Origin and characteristics of endogenous lipid in thoracic duct lymph in rat

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ABSTRACT Thoracic duct lymph of rats eating a fat-free diet contained 7 mg of lipid per hr. The lipid was 70% triglyceride, and largely in the d < 1.006 lipoprotein fraction. Lipid of the d < 1.006 fraction of the lymph was many times more concentrated than that of the blood plasma at the same time. It reached the thoracic duct via lymphatics from the intestine; little entered from the liver. The fatty acid moiety composing over three-fourths of the lymph lipid mass was undoubtedly derived in part from bile lipid—possibly to the extent of roughly 50%, and in some part from other intraluminal materials. Studies with labeled palmitic acid indicated that little circulating free fatty acid was taken up by the intestinal mucosa and incorporated into lymph lipid.

KEY WORDS thoracic duct lymph · endogenous lipid · composition · source of fatty acid · intestine · liver · bile · blood · rat · eicosa-13-enoic acid

It is generally agreed that plasma triglycerides (TG) and phospholipids (PL) in the fasting animal are produced largely by the liver (1, 2), but there is evidence that they continue to be produced to some extent after hepatectomy (2, 3). Several observations suggest that plasma lipids may originate endogenously from the intestine. Thus, Havel and Goldfien (2) observed TG in thoracic duct lymph that were of higher specific activity than those of the plasma in dogs injected with labeled palmitate. Their studies indicated that TG from the liver enter the blood via the hepatic sinusoids rather than via the lymphatics, and they surmised that the lymph TG may have been produced from circulating free fatty acids (FFA) by the intestinal mucosa. Turner et al. (4) found linolenic and arachidonic acids in newly synthesized TG of intestinal mucosa in rats fed diets lacking these acids. Karmen, Whyte, and Goodman (5) and Whyte, Karmen, and Goodman (6), in studies on rats that had received a fatty meal by stomach tube, found that fatty acids of the chyle lipids were to a considerable extent of endogenous origin. Kayden, Karmen, and Dumont (7) made similar observations in human subjects. The present studies, carried out in rats subsisting on a fat-free diet, were made to determine the quantity, character, and mode of origin of the endogenous lipid of thoracic duct lymph.

Abbreviations: TG, triglycerides; PL, phospholipids; FFA, free fatty acids; TL, total lipids.

METHODS

Rats

The studies were made on male Sprague-Dawley rats weighing about 300 g. Some of the rats had been made nephrotic by injecting them with anti-kidney serum (8) about 2 weeks before the lymph studies.

Diet

The rats were fed a 10% water solution of the following fat-free diet mixture: acid hydrolysate of casein (salt-free) 20%, sucrose 76%, Wesson's salt mixture 4%, together with water-soluble vitamins, tryptophane, and choline chloride (8). The solution was offered ad libitum and exclusively, for 1–4 days before starting lymph and bile collections and throughout the period of the collections. The rats drank the solution continually, and often consumed 100 ml or more in 24 hr. Sometimes 0.5% NaCl was added to the diet solution to increase the flow of lymph. Saline was substituted for the diet during certain collection periods.

The diet mixture usually supplied less than 5 mg of fat per rat per day. It was changed frequently to prevent excessive bacterial growth. The rats were not allowed access to feces.

To control the possibility that the rats may have been able to obtain esterified fatty acids by licking the fur and

paws, lymph collections were made in rats fitted with plastic masks. The mask allowed the rat to drink from the diet bottle, but a shield prevented licking the fur and paws.

Collection of Lymph and Bile

The thoracic duct was cannulated below the diaphragm and above the cysterna chyli, following the procedure of Bollman, Cain, and Grindlay (9). In other rats, the intestinal lymph duct draining principally the small intestine was cannulated in its course beside the superior mesenteric artery. Attempts to cannulate the liver lymph duct were largely unsuccessful, so then both the thoracic duct and the intestinal lymph duct were cannulated in the same rat in a number of instances. With the intestinal lymph thus diverted, the lymph obtained from the thoracic duct consisted of liver lymph together with some extraneous lymph. The intestinal lymph always appeared lactescent, except immediately after cannulation, whereas the residual thoracic duct lymph from the liver appeared clear and slightly yellowish. The intestinal lymph was much greater in volume and lower in protein content than the liver lymph. Animals were excluded from the study unless free flow from both cannulae could be maintained.

