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METABOLISM OF CONSTITUENT LIPIDS OF DOG CHYLOMICRONS *

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Chylomicrons are the vehicles for that fraction of absorbed lipid transported in intestinal lymph. They contain mainly triglycerides and lesser amounts of phospholipids, cholesterol esters, free cholesterol, and protein. The phospholipids do not have a major role in the transport of newly absorbed fatty acids, but there is evidence that they and, to some extent, the free cholesterol and protein act to stabilize the chylomicron particle to transport the bulk of the contained lipid (1).

The metabolism of the structural "skeleton" of the chylomicrons might therefore be expected to differ from that of the transported lipid. Labeling with appropriate isotopes has shown that the protein (2), phospholipid (3), and free cholesterol (4) of chylomicrons exchange rapidly with the corresponding lipids in other plasma lipoproteins, whereas little exchange of triglyceride fatty acids (TGFA) and ester cholesterol occurs. Although such reactions have made accurate assessment difficult, the possibility remains that triglycerides may be removed by hydrolysis at cell surfaces, whereas the lipoprotein "skeletons" of higher density are retained in the blood (1).

We have shown previously that in dogs, about one-third of the chylomicron triglycerides removed from blood plasma enters the liver directly (5). Such triglycerides appear to enter the liver without prior hydrolysis (6). It has not been shown conclusively that intact chylomicrons are taken up by the liver, although spaces sufficiently large to permit the passage of chylomicrons have been described in the sinusoidal epithelium of the liver (7), and hepatic parenchymal cells may remove chylomicrons by pinocytosis (8). Extrahepatic tissues are capable of removing TGFA of chylomicrons from the blood (9) and under ordinary conditions are probably the chief sites of removal (5). Extrahepatic uptake of such TGFA is related to the local activity of lipoprotein lipase (10). The liver appears to be the chief site of removal of chylomicron cholesterol from the blood. Biggs (11) injected chyle containing H^3-cholesterol intravenously in rats and recovered about 80% of the label in the liver 30 minutes later. This finding suggests that uptake of chylomicron TGFA occurs in extrahepatic tissues without concurrent uptake of chylomicron cholesterol. The possibility, however, that intact chylomicrons may enter adipose tissue by a process of pinocytosis has been advanced on the basis of *in vitro* incubation studies (12).

This report presents the results of experiments in which the different lipids in chylomicrons were labeled with separate isotopes and injected into both intact and functionally hepatectomized dogs. The rate of removal of the various lipids from plasma and their incorporation into various tissues was then determined.

METHODS

Preparation of labeled chylomicrons. For the experiments on dogs 1, 2, 4, and 5, TGFA, ester cholesterol, and free cholesterol in the chylomicrons were labeled by feeding donor dogs 500 μc of sodium palmitate-9,10-H^3 and 50 μc of cholesterol-4-C^{14} with milk. For the other experiments, the phospholipids in the chylomicrons were also labeled by iv injection of 5 mc of sodium phosphate P^{32} into donor dogs 4 hours before feeding the other isotopes. Lymph was obtained from the donor dogs through a cannula inserted in the thoracic duct. The chylomicrons were separated by layering the lymph under 0.15 M NaCl. After centrifugation for 30 minutes at 20,000 rpm in the 30 rotor of a Spinco model L ultracentrifuge, the supernatant fluid was separated by means of a tube slicer. The chylomicrons were collected and resuspended in saline.

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Experiments in intact dogs. Dogs 1 and 2, weighing 9 kg each and fasted overnight, were anesthetized with iv sodium pentobarbital (30 mg per kg of body weight), and blood pressure was monitored continuously through a catheter placed in a femoral artery. An abdominal incision was made to allow collection of samples of liver and omental adipose tissue. Fifty ml of the chylomicron suspension, containing about 1.5 g of TGFA, was infused over 30 seconds. Samples of blood were collected into chilled, heparinized tubes 2, 5, 10 15, 35, 45, and 60 minutes later. Samples of liver weighing about 500 mg and of omental tissue weighing about 1 g were obtained at 5, 15, 30, 45, and 60 minutes. They were blotted carefully, weighed, and then homogenized in 1:1 acetone: alcohol (vol/vol). At the end of the experiments, the entire liver was removed, blotted, and weighed. Similar experiments were carried out in three additional normal dogs (no. 3, 4, and 5); these studies were terminated 45 minutes after the injection of the chylomicrons, and a single sample of liver was obtained at the end of the experiment.

