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Metabolism of chylomicrons labeled with C^{14}-glycerol-H^3-palmitic acid in the rat*

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SUMMARY

Chylomicrons labeled with C^{14}-glycerol-H^3-palmitic acid were obtained from the cannulated thoracic duct of a rat given C^{14}-glycerol triolein and H^3-palmitic acid. The chylomicrons were injected intravenously into male rats and the labeling of liver, heart, and adipose tissue was studied at various time intervals from 5 to 160 min.

The conclusion is that the major part of the chylomicron glyceride leaves the circulating blood without previous hydrolysis. Evidence for this conclusion is that no appreciable amount of label was found in the plasma di- or monoglycerides and that the label passing through the plasma free fatty acid (FFA) pool during the clearing of the chylomicrons could not account for more than 10–15% of the total fatty acid label cleared.

The liver triglycerides showed a C^{14}/H^3 ratio close to 1.0 during the first 10 min. The ratio then declined rapidly. The conclusion is that chylomicron glyceride is taken up intact by the liver, but is rapidly metabolized with re-esterification of the fatty acids to unlabeled glycerol.

In the heart and the adipose tissue, the C^{14}/H^3 ratio decreased more rapidly. The suggestion is that in these tissues the loss of glycerol may occur during the penetration of the glyceride into the cell, but without mixing of the fatty acids into the plasma FFA pool.

At 20 min, when most of the chylomicron label had disappeared from the blood, 35% of the recovered fatty acid radioactivity was found in the liver and 10% in the adipose tissue.

The mechanism by which chylomicrons, specifically the triglycerides of these, are removed from the circulating blood has been much discussed during recent years. It has been suggested that they are hydrolyzed in the circulating blood and their fatty acids metabolized as plasma free fatty acids (FFA) (1). Havel and Fredrickson (2), however, showed that, although the clearing of chylomicrons is intimately associated with hydrolysis of the triglycerides and retransport of their fatty acids as plasma FFA, the lipolysis probably does not take place in the circulating blood. Fredrickson, McCollester, and Ono (3) later showed that retransport as plasma FFA is not obligatory in the utilization of chylomicron triglyceride fatty acids. These studies thus established that chylomicron triglyceride can be removed as such from the circulating blood. A direct approach to this problem is to label chylomicron triglyceride both in its glycerol and in its fatty acid moiety and study the fate of the two substances. Since the glycerol will have a different fate when liberated from the glyceride, this will permit an evaluation of the role of hydrolysis in the metabolism of chylomicron triglyceride. Such a study was previously reported by Borgström and Jordan (4) and a similar study, using plasma lipoproteins labeled *in vitro*, was reported by Stein and Shapiro (5). Both these studies indicated that the liver can remove triglyceride from the circulating blood without previous hydrolysis, but that the triglycerides taken up are rapidly hydrolyzed and the fatty acids re-esterified to unlabeled glycerol. *In vitro* studies by Rodbell (6) on the uptake of chylomicrons by adipose tissue indicate a similar sequence of events in that tissue.

The present paper represents an extension of the work of Borgström and Jordan.

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EXPERIMENTAL METHODS

Preparation of Double-Labeled Chylomicrons. Glycerol-labeled triolein was synthesized from C^{14}-glycerol (The Radiochemical Centre, Amersham, England) as described by Borgström and Jordan (4). The glyceride obtained was mixed with H^3-palmitic acid (9,10-H^3-palmitic acid, The Radiochemical Centre), emulsified

