The Incorporation of Ricinoleic Acid into Rat Lymph Lipids* (34109)

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(Introduced by F. A. Kummerow)

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One milliliter of ricinoleic acid, triricinolein, or methyl ricinoleate was administered by gastric intubation to thoracic duct–cannulated rats. The lymph was collected over a 48-hr period, the lipids extracted from it, and separated into the various lipid classes by a combination of ion exchange and silicic acid and thin-layer chromatography. The fatty acid composition of each class was determined. The results indicated that ricinoleic acid was present in the triglyceride, diglyceride, monoglyceride, and free fatty acid fractions and reached a peak absorption within 30 hr after administration. Ricinoleic acid was not found in either the phospholipid or cholesterol ester fractions of the lymph lipids. The presence of hydroxy acids in the neutral lipid fractions exerted no particular influence on the general fatty acid composition of the lymph lipids when compared to the lymph lipids from animals that had received a fat-free diet.

Dietary fats which contained long-chain hydroxy acids have been shown to influence the mixed fatty acid composition of rat carcass fat (1). A small percentage of the hydroxy acids was deposited and the remainder converted mainly to monoenoic acids. Both saturated and unsaturated hydroxy acids were apparently converted to monoenes. A larger amount of octadecenoic and hexadecenoic acids seemed to be deposited and a preferential excretion of stearic and linoleic acids appeared to occur in animals fed a source of hydroxy acids in comparison with those fed a source of linoleic acid. The changes in the fatty acid composition which occurred in the lipid classes within the lipids of lymph when ricinoleic acid or its analogs were administered by gastric intubation to thoracic duct–cannulated rats were determined in the present study.

Experimental Methods. A permanent thoracic duct cannulation was carried out according to the method of Bollman (2) on male rats with a minimum weight of 400 g. Intramedic polyethylene tubing (PE 50) which had been previously wetted with a 1% heparin solution was inserted into the main thoracic duct of the rat under anesthesia with Nembutal (5 mg/100 g body weight). The rats were kept in restraining cages and allowed free access to saline solution (0.8% sodium chloride in distilled water), and a fat-free diet (3) for 24 hr.

One milliliter of the test fat was administered with the aid of a stomach tube to a slightly anesthetized rat 24 hr after the operation. The fats were prepared as previously described (1), from concentrates of triricinolein prepared from castor oil[2]. Lymph was collected in 15-ml centrifuge tubes kept in an ice bath and a fresh tube was used after 1, 3, 4, 8, 12, 16, 20, 24, 30, 36, 42, and 48 hr of collection. The lymph was defibrinated with a glass rod and kept under refrigeration.

The lipid was extracted from the lymph by three extractions of 20 vol each of a 2:1:2 chloroform:methanol:diethyl ether solvent system. The combined extracts were dried

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[2] The triricinolein concentrate, as well as the ricinoleic acid and methyl ricinoleate, had the following composition: 16:0, 1.9%; 18:0, 1.2%; 18:1, 4.9%; 18:2, 6.2%; ricinoleic acid, 84.8%.

over anhydrous sodium sulfate, filtered, and the solvent removed under vacuum. Each lipid sample was weighed and stored at 0° under nitrogen until required for further analysis.

The free fatty acids present in the lymph lipids were removed with Amberlite IRA-400, a strong anion-exchange resin, using a modification of Hornstein's method (4). The remaining lymph lipids were fractionated on silicic acid columns (5). Triglycerides containing hydroxy acids were removed from the phospholipid fractions with the aid of a second silicic acid column as previously described (6). The homogeneity of these fractions was determined by thin-layer chromatography.

Methyl esters were prepared from the fatty acids of lymph lipids by transesterification as described for the preparation of methyl ricinoleate (1). The methyl esters were analysed by gas chromatography as previously described (7).

Results and Discussion. The administration of triricinolein and ricinoleic acid to rats resulted in the incorporation of ricinoleic acid into the triglyceride, free fatty acid, and mono- and diglyceride fractions of lymph lipid in varying proportions (Table I). The administration of either methyl ricinoleate, ricinoleic acid, or triricinolein to lymph-cannulated rats appeared to exert very little effect upon the rate of absorption of these materials or the rate of formation of monoglycerides, diglycerides, or triglycerides from the substrate. Methyl ricinoleate appears to be hydrolyzed as easily as the triglyceride. The period of maximum formation of mono- di- and triglycerides from methyl ricinoleate and ricinoleic acid was at 30 hr after ingestion. However, when the maximum appearance of free fatty acid formed by hydrolysis of methyl ricinoleate and triglyceride was measured, it was found to be at 24 hr.