In other rats, the bile duct was cannulated near the liver with a polyethylene catheter similar to that used for the lymphatics. Both the bile duct and the thoracic duct were cannulated in the same animal in some cases so that simultaneous collections of bile and lymph could be made in order to determine the effect on thoracic duct lipid of diverting the bile.

Ether anesthesia was used for the surgical procedures. During the lymph or bile collections, the rats were kept in restraining cages of the type described by Bollman (10) and supplied with the diet solution from an inverted bottle. The lymph or bile was collected continuously from the time of the cannulation into tubes held in an ice bath. The tubes were changed at intervals of from 0.5 to 24 hr.

Studies with Labeled Fatty Acid

Lipids of Lymph and of Bile. While lymph or bile was being collected in six of the experiments, the rat was injected intravenously with 2 μc of palmitic acid-1-^{14}C in 1 ml of rat serum complexed as described by Bragdon and Gordon (11).

Lipid of Intestinal Mucosa. Six rats were injected intravenously each with 1 μc of palmitic acid-1-^{14}C, after they had been fed the fat-free diet for at least 24 hr. After 5 min sodium pentobarbital was injected intravenously. The abdomen was opened, and the rat was bled from the aorta 7–9 min after the palmitic acid injection. Then the small intestine was rapidly removed and irrigated with cold saline. The mucosa was expressed by rolling the intestine, or it was scraped off with a spatula after the intestine had been opened. Liver tissue was obtained at the time of or before and after removing the intestinal mucosa. The tissues were dispersed in chloroform–methanol. In the final experiment saline was substituted for the liquid diet 6 hr before the injection of palmitic acid-1-^{14}C.

Lipid Determination

Lymph, bile, serum, and their fractions (usually 2-ml portions) were extracted with 50 ml of chloroform–methanol 2:1. Liver (about 1.5 g) and the entire intestinal mucosal preparation were homogenized with 50 ml of chloroform–methanol in a Waring Blendor. The extracts were washed according to the procedure of Folch, Lees, and Sloane Stanley (12), usually with 10 ml of 0.02 N H_2SO_4. In the case of bile, water or neutral salt solution was employed, which considerably reduced the amount of oxidizable material ("total lipid") extracted into the chloroform phase without appreciably reducing the lipid P or total fatty acids. The whole lipid extracts were analyzed for total lipid (TL) (13), PL (14), total cholesterol (15), and in some experiments for total fatty acids (16) and TG (17). The lipid classes were examined qualitatively by thin-layer chromatography. Plates of Silica Gel G containing Rhodamine 6G, and a solvent system of hexane–ether–acetic acid 80:20:2 or of benzene–ethyl acetate 4:1 were used. Lymph and serum lipoproteins in many instances were separated into $d < 1.006$ and $d > 1.006$ fractions by overnight centrifugation at 100,000 $\times$ g and 14° prior to lipid analysis.

Fatty acid patterns of total lipids of lymph and bile were determined by gas–liquid chromatography. Methyl esters of the fatty acids were prepared by heating a few milligrams of the lipid at 65° overnight with 2 ml of methanol containing 5% H_2SO_4. After 2 ml of water was added, the methyl esters were extracted five times with hexane. Methylation and extraction of added palmitic acid-1-^{14}C were found to be essentially complete. In some cases the lipid was saponified and the nonsaponifiable material was removed before methylation of the fatty acids. A Packard gas chromatograph was employed, with a hydrogen flame detector and a 10 ft $\times$ 4 mm column containing 14% ethylene glycol succinate polyester on Gas Chrom P at 180°. Nitrogen pressure was 28 psi. Some samples were also examined by means of a column with 2% SE-30 (silicone rubber gum, General Electric) at 200°. NIH and Hormel standards were used, and quantification was made by triangulation. The lymph and bile samples used for GLC analysis were obtained from rats which had been

