Serum lipoproteins in rats with carbon tetrachloride-induced fatty liver

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SUMMARY After the administration of CCl_4 to male rats, liver triglycerides began to increase after a lag period of about 1 hr; the level of serum triglycerides fell sharply during the first 30 min of intoxication.

Three classes of serum lipoproteins were isolated by flotation in the ultracentrifuge and their concentrations and chemical compositions were determined. Within 4 hr of the administration of CCl_4 the level of the very low density (VLD-) lipoproteins fell to 25% of that in the control rats. Smaller decreases in the levels of the other two classes of lipoproteins were evident. The serum concentration of all the components of the VLD-lipoproteins were reduced, but proportionally more lipids were bound to the protein moiety in the CCl_4-treated rats than in the controls. The concentrations of protein and triglycerides of the VLD-lipoproteins declined most steeply during the first hour of intoxication.

The results are interpreted as further evidence that the fatty liver induced by CCl_4 is due to a block in the release of hepatic triglycerides to the plasma, the primary lesion being, very probably, inhibition of the synthesis of the protein moiety of serum lipoproteins.

KEY WORDS liver · serum · lipids · lipoproteins · carbon tetrachloride · fatty liver · rat · pathogenesi

ADMINISTRATION OF CARBON tetrachloride to fasted rats result in a rapid accumulation of triglycerides in the liver (1). Evidence obtained in various laboratories (2–6) supports the hypothesis that such an accumulation is essentially due to a block in the transfer of triglycerides from the liver to the plasma. Since hepatic triglycerides are released into the plasma as lipoproteins (7–9), changes in the level and (or) the composition of circulating plasma lipoproteins would be expected in rats treated with CCl_4. This study was undertaken, therefore, to observe whether such changes do occur during the onset of the fatty liver, and to investigate to what extent different classes of plasma lipoproteins are involved.

MATERIALS AND METHODS

Male albino rats of the Sprague-Dawley strain, maintained on laboratory chow, and weighing between 190 and 220 g were used. The animals were starved for 16 hr before treatment and throughout the subsequent experimental period. Water was allowed ad lib. Carbon tetrachloride was administered by stomach tube, under light ether anesthesia, as a 50% (v/v) solution in mineral oil, at a dosage of 0.5 ml of solution per 100 g of body weight. Control rats received similarly 0.25 ml of mineral oil per 100 g of body weight. Five minutes before sacrifice, 6 mg of pentobarbital per 100 g of body weight was injected intraperitoneally. Blood was withdrawn from the abdominal aorta by means of a dry glass syringe and transferred into glass centrifuge tubes. The liver was rapidly removed and weighed; a tared sample from the left lateral lobe was immediately frozen, and stored at $-15°$ for lipid analyses.

Preparation of Serum

Blood samples were left to clot at room temperature for 3 hr. After removal of the clot, they were centrifuged at room temperature for 15 min at 9000 × g to remove the clot and cell debris. Equal amounts of serum from 2 experimental or 2 control rats were pooled in order to have sufficient material to perform all the analyses.

Separation of Serum Lipoproteins

Lipoproteins were separated from each serum pool as described by Havel, Eder and Bragdon (10), and by Korn (11). Stock solutions of NaCl and NaBr (12) were prepared, and their density was adjusted by pycnometry to $1.0053 ± 0.0005$ and $1.5040 ± 0.0005$ g/ml at $20°$, respectively. Solutions of intermediate density were

prepared by mixing appropriate volumes of the stock solutions. All centrifugations were performed at 15 ± 1° in a Spinco model L ultracentrifuge using a 40.3 rotor. The density of serum, exclusive of protein and serum lipoproteins, was taken to be 1.0073 g/ml (13). Four fractions were isolated as follows: (a) Very low density (VLD) lipoproteins. Three milliliters of serum and 3 ml of saline solution of density 1.0307 g/ml were mixed in siliconized (Siliclad, Clay-Adams, Inc., New York) cellulose tubes (No. 302232, Beckman-Spinco, Palo Alto, Calif.). The tubes were capped and centrifuged at 142,900 × g for 14 hr. After centrifugation, the top 1 ml, containing the lipoproteins of d < 1.019, was transferred into 1 ml volumetric flasks with the aid of a capillary pipette. (b) Low density (LD) lipoproteins. To the infranatant solution from the first ultracentrifugation, 1 ml of saline solution of density 1.2830 g/ml was added; the contents of the tubes were mixed, and the tubes were capped and centrifuged at 142,900 × g for 14 hr. After centrifugation the top 2 ml, containing the lipoproteins of 1.019 > d < 1.063, was transferred into 2 ml volumetric flasks. (c) High-density (HD) lipoproteins. To the infranatant solution from the second ultracentrifugation, 2 ml of stock NaBr solution was added, and the contents of the tubes were mixed and centrifuged at 142,900 × g for 24 hr. The top 2 ml, containing the lipoprotein of 1.063 > d < 1.210, was transferred into volumetric flasks. (d) The final infranatant solution (d > 1.210), containing serum albumin and other proteins and some residual lipids, represented the fourth fraction.

