

Serum and lymph lipids in rabbits with carbon tetrachloride-induced cirrhosis of the liver

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ABSTRACT Lymph flow and the composition of lymph lipids from the hepatic and thoracic ducts of rabbits with cirrhosis of the liver (induced by 46–51 intramuscular injections of a mixture of carbon tetrachloride and olive oil at 4-day intervals) have been compared with those of control animals injected with olive oil only. In cirrhotic animals, the concentration of lymph lipids was not greatly altered, but lymph flow, and consequently the hourly transport of lipids by lymph were greatly increased; the increase in transport of cholesteryl esters, free cholesterol, and phospholipids by way of the thoracic and hepatic duct lymph was particularly striking. The concentration of these lipid fractions in serum from the cirrhotic rabbits was also increased.

The differences normally observed between lipid fatty acid compositions of serum and lymph disappeared in cirrhotic animals; this is interpreted as due to increased hepatic permeability to lipoproteins.

KEY WORDS fatty liver · cirrhosis · rabbits · carbon tetrachloride · lymph · flow · serum · lipid composition · IR spectrometry · fatty acid composition · hepatic permeability

A MARKED INCREASE in lymph flow has been noted in patients with hepatic cirrhosis (1–3). In rats and dogs with CCl₄-induced hepatic cirrhosis (4, 5), a similar increase in lymph flow has been reported. The major acute effects of CCl₄ administration are an accumulation of fat (mainly triglycerides) in the liver, and a decrease in plasma triglycerides (6–12). Brief CCl₄ treatment leads to some increase in cholesterol, free fatty acids, and phospholipids within a few hours (10). The present studies were carried out to determine the chronic effects of CCl₄ administration on lipid levels of serum

Abbreviations: CE, cholesteryl esters; TG, triglycerides; FFA, free fatty acids; FC, free cholesterol; PL, phospholipids.

and lymph, and to evaluate the role of increased hepatic lymph flow in the cirrhotic animal as a pathway for release of liver lipids to the circulation. The fatty acid composition of lipid constituents of serum and of hepatic and thoracic duct lymph was determined in order to ascertain whether this composition changes in hepatic cirrhosis.

METHODS

Animal Treatment

Male and female albino rabbits of about 2 kg body weight at the start of the experiment, fed a pellet diet (RC5, Oriental Yeast Mfg. Ltd., Tokyo) with water ad lib., were divided into three groups, A, B, and C. Animals in groups A and B were given 46–51 intramuscular injections (0.5 ml/kg of body weight) of CCl₄-olive oil 1:4 at 4-day intervals. Control animals (group C) received injections of 0.5 ml/kg of body weight of olive oil. All animals were fasted for 24 hr after the last injection and prior to lymph collection. Thoracic duct lymph was collected from group A and C animals by cannulation in the neck, under sodium pentobarbital anesthesia. Hepatic duct lymph was collected from group B animals by cannulation of a prenodal hepatic lymph duct, under sodium pentobarbital anesthesia. A very small amount of heparin powder was added to the lymph to prevent clotting. Immediately after the lymph collections (which lasted 1.5–5 hr), blood was withdrawn from the hepatic vein and the abdominal aorta. Blocks of liver were excised and fixed in 10% formalin; sections made from them were stained with hematoxylin and eosin.

Extraction of Lipids

Lipids were extracted with chloroform-methanol 2:1, filtered, and washed three times with chloroform-

methanol-water, 3:48:47 by volume (13). The chloroform phase was dried under a stream of N_2 at low pressure. The lipid residue was taken up in 5 ml of petroleum ether (bp 60–70°C) and pipetted onto a column (20 mm i.d. \times 250 mm) packed with 30 g of silicic acid (100 mesh, Mallinckrodt Chemical Works, St. Louis, Mo.).

Column and Thin-Layer Chromatography

Solvents were freshly distilled. 350 ml of diethyl ether-petroleum ether 1:99, 350 ml of diethyl ether-petroleum ether 4:96, 450 ml of diethyl ether, and 300 ml of methanol were used as solvents for the stepwise elution of cholesteryl esters (CE), triglycerides (TG), free fatty acids (FFA) and free cholesterol (FC), and phospholipids (PL), respectively. An aliquot from each fraction was analyzed by IR spectrometry, and another aliquot by gas-liquid chromatography. Each fraction was checked by thin-layer chromatography against a standard mixture consisting of various lipid classes with petroleum ether-diethyl ether-glacial acetic acid 90:10:1 as solvent. Spots were detected by spraying with 50% sulfuric acid and charring.

