Metabolism of chylomicrons by the isolated rat liver

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ABSTRACT Isolated livers perfused with washed corn oil chylomicrons labeled in vivo with palmitic acid-1-^{14}C removed a large proportion of the chylomicrons. Slices from these livers oxidized chylomicron fatty acid esters to both carbon dioxide and acetoacetate. The liver slices also generated free fatty acids from chylomicron lipids and converted chylomicron triglycerides to phospholipids. Similar activities were observed in rat liver slices prepared shortly after the intravenous administration of chylomicrons to intact rats.

The observed chylomicron uptake and lipid conversions were similar in livers from both fed and fasted rats. Fasting increased the oxidation of chylomicron fatty acid esters by livers labeled in vivo and by perfusion.

In livers removed from intact rats given labeled chylomicrons, the triglyceride-^{14}C to phospholipid-^{14}C ratio was high, a finding unexpected if the liver had acquired this ^{14}C by removal of circulating fatty acids formed by extrahepatic lipolysis.

These results demonstrate the ability of the liver to remove and utilize chylomicrons directly and suggest that direct removal accounts for a significant portion of the chylomicron fatty acids utilized by the liver of intact rats.

KEY WORDS chylomicrons · rat · liver · perfusion · slices · fatty acid oxidation · ketogenesis · lipolysis

It is well established that chylomicron fatty acids rapidly accumulate in the liver after the intravenous administration of chylomicrons (1–4). There are several possible mechanisms for the removal of chylomicron fatty acids from the circulation by the liver. Direct removal mechanisms include uptake by liver cells of intact chylomicrons or chylomicron triglyceride molecules, and uptake of free fatty acids after lipolysis of chylomicron fatty acid esters in extracellular compartments of the liver. Chylomicron fatty acids might also reach the liver by an indirect mechanism, namely extrahepatic lipolysis and subsequent transport of free fatty acids to the liver.

Blood circulating through the liver sinusoids is in direct communication with extravascular tissue spaces via fenestrations in the lining of the sinusoids which are of sufficient size to allow the passage of chylomicrons (5, 6). Electron microscopic evidence for the uptake of intact chylomicrons by parenchymal cells has appeared (6–8), but the quantitative significance of this phenomenon remains obscure (9). Measurements on the accumulation of radioactivity in the liver after the administration of doubly-labeled chylomicrons (3, 10) suggest that the liver participates directly in removing chylomicron fatty acids from the circulation. However, as discussed by Dole and Hamlin (11), such results remain equivocal since glycerol released outside the liver can circulate to the liver. Lipoprotein lipase has been implicated in the hydrolysis of chylomicron triglycerides in capillary beds, with subsequent passage of free fatty acids into a variety of tissues (5). The enzyme is rapidly released into the blood by heparinoids and therefore appears to be located in the capillary wall (5). However, since lipoprotein lipase activity was not released from the isolated rat liver perfused with heparin (12–14), this enzyme may not be present in rat liver.

Felts and Mayes (15) observed a low percentage uptake and oxidation of chylomicrons by the perfused liver. These authors therefore suggested that in the intact animal chylomicron fatty acids enter liver cells primarily as free fatty acids formed by hydrolysis of chylomicron triglycerides in extrahepatic capillary beds.

Abbreviations: TLC, thin-layer chromatography.

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In the present study the role of the liver in the metabolism of chylomicrons was studied by a combination of the perfused liver and liver slice techniques. To avoid the release of lipoprotein lipase by heparinoids, we did not use anticoagulants. Under these conditions, the isolated rat liver perfused with washed chylomicrons removed and metabolized chylomicron fatty acids to a significant extent. A preliminary report of this investigation has appeared (16).

MATERIALS AND METHODS

Preparation of Chylomicrons

Thoracic ducts of male Holtzman rats (300–400 g) were cannulated by the procedure of Bollman, Cain, and Grindlay (17). The rats were then placed in restraining cages (18) and 16–24 hr later palmitic acid-1-^{14}C (100–200 μc, purity at least 98%, see below) dissolved in 0.5 ml of corn oil was administered via a gastrostomy tube. When turbidity first appeared, the lymph was collected at room temperature in flasks containing 20 mg of streptomycin and 15,000 units of penicillin. The collection period was 16–20 hr and about 50 ml of lymph was obtained.