Determination of the Level of Serum Lipoproteins

The serum concentrations of the VLD-, LD-, and HD-lipoproteins were determined by measuring their protein and total lipid concentrations. A suitable aliquot of the isolated fractions was extracted for total lipids (see below), and these were then determined by the method of Chiang, Gessert, and Lowry (14). Corn oil (Mazola Corn Products Co., New York), purified by elution from a silicic acid column, was used as a standard. A second aliquot was treated with phosphotungstic acid and MgCl₂ as described by Burstein (15, 16). Of several procedures tried, this was the only one by which a quantitative precipitation of the lipoproteins could be achieved, as well as the removal of serum albumin and amino acid contaminants.[1] Protein was determined in the precipitated lipoproteins by the method of Lowry, Rosenbrough, Farr, and Randall (17). A solution of crystalline

bovine serum albumin (Armour Laboratories, Chicago, Ill.) was used as a standard, after determination of protein nitrogen by a micro Kjeldahl procedure (18). Lipids were not extracted from the lipoprotein precipitates prior to the determination of protein; the possibility of interference of lipids with this determination was excluded by the results of analyzing aliquots of the standard albumin solution in the presence or absence of various amounts of serum total lipids. Precipitation of the isolated lipoprotein by phosphotungstic acid and MgCl₂ was quantitative and was effective in removing the contaminants, as shown by the following tests: (a) triglycerides and total cholesterol analyses were performed on aliquots of each isolated lipoprotein before and after precipitation. Recoveries of the precipitated lipids ranged between 95 and 100%; (b) no precipitate was obtained when solutions of crystalline serum albumin, or portions of the 1.210 infranatant solution (containing the serum albumin), were treated with phosphotungstic acid and MgCl₂; and (c) l-tyrosine-1-¹⁴C and l-phenylalanine-1-¹⁴C (New England Nuclear Corp., Boston, Mass.), together contributing 10,000 cpm, were added to aliquots of each isolated lipoprotein; after treatment with phosphotungstic acid and MgCl₂, the precipitates were plated and counted in a windowless gas-flow counter (Tracerlab, Inc.). In no case did the counts recovered in the precipitates exceed 0.1% of the added counts.

Extraction and Fractionation of Total Lipids

Serum, serum lipoproteins, and liver total lipids were extracted and purified according to the method of Folch, Lees, and Sloane Stanley (19), dried down under N₂ at 40°, and then redissolved in chloroform. Suitable portions of the CHCl₃ extract were applied to small columns of silicic acid (Unisil, Clarkson Chemical Co., Williamsport, Pa.) which had been washed with several portions of CHCl₃ freshly distilled over sodium (Dri-Na, Fisher Scientific, Pittsburgh, Pa.). Neutral lipids plus free fatty acids were eluted with chloroform, and phospholipids with methanol. The lipids in the CHCl₃ eluate were separated further by chromatography on thin layers of Silica Gel G (Merck A.G., Darmstadt, Germany), using a solvent system of n-heptane–isopropyl ether–glacial acetic acid 60:40:2. The plates were air dried, and lipids identified by exposure to iodine vapor. The different fractions were then scraped from the plates and quantitatively transferred into ground-glass stoppered test tubes. Cholesterol esters, free cholesterol, and triglycerides[2] were eluted from the silica gel with

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[1] These contaminants would have led to erroneous estimations of protein by the Lowry method. During similar studies in ethionine-treated rats,[3] a several-fold increase in the level of serum free amino acids was observed, and the isolated VLD-lipoproteins were found to be heavily contaminated with free aromatic amino acids.

[2] Well-defined bands corresponding to di- and monoglycerides were often seen after exposure of the plates to I₂ vapor. However, the amounts of these glycerides were usually too low to be determined.

CHCl₃, and free fatty acids with 0.22% formic acid in CHCl₃. The eluates were filtered through Whatman no. 43 paper, and portions of the filtrates were used for colorimetric analyses after evaporation of the solvents at 40° under N_2. Recovery of different lipid standards (see below) from thin layer plates of silica gel was greater than 95% in all cases.

Other Analytical Procedures

Cholesterol esters and free cholesterol were estimated by the method of Bowman and Wolf (20), using cholesteryl palmitate and cholesterol recrystallized from hot absolute ethanol as standards. Triglycerides were determined by the method of Van Handel and Zilversmit (21–22), with synthetic tripalmitin (a gift of Dr. F. H. Mattson, The Procter and Gamble Co., Cincinnati, Ohio) used as a standard. Free fatty acids were determined by the colorimetric method of Duncombe (23), using chromatographically pure palmitic acid as a standard. Lipid P was determined according to Shin (24) on aliquots of the methanol eluate from the silicic acid columns. A factor of 25 was used to convert lipid P to phospholipid. The sensitivity of some of these methods was increased by reducing the volumes of reagents, and by using microcuvettes in a Coleman Junior Spectrophotometer model-6D.