TABLE 1. Distribution of Radioactivity in the Washed Chylomicron Preparation

	H^3 % total radioactivity	Ratio C^{14}/H^3
NFFA	93.8	1.00
FFA	0.6	—
PLFA	5.6	1.05

in a small volume of an artificial dry milk preparation (Lactovit, AB Mazetti, Malmö, Sweden), and administered intragastrically to a rat on which a thoracic duct cannulation was performed the previous day. Chyle was collected for 24 hr in a flask chilled in ice. It was layered under 1.1% NaCl and centrifuged at approximately 60,000 *g* for 20 min. The chylomicrons were aspirated into a syringe, layered under 1.1% NaCl, recentrifuged, and, after being aspirated into a syringe again, diluted to the desired volume with 0.9% NaCl. The distribution of radioactivity in the washed chylomicrons is shown in Table 1. The injected dose contained 540,000 cpm H^3, 180,000 cpm C^{14}, and 24 mg fat in a volume of 2.0 ml. The washed chylomicrons were stored at 4° until used. All the experiments reported were performed within 8 days after the isolation of the chylomicrons. No visually detectable changes in the state of the chylomicron suspension occurred during this time.

Injection of Chylomicrons and Sampling. Male Sprague-Dawley rats (obtained from AB Anticimex, Stockholm, Sweden), weighing 160–190 g, were used. They were offered 10% glucose in half-strength saline 12–20 hr before and also during the experiment; no other food was given during this time. Under light ether anesthesia, 2.0 ml of the chylomicron suspension was injected into the exposed right jugular vein. At appropriate times, the rats were killed by exsanguination; the liver, the heart, and a piece of the epididymal adipose tissue were removed; and the lipids were extracted in chloroform–methanol 2:1 as previously described (7).

The total lipid-soluble radioactivity remaining was determined on individual rats sacrificed at appropriate times and immediately homogenized in 1,000 ml ethanol. The homogenate was transferred to a 6-liter Erlenmeyer flask, diluted to 4,000 ml with ether, and allowed to stand at room temperature for at least 24 hr. It was then filtered; and an aliquot was taken to dryness, redissolved in chloroform–methanol 2:1, and treated as the other lipid extracts.

Serial blood samples were obtained from separate rats treated and injected as described above. Blood samples (0.2–0.5 ml) were withdrawn from the exposed left jugular vein and transferred to centrifuge tubes containing a small amount of di-sodium-EDTA (ethylenediaminetetraacetate) to prevent clotting. After centrifugation, plasma was withdrawn and an aliquot (50–200 μl) pipetted into 5 ml of chloroform–methanol 2:1 and allowed to stand at room temperature for 24 hr. To the chloroform–methanol extracts was added 2.0 ml of 2% KH_2PO_4. These were shaken, the phases separated, and the upper methanol-water phase was sucked off. The lower chloroform phase was taken to dryness, redissolved in petroleum ether, and transferred to a 1-g silicic acid column prepared and eluted as previously described (7).

Analyses Performed on the Samples. The liver lipids were separated into neutral fat and phospholipids on silicic acid columns as previously described (7). Aliquots of the neutral fat and phospholipid fractions were transferred to liquid scintillation counting vials. Other aliquots were hydrolyzed and their fatty acids and nonsaponifiable lipids extracted with petroleum ether. The samples were dissolved in 10 ml of toluene containing 3 g PPO (2–5-diphenyloxazole) and 100 mg POPOP (1,4-bis-2-(phenyloxazolyl)-benzene) per liter, and counted in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., LaGrange, Ill.). The following settings were routinely used: high voltage tap 6.0 (1,200 volts); split channels, channel 1 = 1–50 volts, channel 2 = 100 volts-∞. With these settings, H^3 counts were found almost exclusively in channel 1. Thus the net cpm in channel 2 were taken as representing only C^{14} counts. The ratio of C^{14} counts in channel 1 to C^{14} counts in channel 2 was determined for each individual sample by use of a C^{14} internal standard, and the net cpm in channel 2 were multiplied by this ratio to give the sample C^{14} counts in channel 1. The net cpm in channel 1 were then reduced by the calculated C^{14} counts in channel 1 to give the sample H^3 counts in channel 1. Finally, the relative efficiencies of the registered counts to those of an unquenched sample were determined by use of internal standards and the values corrected accordingly. The C^{14} counts, recovered in the fatty acids and nonsaponifiable lipids after hydrolysis, were subtracted from the C^{14} counts in the unsaponified sample and the difference, taken as the glycerol-C^{14} value and used in the calculation of the C^{14}/H^3 ratios, reported.

