The uptake of doubly labeled chylomicrons by guinea pig mammary gland and liver

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SUMMARY The uptake of cholesterol-H^3–palmitate-C^{14}-chylomicrons by mammary tissue and liver has been examined. The cholesterol and glyceride components of chylomicrons were taken up at the same rate by both liver and mammary tissue, as demonstrated by the unchanged ratio of C^{14}/H^3 in the tissue lipids compared to the chylomicrons. However, following injection of glycerol-C^{14}–palmitate-H^3-chylomicrons, the H^3/C^{14} ratio of the lipids of mammary tissue was much greater than the H^3/C^{14} ratios of the injected chylomicrons, of the serum lipids, or of the liver lipids, indicating that appreciable hydrolysis of chylomicron glycerides occurred during their uptake by mammary glands. This is consistent with the hypothesis that lipoprotein lipase is involved in the uptake of chylomicron glyceride by mammary gland.

THE RATE OF UPTAKE of chylomicron glycerides by mammary tissue (1) and the lipoprotein lipase activity of this tissue (2) are both much higher in lactating than in nonlactating animals. These facts are compatible with the hypothesis that lipoprotein lipase is involved in the uptake of chylomicron glyceride by mammary tissue. However, these correlations do not prove the hypothesis. A similar relationship is found, for example, for the rate of uptake of free fatty acids by mammary tissue (1), as well as for the activities of several other enzymes of mammary tissue (3, 4).

In order to obtain further information concerning the possibility that lipolysis may occur concomitantly with glyceride uptake in lactating mammary tissue, the metabolism of chylomicrons which were isotopically labeled in two separate parts of the glyceride molecules has been investigated. The chylomicrons were labeled with palmitate-H^3 and glycerol-C^{14}. If glyceride molecules were removed intact, the palmitate-H^3/glycerol-C^{14} ratio should have been the same in the glycerides of the tissue as in the injected material, assuming no subsequent metabolism of the glyceride had occurred in the tissue. Conversely, if partial or total lipolysis did occur during uptake, the ratio of H^3/C^{14} in the glycerides recovered from the tissue should have been altered from that in the injected material unless the rates of reutilization of both labeled moieties by the tissue were precisely equal. The H^3/C^{14} ratio in the tissue glycerides was examined at varying intervals after injection of the chylomicrons in an effort to ascertain whether any potential alteration in the H^3/C^{14} ratio was associated with the process of uptake, or was the result of glyceride metabolism in the tissue subsequent to uptake.

The uptake by mammary tissue and liver of chylomicrons which were isotopically labeled in two different molecular species has also been examined. The injected chylomicrons were labeled, in this case, with cholesterol-H^3 and palmitate-C^{14}. It was anticipated that H^3/C^{14} ratios of the total lipids of the tissue would have been identical with the ratio of the total lipids of the injected material if the chylomicrons were removed intact by the tissue, and neither labeled compound was lost by subsequent metabolism in the tissue.

MATERIALS AND METHODS

Lactating N.I.H. strain guinea pigs which were removed from food and their litters immediately prior to experiments were used (1).

Uniformly tritiated cholesterol (465 mc/mmole), glycerol-1,3-C^{14} (1.6 mc/mmole), and palmitic acid-1-C^{14} (29.7 mc/mmole) were obtained from the Nuclear Chicago Corporation, Chicago, Ill. Palmitic acid-9,10-H^3 (50 mc/mmole) was obtained from the New England Nuclear Corporation, Boston, Mass.

The purity of the cholesterol-H^3 was determined by preparative gas–liquid chromatography (GLC) together

with carrier cholesterol on a column of 1% SE-30. The emerging cholesterol peak was collected in three fractions on Celite coated with silicone oil (5). The cholesterol was eluted with diethyl ether, and 1 aliquot of the solution was used to determine radioactivity while another aliquot was re-chromatographed on an analytical column to determine mass. All of the radioactivity of the original sample was recovered in the cholesterol peak, and the specific activities of the original cholesterol and the three fractions were identical. Another aliquot of the cholesterol-H^3 was co-chromatographed with carrier cholesterol by thin-layer chromatography (TLC) on Silica Gel G, using a solvent system consisting of heptane–diethyl ether–acetic acid, 70:30:1. After staining with I_2 vapor, areas of the silica gel were scraped into scintillation vials and radioactivity was determined. The cholesterol area contained 97% of the radioactivity. The cholesterol-H^3 was therefore used without purification.

The glycerol-1,3-C^{14} was found to be pure by paper chromatography in three systems (6). Palmitate-1-C^{14} and palmitate-9,10-H^3 were each purified from nonacidic contaminants and short chain fatty acids as described previously (7). GLC establishes that both samples were 99% pure.

Albumin-I^{131} (Abbott Laboratories, Chicago, Ill.) was dialyzed extensively against 0.15 M NaCl immediately prior to use, in order to remove any free I^{131}.