Experiments on functionally hepatectomized dogs. In dog 6, the liver was excluded from the circulation by occluding the portal vein and hepatic artery after a portacaval anastomosis had been performed. Immediately thereafter, the chylomicrons were injected (0.15 g TGFA per kg), and samples of blood were taken 5, 12, 25, 40, 60, and 83 minutes later. Blood pressure was about 80 mm Hg during this period. Samples of omental adipose tissue were obtained during the procedure, and a sample of liver was taken at the end of the experiment to determine whether this organ had been successfully excluded from the circulation.

In dogs 7 and 8, the liver was excluded from the circulation by a two-stage operation: first, a portacaval anastomosis was performed; 10 weeks later, the hepatic artery and portal vein were clamped. The immediate discoloration of the liver suggested that the circulation to it had been interrupted. Chylomicrons were then infused (0.15 g TGFA per kg), and samples of blood were collected 2, 5, 10, 15, and 20 minutes later. Samples of liver and omental adipose tissue were taken after the fifth sampling, and no bleeding occurred at the site of liver biopsy. Another blood sample was collected 25 minutes later from dog 8 and 30 minutes later from dog 7; the venous clamps were removed at that time. The improved color of the liver suggested restoration of circulation. Additional samples of blood were collected at 5- to 10-minute intervals for a further ½-hour. Samples of liver and omental tissue were taken at the end of the experiment. Blood pressure remained normal in both animals during the experiments.

Analytical methods. Samples of plasma were centrifuged at their own density for 16 hours at 40,000 rpm in the 40.3 rotor of the ultracentrifuge. The supernatant fluid containing chylomicrons and a small amount of very low-density lipoproteins was separated from the remaining plasma lipoproteins in the infranatant fluid by means of a tube slicer. The lipids in both fractions were then extracted in 2:1 chloroform: methanol (vol/vol). One-fifth vol of 0.02 N HCl was added to produce a two-phase system. The lipids of the chloroform phase were separated into three fractions on silicic acid columns (13). Samples of fraction 1, which contained cholesterol esters, were assayed for radioactivity. Fraction 2 contained triglycerides, free cholesterol, and in the extracts from the infranatant fractions, free fatty acids. The latter were separated from the neutral lipids by the method of Borgström (14). Samples of the neutral lipid fractions were assayed for H^3- (TGFA) and C^{14}- (free cholesterol) radioactivity. P^{32}-radioactivity was measured in phospholipids (fraction 3) when this fraction was labeled. All fractions were dissolved in toluene containing 0.3% diphenyloxazole and assayed for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer. Tritium and C^{14}-radioactivity in fraction 2 were assayed simultaneously (15).

Extracts containing hepatic lipids were treated similarly. Radioactivity associated with TGFA, free cholesterol, and ester cholesterol in adipose tissue was measured in the extracts as follows. Tritium and C^{14}-radioactivity were measured in one sample to determine TGFA and total cholesterol radioactivity, respectively. Free cholesterol was precipitated as the digitonide from another sample, redissolved in methanol, and assayed for C^{14}-radioactivity. Quenching was measured with an internal standard. Radioactivity in ester cholesterol was calculated by difference.

RESULTS

Intact dogs (no. 1–5)

The rates of removal from the chylomicron fraction of plasma of the radioactivity in TGFA, ester cholesterol, and free cholesterol moieties of the infused chylomicrons are shown in Table I.

TABLE I

Initial half-times of removal of radioactivity in triglyceride fatty acids (TGFA), ester cholesterol (EC), free cholesterol (FC), and phospholipids (PL) from the chylomicron fraction of plasma, after iv injection into dogs of chylomicrons labeled with palmitic acid-9,10-H^3, cholesterol-4-C^{14}, and sodium phosphate-P^{32}*

Lipid fraction	Dog†									
	1	2	3	4	5	6	7		8	
							a	b	a	b
TGFA	10	15	6	14	15	38	10	7	10	6
EC	15	15	7	18	38	140	23	9	23	13
FC	15	14	7	16	27	81	12	10	17	14
PL			7			34	9	7	9	8

* Determined from semilogarithmic plots of the radioactivity against time (see Figure 1).