For the removal of radioactive free fatty acids from the chylomicrons, crystalline bovine serum albumin (Pentex, Inc., Kankakee, Ill.) was added to the lymph to a final concentration of 5%. The chylomicrons were then washed at room temperature by layering under a discontinuous aqueous NaCl gradient (19) and centrifuged at 53,500 *g* for 1 hr in the SW 25.1 rotor of the Spinco model L ultracentrifuge. The chylomicrons in the top layers were pooled and dispersed in 10 ml of 0.9% NaCl. This dispersion was overlaid slowly with 20 ml of 0.9% NaCl from a syringe fitted with polyethylene tubing, which minimized mixing of the chylomicrons, and centrifuged at 25,000 rpm (53,500 *g*) in the SW 25.1 rotor for 30 min.

The resulting thick layer of chylomicrons was removed with a spatula and the paste was dispersed in 5 ml of Krebs-Ringer phosphate buffer. In all experiments in which Krebs-Ringer phosphate was used, calcium was omitted. Streptomycin, 2 mg, and penicillin, 1500 units, were added and the chylomicron suspension was kept at room temperature to prevent alterations in structure which might be induced or accelerated by cooling (20).

Liver Slices

1 ml of the chylomicron suspension was administered intravenously for about 1 min into the inferior vena cava of rats anesthetized with ether. The rats were exsanguinated 1–2 min later. The livers were immediately removed and placed in ice-cold saline.

For liver perfusion, rats were anesthetized with ether. After ligation of the abdominal vena cava, just anterior to the kidneys, the coeliac and hepatic arteries and the portal vein about 4 cm posterior to the liver were ligated. A glass cannula was inserted in the portal vein and the liver was immediately perfused with Krebs-Ringer phosphate buffer at room temperature. An exit cannula was tied into the vena cava above the diaphragm and the liver was removed as the perfusion continued. The liver was then placed in a Petri dish containing Krebs-Ringer phosphate buffer at 37°C and perfused with 10 ml of the washed corn oil chylomicrons diluted with Krebs-Ringer phosphate, followed by 40 ml of Krebs-Ringer phosphate at 37°C for the removal of chylomicrons that were freely dispersed in the vascular compartment of the liver. The perfusate, turbid at first, became clear after the first 10 ml of the 40 ml wash. The liver was then placed in ice-cold 0.9% NaCl. In the liver perfusions the chylomicrons were not recirculated. The observed chylomicron uptake, therefore, occurred during a single passage of chylomicrons through the liver.

The ice-cold livers were sliced with Stadie-Riggs microtome blades. A recessed glass plate was used as a guide. The slices were pooled in ice-cold Krebs-Ringer phosphate medium, removed from the medium on a wire loop, and dried by repeated contact with a dry glass plate. They were weighed by suspension of the wire loop from the arm of a torsion balance and placed in 50-ml flasks that contained 5 ml of Krebs-Ringer phosphate. These flasks were equipped with a horizontal Teflon adapter connected to a liquid scintillation vial that contained a roll of filter paper moistened with 0.5 ml of 10% KOH. The flasks containing liver slices were flushed thoroughly with oxygen and stoppered with rubber caps (21).

A Dubnoff shaker was used at an incubation temperature of 37°C in all experiments. At the end of the incubation, total $^{14}CO_2$ was trapped in the scintillation vial by the addition of 1 ml of 62.5% citric acid to the incubation medium followed by 15 min of additional incubation. The scintillation vial was removed and replaced with a new vial which also contained the filter paper roll and 0.5 ml of 10% KOH. To decarboxylate acetoacetic acid (22), we added 1 ml of an aniline citrate solution (3.76 g of aniline hydrochloride in 6 ml of 62.5% citric acid) to the incubation medium. The $^{14}CO_2$ generated was collected for 75 min, and the vials and filter paper were then dried in a vacuum desiccator over P_2O_5. 20 ml of a scintillation solution containing 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene in toluene was then added to the vial and the ^{14}C was counted.