Preparation of Chylomicrons Labeled with Cholesterol-H^3 and Palmitate-1-C^{14}

Repeated attempts to cannulate the guinea pig thoracic duct failed because of its extreme fragility. It was necessary, therefore, to prepare rat chylomicrons.

Palmitic acid-1-C^{14} (0.3 mc) and cholesterol-H^3 (1 mc) were mixed with 1.5 ml of olive oil, and were administered by gastric tube to a rat with a cannulated cisterna chyli. Chyle was collected at 2° during the next 24 hr. After squeezing the chyle (113 ml) through several layers of cheese cloth to remove the fibrin clot, the chyle was centrifuged at 100,000 × *g* for 30 min. The tubes were sliced just below the upper milky layer, and the upper layer of chylomicrons was removed. The chylomicrons were emulsified in 0.15 M NaCl through a No. 15 needle. To remove exchangeable cholesterol-H^3, the chylomicrons were mixed with 25 ml of citrated guinea pig whole blood (diluted 1:1). The mixture was incubated on a Dubnoff shaker for 1 hr at 37°. This preparation was layered under cold 0.9% NaCl and the chylomicrons were reisolated by centrifugation. The whole procedure was then repeated, followed by a third equilibration with blood for 7 hr at 4° and 1 hr at 37°. After the final isolation and emulsification, 2.8 ml of dialyzed radioiodinated serum albumin was added to the chylomicrons. The suspension was mixed by gentle inversion and was used within 2 days. The chylomicrons stayed well emulsified with no aggregation visible under dark-field microscopy. Aliquots were removed at each stage of the isolation procedure and extracted with more than 20 volumes of chloroform–methanol, 2:1 (v/v). Aliquots of these extracts were assayed for C^{14} and H^3.

Preparation of Chylomicrons Labeled with Glycerol-1,3-C^{14} and Palmitate-9,10-H^3

Glycerol-1,3-C^{14} (150 μc, 100 μmoles) was converted to triolein-C^{14} by reacting it with 1 ml of oleoyl chloride in a mixture of 1 ml of pyridine and 1 ml of anhydrous benzene (8). The mixture was shaken for 4 hr at room temperature and at 60° for 1 hr. The reaction mixture was transferred to a separatory funnel in 100 ml of heptane and was washed five times with 100 ml of 0.5 N H_2SO_4 to remove pyridine and three times with 100 ml of alkaline aqueous isopropanol to remove a large portion of the excess free oleic acid. The heptane was removed at 40° under reduced pressure. The glycerol-C^{14}-triolein (1.5×10^8 cpm) and palmitic acid-9,10-H^3 (1.8×10^8 cpm, 10 μmoles) were added to 1.5 ml of olive oil, and traces of solvent were removed by a stream of N_2 at 60°. This mixture was given by gastric tube to a rat with a cannulated cisterna chyli and 133 ml of chyle was collected at 2° during the next 29 hr. The chylomicrons were isolated by ultracentrifugation at 100,000 × *g* for 3 hr, after removal of the fibrin clot. The chylomicrons were emulsified in a small amount of 0.15 M NaCl. Experiments were performed within 7 days after collection of the chylomicrons. There was no apparent change in the physical state of the chylomicrons. Immediately prior to the experiments, 6.8 ml of the chylomicron suspension was mixed with 3.2 ml of fasting normal guinea pig serum, and 2.0 ml of dialyzed radioiodinated serum albumin.

Procedure in the Experiments with Cholesterol-H^3–Palmitate-C^{14}-Chylomicrons

Guinea pigs were anesthetized with diethyl ether, the abdominal cavity was exposed, and 2.0 ml of the suspension of chylomicrons and albumin-I^{131} containing 26 μmoles of glycerides was injected into the inferior vena cava over a 10 sec interval. At a specified time from the mid-point of injection, the left mammary gland was removed, and bleeding vessels were clamped. At a later time, the right mammary gland was removed, and 1–2 ml of blood was simultaneously removed by cardiac puncture. Immediately thereafter, the liver was removed. It was usually possible to remove the mammary glands (sometimes accompanied by some overlying skin) and liver in toto. Only a 5–10 sec interval was required for the removal of each of these tissues.

Each of the tissues was immediately dropped into chloroform–methanol, 2:1 (v/v) in a Waring blendor and homogenized for 2 min; 400 ml of solvent was used in the homogenization of each mammary gland, and 800 ml of solvent for the liver. Whole blood samples were placed directly into graduated conical centrifuge tubes in an ice bucket, and 20 volumes of chloroform–methanol were added. Blood samples were then homogenized in small Waring blendors or in glass homogenizers. A 2 ml aliquot of injected material was similarly extracted. After 24 hr at room temperature, the solutions were filtered. The filter papers were washed with small aliquots of chloroform. The solvent was then removed in a rotary evaporator at 50°, and the lipid residues were redissolved in chloroform containing a little methanol. Aliquots were assayed simultaneously for H^3 and C^{14} in a liquid scintillation spectrometer, using 0.2% diphenyloxazole in toluene. It was possible to exclude more than 99.9% of the H^3 radioactivity from the channel used to measure C^{14} radioactivity. Corrections for quenching were made in all cases using the channels ratio method (9). These corrections were essential since the C^{14} was quenched more than the H^3, and because quenching causes more of the higher energy isotope (C^{14}) to appear in the lower energy channel. An effort was made to minimize quenching by re-counting smaller aliquots of material where necessary. Quench corrections for C^{14} (the more highly quenched isotope) were usually less than 15%, and never exceeded 33%.