† Dogs 1 to 5 = normal controls, dog 6 = complete exclusion of liver from circulation, dogs 7 and 8 = a) partial exclusion of liver from circulation and b) restoration of circulation.

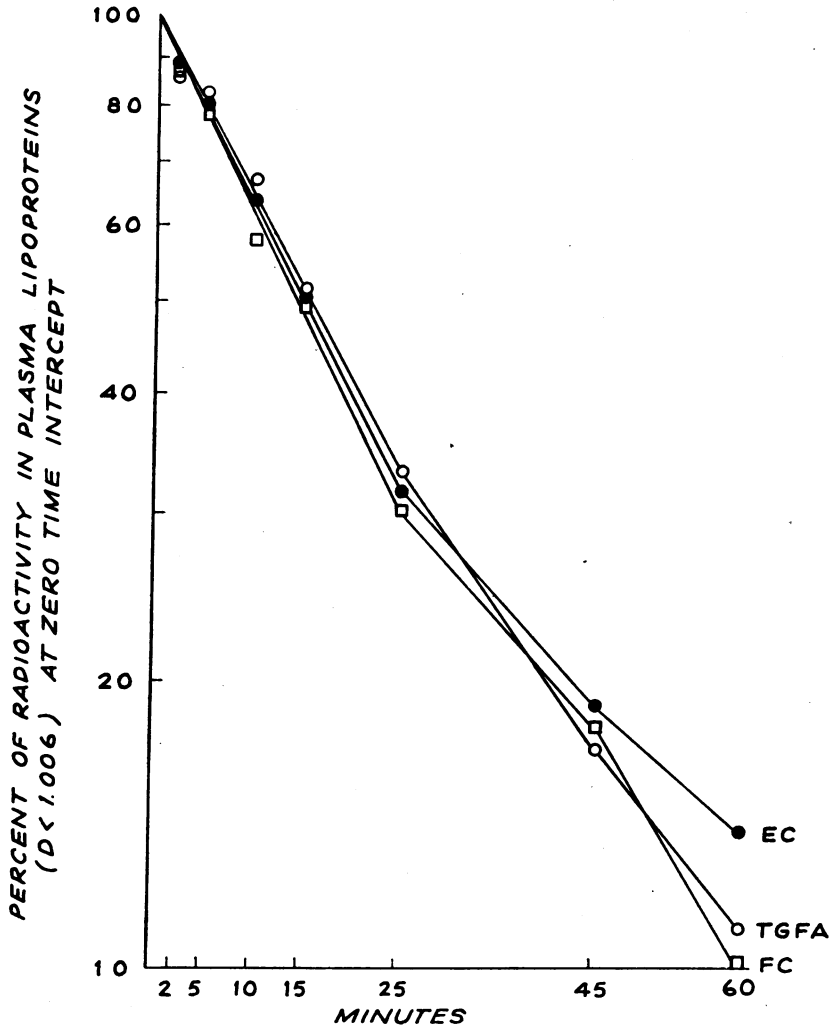

FIG. 1. REMOVAL OF RADIOACTIVITY ASSOCIATED WITH TRIGLYCERIDES, ESTER CHOLESTEROL, AND FREE CHOLESTEROL FROM D < 1.006 LIPOPROTEIN FRACTION OF PLASMA AFTER IV INJECTION OF LABELED CHYLOMICRONS INTO INTACT DOG 2.

In dogs 2 (Figure 1) and 3, radioactivity in all three fractions was removed at similar rates. In dogs 1, 4, and 5, the rate of removal of radioactivity in TGFA was faster than that in ester cholesterol and free cholesterol, but this difference was pronounced only in dog 5.