RESULTS

Level of Serum Lipoproteins

In a first series of experiments the level of serum lipoproteins was determined in control rats and in rats treated with CCl₄ for 4 hr. Total lipids of the unfractionated sera and of d > 1.210 fractions, and liver triglycerides, were also determined (Table 1). Four hours after the administration of CCl₄ there was a 3-fold increase in the level of triglycerides in the liver. At the same time, there was a small but significant ($P < 0.001$) decrease in the concentration of serum total lipids, which was due largely, or solely, to a reduction of the total lipid concentration in the VLD- and HD-lipoproteins. The concentration of protein was decreased in all three classes of lipoproteins. The concentration of protein was decreased in all three classes of lipoproteins, the largest reduction occurring in the VLD-lipoprotein (84%), and the smallest (18%) in the LD-fraction. Thus, in the CCl₄-treated animals there were only small decreases in the level of the LD- and HD-fractions, but the level of the VLD-lipoproteins was reduced to 25% of that in the control. The reduced level of the HD-lipoproteins was due to a proportional decrease of both the protein and lipid moieties, so that the ratio of protein to lipoprotein was essentially the same for control and CCl₄-treated rats.

In the other two fractions, however, the concentration of the protein moiety decreased to a greater extent than that of the lipid moiety, as evidenced by a smaller ratio of protein to lipoprotein in the CCl₄ than in the control group. A small but significant decrease in the total lipids of the d > 1.210 fraction was also observed.

Composition of the Lipid Moiety of Serum Lipoproteins

In a second series of experiments (Table 2) the composition of the lipid moiety of the three classes of serum lipoproteins, as well as of d > 1.210 fraction, was determined. Liver and whole serum lipids were also analyzed. As in the first series, the animals were sacrificed 4 hr after the administration of CCl₄ or of mineral oil. In these experiments a 3.5-fold increase in the level of liver triglycerides was observed. Liver phospholipids, cholesterol, and cholesterol esters were unchanged, whereas free fatty acids were slightly increased. In the serum there was a marked decrease in the level of triglycerides, which was due to a reduced concentration of this lipid fraction in all three classes of lipoproteins, particularly the VLD- and LD-fractions. A small but significant ($P < 0.005$) decrease in the level of serum phospholipids was also present, due to lower concentrations in the VLD- and HD-lipoproteins. Other observed changes were (*a*) a decrease in the level of serum cholesterol esters, which was accounted for by a reduced concentration in the VLD- and HD-lipoproteins, and (*b*) a reduced concentration of free cholesterol in the VLD-lipoproteins.

Time of Onset of Changes in Hepatic and Plasma Triglycerides

Groups of rats were sacrificed 30 min, 1, 2, 3, and 4 hr after the administration of CCl₄. Two additional groups were sacrificed at zero time and at 4 hr after the administration of mineral oil. Liver and plasma triglycerides were determined. Liver triglycerides remained constant during the first hour of intoxication, then rose rapidly at a relatively constant rate for the next 3 hr (Fig. 1). The average rate of increase between the first and the fourth hour was approximately 9 mg/100 g body weight per hr. The levels of liver triglycerides in the two control groups sacrificed at zero time and at 4 hr were the same. The concentration of triglycerides in the plasma decreased very sharply during the first hour of intoxication, then adjusted to a slower rate of decline (Fig. 1). At the end of the first hour, by which time liver triglycerides had not yet increased, the concentration of plasma triglycerides was only 53% that of the zero time control. A further reduction of 13% occurred during the next 3 hr. Administration of mineral oil had no effect on plasma triglycerides.

TABLE 1   Level of Serum Lipoproteins in Control Rats and in Rats Treated with CCl₄ for 4 Hr

Fraction	Number of Samples and Treatment	Total Lipids	Protein*	Lipoprotein	Protein/ Lipoprotein
		mg/100 ml serum			
Whole serum	9 Control	315.3 ± 9.1			%
	9 CCl₄	248.7 ± 13.2			
		P < 0.001			
VLD	9 Control	25.4 ± 0.8	2.5 ± 0.2	27.9 ± 2.6	8.9 ± 0.3
	9 CCl₄	6.5 ± 0.3	0.4 ± 0.5	6.9 ± 0.4	6.2 ± 0.8
		P < 0.001	P < 0.001	P < 0.001	P < 0.001
LD	9 Control	60.6 ± 3.4	15.5 ± 0.5	76.1 ± 3.7	20.5 ± 0.9
	9 CCl₄	57.9 ± 4.4	12.7 ± 0.9	70.6 ± 5.3	17.9 ± 0.4
			P < 0.025		P < 0.025
HD	9 Control	132.2 ± 6.2	63.4 ± 3.1	195.6 ± 9.1	32.4 ± 0.5
	9 CCl₄	101.0 ± 2.8	50.7 ± 2.4	151.7 ± 7.4	33.5 ± 0.5
		P < 0.001	P < 0.001	P < 0.001	
d > 1.210	9 Control	51.3 ± 1.7			
	9 CCl₄	45.0 ± 1.8			
		P < 0.025			

* Milligrams per fraction in 100 ml of serum. Each value represents the mean ± SEM.
  The mean concentration of triglycerides in the liver, in mg/100 g body weight, was 16.1 ± 5.1 for the controls (18 rats) and 47.1 ± 7.5 for the carbon tetrachloride-treated rats (18) (P < 0.005).