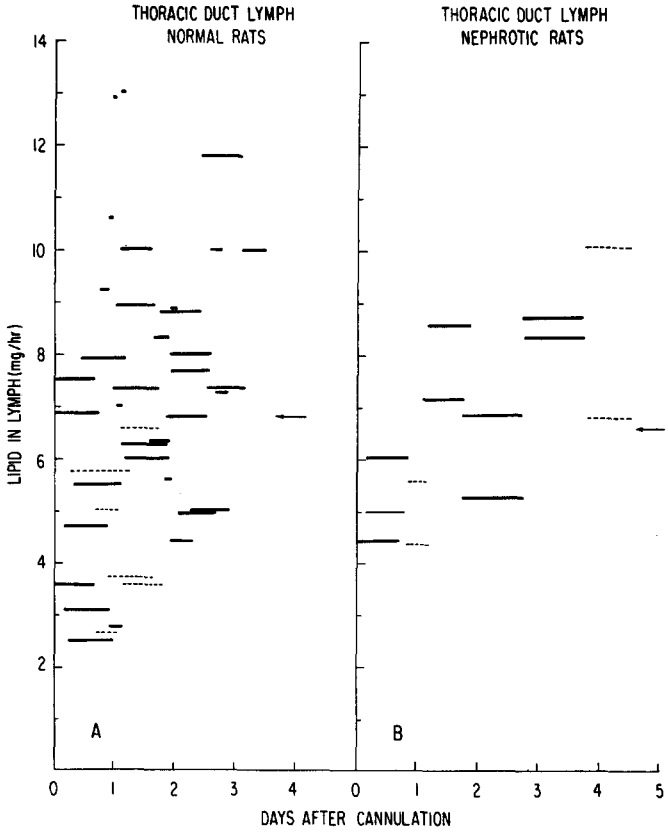

FIG. 1. Lipid content of thoracic duct lymph of normal rats (A) and of nephrotic rats (B) expressed in milligrams of lipid per hour of collection. The length of the bars in the graph shows the length of the individual collection periods. The dashed bars indicate period during which saline was substituted for the fat-free diet. The arrows indicate the approximate mean values.

fed the fat-free diet for 1–4 days and in which drainage of lymph or bile had been in progress for 0–36 hr.

The lipid extracts were analyzed for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer. The samples were taken to dryness in a counting vial and dissolved in 15 ml of toluene containing 0.5% diphenyloxazole.

RESULTS

Lipid Content of Thoracic Duct Lymph

The flow rate of thoracic duct lymph in experiments employing the liquid diet was about 0.5–2.0 ml/hr, usually a little over 1 ml/hr. Values for total lipid in the lymph of normal rats in milligrams per hour of collection are shown in Fig. 1A. The values varied widely—from 2.5 to 13 mg/hr—with a mean value of 6.8 mg/hr for 42 collections in 11 rats. The lipid values appeared to be independent of the lymph flow rate. There seemed to be a tendency for the lipid values to increase during the course of successive collection periods. Possibly the initial values were depressed as a result of the surgical procedure. It should be kept in mind also that the external drainage of lymph results in loss of proteins, lipids, and electrolytes, which may have influenced the results.

In Fig. 1B are shown the TL values for thoracic duct lymph obtained from nephrotic rats. At the time of the thoracic duct cannulations, serum lipids of the rats were very high and the serum was lactescent.[1] The lymph lipid values did not differ significantly from those of normal rats.