The radioactivity in TGFA, ester cholesterol, and free cholesterol in the samples of liver was calculated for each time interval after injection. In Table II these data are expressed as the percentage of the initially injected radioactivity that had left the plasma chylomicron compartment (see Figure 1). No correction was made for the presence of contaminating plasma chylomicrons.

In dogs 1 and 2 (Figure 2), radioactivity in hepatic TGFA increased rapidly in the first 5 minutes; thereafter it rose less rapidly. The radioactivity associated with ester cholesterol and free cholesterol in the liver also increased rapidly in the first 5 minutes, then continued to rise more slowly during the remainder of the experiment.

In dogs 1 and 2, virtually all the radioactivity associated with the ester cholesterol and free cholesterol removed from the chylomicron fraction of plasma during the first 5 minutes was recovered in the liver. Of the radioactivity that had left the chylomicron fraction of plasma, no less than 75 to 90% of that in ester cholesterol and 83 to 100%

TABLE II

Radioactivity found in triglyceride fatty acids (TGFA), ester cholesterol (EC), and free cholesterol (FC) fractions of liver after iv injection of chylomicrons labeled with palmitic acid-9,10-H^3 and cholesterol-4-C^{14} into five normal dogs

Dog	Lipid fraction	Radioactivity in liver				
		5 min	15 min	30 min	45 min	60 min
		Percentage of radioactivity removed from plasma chylomicrons				
1	TGFA	70	35	48	28	27
	EC	100	84	76	75	77
	FC	100	90	84	85	85
2	TGFA	100	75	61	60	59
	EC	89	78	85	85	90
	FC	100	100	83	90	
3	TGFA				30	
	EC				80	
	FC				85	
4	TGFA				18	
	EC				70	
	FC				77	
5	TGFA				33	
	EC				90	
	FC				90	

of that in free cholesterol were found in samples of liver taken during the remainder of the experiment. With the exception of the 5-minute sample, in which contaminating plasma chylomicrons contributed most significantly to the values obtained, the radioactivity associated with both hepatic ester cholesterol and free cholesterol was a fairly constant fraction of the corresponding radioactivity removed from plasma. In contrast, in hepatic TGFA the fraction of radioactivity removed from TGFA of plasma declined progressively with time. Considerably more TGFA radioactivity was found in the liver of dog 2 than in the liver of dog 1. Similar results were obtained in determinations on samples of liver taken at 45 minutes in dogs 3, 4, and 5.

Radioactivity associated with ester cholesterol and free cholesterol was measured in omental adipose tissue in dogs 1 and 2. Assuming that adipose tissue represents 5% of body weight and that radioactivity is distributed evenly within all adipose tissue, it was calculated that in both ani-