IR Spectrometry

The IR spectrophotometer (Type EPI-S, Hitachi Ltd., Tokyo) was equipped with a sodium chloride prism. The sample, in a measured volume of CS_2 (14), was measured in an absorption cell that had an optical path of 0.9 mm. The concentrations of TG, CE, FFA, PL, and FC were determined by comparison of the peak absorbance of bands at 5.75, 5.8, 5.85, 9.35, and 9.5 μ , respectively, with calibration curves obtained from pure cholesteryl stearate, cholesterol, tristearin, stearic acid, and egg lecithin (Tokyo Kasei Co. Ltd., Tokyo).

Gas-Liquid Chromatography

An aliquot of each lipid fraction was saponified with 0.5 N KOH-ethanol by the method of Böttcher, Woodford, Boelsma-van Houte, and Van Gent (15) and methylated with BF_3 -methanol according to the method of Metcalfe and Schmitz (16). The methyl esters were analyzed on a gas-liquid chromatograph (Type GC-1B, Shimadzu Seisakusho Ltd., Kyoto) with a column 2.25 m \times 4 mm i.d. containing 15% ethylene glycol succinate polyester coated on 60–80 mesh Shimalite B. The column was kept at 215°C, and the flame ionization detector at 280°C. Arachidic acid was used as internal standard and the peak areas were determined by triangulation. Peaks were identified by comparison with reference esters (Applied Science Laboratories Inc., State College, Pa.).

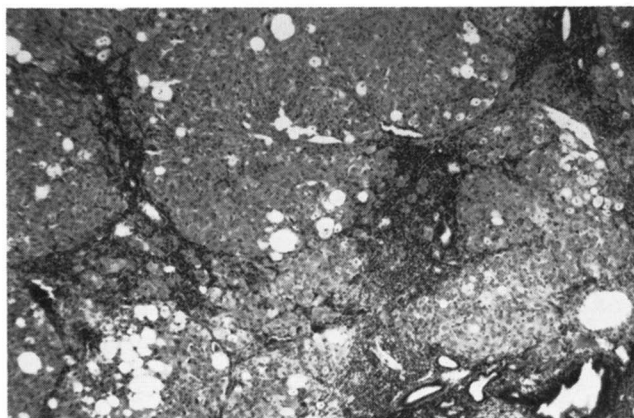


FIG. 1. Section of rabbit liver showing cirrhotic changes induced by chronic injections of CCl_4 . (Hematoxylin-cosin \times 90.)

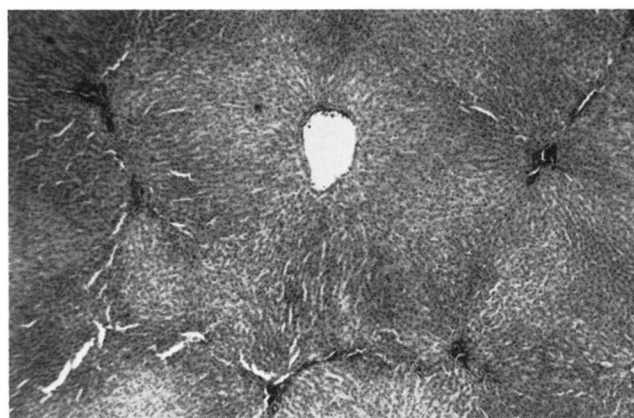


FIG. 2. Section of normal control rabbit liver. (Hematoxylin-cosin \times 90.)

RESULTS

Gross and Microscopic Observations

On autopsy, all of the CCl_4 -treated rabbits showed evidence of ascites; the amount of free fluid was variable. The livers of CCl_4 -treated animals were uniformly irregular and nodulated, as compared with the smooth and regular surface of the control rabbits. The liver lobules of treated animals were irregular in shape and size, and were surrounded by abundant interlobular connective tissue infiltrated with mononuclear cells (Fig. 1); in the livers of control animals, there was only a scant amount of interlobular connective tissue (Fig. 2). The liver cells of treated animals showed the “hydropic degeneration” or the “balloon-like” changes described previously (17).