The heart lipids were similarly separated into neutral fat and phospholipids. The FFA were separated from the neutral fat as previously described (7). No aliquots were hydrolyzed and therefore the C^{14} counts used in this case are those for the unhydrolyzed samples without correction.

The adipose tissue lipids were not separated into their classes. An aliquot was counted directly, and another aliquot was counted after hydrolysis and extraction of the fatty acids and nonsaponifiable lipids. The C^{14} counts were corrected as described for the liver lipids.

The total recovery was determined by counting an aliquot of the lipid extract from the homogenized whole rats. No hydrolyzed samples were counted. The C^{14} counts of these samples were not considered in this report.

The plasma lipids were separated into three fractions on silicic acid columns (7). The first fraction, containing the cholesterol esters, and the third, containing the phospholipids, were counted directly. At all times, only small amounts of radioactivity could be found in the cholesterol ester fraction, which is therefore not included in the results reported. The second fraction, containing the glycerides, free cholesterol, and FFA, was separated into a FFA fraction and a neutral fat fraction on ion exchange columns (8). In most cases, these fractions were counted directly. In some cases, however, the neutral fat fraction was further fractionated into tri-, di-, and monoglyceride fractions by thin-layer chromatography using silica gel (Kiselgel G, Merck AG, Darmstadt, Germany) with a developing system of 85% petroleum ether, 14% diethyl ether, and 1% glacial acetic acid. The spots were scraped off and eluted with chloroform–methanol 2:1, and the eluates were counted.

Altogether 34 rats were included in this study. All points on the curves represent values from single rats.

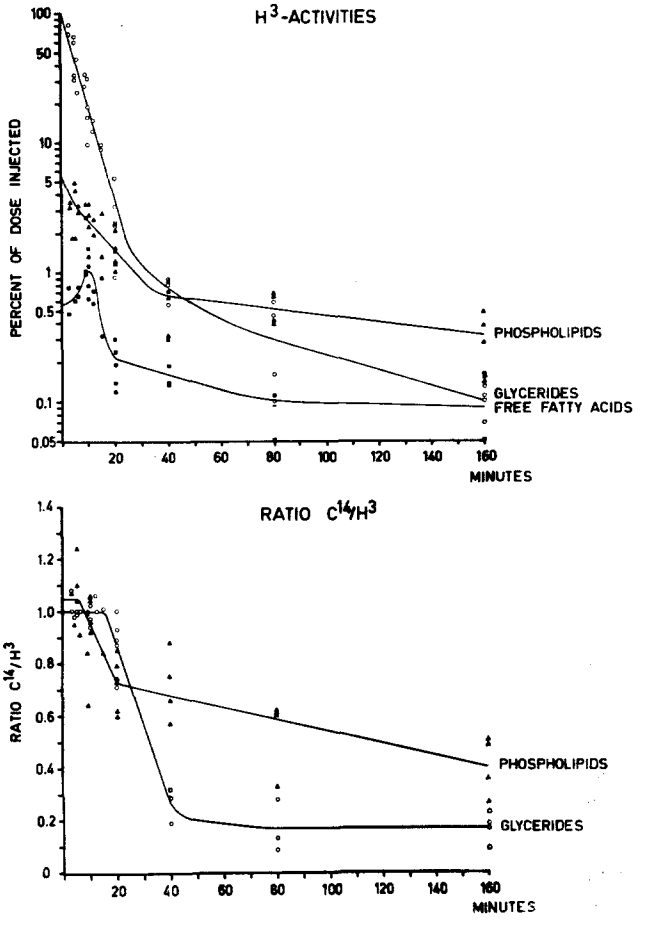

FIG. 1. Plasma radioactivities of male rats injected with chylomicrons labeled with C^{14}-glycerol-H^3-palmitic acid. ○ glycerides, ■ free fatty acids, ▲ phospholipids.