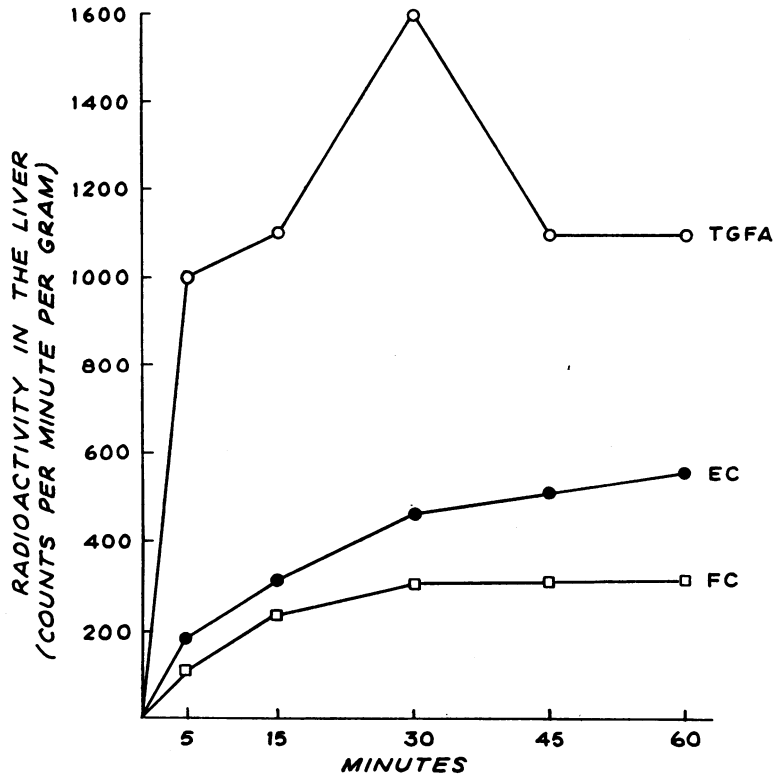

FIG. 2. UPTAKE BY THE LIVER OF RADIOACTIVITY IN TRIGLYCERIDES, ESTER CHOLESTEROL, AND FREE CHOLESTEROL AFTER INJECTION OF LABELED CHYLOMICRONS INTO INTACT DOG 2.

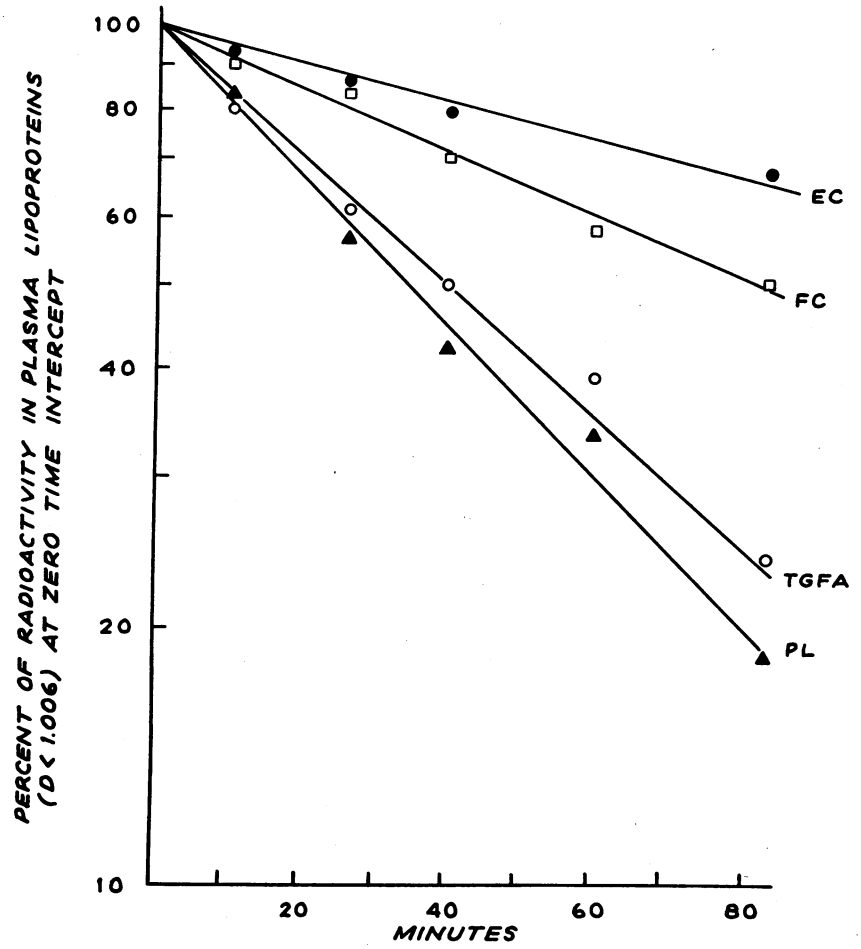

FIG. 3. REMOVAL OF RADIOACTIVITY IN TRIGLYCERIDES, PHOSPHOLIPIDS, ESTER CHOLESTEROL, AND FREE CHOLESTEROL FROM D < 1.006 LIPOPROTEIN FRACTION OF PLASMA AFTER IV INJECTION OF LABELED CHYLOMICRONS INTO COMPLETELY FUNCTIONALLY HEPATECTOMIZED DOG 6.

mals approximately 4 to 5% of the infused radioactivity in total cholesterol was present in adipose tissue at 15 to 30 minutes and 8 to 10% at 45 and 60 minutes. At the end of 1 hour, 21% of the radioactivity infused in TGFA was in the adipose tissue of dog 1, but only 9% in dog 2.