The tissue residues from the extractions with chloroform–methanol were dissolved in four volumes of 25% aqueous KOH, and I^{131} was determined using a thallium-activated sodium iodide scintillation crystal. The amount of H^3 and C^{14} remaining in sera at the end of the experiment could then be calculated from the ratios of these isotopes to albumin-I^{131} in the injected material and in the sera, making the assumption that none of the injected albumin-I^{131} left the serum. Similarly, the contribution to the H^3 and C^{14} in the tissue extracts by the blood present in the tissues was calculated from the I^{131} content of the tissue residue. Ester assays were performed by the method of Rapport and Alonzo (10); phosphorus by a modification of the procedure of Fiske and Subbarow (11); and cholesterol by the method of Sperry and Webb (12).

Procedure in the Experiments with Glycerol-C^{14}–Palmitate-H^3-Chylomicrons

A 1 ml aliquot of the chylomicron suspension containing 30 μmoles of glyceride was injected into the inferior vena cava over a 10 sec interval. Part of one mammary gland was rapidly excised at the specified time, and blood was simultaneously withdrawn from the heart or aorta. Part of the liver was then removed. All effort was directed toward the removal of tissues as rapidly as possible (2–5 sec), and consequently no effort was made to remove the tissues in toto. After removal of the tissues, they were dropped directly into liquid nitrogen to halt further metabolism. Subsequently, they were weighed while frozen, partially pulverized, and homogenized in 20 volumes of chloroform–methanol in a Waring blendor. The lipids were extracted by the method of Folch et al. (13). Specimens of blood were transferred directly into tubes in an ice bucket and permitted to clot. Serum was removed at 2° by centrifugation. The sera and an aliquot of the injected chylomicrons were extracted by the procedure used for the tissues. The extracted lipids were recovered, and C^{14} and H^3 were determined as just described. Corrections for contributions of H^3 and C^{14} by blood remaining in the tissues were also made as just described.

Lipids of the tissues, serum, and injected material were chromatographed on columns of silicic acid, usually by the method of Hirsch and Ahrens (14). In certain instances, the lipids were fractionated only into neutral lipids and phospholipids by silicic acid chromatography according to the method of Borgström (15). The neutral lipids prepared by both methods were partitioned between petroleum ether and alkaline aqueous isopropanol to remove the free fatty acids. The free fatty acids were recovered from the acidified aqueous phase by two extractions with petroleum ether.

Thin-layer chromatography was performed as described previously (7). Lipids were stained with iodine vapor. After allowing most of the iodine to sublime, the areas containing lipids were scraped into disposable pipettes which were plugged with glass wool. The lipids were then eluted with small aliquots of petroleum ether–diethyl ether, or chloroform–methanol.

RESULTS

Cholesterol-H^3–Palmitate-1-C^{14}-Chylomicrons

During the preparation of the chylomicrons, there was a progressive and unequal loss of cholesterol-H^3 and palmitate-C^{14} (Table 1), probably due to exchange between the chylomicrons and the soluble lipoproteins of chyle and blood. The distribution of radioactivity among the lipids of the final preparation is shown in Table 2. About 72% of the H^3 was found in cholesterol esters, while the remainder was present as free cholesterol. Most of the C^{14} activity was present in triglycerides with only small amounts in partial glycerides, as has also been found by other investigators. Only 0.6% of the C^{14} was present as free fatty acids. Thus, in experiments with these chylomicrons, the uptake of C^{14} would represent primarily uptake of triglyceride fatty acids, while H^3

TABLE 1 ANALYSIS OF THE RADIOACTIVITY IN THE CHOLESTEROL-H^3–PALMITATE-C^{14}-CHYLOMICRONS AT DIFFERENT STAGES OF PREPARATION

Fraction	Total Counts ($\times 10^{-6}$)		% Recovery		C^{14}/H^3 Ratio
	C^{14}	H^3	C^{14}	H^3	
Whole chyle	228.0	153.4	100	100	1.49
Chylomicrons	188.6	106.8	82.7	69.6	1.77
1st equilibration with blood	181.2	91.3	79.5	59.5	1.98
2nd equilibration with blood	145.9	68.8	64.0	44.9	2.12
3rd equilibration with blood	120.9	51.6	53.0	33.6	2.34

uptake would represent esterified, or free cholesterol, or both.