TABLE 2   Concentration of Lipids in the Liver, Whole Serum, and Serum Lipoproteins of Control Rats and of Rats Treated with CCl₄ for 4 Hr

Fraction	Number of Samples and Treatment	Triglycerides	Phospholipids	Free Fatty Acids	Cholesterol Esters	Free Cholesterol
		mg/100 g body weight				
Liver	14 Control	11.2 ± 0.9	110.9 ± 8.4	3.7 ± 0.3	3.5 ± 0.4	6.3 ± 0.1
	16 CCl₄	39.0 ± 2.1	114.3 ± 3.1	5.7 ± 0.5	3.4 ± 0.2	6.2 ± 0.1
		P < 0.001		P < 0.001		
		mg/100 ml serum				
Whole serum	6 Control	12.0 ± 1.2	76.8 ± 3.5	13.2 ± 0.6	113.5 ± 5.7	10.4 ± 1.2
	8 CCl₄	4.7 ± 0.3	61.6 ± 2.5	13.8 ± 0.9	82.8 ± 5.3	11.4 ± 0.3
		P < 0.001	P < 0.001		P < 0.025	
VLD	6 Control	8.3 ± 0.4	4.3 ± 0.8	0.2 ± 0.1	4.6 ± 0.5	1.2 ± 0.1
	8 CCl₄	2.9 ± 0.2	1.0 ± 0.3	0.2 ± 0.1	0.9 ± 0.9	0.3 ± 0.1
		P < 0.001	P < 0.005		P < 0.001	P < 0.025
LD	7 Control	2.6 ± 0.4	13.4 ± 1.4	0.4 ± 0.1	26.2 ± 3.1	3.2 ± 0.5
	8 CCl₄	1.0 ± 0.1	16.7 ± 1.7	0.4 ± 0.1	25.7 ± 2.9	4.3 ± 0.3
		P < 0.001				
HD	7 Control	0.9 ± 0.1	44.3 ± 2.3	1.0 ± 0.1	80.3 ± 4.9	3.6 ± 0.1
	8 CCl₄	0.5 ± 0.1	33.3 ± 2.1	0.7 ± 0.1	54.6 ± 5.2	4.7 ± 0.4
		P < 0.001	P < 0.005		P < 0.005	
d > 1.210	6 Control	0.5 ± 0.1	17.2 ± 2.8	11.7 ± 0.7	2.3 ± 0.3	1.0 ± 0.1
	8 CCl₄	0.5 ± 0.1	10.9 ± 1.3	12.5 ± 0.7	1.9 ± 0.2	1.1 ± 0.1

Each value represents the mean ± SEM.
Body weights in g, mean (range): control, 210 (194–220); CCl₄, 208 (190–220).
Liver weights in g, mean (range): control, 6.62 (5.90–7.50); CCl₄, 6.91 (6.03–7.40).

Protein and Triglyceride Concentrations in VLD-Lipoproteins: Changes with Time after the Administration of CCl₄

In another experiment, groups of rats were sacrificed at 0, 1, 2½, and 4 hr after the administration of CCl₄.

VLD-lipoproteins were isolated, and their protein and triglyceride concentrations were determined. A total of 16 rats were used. The results are presented in Fig. 2. A marked decrease in the concentration of both protein and triglycerides occurred within 1 hr after the administration of CCl₄. Smaller, consecutive decreases
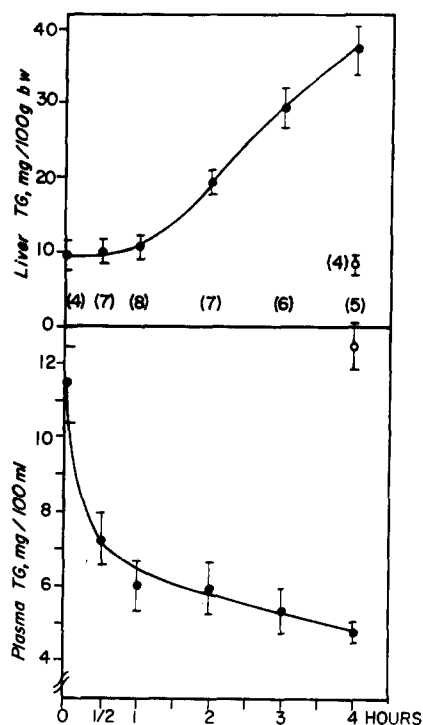
FIG. 1. Time of onset of hepatic and plasma triglyceride changes after the administration of CCl₄ (●) or mineral oil (○). Each point represents the mean ±SEM. Number of rats per group in parenthesis. (b w, body weight)