Lipid Content of Intestinal Lymph

Studies to determine the source of the lipid in thoracic duct lymph were made on lymph from the intestinal lymph duct. The mean value for 25 TL determinations in four rats was 7.2 mg/hr (Fig. 2A). Since the intestinal lymph normally enters the thoracic duct, it is evident that the lipid of thoracic duct lymph derives almost

[1] Serum lipid values of similar rats from the same group averaged: TC 375, PL 550, and TL 1870 mg/100 ml. However, the values fell considerably during the course of lymph drainage.

entirely from the intestinal lymph. It follows that the lymph from other organs drained by the thoracic duct, including that from the liver, must contain very little lipid. The correctness of this conclusion was established by studying the thoracic duct lymph after the intestinal lymph had been diverted. The residual thoracic duct lymph was small in volume and contained never more than 0.5 mg of lipid per hr. Most of this lipid was in the $d > 1.006$ fraction, in contrast to that of the intestinal lymph.

Influence of Licking

Because the rat may obtain some lipid by licking the fur and paws, studies were made on rats fitted with a mask that prevented licking. The results thus obtained for thoracic duct TL are shown in Fig. 2B. The mean value of 5.7 mg/hr for 21 collections in three rats is a little lower than that obtained without the mask. However, the rats with the mask were very uncomfortable.

Effect of Food

During 30 alternate periods of 2–30 hr each, saline was substituted for the liquid diet. Lymph lipid values during some of these periods are shown by the dashed bars in Figs. 1 and 2. Other values are not included because the experimental conditions were somewhat different. Although the results with saline were variable and slightly higher on average, they were not significantly different from those obtained with the diet.

Composition of Thoracic Duct and Intestinal Lymph Lipid

Lipid of thoracic duct lymph and lipid of intestinal lymph were similar in composition. Mean values for 17 samples of thoracic duct lymph were: TL 6.5, PL 1.5, and total cholesterol 0.4 mg/hr. The lipid was approximately 70% TG. Esterified fatty acids made up about three-fourths of the lipid mass, amounting to about 5 mg/hr. The individual fatty acids are discussed later.

Eighty-two per cent of the lymph lipid (12 determinations) was present in the $d < 1.006$ lipoprotein fraction. This very low density fraction, therefore, is the one of major interest. It was higher in TG and lower in PL and total cholesterol than the lipid of whole lymph. It contained lactescent material that rose to the top of the tube during a 0.4×10^6 *g*-min centrifugation (18). However, a considerable quantity of lactescent material remained distributed throughout the tube, indicating presumably that smaller lipid-containing particles may also originate from the intestine.

Comparison of Lipid Levels in Plasma and Lymph

After periods of 4 hr–4 days of thoracic duct or intestinal lymph drainage in a number of experiments, a final brief

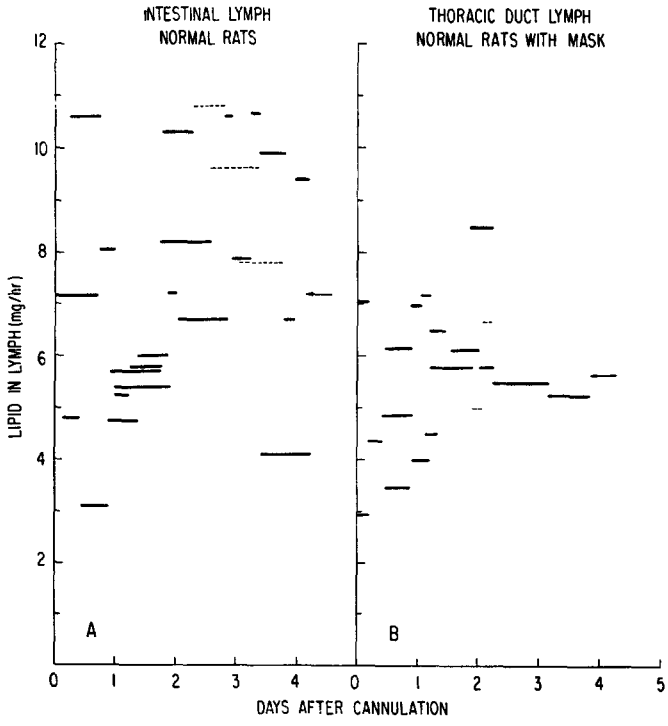

FIG. 2. Lipid content of intestinal duct lymph (A) and of thoracic duct lymph of animals fitted with mask (B). See legend to Fig. 1 for explanation of symbols.