The uptake of C^{14} and H^3 by mammary glands and liver, the amount remaining in serum, and the C^{14}/H^3 ratio in each tissue are shown in Table 3. All C^{14}/H^3 ratios have been related to that of the injected chylomicrons which was arbitrarily assigned a value of 1.00. The values for mammary gland and liver have been corrected for the amount of C^{14} and H^3 in the blood remaining in the tissues. The removal of triglyceride (C^{14}) from the blood was only slightly more rapid than the removal of cholesterol and/or cholesterol esters (H^3). About 10–17% of the C^{14} was removed from the blood in 2 min, and 20–50% was removed in 6 min. The ratio of C^{14}/H^3 in the blood samples at the end of the experiments was only slightly less than the C^{14}/H^3 ratio in the injected chylomicrons. In experiment 6, the H^3 and C^{14} activities of serum and blood were compared. If significant exchange of cholesterol between chylomicrons and red blood cells had occurred during the course of the experiment, the C^{14}/H^3 ratio would have been higher in the serum than in the blood. This was not found.

TABLE 2 DISTRIBUTION OF C^{14} AND H^3 AMONG THE LIPID FRACTIONS OF THE CHOLESTEROL-H^3–PALMITATE-C^{14}-CHYLOMICRONS

Fraction	C^{14}		H^3		Ester
	cpm	%	*cpm*	%	*μmoles/ml, of chylomicron suspension*
Cholesterol ester	25,210	0.3	2,396,460	71.55	
Triglyceride	7,645,100	92.43	0		35.7
Cholesterol	114,895	1.39	873,230	26.07	
Diglyceride	263,165	3.18	6,165		1.32
Monoglyceride	20,335	0.25	36,360		0.79
Phospholipid	153,585	1.86	35,860		1.39
Free fatty acids	49,305	0.60	1,085		

Lipids were separated on silicic acid by stepwise elution with the following percentages of diethyl ether in petroleum ether: cholesterol esters, 1%; triglycerides, 4%; free cholesterol, 4–8%; diglycerides, 25%; monoglycerides, 100% diethyl ether; and phospholipids with 100% methanol. Free fatty acids were recovered by partitioning the neutral lipid fractions. There was some tailing of the cholesterol into later fractions.

The uptake of both the cholesterol and glyceride moieties of the chylomicrons was as rapid by mammary tissue as by liver. Both moieties were taken up at about the same rate by both tissues, as demonstrated by the fact that, in most cases, the C^{14}/H^3 ratios of the tissue lipids were not significantly different from those of the injected chylomicrons. In experiment 9 the total uptakes by both mammary glands and by liver were measured in an animal which had been removed from the suckling litter 48 hr prior to the experiment. The average uptake of C^{14} by one mammary gland in this animal was only 1.4% compared to the uptakes of 6–15% in the lactating animals. This difference in chylomicron uptake is qualitatively similar, but quantitatively smaller, than the difference observed previously (1) between mammary glands of pregnant and lactating guinea pigs.

Glycerol-C^{14}–Palmitate-H^3-Chylomicrons

A lipid extract of this preparation of chylomicrons was fractionated by silicic acid chromatography (Table 4). About 96% of the recovered C^{14} and 95% of the recovered H^3 were found in the glycerides. However, only about 70–75% of the C^{14} and H^3 was present in triglycerides, the remainder being in diglycerides and monoglycerides. This differs from the distribution found in the other batch of chylomicrons in which 92% of the glyceride radioactivity was in triglyceride. Only 1% of the H^3 was present as free fatty acids. The results of the 10 experiments in which these chylomicrons were used are summarized in Tables 5, 6, and 7.

The distribution of H^3 and C^{14} in the serum lipids at the end of the experiments may be seen in Table 5. Only about 60–70% of the total recovered C^{14} and H^3 was present in the form of triglycerides at any of the times from 1 to 5 min following injection. The percentage of the total H^3 and C^{14} found in the partial glycerides in the serum was slightly greater than that present in the original chylomicrons; there was about 25–30% in diglycerides, and 5–10% in monoglycerides. The percentage of H^3 found in the sera as free fatty acids varied from 3.6 to 18.5% of the total H^3, but this represented only 2–5% of the injected H^3.

The distributions of H^3 and C^{14} among the lipids of mammary tissue and liver are shown in Table 6. Several differences are apparent when the distribution of radioactivity among the tissue lipids is compared to the distribution of radioactivity among the lipids of the sera (Table 5) and of the injected chylomicrons (Table 4). For example, the partial glycerides of mammary tissue

contained a smaller percentage of H^3 and C^{14} and the phospholipids a greater percentage than did the chylomicrons of serum. There was very little unesterified palmitate-H^3 in the mammary tissue at any time No unesterified palmitate-H^3 was found in the lipid extracts of liver.

In Table 7, the H^3/C^{14} ratios of the various lipids of mammary gland, serum, and liver are compared to the H^3/C^{14} ratios of the corresponding fractions of the injected chylomicrons which were arbitrarily assigned a value of 1.00. The H^3/C^{14} ratios of the total lipids of serum, of the serum glycerides, and of the serum triglycerides did not deviate greatly from unity (0.95–1.21). The H^3/C^{14} ratios of the partial glycerides and phospholipids of serum fluctuated considerably, but with no discernible pattern. The H^3/C^{14} ratios of the total lipids and of the glycerides of the livers were very close to 1.0 in all of the experiments. The H^3/C^{14} ratios of the liver phospholipids were frequently significantly greater than 1.0.