occurred thereafter. The effect on the protein was relatively more pronounced than that on the triglycerides. The ratios of triglycerides to protein at zero time, 1, $2^1/_2$, and 4 hr were 3.6, 7.1, 7.6, and 6.0, respectively. This indicates that a marked change in the over-all composition of this serum lipoprotein fraction occurs soon after CCl₄ administration, the protein content decreasing relatively more than that of the triglyceride (see also Table 1).

DISCUSSION

The most cogent hypothesis advanced in the past, to explain the effect of carbon tetrachloride on hepatic triglycerides, has been that of an impairment in fatty acid oxidation due to a number of specific lesions in the mitochondria of the liver cells (25, 26.) This hypothesis, however, proved untenable when it was shown that the mitochondrial lesions did not become established until several hours after the onset of the fatty liver, and therefore cannot be considered as pathogenetic (27–29). On the basis of electron microscopic and enzymatic studies, it now appears that the endoplasmic reticulum, rather than the mitochondria, is affected in the early stages of carbon tetrachloride intoxication (29–33).

Results recently obtained in a number of laboratories (2–6) have indicated that in CCl₄-treated rats there is a

direct relationship between the fall in the concentration of plasma triglycerides and the accumulation of triglycerides in the liver. The findings in all these studies are consistent with the hypothesis (2) that the development of fatty liver in CCl₄-treated rats is due primarily to a block in the release of hepatic triglycerides to the plasma. According to this thesis, the liver rapidly converts to triglycerides free fatty acids coming to it from the adipose tissue, but fails to release triglycerides into the plasma. As a consequence, triglycerides accumulate within the liver and decrease in the plasma. That the liver triglyceride fatty acids originate in the adipose tissue, in CCl₄-treated rats, has been shown by Horning, Earle, and Maling (34), and Barret, Best, and Ridout (35). Recently, Maximchuk and Rubenstein (36) have reported that the level of plasma free fatty acids is increased in rats intoxicated with CCl₄, and took this as an indication of an increased mobilization from adipose tissue. We have not seen such an increase after 4 hr of intoxication (Table 2), and Schotz and Recknagel (37) found no increase in the release in vitro of free fatty acids by adipose tissue of rats treated with CCl₄ for various times up to 9 hr. It therefore appears doubtful that increased mobilization of adipose tissue triglycerides is per se the cause of the deposition of triglycerides in the liver (6, 38).

In fasting animals the liver is the major source of plasma triglycerides (8, 9, 39, 40), which are synthesized chiefly from plasma free fatty acids (7–9, 41–44), and are released into the plasma as a moiety of lipoproteins (7–9). It is also known that lipids are present in the plasma almost entirely as lipoproteins (45–48), and the
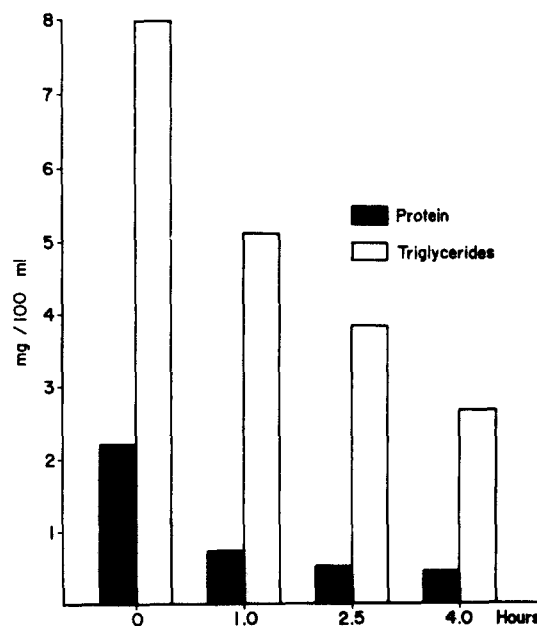

FIG. 2. Protein and triglyceride concentrations in VLD-lipoproteins. Changes with time after the administration of CCl₄.