RESULTS

Plasma. Fig. 1 shows the time-course of H^3 content and C^{14}/H^3 ratio in plasma lipids. The values showed considerable variation from rat to rat. The initial straight line for the decline of glyceride fatty acid radioactivity was fitted visually. Its slope corresponds to a half-life of 4.3 min. During the first 10–15 min, the values for the ratio C^{14}/H^3 were close to unity, identical to that in the injected chylomicrons. The ratio then rapidly declined to low values.

The decline of plasma phospholipid fatty acid radioactivity was slower than that for glyceride fatty-acid radioactivity. This agrees well with previous results (9, 10). Due to variation between individual values, the curve cannot be precisely defined, but seems to correspond to a half-life of 10 min or slightly more. The ratio C^{14}/H^3 radioactivity in the plasma phospholipids began to decline by 5 min.

The FFA radioactivity rose to a maximum at about 10 min and then fell off.

In some of the experiments, the neutral fat fraction was separated into tri-, di-, and monoglycerides using thin-layer chromatography. The results were similar to previous results summarized elsewhere (9). The triglyceride radioactivity curve followed approximately the same course as the total glyceride radioactivity in Fig. 1. There was initially a slight increase in the monoglyceride fatty acid radioactivity. Both the mono- and the diglyceride radioactivity then fell off at rates slightly faster than that for the triglycerides.

Liver. The fatty acid radioactivity in the liver glycerides rapidly rose to a maximum at 20 min and then fell off again (Fig. 2). During the first 10 min, the ratio C^{14}/H^3 in the liver glycerides was only slightly lower than that of the injected chylomicron glycerides. The ratio declined after 10 min, but seemed to level off at values around 0.35 at 80 min.

The fatty acid radioactivity in the liver phospholipids rose more slowly and had its maximum later than the glyceride fatty acid radioactivity. The ratio C^{14}/H^3

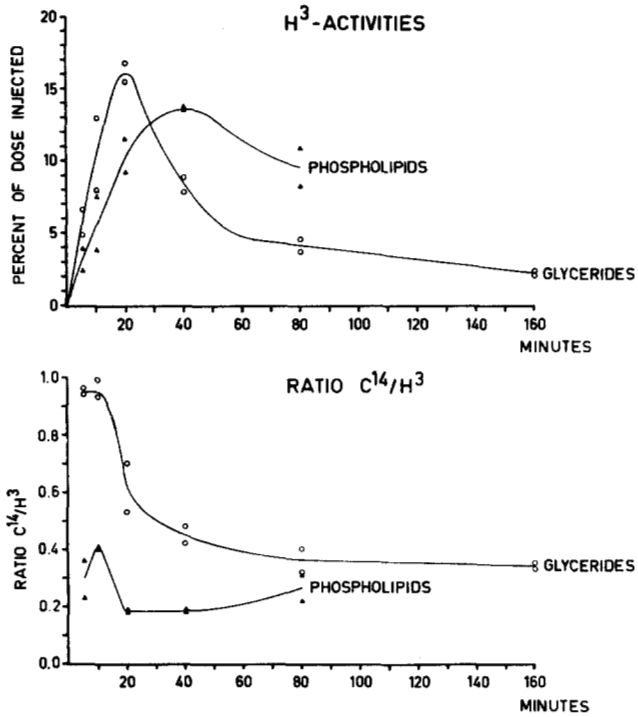

FIG. 2. Liver radioactivities of male rats injected with chylomicrons labeled with C^{14}-glycerol-H^3-palmitic acid. O glycerides, ▲ phospholids.

was never higher than 0.45, compared to 1.05 in the injected chylomicron phospholipids.