Flow Rate and Output of Lymph

Table 1 shows that the flow rate of thoracic duct lymph was 12.9 ml/hr in cirrhotic as compared with 2.0 ml/hr in control animals. In animals of group B the hepatic

TABLE 1 FLOW RATE AND LIPID CONCENTRATIONS OF SERUM AND LYMPH LIPIDS IN CONTROL AND CARBON TETRACHLORIDE-TREATED RABBITS

Group	Lymph	Flow Rate	Lipid Composition* of Lymph					Lipid Composition* of Serum				
			CE	FC	TG	FFA	PL	CE	FC	TG	FFA	PL
		ml/hr	mg/100 ml					mg/100 ml				
A (6)†	Thoracic	12.9 ± 2.1‡	47.9 ± 9.6	18.3 ± 3.2	59.9 ± 11.9	8.7 ± 1.8	73.6 ± 13.1	138.6 ± 20.2	49.7 ± 19.9	53.0 ± 11.2	17.2 ± 3.5	110.1 ± 16.3
B (5)	Hepatic	1.8 ± 0.2	116.2 ± 24.9	49.2 ± 10.1	87.7 ± 13.2	8.8 ± 1.8	86.3 ± 18.0	133.3 ± 45.0	44.2 ± 10.3	90.5 ± 15.6	7.8 ± 1.0	138.7 ± 20.0
C (7)	Thoracic	2.0 ± 0.3	40.5 ± 2.4	13.7 ± 2.5	100.9 ± 10.5	21.9 ± 2.2	44.2 ± 9.1	31.5 ± 4.0	11.0 ± 1.2	49.0 ± 5.3	15.1 ± 3.2	31.2 ± 6.5

* CE, Cholesteryl esters; FC, free cholesterol; TG, triglycerides; FFA, free fatty acids; and PL, phospholipids.

† Groups A and B, CCl₄-treated; group C, control. Number of animals in parentheses.

‡ Mean ± SEM.

lymphatic vessels found to be dilated were ordinarily 4–6 in number. Lymph was collected from one of those dilated vessels in sufficient volume to permit analysis of lipids. Lymph flow in one of the lymphatic vessels from the liver of group B animals was 1.8 ml/hr. Attempts to collect hepatic lymph from animals injected with olive oil were not successful.

Lymph and Serum Lipid Concentration

The lipid compositions of lymph from thoracic and hepatic ducts are presented in Table 1. Although the concentrations of TG and FFA in thoracic duct lymph were lower in group A than in group C, the amount of these lipids transported per hour increased rather markedly (because of the higher flow rate) in group A; relative increase was greater for TG than for FFA. All other lipids were found in slightly higher concentrations in group A than in group C and the increase in their transport during cirrhosis was therefore striking. The concentrations of CE and FC were still higher in hepatic lymph from group B than in thoracic lymph from group A.

The lipid composition of serum from the abdominal aorta of animals from groups A–C is also shown in Table 1. The concentrations of CE, FC, and PL were higher in the cirrhotic animals than in the controls. The concentrations of TG and FFA were not different in cirrhotic animals. As there was no significant difference between the lipid concentration of serum from the abdominal aorta and hepatic, vein, values for the latter are not shown.

Lymph and Serum Fatty Acid Compositions

Table 2 shows the percentage composition of major fatty acids of lymph from thoracic and hepatic ducts of animals from groups A, B, and C. The percentages of major fatty acids of serum from the hepatic vein are also shown in Table 2. The fatty acid composition of serum lipids from the abdominal aorta did not differ from that of serum lipids from the hepatic vein in each group. In the cirrhotic animals, the fatty acid composition of lymph lipids was similar to that of serum