The H^3/C^{14} ratios of the lipids of mammary tissue were markedly different, however, from the values found for serum and liver. Although there was considerable variability, the H^3/C^{14} ratios of the total lipids, of the total glycerides, and of the triglycerides of mammary tissue were always significantly greater than 1.0, even as early as 1 min following injection. The highest H^3/C^{14} ratios were found in the diglycerides and phosphatides of mammary tissues with values usually in the range of 2 to 4, irrespective of the duration of the experiment. The H^3/C^{14} ratios were also consistently elevated in the monoglycerides of mammary tissue.

Further Characterization of Fractions from Silicic Acid Chromatography

The data shown in Tables 5–7 were obtained on lipid fractions prepared by silicic acid column chromatography. In order to confirm the composition of each fraction, aliquots were analyzed by TLC; in all cases the results confirmed the data obtained by silicic acid column chromatography.

The percentage of radioactivity in the diglycerides of the original chylomicrons was surprisingly high (Table 4). To confirm the composition of this fraction, an aliquot of the chylomicron diglycerides was analyzed by TLC before and after acylation with oleoyl chloride. Standard 1,2-diolein was also chromatographed before and after acylation. The unacylated chylomicron diglycerides migrated in the area expected for diglycerides, whereas after acylation the material migrated predominantly in the area expected for triglycerides. There was some overlap, probably attributable to the overloading of the plate with free fatty acids, and to incomplete acylation of the diglyceride. (It was found that most of the control 1,2-diolein had been acylated and migrated as triglyceride, but a small amount of diglyceride persisted.) The H^3/C^{14} ratios of the unacylated and acylated

TABLE 3 Uptake of Cholesterol-H^3–Palmitate-C^{14}-Chylomicrons by Mammary Glands and Liver of Lactating Guinea Pigs

Expt.	Time of Sample	% of Injected C^{14}			C^{14}/H^3 Ratio*		
		Blood	Mammary Gland†	Liver	Blood	Mammary Gland	Liver
	min						
1	1.2		0.65			0.95	
	2.2	90.2	0.32	4.66	1.00	1.14	0.99
2	1.2		2.47			1.06	
	2.2	83.0	9.43	4.92	0.94	1.06	1.17
3	2.2		0.67			1.03	
	4.2	78.5	1.44	7.22	0.97	0.97	1.11
4	2.2		1.82			1.09	
	4.1	74.0	7.87	12.60	0.90	1.26	0.98
5	2.1		3.38			1.05	
	4.2	75.0	6.05	4.43	0.93	1.18	1.03
6	3.1		9.77			1.15	
	6.1	60.4	15.45	7.44	0.89	1.45	0.91
		55.9‡			0.88‡		
7	3.0		4.28			1.04	
	6.1	81.7	6.34	11.18	0.95	1.33	0.91
8	3.0		4.75			1.00	
	6.1	47.3	5.88	10.35	0.91	1.14	1.02
9§	6.0	≈100	2.81†	1.71	0.92	1.25	1.17

* C^{14}/H^3 ratios are relative to the C^{14}/H^3 of the injected chylomicrons, which was arbitrarily assigned a value of 1.0.
† Values are for one mammary gland at each time except for experiment 9, where the value is for both mammary glands.
‡ Values for serum.
§ Animal removed from suckling litter 48 hr prior to the experiment.

TABLE 4 DISTRIBUTION OF RADIOACTIVITY AMONG THE LIPIDS OF THE GLYCEROL-1,3-C^{14}–PALMITATE-9,10-H^3-CHYLOMICRONS

Fraction	C^{14}		H^3		H^3/C^{14}
	cpm	%	*cpm*	%	
Total lipids	1,107,234	100	3,124,043	100	2.82
Cholesterol ester	191	0.02	13,074	0.42	—
Triglyceride	756,809	68.4	2,115,319	67.7	2.80*
Diglyceride	194,521	17.6	486,418	15.6	2.50*
Monoglyceride	45,638	4.1	106,862	3.4	2.34*
Phospholipid	41,986	3.8	116,259	3.7	2.77
Free fatty acid	1,206	0.1	31,649	1.0	—
Total of fractions	1,040,351	94.0	2,869,581	91.8	2.76

* Mean ratio of total glycerides = 2.72.

chylomicron diglycerides were the same, indicating that the change in chromatographic distribution of the radioactivity was not due to specific loss of a more slowly migrating component during the acylation procedure. Furthermore, recovery of radioactivity in the acylation procedure was essentially quantitative.