In dogs 1 and 2, the ratio of radioactivity in ester cholesterol to that in free cholesterol in the infused chylomicrons was 1.8; this was maintained in the chylomicron fraction of plasma up to 60 minutes. In the liver of both dogs, the ratio was slightly less (1.6), indicating that little hydrolysis had taken place, but in the samples of adipose tissue taken last, the ratio was decreased to 1.1.

It is not possible to assess precisely to what extent exchange or transfer of lipids occurred between the plasma chylomicrons and the higher-density lipoproteins, since no data on specific activities were obtained. Assuming plasma volume to be 4% of body weight, it was calculated that higher-density lipoproteins never contained more than 4% of the injected ester cholesterol radioactivity and 9% of the injected free cholesterol radioactivity. In dog 3, higher-density lipoproteins contained 30% of the injected P^{32}-radioactivity 20 minutes after injection, suggesting that extensive exchange within the plasma compartment had occurred. About 5% of injected TGFA radioactivity was recovered in higher-density lipoproteins.

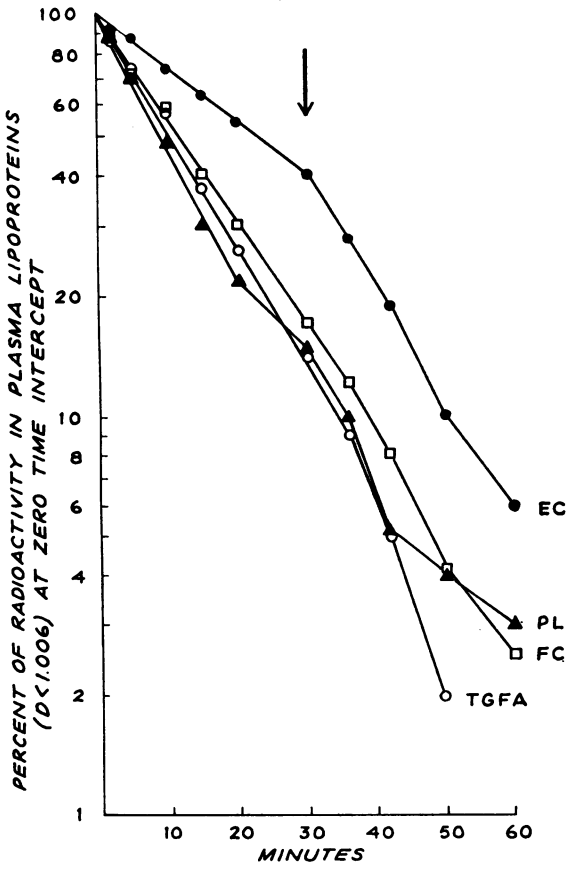

FIG. 4. REMOVAL OF RADIOACTIVITY IN TRIGLYCERIDES, PHOSPHOLIPIDS, ESTER CHOLESTEROL, AND FREE CHOLESTEROL FROM D < 1.006 LIPOPROTEIN FRACTION OF PLASMA AFTER IV INJECTION OF LABELED CHYLOMICRONS INTO PARTIALLY FUNCTIONALLY HEPATECTOMIZED DOG. 7. The liver was excluded from the circulation during the first 30 minutes and then returned to the circulation at the time indicated by the arrow.

Functionally hepatectomized dogs

Complete hepatic exclusion (dog 6). In this animal, the circulation to the liver was totally interrupted at a single operation immediately before the infusion of chylomicrons. The rates of disappearance of radioactivity from the four lipid moieties of the chylomicron fraction of plasma from dog 6 are shown in Figure 3 and expressed as half-times in Table I. The rates of removal were considerably slower than those in the five intact dogs. Radioactivity associated with ester cholesterol and free cholesterol was removed very slowly; radioactivity in TGFA and phospholipids was removed much more rapidly. No radioactivity was found in the sample of liver taken at the end of the experiment.

Samples of omental adipose tissue obtained at the end of the experiment contained 40% of the small amount of radioactivity associated with the cholesterol fractions removed from plasma chylomicrons. The radioactivity was evenly divided between the ester cholesterol and the free cholesterol fractions, although the ratio of the two fractions in the infused chylomicrons was 2:1. About 40% of the radioactivity in TGFA removed from plasma was also present in the adipose tissue.