TABLE 1 CONCENTRATION OF LIPIDS IN BLOOD SERUM AND IN LYMPH

Lipoprotein Fraction	Concentration of Total Lipid					
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6
	mg/ml					
Lymph d < 1.006	7.24	6.15	12.21	10.38	9.55	12.50
Serum d < 1.006	0.50	0.10	0.30	0.29	0.31	0.18
Lymph d > 1.006	0.72	0.57	1.29	1.38	1.35	1.53
Serum d > 1.006	2.92	2.27	4.46	1.93	—	2.06
	hr					
Total period of lymph drainage	96	52	28	4.5	4.5	96
Length of this lymph collection	4	1	4	1	0.75	5

The lymph in Expts. 1 and 2 was intestinal lymph, and that in the other experiments thoracic duct lymph.

TABLE 2 LIPID CONTENT OF SUCCESSIVE COLLECTIONS OF BILE

Collection No.	Length of Collection Period	Expt. 1			Expt. 2			Expt. 3	
		TL	PL	TFA	TL	PL	TFA	TL	PL
	hr		*mg/hr*			*mg/hr*			*mg/hr*
1	1	2.52	2.28	1.62	2.82	2.36	1.96	3.45	3.30
2	1							3.11	3.06
3	1	2.44	2.22	1.56	2.39	2.11	1.64	3.34	3.14
4	1							2.94	3.48
5	4	1.41	1.46	0.94	1.58	1.12	0.92	1.96	1.97
6	5	1.07	1.02	0.65	0.37	0.27	0.20	1.14	1.09
7	10	1.01	0.97	0.63	0.77	0.66	0.58	1.00	0.92
8	8, 4*	(0.55)	(0.53)	(0.34)	0.79	0.64	0.53	0.82	0.79
9	10, 3*	1.62	1.40	(0.91)	0.88	0.79	0.58	1.36	1.22
10	10				1.11	1.02	0.87		

TL, total lipids; PL, phospholipids; TFA, total fatty acids.
* These shorter periods apply to Expt. 3.

lymph collection was made and then the animal was bled from the aorta. The lymph was slightly lactescent, but the blood serum in all cases appeared entirely clear. Lipid analyses of the lymph and serum specimens are shown in Table 1. The average concentration of d < 1.006 lipoprotein lipid in the lymph was many times higher than that in the serum. The d > 1.006 lipid was higher in the serum, and may therefore have passed from serum into lymph.

Lipid in Bile

Bile was collected and analyzed to estimate how much TFA it contributes to the intestinal contents for possible esterification and transfer to the lymph by the intestinal mucosa. In the early experiments, bile collected during the first 6 hr after the bile duct had been cannulated was discarded. Subsequent collections often contained only about 1 mg of TL per hr of collection (excluding bile acids). Later it became evident that bile lipid fell off appreciably during the initial 6 hr of drainage (Table 2). This decline probably should be regarded as an aberration produced by the drainage procedure, but this is not certain. Most of the bile lipid was PL, as shown by thin-layer chromatography; however, for technical reasons, the differences between TL and PL values shown in Table 2 do not accurately reflect the quantity of the other lipids present. Total cholesterol in one experiment was 10–15% of TL. TL values for collection periods (up to 6 hr in length) starting immediately following bile duct cannulation in 14 rats averaged 3.0 mg/hr. Using only the 10 periods of 3 hours' duration or less, the average TL value was 3.4 mg/hr. About two-thirds of this was fatty acid, which is roughly half the fatty acid found in the lymph.