The amount of $^{14}CO_2$ generated from the carboxyl group of acetoacetic acid was multiplied by 2 in all cases,

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since the carbonyl and carboxyl carbon atoms of acetoacetate generated in the liver from palmitic acid-1-^{14}C contain an equal amount of ^{14}C (23). The radioactivity in β-hydroxybutyric acid is not measured by the aniline citrate procedure. The observed oxidation of chylomicron palmitic acid-1-^{14}C is therefore a minimum quantity.

Lipid Analyses

Radioactivity in lipid fractions was determined on additional liver samples incubated like the others, but then placed directly in 20 volumes of chloroform–methanol 2:1. After filtration and washing according to the method of Folch, Lees, and Sloane Stanley (24), aliquots were evaporated to dryness under N_2 and the scintillation solution was added, for measurement of the total lipid radioactivity. The distribution of radioactivity in liver and chylomicron phospholipids was determined in other aliquots that were evaporated to dryness, dissolved in chloroform, and placed on a silicic acid column. The neutral lipids were eluted with chloroform, the phospholipids with methanol. Both fractions were evaporated to dryness and dissolved in a small amount of chloroform–methanol 2:1.

The phospholipids were placed on 20 × 20 cm thin-layer chromatographic plates of alkaline silica gel (Camag, D.O., Arthur H. Thomas Co., Philadelphia, Pa.) containing the fluorescent agent Ultraphor (25), and separated with chloroform–methanol–glacial acetic acid–water 25:15:4:2 (26). The phosphatidyl inositol and phosphatidyl serine migrate between lecithin and phosphatidyl ethanolamine and are not well separated in this system. The combined phosphatidyl inositol and phosphatidyl serine fraction was, therefore, eluted and rechromatographed with a solvent system of diisobutyl ketone–glacial acetic acid–water 40:25:5 on an alkaline silica gel plate containing Ultraphor (27). Standard phospholipids were concurrently chromatographed. The radioactivity in the different phospholipids separated by TLC was measured as described below.

Lipids in other aliquots of the filtered and washed total lipid extracts were separated on Silica Gel G containing Ultraphor with a solvent system of Skellysolve B[1]–diethyl ether–glacial acetic acid 80:20:1. When the quantity of free fatty acids was extremely low and the triglyceride level high, a newly developed two-dimensional thin-layer chromatographic system was used. The lipids were dissolved in Skellysolve B containing 0.05% diethylamine. This extract was applied to an 8 × 10 cm thin-layer plate. After development with chloroform–acetone 60:40, the plate was air-dried and the sample was subjected to migration in the second dimension in Skellysolve B–diethyl ether–glacial acetic acid 40:60:2. The free fatty acids remained at the origin in the first solvent system and migrated with an R_f of about 0.6 in the second. When palmitic acid-1-^{14}C, mixed with unlabeled palmitic acid or with a total liver lipid extract, was chromatographed by this procedure, 99% of the radioactivity was found in the free fatty acid spot. When the quantity of free fatty acids was too low to be detected with the Ultraphor, a standard free fatty acid in 0.05% diethylamine in Skellysolve B was placed on the plate next to the original point of sample application, prior to development in the second solvent system, to aid in locating the free fatty acids after solvent flow in the second dimension.

The silica gel scraped from appropriate spots on the plates was air-dried and added to counting vials. The scintillation solution added was that of Kinard (28) except that α-naphthylphenyloxazole was replaced by 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene and 4% Cab-O-Sil was added as suggested by Gordon and Wolfe (29). The vials containing silica gel and Cab-O-Sil

[1] A crude fraction of petroleum ether, bp 60–70 °C (Skelly Oil Company, Kansas City, Mo.).