DISCUSSION

Uptake of Cholesterol-H^3–Palmitate-C^{14}-Chylomicrons

Studies both in vivo (16, 17) and in vitro (18, 19) have demonstrated that the free cholesterol of chylomicrons, plasma, and red blood cells are readily exchangeable. Cholesterol esters also exchange, but this equilibration is less rapid than that involving free cholesterol. These phenomena create a serious problem in interpreting results concerning the rate of uptake by tissues of chylomicron cholesterol. One way of minimizing this difficulty is to equilibrate the chylomicrons with blood in vitro before use (18). In the present investigation, the chylomicrons were equilibrated three times with citrated whole guinea pig blood (Table 1). During the last in vitro equilibration, there was only a 10% change in the C^{14}/H^3 ratio. This occurred over an 8 hr interval including 1 hr at 39°. Consequently, it is unlikely that any significant change in the C^{14}/H^3 ratio of the injected chylomicrons in vivo could be attributable to exchange of cholesterol with other fractions of blood. None of the experiments lasted longer than 6 min. This conclusion is supported by the fact that, in the one epxeriment in which they were both examined (Table 3), the C^{14}/H^3 ratio was the same for the lipids of serum and blood, indicating that there was no significant exchange of cholesterol-H^3 between chylomicrons and red blood cells.

It was found that the cholesterol-H^3 was removed from the blood slightly less rapidly than was the palmitate-C^{14}. However, the rates of removal of the two moieties were not greatly different, as demonstrated by the fact that C^{14}/H^3 ratios in blood never decreased to less than 0.89 even when as much as 50% of the chylomicrons had been removed from the blood. Liver removed chylo-

TABLE 5 DISTRIBUTION OF RADIOACTIVITY AMONG THE LIPIDS REMAINING IN SERUM AFTER THE INJECTION OF GLYCEROL-C^{14}–PALMITATE-H^3-CHYLOMICRONS

Expt.	Time of Expt.	Total Lipids		Triglycerides		Diglycerides		Monoglycerides		Free Fatty Acids	Phospholipids	
		H^3	C^{14}	H^3	C^{14}	H^3	C^{14}	H^3	C^{14}	H^3	H^3	C^{14}
	min	*% of injected radioactivity*		*% of radioactivity in total lipids**								
1	1	95.8	92.5	52.6	55.2	21.3	26.4	2.7	4.2	4.0	1.5	2.2
2	1	53.7	49.9	64.2	62.8	20.5	23.7	4.1	4.6	4.5	2.3	3.3
3	2	48.3	43.0	62.1	58.9	23.6	29.5	4.1	6.0	6.3	2.5	3.8
4	2	58.5	52.6	59.3	57.0	21.2	34.7	3.9	6.8	5.5	1.5	1.7
5	3	93.5	78.9	60.2	57.6	5.4	12.8	3.6	6.0	8.0	—	—
6	3	41.2	30.5	46.4	49.9	20.7	27.4	5.8	11.7	14.3	2.0	4.5
7	4	28.9	22.5	52.0	48.2	24.4	33.9	6.9	10.5	8.8	2.5	3.6
8	4	50.5	44.2	76.1†	81.5†	—	—	—	—	9.9	7.4	13.6
9	5	24.8	19.1	41.8	46.2	19.0	27.8	3.9	5.8	18.5	2.5	1.9
10	5	51.9	47.4	57.5	51.6	23.7	29.7	5.9	8.8	3.6	8.2	12.6

* Total recovery of radioactivity varied from 85 to 103% and was usually better than 95%.
† Values are for total glycerides, which were not subfractionated in this experiment.

TABLE 6 DISTRIBUTION OF RADIOACTIVITY AMONG THE LIPIDS OF MAMMARY GLAND AND LIVER FOLLOWING THE INJECTION OF GLYCEROL-C^{14}–PALMITATE-H^3-CHYLOMICRONS

		Mammary Gland									Liver				
		Triglycerides		Diglycerides		Monoglycerides		Free Fatty Acids	Phospholipids		Glycerides		Phospholipids		Free Fatty Acids
Expt.	Time of Expt.	H^3	C^{14}	H^3	C^{14}	H^3	C^{14}	H^3	H^3	C^{14}	H^3	C^{14}	H^3	C^{14}	H^3
	min	*% of radioactivity in total lipids**									*% of radioactivity in total lipids†*				
1	1										92.9	90.2	5.4	3.9	0
2	1	66.9‡	56.7‡								96.7	94.0	1.9	<0.1	0
3	2	76.9	84.6	9.9	9.2	1.4	1.9	2.8	7.3	3.9	97.1	92.1	8.5	7.3	0
4	2	67.1	75.0	9.7	5.0	2.6	3.9	3.6	15.0	14.3					
5	3	75.5	74.7	12.5	12.8	1.9	2.6	0.9	8.3§	6.8§	87.0	93.3	10.3	13.3	0
6	3	74.3	77.8	13.4	12.6	1.1	1.4	1.5	9.6	6.5	103.5	99.2	7.0	5.6	0
7	4	70.2	71.9	7.3	6.0	1.1	1.6	1.1	13.3	11.8	96.8	90.4	4.3	3.0	0
8	4	73.0§	75.2§	10.1§	10.2§	1.4§	2.0§	1.5§	10.4§	8.5§	94.2	99.2	10.8	7.9	0
9	5	78.5	77.6	9.5	9.6	2.1	2.8	1.2	7.2	6.8	102.1	86.5	8.5	8.9	0
10	5	68.6‡	68.1‡								87.1	97.0	10.2	8.0	0.8