For further study of the lipid contribution of bile, thoracic duct lymph was collected from rats after the bile had been diverted from the gut. The volume of lymph was markedly reduced, and its lipid content per hour was also reduced. Collections were often unsatisfactory because of clotting in the catheters. Some specimens seemed to be complete and satisfactory, however, and the four highest TL values found in analyzing many specimens from four rats were: 2.65, 2.33, 1.95 and 1.73 mg/hr. Most other values were much lower. Thus, the

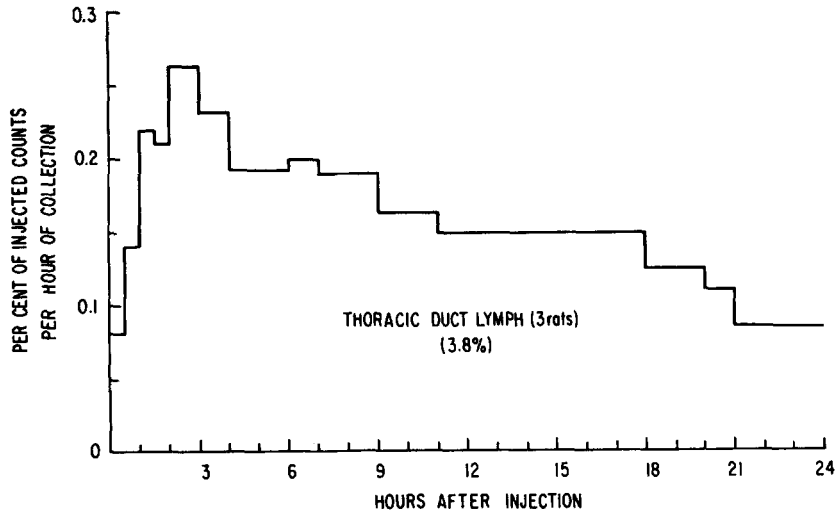

FIG. 3. Percentage of counts from injected palmitic acid-1-^{14}C appearing in thoracic duct lymph per hour of collection.

thoracic duct lymph seems to contain at most less than half of its normal lipid component, after bile flow into the intestine has been diverted.

Incorporation of FFA into Lipid of Lymph

After intravenous injection of palmitic acid-1-^{14}C, radioactivity of the thoracic duct lymph lipid usually reached a peak during the 3rd hr and then slowly declined. In 24 hr, about 4% of the injected counts were collected in the TL of the lymph (Fig. 3). When the lymph flow rate was increased to 4–6 ml/hr by increasing the NaCl content of the diet, counts appearing in the lymph lipid during the first hour were only slightly increased, indicating that lymphatic dead space was small. Almost all of the counts were in fatty acids (extracted after saponification), and most of them were in the $d < 1.006$ fraction of the lymph. It is of particular interest that in the first 4 hr after the palmitic acid-1-^{14}C injection, less than 1% of the label appeared in the lymph lipid. The fact that a small part of this label was in FFA that presumably passed directly into the lymph only strengthens the conclusions to be drawn from the smallness of the amount of label found in the lymph. The counts appearing in lymph lipid from labeled palmitic acid were greatly decreased as a result of diverting the bile from the intestine, but lymph flow was unsatisfactory in these experiments.

Incorporation of FFA into Lipid of Bile

When labeled palmitic acid was injected intravenously soon after the bile duct had been cannulated, the percentage of injected counts appearing in the bile lipid during the first few hours was sometimes as great as that appearing in the lymph lipid (of other rats) in the same period, and the specific activity of the bile lipid was higher. However, after 5 or 6 hr, the bile lipid counts fell far below the lymph lipid counts, and the sum of the bile lipid counts in 24 hr was in all cases less than half the sum of the lymph lipid counts. For reasons already discussed, these results do not tell what fraction of the lymph lipid counts is derived from bile when the bile flows normally into the intestine.

Uptake of FFA by the Intestinal Mucosa

The counts found in intestinal mucosal preparations from animals killed 8 min after injections of palmitic acid-^{14}C varied widely depending on the amount of deeper tissue removed with the mucosa. However, values for the mucosa itself probably were <0.2% of the injected counts (Table 3). The specific activity of the extracted lipid did not vary appreciably with the quantity of extraneous tissue included with the mucosa, and was only about 20% of that of the liver lipid at the same time.