TABLE 1 OXIDATION OF ^{14}C-LABELED CHYLOMICRONS BY LIVER SLICES FROM FED RATS

Liver slices, 200–400 mg, were placed in 5 ml of Krebs-Ringer phosphate buffer. In each experiment duplicate flasks were incubated for 3 hr at 37 °C in a gas phase of oxygen. The total weight of the injected chylomicrons was measured gravimetrically.

	Route of Chylomicron Administration	Injected Dose		Liver Wt.	Chylomicron Uptake	Total Lipid ^{14}C*	$^{14}CO_2$ Produced	Acetoacetate-^{14}C Produced	Chylomicron-^{14}C Oxidized†
		mg	*cpm × 10⁻³*	*g*	*%*	*cpm/100 mg liver*			*%*
Expt. 1	Liver perfusion	54.3	10,692	17.16	21.3	13,250	319	91	3.1
	Intravenous infusion	54.3	10,692	13.50	4.3	3,385	160	98	7.6
Expt. 2	Liver perfusion	165.8	26,940	16.80	6.7	10,670	463	281	7.0
	Intravenous infusion	165.8	26,940	15.78	6.0	10,160	293	142	4.3
Expt. 3	Perfusion of isolated liver	87.4	21,520	15.25	13.5	19,100	619	291	4.8
	Intravenous infusion	87.4	21,520	14.87	4.4	6,378	333	179	8.0

* Before incubation.
† $^{14}CO_2$ plus acetoacetate-^{14}C times 100 ÷ lipid-^{14}C.

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were sonicated. All samples were counted in a Packard Tri-Carb liquid scintillation spectrometer and corrected for quenching.

The palmitic acid-1-^{14}C used in this study was obtained from New England Nuclear Corp., Boston, Mass., and its radiochemical purity was evaluated by TLC on Silica Gel G developed with Skellysolve B–diethyl ether–glacial acetic acid 80:20:1. The radioactivity in each area of the plate was determined as described above. The radiochemical purity of the palmitic acid-1-^{14}C was 98% or higher.

RESULTS

Livers from rats on an ad lib. food intake, when perfused with washed corn oil chylomicrons, yielded slices which oxidized to $^{14}CO_2$ and acetoacetate-^{14}C between 3.1 and 7.0% of the palmitic acid-1-^{14}C present in the liver (Table 1). Similar percentages of palmitic acid radioactivity were oxidized by liver slices that had been labeled in vivo by injection of chylomicrons into the inferior vena cava 1–2 min before removal of the liver. In experiments 1 and 2 (Table 1) the livers were perfused before removal from the animal; in experiment 3 (and in subsequent perfusion experiments), afterwards.

TABLE 2 Distribution of Radioactivity in Lipids of Liver Slices from Fed Rats before and after Incubation

Slices prepared from rat livers in expt. 3 were incubated as described in Table 1. The lipids were extracted in chloroform–methanol 2:1 and separated by TLC.

	Chylomicrons Administered	Liver Labeled by: Perfusion, Before Incubation	Perfusion, After Incubation	I. V. Infusion, Before Incubation	I. V. Infusion, After Incubation
			%		
Cholesteryl esters	0.5	0.6	0.7	0.6	0.7
Triglycerides	96.6	90.6	66.8	90.8	57.7
Free fatty acids	0.04*	0.7	6.3	1.0	6.0
Partial glycerides	1.0	1.2	1.6	2.9	3.4
Phospholipids	2.0	7.6	22.2	6.4	30.9

* Measured by two-dimensional TLC.

In experiment 3 the distribution of radioactivity in the various lipids before and after uptake by liver slices was measured (Table 2). Even though the livers were exposed to chylomicrons for only 2 min before being rapidly cooled in ice-cold saline, a considerable alteration in the distribution of labeled lipids occurred during this period. During the 3 hr incubation the percentage of labeled triglyceride decreased markedly; that in free fatty acids and phospholipids increased.