available evidence indicates that the liver is the source of these lipoproteins (49–55). Thus, an interference with the hepatic synthesis and/or secretion of lipoproteins appears as the most probable mechanism by which CCl_4 affects the concentration of both liver and plasma lipids. This conclusion receives strong further support from the results of the present study, in which a large decrease in the level of circulating serum lipoproteins was found in rats with a fatty liver due to CCl_4-intoxication (Table 1). Of the fractions studied, the level of the VLD-lipoproteins was the most reduced, followed by that of the HD-fraction, while the level of the LD-lipoproteins was essentially normal. This difference is undoubtedly a reflection of the different role of each of these lipoproteins in the transport of fatty acids in the body (48). Under normal conditions the VLD-lipoproteins, which carry over 65% of the serum triglycerides (Table 2), represent the major vehicle by which triglycerides are transported away from the liver (9, 48). Isotopic studies of Havel, Felts, and Van Duyne (9) have shown clearly that hepatic triglycerides are the immediate precursors of those of the VLD-lipoproteins, while it was doubtful whether the lipoproteins of higher density derive their triglycerides from the liver or from the VLD-lipoproteins. Therefore, the finding of a greatly reduced (25% of the control) level of VLD-lipoproteins is of major significance for the understanding of the pathogenesis of the CCl_4-induced fatty liver. As shown in Fig. 2, the concentration of protein and of triglycerides in the VLD-lipoproteins is greatly reduced even 1 hr after intoxication, and at a time prior to the onset of the fatty liver (Fig. 1). This finding is just what one would expect were the accumulation of triglycerides in the liver due to a block of their release into the plasma as lipoproteins. That triglycerides fall in the plasma before any increase can be detected in the liver may be explained by the observations that (a) only a fraction of liver triglycerides is the immediate precursor of plasma triglycerides (9, 41, 44), (b) the pool of liver triglycerides is some twenty times larger than that of the plasma, and (c) the turnover of plasma triglycerides is faster than the over-all turnover of liver triglycerides (44).

Recently Ribeiro and McDonald (56) have reported an *increased* level of low-density lipoproteins after the administration of CCl_4 to rats. Differences in experimental conditions are probably responsible for the discrepancy between their results and those reported in this paper. For example, in their studies, (a) the animals were not starved before receiving the toxin, (b) the dose administered was smaller (0.1 ml/100 g body weight, subcutaneously, versus 0.25 ml by forced feeding), (c) the first analyses were performed 24 hr after administration, and (d) lipoprotein levels were estimated by electrophoretic techniques.

The effects of CCl_4 on serum lipoproteins, observed in our study, could result from interference with any of the following steps: synthesis of triglycerides and other lipid moieties; synthesis of the protein moiety; conjugation of the various moieties; release of the lipoproteins into the plasma.

Several alterations in the hepatic lipid synthesis have been shown to follow the administration of CCl_4 to animals (57–64). At present, however, there is no convincing evidence that an impairment in the synthesis of a lipid moiety is the primary effect of CCl_4 on lipoproteins. Whether a restriction exists in the availability of hepatic lipids for incorporation into lipoproteins is not known. On the other hand, a block in protein synthesis appears to be one of the earliest effects of CCl_4 on the liver (33, 65). Seakins and Robinson (5) have shown recently that such a block includes also the protein moiety of serum lipoproteins, for $2^1/_2$ hr after the administration of CCl_4 the extent of in vivo incorporation of leucine-^{14}C into these proteins was only about one-tenth that in the controls, the inhibition being somewhat greater for the protein of low-density lipoprotein than for that of the high-density fraction. The analysis of these data, as well as of others obtained in similar studies on the incorporation of ^{32}P and acetate-1-^{14}C into liver and plasma lipids, led these workers to conclude that the primary effect of CCl_4 on lipoproteins is an inhibition of the synthesis of their protein moiety. The finding, in the present study, of decreasing levels of circulating lipoprotein protein in rats treated with CCl_4 is consistent with these results. Also the finding that the protein of the VLD-fraction is the most reduced, is in agreement with what is known about the relative rate at which the various lipoprotein proteins are synthesized (53). It is significant to note that the greatest reduction in the level of the VLD-lipoprotein protein occurs during the first hour of intoxication (Fig. 2), that is, prior to the accumulation of triglycerides in the liver. Therefore, it appears very probable that the primary effect of CCl_4 on lipoproteins is a block in the synthesis of their protein moiety (5), and that the changes in plasma and liver lipids are secondary to this block. However, other possibilities must be kept in mind, such as that the protein is synthesized but is not available for incorporation into the lipoprotein molecule.

Absolute measurements of the rate of turnover of the protein moiety of serum lipoproteins, especially of the VLD-fraction, are not available for the rat. In other animal species it has been estimated that their circulating half-life is of the order of a few days (66–68). The results presented in this paper (as well as similar ones obtained in ethionine-treated rats)[3] indicate that, either the turn-

[3] G. Ugazio, and B. Lombardi. Paper submitted for publication in *Lab. Invest.*

over of the lipoprotein proteins in the rat is considerably faster than in other species, or that other factors besides a block in synthesis contribute to the reduction in the level of circulating lipoprotein proteins after administration of CCl_4 to rats. Recently Eder, Roheim, Gidez, and Switzer (69) have reported the presence in rat plasma of a lipid acceptor protein, which is said to be turning over "very rapidly," and can act as a precursor for the protein moiety of the VLD-lipoproteins. Whether and to what extent this protein is involved in the pathogenesis of the CCl_4-induced fatty liver remains to be determined.