Fatty Acid Patterns of Lymph and of Bile Lipid

In Table 4 the fatty acid composition of lymph and of bile lipid is compared. Rather marked differences are apparent, indicating that a substantial amount of the lymph lipid fatty acid was probably derived from sources other than bile lipid. Differences in fatty acid composition of the $d < 1.006$ and the $d > 1.006$ fractions of the lymph lipoproteins were less than might have been expected from differences in their composition according to lipid classes. It should be noted that fatty acid composition of both lymph and bile lipids varied considerably from animal to animal, and that lymph and bile specimens were obtained from different animals under slightly different conditions. The origin of the 20:1 fatty acid deserves further study. This component also appeared

TABLE 3 RADIOACTIVITY OF TISSUE LIPIDS AFTER INJECTION OF PALMITATE-1-^{14}C

Expt. No.	Intestinal Mucosa Lipid		Serum Lipid		Liver Lipid	
	mg	*% of inj. cpm*	*mg/ml*	*% of inj. cpm/ml*	*mg*	*% of inj. cpm*
1	14					
2	11					
3	40*	0.44*		0.04		
4	13	0.13		0.09		
5	8	0.06	1.85	0.08		
6	15	0.14		0.08	438	24
7	18	0.14	2.03	0.07	559†	27†
					559†	28†
8	14*	0.13*	1.96	0.08	475	29
	19*	0.19*				
			Fasting for 6 hr			
9	15*	0.08*	1.99	0.08	460†	20†
	22*	0.17*			460†	20†

* In Expt. 3, a considerable amount of deeper tissue was included with the mucosa. In Expts. 8 and 9, first the mucosa and then a deeper layer of tissue were removed in succession and analyzed separately.

† Liver samples were taken before and after removing the intestinal mucosa.

TABLE 4 PERCENTAGE FATTY ACID COMPOSITION OF LYMPH AND OF BILE LIPIDS*

	Fatty Acid						
	16:0†	16:1	18:0	18:1	18:2	20:1‡	20:4
Lymph TL (7)	25.5 22.6–28.5	6.6 3.1–13.6	12.0 9.8–13.5	24.4 20.5–28.9	16.4 8.4–21.5	7.4 2.2–11.8	7.8 3.2–14.4
Lymph d < 1.006 (3)	25.2 22.6–27.1	6.4 3.7–10.8	13.0 11.9–13.9	23.6 21.5–27.1	15.4 9.1–20.8	7.5 3.5–11.0	8.9 6.0–12.2
Lymph d > 1.006 (2)	24.8 23.6–26.1	8.1 3.4–12.8	13.1 13.0–13.2	20.0 17.9–22.1	15.8 10.7–20.9	3.0 2.9–3.1	15.2 14.7–15.7
Bile TL (5)	36.2 31.6–40.6	4.1 1.3–11.0	5.7 3.8–7.4	13.7 10.0–21.4	26.7 12.5–33.9	— —	13.7 9.4–16.0

TL, total lipid.

* Principal components with retention time equal to that of 20:4 or less are shown. Minor components are omitted. Mean values and ranges are given, with number of samples analyzed in parentheses.

† Number of carbon atoms:number of double bonds.

‡ Double bond at C_{13} (*see* text).

to be present in significant amounts in the intestinal contents. Examination[2] of its methyl ester in a mass spectrometer showed the expected molecular weight of 324 and a fragmentation pattern compatible with that of an unsaturated fatty acid. Hydrogenation produced the normal 20:0 acid. Oxidative degradation[2] produced a C_{13} dicarboxylic acid and a C_7 monocarboxylic acid, indicating that the original fatty acid was 20:1 [13].

DISCUSSION

Quantities of lipids have been observed by others in post-absorptive thoracic duct lymph (19–23). Rampone (22) found 124 mg of lipid per hr in the thoracic duct lymph of dogs fasted for 24–48 hr. Gottenbos and Thomasson (23) found the lymph of fasting rats to contain 114 mg of lipid per day, of essentially the same composition reported here. In addition, it has been noted that the feeding of TG to rats in the post-absorptive state increases the quantity not only of TG in the thoracic duct lymph but also of cholesterol (19, 21) and of phospholipids (19). Karmen et al. (5) interpreted their results to indicate that the feeding of TG greatly increases endogenous as well as exogenous TG in thoracic duct lymph, but they found little thoracic duct lipid in fasting rats.