Further data obtained for the distribution of ricinoleic acid among the lipid classes in total lymph samples 48 hr after sample administration is shown in Table II. Ricinoleic acid was incorporated more extensively into the triglyceride and mono- and diglyceride fraction than when the triglyceride moiety was fed, or 24 and 41.5% respectively. These differences in incorporation may be due to the different mechanisms of digestion and absorption of ricinoleic acid and triricinolein. Triricinolein may undergo inadequate emulsification in the intestine and subsequently limit absorption in the intestinal mucosa while dietary ricinoleic acid may undergo limited absorption into the intestinal mucosa but once within the mucosal wall is apparently transformed into triglycerides as readily as

TABLE I. Incorporation of Ricinoleic Acid into Lymph Lipids.[a]

Collection of lymph lipids (time in hr)	Substrate administered							
	Methyl ricinoleate (%)			Ricinoleic acid (%)		Triricinolein (%)		
	TG[b]	M&DG[c]	FFA[d]	TG	M&DG	TG	M&DG	FFA
12[e]	—	—	5.4	—	—	—	—	6.1
16	3.9	3.6	21.1	6.3	6.0	5.8	4.7	10.4
20	19.0	—	36.8	11.6	16.9	9.7	8.8	16.6
24	—	20.2	49.9	19.9	24.6	15.8	13.9	22.7
30	32.9	27.9	34.3	26.1	38.5	20.5	18.3	17.3
36	24.3	22.2	18.8	17.2	20.9	13.6	11.4	10.2
42	10.9	7.6	3.2	9.9	7.3	6.3	5.9	4.2

[a] The results are the average of the three determinations. Maximum variation between determinations was ± 0.6%.

[b] Triglycerides.

[c] Mono- and diglycerides.

[d] Free fatty acids.

[e] Significant amounts of hydroxy esters were not present in earlier samples.

TABLE II. Incorporation of Ricinoleic Acid into Lymph Lipids.[a]

Fat administered	Triglyceride (%)	Free fatty acid (%)	Mono- and diglycerides (%)
Triricinolein	10.6	10.2	10.0
Ricinoleic acid	23.9	12.6	41.5
Methyl ricinoleate	21.9	48.3	17.8

[a] Percentage of occurrence within the total fatty acid composition of that fraction after 48 hr. Numerical averages of three animals from each group.

the mono- and diglycerides. Methyl ricinoleate appears to be handled in much the same way as the triglyceride, and it is apparently more readily hydrolyzed to the free acid.

The observation that ricinoleic acid was not found in the cholesterol ester fraction of the lymph from rats fed triricinolein, ricinoleic acid, and methyl ricinoleate may be explained by the results obtained by Whyte, Karmen, and Goodman (8). They determined the distribution of the total chylomicron fatty acids among the liver lipid classes after feeding a labeled dietary fat and found that the glycerides contained 92% of all fatty acid, lecithin 6%, and cholesterol esters 1.8%. These percentages were remarkably similar, regardless of the type of fat administered; that is, labeled acids in olive oil or in corn oil. However, less than 2% of exogenous fatty acids were incorporated into the cholesterol ester fraction of the chylomicron. A limited absorption of ricinoleic acid from the small intestine would tend to further inhibit its esterification and reduce the amounts of ricinoleic acid in the cholesterol ester fraction.

The absence of ricinoleic acid in the phospholipids may be explained in a similar manner. During the intestinal absorption of long-chain fatty acids, a small fraction of the absorbed fatty acids usually appears in lymph as chylomicron phospholipid. The extent to which this occurs for each major fatty acid under different dietary conditions is not entirely clear. However, from the results of studies (9–15) on both rats and human subjects, about 2–6% of the administered exogenous fatty acid radioactivity was usually incorporated into lecithin. Clement and Mead (16) also have reported a low recovery of radioactivity (1–6%) in lymph phospholipids after feeding oleic acid ^{14}C.

Since such a small fraction of absorbed fatty acids appears in the phospholipid, and especially since the rate of absorption of ricinoleic acid is slow, the presence of ricinoleic acid in the phospholipid fraction of lymph lipids is unlikely and this was indeed shown to be the case when dietary triricinolein, ricinoleic acid, and methyl ricinoleate were fed. A similar observation was reported by Stewart and Sinclair (17) who indicated that ricinoleic acid was not present in the phospholipids of the small intestine, liver, or muscle. The hydroxy acids, when fed via stomach tube, exerted no significant influence on the

TABLE III. Fatty Acid Composition of Lymph Lipid Fractions before and after Administration of Ricinoleic Acid in Rats.[a]

	Endogenous lipid fractions (%)		Lipid fractions after feeding ricinoleic acid (%)	
Lipid class	Triglyceride	Free fatty acid	Triglyceride	Free fatty acid
C12:0	0.5	1.6	0.6	0.8
C14:0	1.2	1.7	1.5	1.1
C15:0	0.8	1.5	0.4	0.7
C16:0	38.6	39.2	31.9	32.3
C16:1	1.9	1.7	2.2	1.6
C18:0	16.8	16.0	14.3	15.5
C18:1	31.1	28.2	24.4	25.5
C18:2	5.3	6.9	4.4	5.7
C18:3	3.8	3.2	3.1	2.3
R-A	—	—	17.2	14.5

[a] Percentage of total fatty acid composition in that fraction collected for 36 hr. The results reported are the average of three determinations in each group.

general fatty acid composition of the lipid classes of lymph lipid as shown by the comparison of the triglyceride and free fatty acid fractions with and without administered ricinoleic acid (Table III). The fatty acid composition of the lymph lipids obtained in this way was, with the exception of the presence of ricinoleic acid, comparable to that obtained from animals fed a fat-free diet during the experimental period.

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