Heart. The fatty acid radioactivity in the heart glycerides was at its maximum at the shortest time studied, 5 min, and rapidly fell off. The ratio C^{14}/H^3 was initially 0.3 and then fell further (Table 2). The C^{14} counts in the heart glycerides were not corrected for C^{14} incorporation into fatty acids or nonsaponifiable lipids as was done for the liver glycerides. Therefore, it seems possible that part of the C^{14} radioactivity measured was contained in fatty acids, especially since the heart FFA showed a rapid increase in the ratio C^{14}/H^3. It is also possible that, at the shortest times, part of the C^{14} radioactivity measured derived from glycerides in the plasma remaining in the heart. It is thus probable that the C^{14}/H^3 ratios in the heart glycerides are erroneously high.

Body Distribution of Label. Fig. 3 shows the body distribution of fatty acid label and some of the ratios studied. The fatty acid radioactivity curves for plasma and liver are the sum of the radioactivities in the different fractions as described above. No correction for label in blood lipids remaining in the tissues was made. The values for adipose tissue were calculated assuming that 70% of the total body fatty acids were contained in adipose tissue and that all adipose tissue

TABLE 2. RADIOACTIVITIES IN HEARTS OF MALE RATS INJECTED WITH CHYLOMICRONS LABELED WITH C^{14}-GLYCEROL-H^3-PALMITIC ACID

		Neutral Fat		FFA	
Time (min)	Rat No.	H^3 % of dose injected	Ratio C^{14}/H^3	H^3 % of dose injected	Ratio C^{14}/H^3
4	1	1.7	0.43	0.56	0.55
5	2	1.5	0.46	0.72	0.64
10	3	0.86	0.43	0.19	0.63
10	4	0.86	0.37	0.17	1.7
20	5	0.67	0.18	0.15	3.4
20	6	0.62	0.14	0.13	3.3
40	7	0.36	0.17	0.12	3.7
40	8	0.67	0.11	—	—
80	9	0.13	1.3	0.11	2.9
80	10	0.06	1.0	—	—
160	11	0.06	1.7	0.11	2.2
160	12	0.06	1.0	0.14	1.3

fatty acids had the same specific activity (10). The values for total recovery were determined from separate rats.

DISCUSSION

The present results yield information on three aspects of chylomicron metabolism, namely, the removal of chylomicron glyceride from the circulating blood, its tissue distribution, and its fate after removal from the circulating blood.

Removal of Chylomicron Glyceride from the Blood Stream. As pointed out in the introduction, there is ample evidence that chylomicron glyceride can leave the circulating blood without prior hydrolysis. Further evidence to this point has been presented in a recent paper from this laboratory (9), and the present results (i.e., that the ratio C^{14}/H^3 in the plasma glycerides remained close to unity during the first 15 min, that only small amounts of label appeared in the plasma di- and monoglycerides, and that the peak of radioactivity in the plasma FFA occurred relatively late) confirm the conclusions previously drawn. Furthermore, it can be calculated, assuming the fractional turnover rate of plasma FFA to be 1.0/min (11), that no more than 10–15% of the injected label could have passed through the plasma FFA pool during the first 20 min. Since, during the same time, the plasma glyceride radioactivity declined from 94% to 4% of the injected dose, the major part of the chylomicron glyceride must have been removed from the circulating blood without prior hydrolysis. This evidence does not argue against the possibility that they are hydrolyzed in passing from the

circulating blood into tissues. If the hydrolysis occurred at or in the cell membrane, the materials taken up by the cell might be FFA and free glycerol, but this would not necessarily be reflected by the appearance of partial glycerides or of FFA in the circulating blood. Indeed it will be argued later in this discussion that such hydrolysis at or in the cell membrane is probable for some extrahepatic tissues.