To determine if the liver preferentially removes chylomicrons of a certain size range under these experimental conditions, we subjected samples of the washed chylomicrons, before and after passage through the liver, to sucrose density gradient centrifugation (30). We found no detectable selection of chylomicrons on the basis of size. Further, the chylomicrons in the perfusate had (as judged by TLC) very nearly the same relative percentages of radioactivity in the cholesterol ester, triglyceride, partial glyceride, and phospholipid fractions as the chylomicrons infused, so that no preferential uptake of labeled phospholipids had taken place. Liver slices from fasted rats (Table 3) oxidized a greater percentage of the labeled chylomicrons to $^{14}CO_2$ and acetoacetate-^{14}C than those from well-nourished rats (Table 1). The liver slices from fasted rats also converted chylomicron triglycerides to free fatty acids and phospholipids (Table 4).

The uptake of chylomicrons by the liver 1–2 min after intravenous infusion was 3–6% of the injected dose, and the isolated liver perfused with the same dose removed 7–22% of the chylomicrons (Tables 1, 3). Calculations from these data reveal that the perfused livers removed a quantity of chylomicrons, 0.7–0.8 mg/g of liver, which was independent of the dosage used.

About 81% of the chylomicron phospholipid radioactivity was in the lecithin fraction, about 12% in the phosphatidyl ethanolamine, and 4% in the phosphatidyl

TABLE 3 Oxidation of ^{14}C-Labeled Chylomicrons by Liver Slices from Fasted Rats

The incubation conditions were the same as described in Table 1 except that the incubation period in expt. 4 was 90 min. The total weight of the injected chylomicrons was measured gravimetrically.

	Route of Chylomicron Administration	Injected Dose		Liver Wt.	Chylomicron Uptake	Total Lipid ^{14}C*	$^{14}CO_2$ Produced	Acetoacetate-^{14}C Produced	Chylomicron-^{14}C Oxidized†
		mg	*cpm × 10⁻³*	*g*	*%*	*cpm/100 mg of liver*			*%*
Expt. 1	Perfusion of isolated liver	71.6	32,194	17.56	20.5	37,500	1816	2866	12.5
	Intravenous infusion	71.6	32,194	15.08	3.9	8,230	585	321	11.0
Expt. 2	Perfusion of isolated liver	47.0	20,412	13.34	22.2	34,020	2605	2513	15.0
	Intravenous infusion	47.0	20,412	13.21	3.0	4,660	613	430	22.4

* Before incubation.
† $^{14}CO_2$ plus acetoacetate-^{14}C times 100 ÷ lipid-^{14}C.

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TABLE 4 Distribution of Radioactivity in Lipids of Liver Slices from Fasted Rats before and after Incubation

Liver slices prepared from rat livers in expt. 5 (Table 3) were incubated as described in Table 1.
Lipids were extracted in chloroform–methanol 2:1 and separated by TLC.

	Chylomicrons Administered	Liver Labeled by: Perfusion, Before Incubation	Perfusion, After Incubation	I. V. Infusion, Before Incubation	I. V. Infusion, After Incubation
			%		
Cholesteryl esters	0.5	0.9	1.1	0.8	1.2
Triglycerides	94.3	78.4	63.1	81.4	56.8
Free fatty acids	0.04*	6.7*, 7.0	4.4*, 4.9	0.8	6.7
Partial glycerides	1.8	0.6	1.8	1.0	1.6
Phospholipids	1.6	11.0*, 11.3	22.3*, 25.4	13.8	30.4

* Measured by two-dimensional TLC.

TABLE 5 Phospholipid Radioactivity in Liver Slices before and after Incubation

The phospholipids in extracts of liver slices incubated in expts. 3 and 5 (Tables 1 and 3) were separated by TLC.
For comparison the phospholipid radioactivity in the washed chylomicrons used in expt. 5 was also measured.