Courtice and Morris (20) believed that lymph lipid in general arises in large part from passage of plasma lipids through capillary walls. However, they described post-absorptive thoracic duct lymph samples that contained

[2] I am indebted to Dr. Robert J. Highet for studying the mass spectrum and to Dr. Edward D. Korn for determining the position of the double bond.

more lipid than the corresponding samples of plasma. Several investigators have observed an increase in quantity or a labeling of thoracic duct lipids following intravenous infusions of chyle or serum lipids (19, 22, 24, 25). Usually the magnitude of the apparent passage into the lymph was of the order of 1% of the infused dose in 3–6 hr. However, Reinhardt, Fishler, and Chaikoff (24) reported that 8–20% of infused phospholipid passed into thoracic duct lymph of dogs in 3–6 hr.

The thoracic duct lymph lipid under the conditions of the present experiments amounted to 6–7 mg/hr, and was largely TG. Most of it arose in some manner from the intestine, but its exact mode of origin was not entirely clear. This lipid would not be measured as a plasma–lipid increment across the splanchnic area in studies such as those of Carlson and Ekelund (26). It is well known that the intestine is able to produce glycerol and to esterify fatty acids. The chief question, then, concerns the source of the fatty acids. The small cholesterol component was not studied in detail.

It is evident that the $d < 1.006$ lipoproteins of the lymph, which contain most of the fatty acid present, could not have been derived passively from the plasma. The concentration gradient was such that any net movement would have been from the lymph to the plasma.

Bile has been recognized as a source of endogenous intestinal lipid (27). If the bile collected during the first few hours after bile duct cannulation is representative of that which normally flows into the intestine, the bile lipid probably supplies about half of the fatty acid for the $d < 1.006$ thoracic duct lymph lipid. This estimate may be high. On the other hand, it could be low, since the lipid content of the bile per hour may have been reduced by the operative procedure. In support of the latter possibility was the finding that less than half—usually much less than half—the normal quantity of lipid appeared in the thoracic duct lymph after the bile had been diverted from the intestine. This result may have been explained in part by technical difficulties, and by the requirement of bile for the absorption of intraluminal lipids from other sources and possibly for the esterification of fatty acids by the mucosal cells (28).

If only about half of the lymph lipid fatty acid is derived from bile, where does the remainder come from? Some is licked from the fur, and some of the $d > 1.006$ lipid may pass from the serum. It is possible that significant quantities of lipid are present in other intestinal secretions. Also, desquamated cells and bacteria may contribute some lipid, although complete desquamation of the mucosa in 24 hr could apparently supply only 1 mg of lipid per hr, and the role of bacteria is unknown. Finally, possibly FFA or TG are taken up from the plasma by the mucosa, or possibly fatty acids are synthesized de novo by the mucosa. Calculations based on a plasma FFA level in the rat of 0.3 μeq/ml and on a turnover time of 0.9 min (29), indicate that for the intestine to make 3 mg of TG per hr from circulating FFA, over 4% of all circulating FFA must be utilized for this purpose. This estimate may be much too high. However, following the injection of palmitic acid-^{14}C, less than 1% of the label appeared in the thoracic duct lymph in 4 hr, and much of this undoubtedly was derived from recirculated FFA as well as from fatty acids of bile lipids and other intraluminal lipids. Considering the relatively small pool of lipid in the mucosal cells and the small lymph dead space, these observations suggest that the quantity of lymph TG made by the intestine from circulating FFA must be very small. This conclusions is supported by the finding of much less than 0.5% of the injected dose of labeled palmitic acid in the intestinal mucosa about 8 min after the injection, at a time when little of the label had appeared in the lymph. The results obtained in nephrotic animals make it appear unlikely that TG are taken up in appreciable quantities by the mucosa. The possibility that fatty acids are synthesized by the intestinal mucosa was not studied.

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