There are some differences between the present results and those of Borgström and Jordan (4). They reported ratios of glycerol radioactivity to fatty acid radioactivity above 1 both in the blood and in the liver during the first 20 min of the experiment. In the present study, no significant rise in the ratio above 1 was observed either in the blood or in the liver. Stein and Shapiro (5), in a similar experiment using plasma lipoproteins labeled *in vitro*, found no ratio of glycerol radioactivity to fatty acid radioactivity above 1 in the liver at any time. The reasons for the difference between our results and those of Borgström and Jordan are not clear at this moment. Some other differences that may have a bearing on this problem are that the half-life of the injected chylomicron triglyceride fatty acid was different in the two studies as was also the nutritional state of the rats. Our rats were less rigorously carbohydrate-fed than their rats, which means that oxidation of the fatty acids probably occurred more rapidly in our rats. At 160 min, the total recovery in our rats was only about 44%, indicating a rather rapid oxidation.

Tissue Distribution of Fatty Acid Label. At 20 min, when most of the fatty acid label had disappeared from the blood, 26% of the injected fatty acid radioactivity was present in the liver. This does not give a true figure for the fraction of chylomicron glyceride taken up by the liver since, during these 20 min, some fatty acids were oxidized in the liver and probably some were retransported from the liver to other tissues or to the liver from other tissues. If it is assumed that oxidation and retransport had the same influence on fatty acid label in all tissues during these 20 min, then the ratio of liver uptake to total uptake should be the same as the ratio of fatty acid radioactivity in liver to total fatty acid radioactivity. Calculated in this way, the ratio comes out to about 0.35. This value seems to be in agreement with values previously reported (4, 10, 12, 13). Borgström and Jordan (4) pointed out that, in these experiments, the initial concentration of chylomicrons in the blood is higher than it is during fat absorption, and suggested that, under physiological conditions, the liver might take up a much larger fraction of chylomicron glyceride. The present results do not yield information concerning this point; thus, the estimate that the liver takes up

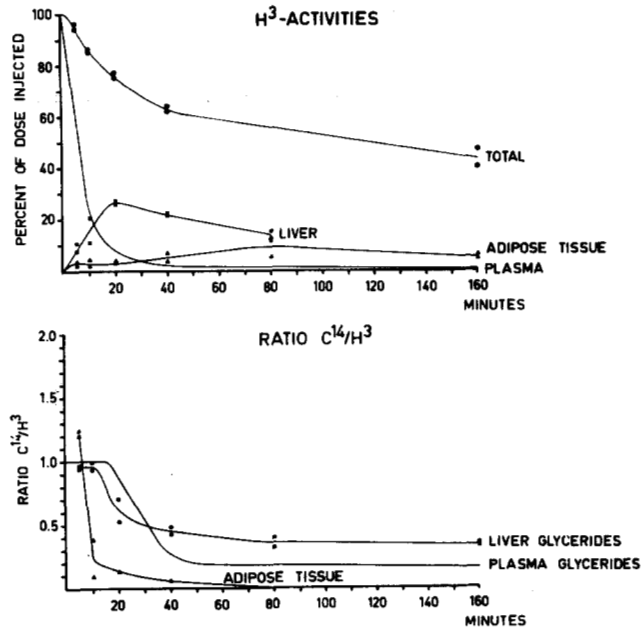

FIG. 3. Body distribution of label in male rats injected with chylomicrons labeled with C^{14}-glycerol-H^3-palmitic acid. The plasma values are the sum of the values for the different fractions as shown in Fig. 1. To avoid crowding of the fig., the individual points have not been plotted here. ● total, ■ liver, ▲ adipose tissue.

35% of injected chylomicron glyceride is valid only for these experimental conditions and does not necessarily reflect the fraction taken up under physiological conditions. Furthermore, it should be stressed that the estimate is correct only if the assumption is valid that oxidation and retransport had the same influence in all tissues. It seems possible that the rate of loss of fatty acid label from the liver was higher than from most other tissues. If this is the case, then 35% is an underestimate of the fraction taken up by the liver, and the true figure might be 40–50%.