At present there is no evidence that CCl_4 interferes with the conjugation of the various moieties and/or the secretion of serum lipoproteins by the liver. Relatively more total lipids and triglycerides were found to be bound to the protein of the VLD- and LD-lipoproteins of the CCl_4-treated rats, than to that of the controls (Table 1, Fig. 2). This finding may represent further evidence that the primary effect is an inhibition of the protein moiety synthesis, if interpreted as the result of an attempt by the liver to release as much lipid as possible with whatever protein is available for lipoprotein synthesis. An alternative explanation which must be considered, however, is that the uptake of plasma triglycerides by the liver and the extrahepatic tissues (6) of CCl_4-treated rats is reduced, resulting in a higher ratio of triglyceride to protein in the circulating lipoproteins. If the first interpretation is correct, one could then infer, also, that CCl_4 probably does not interfere with the conjugation of the lipid and protein moieties, or with the release by the liver of preformed lipoproteins.

REFERENCES

1. Scholtz, M. C., and R. O. Recknagel. *Biochim. Biophys. Acta* **41**: 151, 1960.
2. Recknagel, R. O., B. Lombardi, and M. C. Schotz. *Proc. Soc. Exptl. Biol. Med.* **104**: 608, 1960.
3. Maling, H. M., A. Frank, and M. G. Horning. *Biochim. Biophys. Acta* **64**: 540, 1962.
4. Heimberg, M., I. Weinstein, G. Dishman, and A. Dunkerley. *J. Biol. Chem.* **237**: 3623, 1962.
5. Seakins, A., and D. S. Robinson. *Biochem. J.* **86**: 401, 1963.
6. Schotz, M. C., N. Baker, and M. N. Chavez. *J. Lipid Res.* **5**: 569, 1964.
7. Havel, R. J., and A. Goldfien. *J. Lipid Res.* **2**: 389, 1961.
8. Borgström, B., and T. Olivecrona. *J. Lipid Res.* **2**: 263, 1961.
9. Havel, R. J., J. M. Felts, and C. M. Van Duyne. *J. Lipid Res.* **3**: 297, 1962.
10. Havel, R. J., H. A. Eder, and J. H. Bragdon. *J. Clin. Invest.* **34**: 1345, 1955.
11. Korn, E. D. *Methods Biochem. Analy.* **7**: 145, 1959.
12. Del Gatto, L., F. T. Lindgren, and A. V. Nichols. *Anal. Chem.* **31**: 1397, 1959.
13. De Lalla, O. F., and J. W. Gofman. *Methods Biochem. Analy.* **1**: 459, 1954.
14. Chiang, S. P., C. F. Gessert, and O. H. Lowry. *Air Force School of Aviation Medicine, Research Report No. 56,113* June, 1957.
15. Burstein, M. *J. Physiol. (Paris)* **54**: 647, 1962.
16. Burstein, M. *Nouvelle Rev. Franc. Hematol.* **3**: 139, 1963.
17. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. *J. Biol. Chem.* **193**: 265, 1951.
18. Kirk, P. L. *Anal. Chem.* **22**: 354, 1950.
19. Folch, J., M. Lees, and G. H. Sloane Stanley. *J. Biol. Chem.* **226**: 497, 1957.
20. Bowman, R. E., and R. C. Wolf. *Clin. Chem.* **8**: 302, 1962.
21. Van Handel, E., and D. B. Zilversmit. *J. Lab. Clin. Med.* **50**: 152, 1957.
22. Van Handel, E. *Clin. Chem.* **7**: 249, 1961.
23. Duncombe, W. G. *Biochem. J.* **88**: 77, 1963.
24. Shin, Y. S. *Anal. Chem.* **34**: 1164, 1962.
25. Christie, G. S., and J. D. Judah. *Proc. Roy. Soc. London, Ser. B.*, **142**: 241, 1954.
26. Dianzani, M. U., and U. Marinari. *Biochim. Biophys. Acta* **48**: 552, 1961.
27. Calvert, D. N., and T. M. Brody. *J. Pharmacol. Exptl. Therap.* **124**: 273, 1958.
28. Recknagel, R. O., and D. D. Anthony. *J. Biol. Chem.* **234**: 1052, 1959.
29. Recknagel, R. O., and B. Lombardi. *J. Biol. Chem.* **236**: 564, 1961.
30. Oberling, C., and C. Rouiller. *Ann. Anat. Pathol.* **1**: 401, 1956.
31. Bassi, M. *Exptl. Cell Res.* **20**: 313, 1960.
32. Neubert, D., and D. Maibauer. *Arch. Exptl. Pathol. Pharmakol.* **235**: 291, 1959.
33. Smuckler, E. A., O. A. Iseri, and E. P. Benditt. *J. Exptl. Med.* **116**: 55, 1962.
34. Horning, M. G., M. J. Earle, and H. M. Maling. *Biochim. Biophys. Acta* **56**: 175, 1962.
35. Barret, H. M., C. H. Best, and J. H. Ridout. *J. Physiol.* **93**: 367, 1938.
36. Maximchuk, A. J., and D. Rubenstein. *Can. J. Biochem. Physiol.* **41**: 525, 1963.
37. Schotz, M. C., and R. O. Recknagel. *Proc. Soc. Exptl. Biol. Med.* **103**: 398, 1960.
38. Lombardi, B., and R. O. Recknagel. *Am. J. Pathol.* **40**: 571, 1962.
39. Harper, P. V., Jr., W. B. Neal, Jr., and G. R. Hlavacek. *Metabolism* **2**: 69, 1953.
40. Byers, S. O., and M. Friedman. *Am. J. Physiol.* **198**: 692, 1960.
41. Stein, Y., and B. Shapiro. *Am. J. Physiol.* **196**: 1238, 1959.
42. Laurell, S. *Acta Physiol. Scand.* **46**: 97, 1959.
43. Olivecrona, T. *Acta Physiol. Scand.* **54**: 295, 1962.
44. Baker, N., and M. C. Schotz. *J. Lipid Res.* **5**: 188, 1964.