* Uptake of H^3 was 11, 2, 22, 7, 14, 12, 9, 24, 24 and 1% in experiments 1 through 10, respectively.
† Uptake of radioactivity was 6, 2, 6, 1, 1, 6, 20, 7, 8 and 5% in experiments 1 through 10, respectively.
‡ Values are for total glycerides, which were not subfractionated in these experiments.
§ Values not corrected for contamination by blood lipids. Corrections would generally be about 1–2%.

micron cholesterol and glycerides at the same rate (C^{14}/H^3 in liver = 1.0) in agreement with the concept that chylomicrons are removed intact by the liver by the process of pinocytosis (20, 21).

There was also very rapid uptake of both chylomicron cholesterol and chylomicron glycerides by mammary tissue. The rates of uptake of both components by mammary tissue were very similar (except in experiment 6, Table 3) as indicated by the fact that the C^{14}/H^3 ratio in this tissue also remained near unity throughout the interval studied. This result is consistent with the possibility that chylomicrons may also be removed intact by mammary tissue. However, it can be stated with certainty only that both components of the chylomicrons are removed by mammary tissue at approximately equal rates, whatever the mechanism may be.

Goodman (18) has studied the kinetics of uptake of chylomicrons labeled with cholesterol-C^{14} by rats in vivo. He found that the liver was almost exclusively responsible for the initial removal of chylomicron cholesterol esters and free cholesterol. A similar interpretation has been offered by Borgström (22). The results of the present investigation demonstrate that the free and esterified cholesterol of the chylomicrons may be removed by at least one other tissue without prior passage through the liver. Lossow, Brot, and Chaikoff have also studied the uptake of cholesterol-C^{14}-labeled chylomicrons by rat tissues in vivo (17) and in vitro (23) and have concluded that chylomicron cholesterol may be removed directly from the blood by a variety of tissues in addition to liver, even though liver is the principal site of uptake under most circumstances.

Uptake of Glycerol-C^{14}–Palmitate-H^3-Chylomicrons

About 20% of the H^3, and about 23% of the C^{14} present in this chylomicron preparation were found in partial

TABLE 7 H^3/C^{14} RATIOS OF LIPID FRACTIONS OF SERUM (S), MAMMARY TISSUE (M), AND LIVER (L) RELATIVE TO CORRESPONDING FRACTIONS OF INJECTED GLYCEROL-C^{14}–PALMITATE-H^3-CHYLOMICRONS

		Total Lipids			Glycerides			Triglycerides		Diglycerides		Monoglycerides		Phospholipids		
Expt.	Time	S	M	L	S	M	L	S	M	S	M	S	M	S	M	L
	min															
1	1	1.01	1.45	0.88	0.92		0.94	0.95		0.92		0.83		0.76		1.36
2	1	1.06	1.97	0.92	1.05	2.36	0.98	1.06		1.02		1.19		0.80		
3	2	1.10	1.52	0.97	1.07	1.42	1.06	1.14	1.36	0.99	1.85	0.95	1.41	0.80	3.08	1.23
4	2	1.09	1.80		0.95	1.73		1.11	1.58	0.75	3.91	0.79	1.54	1.06	2.07	
5	3	1.16	1.93	1.06	1.07	1.96	1.03	1.19	1.92	0.55	2.13	0.89	1.77		2.60	0.89
6	3	1.32	1.64	1.01	1.10	1.61	1.03	1.21	1.54	1.12	1.96	0.83	1.67	0.66	2.64	1.30
7	4	1.26	2.66	0.91	1.05	2.68	1.01	1.21	2.55	0.93	3.60	0.95	2.51	0.87	3.30	1.41
8	4	1.12	1.91	1.10	1.08	1.83*	1.03		1.77*		2.06*		1.69*	0.67	2.51*	1.53
9	5	1.27	1.74	1.02	1.05	1.77	1.24	1.13	1.73	0.98	1.94	1.09	1.63	1.84	2.02	1.06
10	5	1.07	1.26	1.18	1.06	1.29	1.04	1.18		0.96		1.08		0.77		1.53

* Not corrected for contamination by blood lipids. The correction would increase the ratios by about 5–10%.

glycerides (Table 4), whereas only 4% of the radioactive fatty acids were in the partial glycerides of the cholesterol-H^3–palmitate-C^{14}-chylomicrons. The reason for this difference is not known. Slightly more total lipid and much more free fatty acid were given to the rat used in the preparation of the glycerol-C^{14}–palmitate-H^3-chylomicrons.