Chylomicron Administration			Lecithin	Phosphatidyl Ethanolamine	Phosphatidyl Inositol	Sphingo-myelin
				% of phospholipid radioactivity		
Expt. 3	Perfusion:	before incubation	62.6	33.3	3.7	<1
		after "	70.1	21.4	4.6	1.5
	Intravenous:	before incubation	74.8	23.3	1.9	<1
		after "	71.2	19.3	5.6	1.9
Expt. 5	Perfusion:	before incubation	65.2	28.0	5.1	<1
		after "	75.9	16.8	4.0	1.6
	Intravenous:	before incubation	75.5	22.0	2.0	<1
		after "	76.4	16.6	3.3	2.7
	Chylomicrons		80.9	12.2	4.1	<1

inositol fraction (Table 5). The presence of the latter in chylomicrons has not been reported previously. The sphingomyelin, lysolecithin, and phosphatidyl serine fractions contained lower percentages of the total radioactivity in all chylomicron and liver phospholipid samples analyzed.

Chylomicrons Added In Vitro

Chylomicrons, 675 μg and 6.75 mg, labeled with palmitate-^{14}C in vivo were added to 200–300 mg of liver slices in 2.0 ml of Krebs-Ringer phosphate. The slices oxidized 0.1 and 0.03% respectively of the chylomicron palmitic acid-1-^{14}C to $^{14}CO_2$ and acetoacetate-1-^{14}C per 100 mg of tissue in 3 hr. This low percentage of oxidation of chylomicrons added in vitro to liver slices was decreased 80–90% by the addition of 4% bovine serum albumin prior to incubation. Albumin probably decreases the oxidation of labeled chylomicron lipids by binding radioactive free fatty acids, which may be generated by lipolysis of chylomicron fatty acid esters during metabolism of the chylomicrons by the liver slices.

DISCUSSION

Under the conditions of these experiments, the initial level of free fatty acids in the chylomicrons administered was very low, namely between 0.01 and 0.04% of the chylomicron radioactivity. The rise in free fatty acid radioactivity in the liver after chylomicron administration (Tables 2, 4) clearly indicates hepatic lipolysis of chylomicron fatty acid esters, in agreement with the observation that isolated liver cells firmly bind chylomicrons and hydrolyze chylomicron triglycerides (31). Since a portion of the free fatty acids is probably oxidized and incorporated into various lipids, lipolytic activity is probably greater than is indicated by the quantity of free fatty acids present. Partial glyceride-^{14}C does not accumulate (Tables 2, 4), in agreement with observations on partial glyceride radioactivity in liver after the intravenous injection of doubly-labeled chylomicrons (32).

The accumulation of labeled phospholipids in the isolated perfused liver is apparently very rapid (Tables 2, 4). This is not the result of transfer of phospholipid from the

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perfused chylomicrons to the liver or exchange between the liver and the perfused chylomicrons, since the phospholipid-^{14}C to triglyceride-^{14}C ratio of the chylomicrons emerging from the liver was not lower than in the chylomicrons perfused into the liver. This agrees with the observation that isolated livers perfused with chylomicrons, labeled with triglyceride-^{14}C and phospholipid-^{32}P, removed ^{14}C and ^{32}P in the same proportions as were present in the chylomicrons in the perfusion medium (33). Further increases in phospholipid radioactivity occurred during subsequent incubation of liver slices labeled with chylomicrons (Tables 2, 4). Apparently the fatty acid of chylomicron triglyceride readily undergoes incorporation into lecithin, phosphatidyl ethanolamine, phosphatidyl inositol, and sphingomyelin (Table 5).

The distributions of radioactivity in the lipid fractions of livers labeled by intravenous infusion and by perfusion were very similar (Tables 2, 4). The observed ratio of liver triglyceride-^{14}C to liver phospholipid-^{14}C was about 12–14 to 1 in the fed rats and 6–7 to 1 in the fasted rats prior to incubation and was not affected by the method of chylomicron administration. Soon after the intravenous administration of albumin-bound radioactive free fatty acids, the liver triglycerides and phospholipids are about equally labeled (34–36). If chylomicron fatty acid-^{14}C in the intact rat reached the liver predominantly as free fatty acids after hydrolysis of chylomicron triglycerides in extrahepatic capillary beds (15), the distribution of radioactivity in the livers of rats given chylomicrons intravenously would have been quite different from that observed in the perfused liver. Therefore, the observed distribution of radioactivity in the livers prior to incubation suggests that the major portion of chylomicron-^{14}C in liver is a result of some type of direct removal of chylomicrons from the plasma by the liver in vivo.

