liver is the major, if not the sole, source of plasma triglycerides in the fasting rat,[21, 22] the data indicate that the poisoned rat liver is excreting a different type of triglyceride into the circulation. Plasma triglycerides in the fasting rat are derived from liver triglycerides and not vice versa. The composition of plasma triglycerides, however, never resembled that of the liver. This observation may, therefore, indicate that in the treated rat an unexplicable secretion mechanism(s) is operative. The fatty-acid composition of plasma free-fatty acids also did not change in rats given a 24-hr pretreatment of ethionine, but the composition of plasma triglycerides was altered.[23, 24] Thus, in ethionine-intoxicated rats there were significant increases in the percentage of palmitic, palmitoleic and stearic acid and a significant decrease in the percentage of linoleic and arachidonic acid. Livers from ethionine-treated rat contained more palmitic acid at the 1,3-positions (in this case, however,

oleic acid, instead of linoleic acid, decreased at these loci).[4] In this respect, alteration in the metabolism of palmitic acid is the common phenomenon of these two hepatotoxic reagents.

Finally, it appears that the interplay of a number of factors controls the composition and structure of liver triglycerides. This includes the enzyme systems involved in the synthesis and hydrolysis of the triglycerides, the nature of the fatty acid pool available for synthesis, and the rate of release of hepatic triglycerides into the circulation. It is clear that all these factors must be taken into consideration in evaluating the metabolism of liver triglycerides from rats dosed with CCl_4. More direct, precise knowledge will be given by the analyses of molecular families of triglycerides and by the use of labeled fatty acids to monitor the process of triglyceride synthesis. These studies are in progress in our laboratory.

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