TABLE 2 PERCENTAGES OF MAJOR FATTY ACIDS OF SERUM AND LYMPH LIPIDS IN CONTROL AND CARBON TETRACHLORIDE-TREATED RABBITS

	Controls*		CCl ₄ -treated†			
	Thoracic Lymph	Serum	Thoracic Lymph	Serum	Hepatic Lymph	Serum
Cholesteryl esters						
16:0	31.2	18.0	17.4	19.8	21.1	22.2
16:1	5.9	3.6	4.0	4.0	2.7	3.3
18:0	10.2	6.0	6.6	5.9	7.3	7.5
18:1	18.5	18.5	30.2	28.9	23.8	23.1
18:2	22.4	40.7	27.7	26.0	33.1	32.2
18:3	4.4	4.2	7.4	6.7	6.1	6.4
20:4	0.5	2.4	0.9	1.1	1.4	1.6
Triglycerides						
16:0	30.0	40.4	32.2	34.4	38.4	38.6
16:1	3.9	5.0	3.4	4.0	2.8	3.1
18:0	11.4	4.4	10.1	6.4	8.0	6.6
18:1	16.7	22.7	21.2	22.0	20.9	19.7
18:2	26.2	17.7	18.7	18.8	18.2	20.4
18:3	5.4	5.5	6.4	7.2	5.8	6.3
20:4	0.3	—	tr.	0.5	0.3	0.3
Free fatty acids						
16:0	30.6	34.0	27.1	30.2	36.5	31.9
16:1	4.4	4.9	4.7	3.8	3.5	2.8
18:0	7.5	8.3	5.9	9.4	11.8	8.3
18:1	20.6	21.5	16.5	19.8	18.8	18.1
18:2	19.8	17.4	20.0	14.1	11.5	19.4
18:3	8.3	9.0	14.1	9.4	5.9	8.3
20:4	0.4	0.7	2.4	1.9	1.2	1.4
Phospholipids						
16:0	19.4	20.5	25.9	22.8	24.2	24.7
16:1	1.6	1.3	1.0	0.9	0.9	1.0
18:0	22.6	25.9	20.3	20.5	22.4	20.7
18:1	11.3	9.2	13.8	14.6	11.4	12.2
18:2	29.8	29.7	27.6	28.5	28.5	29.0
18:3	4.5	2.5	2.4	2.8	2.7	3.0
20:4	3.6	6.3	4.8	5.0	6.3	5.6

Fatty acids are designated by number of carbon atoms: number of double bonds.

* Group C.

† Group A (thoracic) and Group B (hepatic).

lipids in every fraction, whereas in the controls, the proportion of some major fatty acids in CE and TG was different for lymph and serum.

DISCUSSION

The livers of CCl₄-treated animals showed the intensive histological changes characteristic of cirrhosis (17–21).

Ascites was present. The protein concentrations of hepatic and thoracic duct lymph of the treated animals and of thoracic duct lymph of the control animals were 6.80, 4.62, and 4.25 g/100 ml, respectively. The albumin:globulin ratios were 0.84, 1.17, and 1.76, respectively. About seven times as much protein was transported per hour in the thoracic duct lymph of the treated animals as in that of the controls. These results compel us to accept the view (4, 5) that the permeability of the cirrhotic liver is greater than that of the control liver in that a larger volume of lymph is formed and also that the lymph contains more protein and relatively more globulin than that of the control animals. The albumin:globulin ratio of blood serum was lower in the cirrhotic animals (1.00) than in the controls (1.75). In treated animals the amounts of α - and β -globulins transported in thoracic duct lymph per hour were 88 and 114 mg, respectively, and in controls 13 and 11 mg. In the hepatic lymph of treated animals, the percentages of α - and β -globulins in the total proteins were 17.7% and 21.3%—both higher than in thoracic duct lymph (α , 14.8%; β , 19.2%). These higher proportions were probably due to the presence of increased concentrations of α - and β -lipoproteins, since some lipid concentrations in lymph were increased (Table 1).

In normal animals, the fatty acid compositions of different lipid classes in serum are different from those in thoracic duct lymph (Table 2); this difference between serum and lymph disappears in the CCl₄-treated animals. The similarity between lymph and serum in the treated animals could be explained on the basis of permeability changes in the liver.

It has been demonstrated that the lipid content of the cirrhotic liver of mice after long-term CCl₄ treatment is much lower than that of normal liver (19). The cholesterol concentration of plasma immediately after brief CCl₄ administration did not increase, or did so very slightly (8, 10). In the rabbits of the present study, hypercholesterolemia developed after long-term CCl₄ treatment (Table 1); such an increase in the cholesterol concentration of serum after long-term CCl₄ administration has not been reported before. It may depend on the animal species, and also on the involvement of organs other than the liver, e.g., the adrenals (20). In human

cirrhosis, plasma cholesterol concentration is lower than normal (22).

We wish to thank Dr. F. Ichida, Associate Professor in the Virus Institute of Kyoto University, and Dr. W. O. Reinhardt, Professor of Anatomy of the University of California San Francisco Medical Center for their invaluable advice in connection with this work.

Manuscript received 28 June 1966; accepted 19 December 1966.

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