About 10% of the total recovered fatty acid radioactivity at 20 min was found in the adipose tissue. This value agrees well with values reported by French and Morris (12) and from this laboratory (10). The value is appreciably less than that reported by Bragdon and Gordon (13) for rigorously carbohydrate-fed rats, but higher than the value they reported for fasted rats. As was pointed out above, the rats in the present study were not rigorously carbohydrate-fed and thus the results do not contradict the conclusion of Bragdon and Gordon that the adipose tissue is a major site of chylomicron uptake in carbohydrate-fed rats.

Fate of the Chylomicron Glyceride After Removal from the Blood Stream. As discussed above, it seems quite clear that chylomicron glycerides are largely removed from the circulation without previous hydrol-

ysis. Since the liver uptake, plus the amount of fatty acids recirculated as FFA during the first 20 min, was considerably less than the total amount of chylomicron glyceride removed from the circulation during this time, it follows that chylomicron glyceride must have been removed from the circulating blood by extrahepatic sites, without prior hydrolysis in the circulating blood.

After the removal of chylomicron glycerides from the circulating blood, the glycerides were rapidly hydrolyzed and their fatty acids re-esterified with unlabeled glycerol. This is evident from the rapid decline of the ratio C^{14}/H^3 and from the appearance of label in the phospholipids, although only small amounts of labeled phospholipids were injected. The latter point is well demonstrated in the liver, where the radioactivity in the glycerides rose rapidly and declined rather rapidly, simultaneous with a rise of the phospholipid radioactivity. After 30 min, the liver phospholipids actually contained more label than the liver glycerides. Whether the transfer of fatty acids from the glycerides to the phospholipids proceeded via the formation of FFA or via a transesterification reaction cannot be decided from the present data.

In the adipose tissue, the ratio C^{14}/H^3 soon fell to zero, indicating complete loss of the glycerol. The same was probably true for the heart, although the rapid incorporation of C^{14} from glycerol into the fatty acids in this tissue somewhat obscured the picture. In the liver, the ratio C^{14}/H^3 leveled off to about 0.35 at 80 min, indicating that some of the glycerides were not completely hydrolyzed prior to the incorporation of their fatty acids into the bulk of liver fat, or that some of the glycerol was reincorporated into the glycerides.

As judged from the ratio C^{14}/H^3, the rate of hydrolysis was high both in the heart and in the adipose tissue. These tissues are known to be rich in lipoprotein lipase (14), for which no physiological function has been definitely established. Possibly this enzyme is responsible for the rapid hydrolysis of chylomicron glycerides in these tissues. Robinson and Harris (15) have presented evidence that the enzyme is, at least partly, localized in the immediate vicinity of the circulating blood, perhaps the walls of the blood vessels. If lipoprotein lipase is responsible for the hydrolysis of the chylomicron glycerides, this hydrolysis might proceed at the cell membrane with the chylomicron adsorbed to it, or the cell membrane might first be folded into a pinocytotic vesicle, inside which hydrolysis proceeds. Thus, in these tissues, most, if not all of the chylomicron glyceride may have been hydrolyzed before the fatty acids penetrated into the cell itself, *but without these fatty acids having been circulated through the blood as free fatty acids.*

The liver shows many differences from the heart and the adipose tissue. It probably lacks lipoprotein lipase (16), the glycerol is lost less rapidly from the glycerides, and part of the glycerol seems to be incorporated into the bulk of liver fat. Another feature, specific for the liver and the intestinal mucosa, is the capacity of transferring triglycerides to the plasma (17, 18, 19). Thus it seems that there is present in the liver some mechanism that permits the passage of esterified fatty acids, or perhaps even complete lipoprotein entities, through the cell wall without breakdown.

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