The observation that liver slices from fasted rats (Table 3) oxidized chylomicron fatty acids to a greater extent than liver slices from fed rats (Table 1) is consistent with reports that fasting increases the oxidation of palmitic acid-1-^{14}C by liver slices (37) and the oxidation of fatty acids by the perfused liver (38).

On the basis of rat liver perfusion experiments, Felts and Mayes suggested that extrahepatic lipolysis precedes oxidation of chylomicron fatty acids by the liver (15). In our experiments slices from livers, labeled by perfusion or by intravenous injection of chylomicrons, oxidized chylomicron fatty acids to a significant and similar extent (Tables 1, 3). The isolated liver can therefore utilize chylomicron fatty acids directly. The similar extent of oxidation of chylomicron fatty acids by liver slices labeled in vivo and by liver perfusion indicates that the perfused chylomicrons reached physiologically active sites of metabolism in the liver. Most of the labeled chylomicrons added in vitro to unlabeled liver slices apparently did not reach such sites since, under these conditions, an extremely low percentage of added chylomicrons was oxidized. It is possible that chylomicrons added to liver slices in vitro mainly encounter the inner surface of the membranes of ruptured liver cells, whereas both in vivo and in the perfused liver, chylomicrons first come into contact with the outer surface of the cell membrane; the difference in results therefore suggests that the liver cell membrane actively participates in the utilization of chylomicron lipids by the liver. Now, in the interpretation of the oxidation of chylomicrons added to liver slices in vitro, or circulated through the liver as done by Felts and Mayes (15), it is important to consider that a variable portion of the added chylomicron-^{14}C is taken up. Expressing the oxidation of lipid as a percentage of the added dose does not adequately reflect the activity of the tissue, especially when the added dose is high. This oxidation should be calculated from the chylomicrons taken up, not from the administered dose. For example, Felts and Mayes observed that only about 0.5% of the chylomicrons in their liver perfusion medium was oxidized (15). However, this represents about 20–60% of the chylomicrons taken up by the perfused liver in 90 min, and the oxidation of about 0.5 mg of chylomicrons by the liver per hr. A similar rate can be calculated from our data (Table 3).

The interpretation of these data may be facilitated by estimating whether the observed rate of utilization of chylomicrons by the liver accounts for a significant portion of the total utilization by the intact rat. As calculated from the data in Tables 3 and 4, a total of about 40% of the chylomicron triglyceride was converted to oxidation products and to other lipids by slices of isolated livers perfused with chylomicrons. About half of this conversion represents phospholipid formation. The data in Table 4 represent the lipid conversions in experiment 5. The observed extent of chylomicron triglyceride conversion was similar in experiment 4. Since free fatty acids are converted about equally to phospholipids and triglycerides in the liver (34–36), it is likely that a portion of the triglyceride radioactivity at the end of the incubation is resynthesized triglyceride. The liver may therefore process about 60% of the chylomicron triglycerides in 90 min in experiment 4 and in 3 hr in experiment 5. Since the lipid conversions occur rapidly after the removal of chylomicrons from the perfusion medium (Tables 2, 4), the calculated rate of utilization in experiment 4 probably reflects the activity of the tissue more closely than the rate observed when liver slices are incubated for a longer time. The rate of utilization in experiment 4 was 40% per hr, or 6 mg of chylomicrons per hr. Rats that daily consume 20 g of food containing 4% fat utilize about 800 mg of chylomicrons per day, or

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about 30 mg/hr. Our data therefore suggest that the direct removal of chylomicrons by the liver accounts for a significant portion (perhaps 20%) of the chylomicron fatty acids utilized by the intact rat.

The authors thank Mrs. Rita Russell for capable technical assistance.

This study was supported by USPHS Training Grant HE-5279 and Research Grant HE-1238.

Manuscript received 16 July 1966; accepted 1 November 1966.

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