Partial hepatic exclusion (dogs 7 and 8). In these two animals, portacaval anastomoses were performed 10 weeks before the infusion of chylomicrons, which were injected immediately after the portal vein and hepatic artery were clamped. The color of the liver had indicated that the circulation had been successfully interrupted; nevertheless, one-quarter of the total cholesterol radioactivity removed from the chylomicron fraction of plasma during the first 30 minutes was found in the liver.

During hepatic exclusion in both dogs, the radioactivity associated with the phospholipid fraction was removed from plasma chylomicrons most rapidly (Figure 4 and Table I). TGFA radioactivity disappeared from the chylomicron fraction of plasma more rapidly than radioactivity associated with free cholesterol and considerably more rapidly than that associated with ester cholesterol. After the clamps were removed from the hepatic artery and portal vein, the rates of disappearance of radioactivity in all four fractions increased, particularly in that associated with ester cholesterol. In both dogs, comparison of the concentration of radioactivity in the samples of liver obtained before the clamps were released and at the end of the experiment showed that approximately three-quarters of the cholesterol radioactivity found in the liver at the end of the experiment had been removed after restoration of the hepatic circulation. At that time, the liver contained about half the radioactivity removed from the plasma chylomicrons.

From the concentration of radioactivity in samples of adipose tissue taken at the end of the experiment, it was calculated that a third of cholesterol radioactivity removed from plasma chylomicrons had been deposited in adipose tissue.

DISCUSSION

The rate of removal of radioactivity was determined in five intact dogs after injection of chylomicrons in which the different lipid components had been individually labeled (Table I). In two dogs, radioactivity in TGFA, ester cholesterol, and free cholesterol was removed from the circulation at similar rates. In the other three animals, radioactivity in TGFA was removed slightly faster (two dogs) or considerably faster (one dog) than the radioactivity in ester cholesterol and free cholesterol.

Conversely, in the one experiment (dog 6) in which the liver was entirely excluded from the circulation, cholesterol radioactivity, and in particular, that associated with ester cholesterol, was removed from plasma chylomicrons very slowly (Figure 3); radioactivity in TGFA and phospholipids was removed considerably more rapidly. The relatively slow removal of TGFA in this dog may have resulted from reduced perfusion of tissues, since the blood pressure was only about 80 mm Hg.

In the two dogs in which the liver was only partly excluded from the circulation, probably owing to the presence of collateral vessels at the diaphragmatic surface, the findings were less conclusive. Nevertheless, the removal of radioactivity in ester cholesterol was considerably slower than that in TGFA; in this respect, the findings in these partially hepatectomized dogs differ from those in four of the five normal dogs.

These findings are consistent with the high rate of incorporation of cholesterol radioactivity into hepatic cholesterol in the intact dogs (Table II). At least 70% of the ester cholesterol radioactivity and 77% of the free cholesterol radioactivity removed from plasma chylomicrons were recovered in the liver. Therefore, in intact dogs the liver appears to be the major site of removal of chylomicron cholesterol. This is in agreement with similar observations in the rat by Biggs (11) and by Borgström, Lindhe, and Wlodawer (16), who were able to recover the greater part of an orally administered load of labeled cholesterol in the liver. After the completion of our experiments, Lossow, Brot, and Chaikoff (17) and Goodman (18) published the results of studies in the rat that confirm and extend Biggs's findings. They also found 6 to 18% (17) and 1 to 3% (18) of the cholesterol in adipose tissue 10 to 20 minutes after injection of chylomicrons labeled with C^{14}-cholesterol.

In our intact animals, about 5% of the radioactivity associated with the cholesterol removed from plasma chylomicrons in 15 to 30 minutes was present in adipose tissue. This could have been derived from contaminating plasma chylomicrons or from other plasma lipoproteins becoming labeled either in the liver, or by direct transfer or exchange of cholesterol within the plasma compartment.