45. Gofman, J. W., O. DeLalla, F. Glazier, N. K. Freeman, F. T. Lindgren, A. V. Nichols, B. Strisover, and A. R. Tamplin. *Plasma* **2:** 413, 1954.

46. Fredrickson, D. S., and R. S. Gordon, Jr. *Physiol. Rev.* **38:** 585, 1958.

47. Oncley, J. L. In *Chemistry of Lipids as Related to Atherosclerosis*, edited by I. H. Page. Charles C Thomas, Springfield, 1958, pp. 114–129.

48. Olson, R. E., and J. W. Vester. *Physiol. Rev.* **40:** 677, 1960.

49. Marsh, J. B. *Federation Proc.* **17:** 270, 1958.

50. Radding, C. M., J. H. Bragdon, and D. Steinberg. *Biochim. Biophys. Acta* **30:** 443, 1958.

51. Marsh, J. B., and A. F. Whereat. *J. Biol. Chem.* **234:** 3196, 1959.

52. Radding, C. M., and D. Steinberg. *J. Clin. Invest.* **39:** 1560, 1960.

53. Haft, D. E., P. S. Roheim, A. White, and H. A. Eder. *J. Clin. Invest.* **41:** 842, 1962.

54. Roehim, P. S., D. E. Haft, L. I. Gidez, A. White, and H. A. Eder. *J. Clin. Invest.* **42:** 1277, 1963.

55. Marsh, J. B. *J. Biol. Chem.* **238:** 1752, 1963.

56. Ribeiro, L. P., and H. J. McDonald. *Clin. Chim. Acta* **8:** 727, 1963.

57. Severi, C., and A. Fonnesu. *Proc. Soc. Exptl. Biol. Med.* **91:** 368, 1956.

58. Rossi, F., and M. Zatti. *Experientia* **16:** 513, 1960.

59. Wilgram, G. F., S. W. Smith, D. J. Ingle, and E. P. Kennedy. *Federation Proc.* **20:** 290, 1961.

60. Rossi, F., and M. Zatti. *Brit. J. Exptl. Pathol.* **44:** 131, 1963.

61. Horning, M. G., M. Wakabayashi, and H. M. Maling. In *Effects of Drugs on Synthesis and Mobilization of Lipids*, edited by E. C. Horning. Macmillan, New York, 1963, pp. 13–27.

62. Rees, K. R., and V. L. Shotlander. *Ann. N. Y. Acad. Sci.* **104:** 905, 1963.

63. Aiyar, A. S., P. Fatterpaker, and A. Sreenivasan. *Biochem. J.* **90:** 558, 1964.

64. Rubinstein, B., and D. Rubenstein. *Can. J. Biochem.* **42:** 1263, 1964.

65. Smuckler, E. A., O. A. Iseri, and E. P. Benditt. *Biochem. Biophys. Res. Commun.* **5:** 270, 1961.

66. Volwiler, W., P. D. Goldsworthy, M. P. MacMartin, P. A. Wood, I. R. MacKay, and K. Fremont-Smith. *J. Clin. Invest.* **34:** 1126, 1955.

67. Avigan, J., H. A. Eder, and D. Steinberg. *Proc. Soc. Exptl. Biol. Med.* **95:** 429, 1957.

68. Gitlin, D., and D. Cornwell. *J. Clin. Invest.* **35:** 706, 1956.

69. Eder, H. A., P. S. Roheim, L. I. Gidez, and S. Switzer. In *Proceedings of an International Symposium on Lipid Transport*, edited by H. C. Meng. Charles C Thomas, Springfield, 1964, p. 202.