It has usually been reported that chylomicrons contain less than 10% partial glycerides (24), and usually less than 10% of the radioactivity has been found in the partial glycerides of isolated chylomicrons following the oral ingestion of radioactive free fatty acids (25–27). However, Carlson and Wadström (28) found about 13% diglycerides in human chylomicrons isolated from a patient with chylothorax, and about 10% diglycerides in human serum during alimentary lipemia. Mead and Fillerup (29) found about 20% of the C^{14} in the serum of rats to be present in di- and monoglycerides at $^1/_2$ to 4 hr intervals following the oral ingestion of fatty acid-1-C^{14} methyl esters, but they were unable to determine whether these partial glycerides resulted from hydrolysis of triglycerides in vivo or from the presence of partial glycerides in the chyle. However, Olivecrona et al. (27) have reported that during the clearing of glycerol-C^{14}–palmitate-H^3-chylomicrons by rats the H^3 and C^{14} in the partial glycerides of serum remain constant. This suggests that in the studies by Mead and Fillerup (29) the partial glycerides were, in fact, present in the chyle.

There can be no doubt of the high percentage of mono- and diglycerides in the chylomicrons the analysis of which is given in Table 4. Duplicate analysis by silicic acid column chromatography gave the same results. These were confirmed by TLC, and the diglyceride fraction was capable of being acylated to triglyceride. The possibility that the partial glycerides resulted from hydrolysis of the triglycerides during isolation of the chylomicrons or chromatography of the lipids can also be eliminated. If this had occurred, the H^3/C^{14} ratios of the di- and monoglycerides would have been $^2/_3$ and $^1/_3$, respectively, of the H^3/C^{14} ratio of the triglycerides, and this was not the case. Furthermore, a proportionate increase in free fatty acids in the chylomicrons would have resulted and this was not found.

The data indicate that chylomicron triglycerides are taken up by liver without any hydrolysis. The H^3/C^{14} ratios of the total lipids and of the glycerides of liver were unchanged from the corresponding H^3/C^{14} ratios of the injected chylomicrons in all ten experiments. Similar results have been obtained by others using doubly labeled chylomicrons (30) or plasma lipoproteins which had been labeled in vitro (31). Our results confirm those earlier reports, and serve as a control for the data obtained with mammary tissue.

The H^3/C^{14} ratios of the serum total lipids, of the total glycerides, and of the triglycerides increased very little during the time periods studied, while the ratios of the partial glycerides of serum fluctuated. There was only a small increase in the percentage of total radioactivity in the partial glycerides of the serum compared to the partial glycerides of the injected chylomicrons. These results indicate that no extensive hydrolysis of glycerides occurred within the bloodstream.

The most impressive finding was the dramatic increase in the H^3/C^{14} ratio of the total lipids, of the total glycerides, and of the tri-, di-, and monoglycerides of mammary tissue compared to the corresponding fractions of the injected chylomicrons. These increases appeared to be largely independent of the length of time during which uptake had been proceeding. These data strongly indicate that hydrolysis of chylomicron glycerides did occur during their uptake by mammary tissue, and that the free fatty acid moiety was reutilized to a greater extent than were the other products of hydrolysis. For example, where the H^3/C^{14} ratio was 1.50 in the triglycerides of mammary tissue relative to the triglycerides of chylomicrons, at least $^1/_3$ of the glycerides must have undergone partial or complete hydrolysis during uptake. However, it should be pointed out that the percentage of material which had undergone hydrolysis during uptake cannot be determined quantitatively from these ratios since it has been shown (6, 7) that all of the products of hydrolysis of triglycerides can be used to some extent by mammary tissue for the resynthesis of triglycerides. Therefore, any estimate of the degree of hydrolysis must, of necessity, represent the minimum amount of hydrolysis which actually occurred.

It had been anticipated that considerable hydrolysis might occur during uptake without causing any change in the H^3/C^{14} ratio of the total glyceride fraction since an increase in the H^3/C^{14} ratio of the triglycerides might be counterbalanced by a decrease in the H^3/C^{14} ratio of the di- and monoglycerides resulting from partial hydrolysis. However, it was found that the increase in the H^3/C^{14} ratio of tissue diglycerides relative to the chylomicron diglycerides was even greater than that of the triglycerides. This suggests that a large proportion of the partial glycerides in mammary tissue at the end of the experiments resulted from acylation of unlabeled glycerol, α-glycerophosphate, or monoglyceride, with palmitate-H^3, rather than from hydrolysis of triglycerides.

It is certain that the elevated H^3/C^{14} ratio in mammary tissue was not simply the result of uptake of radioactive free fatty acids from the plasma. This can be calculated from the H^3 activity of the plasma free fatty acid fraction, and the rate of uptake of free fatty acids by mammary gland (1). More simply, the unchanged H^3/C^{14} ratio

of the liver glycerides serves as an internal control. Radioactive free fatty acids, if present in significant quantity, would have been taken up by liver and incorporated into glycerides at least as efficiently as by mammary tissue.

Results similar to these have been found by Olivecrona (30), who studied the uptake of chylomicrons by adipose tissue in vivo. The present study adds further support to the conclusion that lipoprotein lipase may be involved in chylomicron glyceride uptake by certain extrahepatic tissues. Whether the site of action is at a capillary surface, cell surface, or elsewhere, remains undetermined.

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