The mechanism involved in extrahepatic uptake of chylomicron cholesterol is uncertain. The liver is not essential for this process, since it occurred in the hepatectomized dog. This process is apparently less efficient that that responsible for uptake of TGFA. In the functionally hepatectomized animals, the rate of removal of TGFA radioactivity was considerably more rapid than that of ester cholesterol. In the intact dog, extrahepatic tissues have a major role in removal of chylomicron TGFA from the blood; two-thirds of the radioactivity associated with TGFA was found to be removed directly by extrahepatic routes when moderate amounts of triglyceride were continuously infused at a slow rate (5). In the present experiments in which large amounts of lipid were injected as a single dose, more TGFA radioactivity was found in the liver than when less lipid was delivered by slow, constant infusion. Nevertheless, less radioactivity in TGFA than in cholesterol was recovered in the liver of intact dogs (Table II), suggesting more extensive removal by extrahepatic tissues of TGFA than of cholesterol.

The more rapid removal of TGFA than of ester cholesterol radioactivity in three normal dogs was probably related to more extensive extrahepatic uptake of TGFA. This is supported by the findings in the first two dogs in which samples of liver were obtained early in the experiment. In dog 1, radioactivity in TGFA was removed more rapidly than that in ester cholesterol; in dog 2, the rates of removal were similar. In samples of liver obtained 15 minutes after the injection of chylomicrons, 35% of the radioactivity removed from plasma TGFA was found in the liver of dog 1, whereas 75% was present in the

liver of dog 2. In contrast, 84 and 78% of the radioactivity removed from the plasma ester cholesterol of the two dogs after 15 minutes was recovered in the corresponding samples of liver. Moreover, at the end of 1 hour, 21% of the radioactivity removed from chylomicron TGFA was recovered in the adipose tissue of dog 1, but only 10% in dog 2. In both, 10% of the total infused cholesterol radioactivity was present in adipose tissue at the end of 1 hour. It appears, therefore, that when chylomicron removal occurs primarily in the liver, the TGFA and ester cholesterol moieties of chylomicrons are removed at similar rates. TGFA, however, are removed more rapidly than ester cholesterol when extrahepatic tissue participates more extensively in the removal of TGFA.

P^{32} was consistently removed from the chylomicron fraction of plasma at a rate comparable to that of radioactivity in TGFA. The similar rates cannot be taken to suggest a similar mechanism for removal, since a large fraction of the injected P^{32} was rapidly transferred to higher-density lipoproteins. This finding agrees with the earlier observation by Havel and Clarke (3) of the rapid exchange of P^{32} in chylomicron phospholipids with those of higher-density lipoproteins upon incubation *in vitro*.

On the basis of our data and other available evidence, the most probable mechanism for removal of chylomicrons from the blood is as follows. In extrahepatic tissues, part of the triglyceride moiety is removed, probably after prior hydrolysis, and uptake of intact chylomicrons is limited. In the liver, intact chylomicrons and chylomicrons from which variable quantities of triglycerides have been removed are taken up intact into the parenchymal cells, presumably by pinocytosis.

SUMMARY

The metabolism of the individual lipid constituents of chylomicrons was studied in the dog. Chylomicrons in which the different lipids had been labeled by separate isotopes were injected intravenously into five normal dogs. The radioactivity in triglyceride fatty acids, ester cholesterol, and free cholesterol was removed from the chylomicron fraction of plasma lipids at similar rates in two animals. Radioactivity in the cholesterol fractions was removed slightly more slowly in two of the remaining three animals and considerably more slowly in the third. In a single experiment in which phospholipids were also labeled, radioactivity in phospholipids was removed at a rate similar to that in triglyceride fatty acids.

When similarly labeled chylomicrons were infused into dogs in which the liver was either completely or partially excluded from the circulation, radioactivity was removed considerably more slowly in the cholesterol fractions than in the triglyceride fatty acids or phospholipids.

In normal dogs, the liver removed almost all the infused chylomicron cholesterol radioactivity, but only a smaller and variable fraction of the triglyceride fatty acid radioactivity. In the functionally hepatectomized dogs, about one-third of the radioactivity removed from chylomicron cholesterol was recovered in adipose tissue.

The mechanisms of uptake of chylomicron constituents in liver and extrahepatic tissues are discussed in light of this information.

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