The role of the liver and of extrahepatic tissues in the transport and metabolism of fatty acids and triglycerides in the dog[*][†]

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SUMMARY

Labeled free fatty acids (FFA) and chylomicron triglycerides were injected intravenously into intact and hepatectomized dogs. Measurements were then made of the rate of their removal from the circulation, conversion to other circulating lipids, oxidation, and distribution in certain tissues. Hepatectomy reduced the rate of removal of FFA from the circulation moderately, but almost abolished the appearance of radioactivity in circulating triglycerides, indicating that the liver is the chief site of conversion of plasma FFA to plasma triglycerides. Experiments in dogs with thoracic duct fistulae showed that these triglycerides enter the circulation through the hepatic sinusoids. In hepatectomized animals a small quantity of triglycerides continues to enter the blood stream, probably from the intestinal mucosa by way of the thoracic duct, and possibly from other sites as well. Hepatectomy or temporary exclusion of the liver from the circulation reduced the rate of removal of chylomicron triglycerides variably. Hydrolysis to form circulating FFA occurred as in intact dogs. Most of the triglycerides removed from the circulation of hepatectomized dogs appeared to enter adipose tissue. Evidence was obtained that these triglycerides were hydrolyzed prior to entrance of their constituent fatty acids into adipose tissue cells.

Chylomicron triglycerides and FFA[1] bound to albumin have been shown to have rapid turnover rates in the blood (1). The liver is known to play an important role in their removal from the circulation. Bragdon and Gordon (2) injected fasting rats intravenously with albumin-bound palmitic acid-1-C^{14} and with chylomicrons obtained from the thoracic duct lymph of rats fed palmitic acid-1-C^{14}. Ten minutes after the injection they found that 12% and 21%, respectively, of the radioactivity cleared from the blood was present in liver lipids. Removal of these lipid moieties from the circulation by the liver has also been demonstrated by measurement of portal-hepatic venous differences (3, 4, 5).

In the present investigation we studied the metabolism of chylomicron triglycerides and FFA in intact and hepatectomized dogs. The results obtained provide further information regarding the role of the liver, and emphasize the importance of extrahepatic tissues in the transport and metabolism of these constituents.

METHODS

Preparation of Animals. Male mongrel dogs, weighing 20 to 24 kg, which had fasted overnight, were anesthetized with sodium pentobarbital given intravenously in a dose of 30 mg per kilogram body weight. A side-to-side portocaval anastomosis was created, and a large polyethylene cannula was inserted into the inferior vena cava as described by Dakin *et al.* (6). Respiration was maintained with a pump connected to an endotracheal tube. The liver was then either excised or excluded from the circulation temporarily by placing ligatures around the portal vein and hepatic

[1] Abbreviations: FFA = free fatty acids; TGFA = triglyceride fatty acids.

artery, and about the caval catheter above and below the liver. After hepatectomy and during exclusion of the liver, the animals were given a constant infusion of glucose, 0.1 g per kilogram body weight per hour, through a catheter placed in a femoral vein. Blood samples were taken and blood pressure measured through a catheter placed in the abdominal aorta via a femoral artery. Expired air was collected in polyvinyl beach balloons from the outlet of the respiration pump.

Preparation of Lipids for Injection. Palmitic acid-1-C^{14}, specific activity 5 mc per millimole, was complexed with lipoprotein-free proteins from dog serum as described previously (1). Chylomicrons in which the triglycerides were labeled in the fatty acid moiety were isolated from thoracic duct lymph of dogs fed palmitic acid-1-C^{14} in cream as described previously (1), except that the thoracic duct was cannulated directly with a plastic catheter. The radioactive preparations were injected intravenously over a period of 10 to 15 seconds in a volume of 10 to 20 ml.

Analytical Methods. Blood samples were mixed with 1 mg of sodium oxalate per milliliter, chilled immediately in ice water, and centrifuged at 3°. Lipids were extracted from plasma by the method of Davis (7) to provide an extract practically free of phospholipids.[2] In experiments using chylomicrons and for analysis of thoracic duct lymph, the samples were centrifuged at 140,000 × g (maximum) for 15 hours at 10°; the supernatant very low density lipoproteins, including chylomicrons, were then recovered with the aid of a tube slicer. The lipids were extracted from these lipoproteins in chloroform-methanol 2:1 (v/v); after at least 30 minutes the extract was equilibrated with one-fifth volume of water. Lipids were extracted from the infranatant plasma by the method of Davis (7). Adipose tissue lipids were extracted with at least 25 volumes of ethanol-acetone 1:1 (v/v) in a Waring Blendor for 1 minute. Lipids from other tissues were extracted for 15 hours with the same solvent in a Sohxlet apparatus. FFA were separated from neutral lipids by the method of Borgström (9), and titrated by Dole's procedure (10). In the case of adipose tissue, the separation was performed twice prior to analysis of FFA. Cholesterol esters were separated from glycerides on silicic-acid columns containing one-third celite by weight. Cholesterol esters were eluted with heptane-diethyl ether 100:2 (v/v), and glycerides and cholesterol with chloroform. Glyceride glycerol was determined by Carlson's

modification of the Lambert-Neish method (11). C^{14} assays were carried out in a Packard liquid scintillation spectrometer, with 0.3% diphenyloxazole in toluene as the phosphor. Carbon dioxide was collected from the samples of expired air in a methanolic solution of hyamine and assayed for CO_2 and $C^{14}O_2$ as described by Fredrickson and Ono (12). The volume of air was recorded with a Collins' spirometer. Plasma glucose concentration was measured by a glucose oxidase method.[3] For convenience in comparing the radioactivity of TGFA with that of FFA, the former were calculated as three times the molar concentration of glyceride glycerol.

RESULTS

Free Fatty Acid Metabolism. When palmitic acid-1-C^{14} was injected intravenously into fasted intact dogs, radioactivity disappeared exponentially from the plasma FFA, with a half time of about 2 minutes for approximately 15 minutes, after which the rate slowed. In three studies the specific radioactivity of expired CO_2 was maximal in about 15 minutes, and 22% to 34% of the C^{14} injected was found in expired CO_2 in 3 hours. Radioactivity appeared rapidly in plasma neutral lipids. In preliminary experiments practically no radioactivity was found in cholesterol esters separated from the other neutral lipids on silicic-acid columns. Therefore radioactivity in neutral lipids was assumed to be contained exclusively in TGFA. The specific activity of TGFA exceeded that of FFA after 30 minutes (Fig. 1).

When these studies were performed on four hepatectomized dogs, the specific activity-time curves for FFA were similar to those observed in intact dogs, except that the initial half times of disappearance were 2.5 to 3.5 minutes. In these animals specific activity of expired CO_2 was maximal in about 15 minutes, and 10% to 16% of the C^{14} injected was found in expired CO_2 in 3 hours. Very little radioactivity appeared in plasma TGFA of the four dogs during the first hour; in two of the animals, however, considerably more radioactivity was present after 2 hours (Fig. 2). In one of these animals an increase in plasma triglyceride concentration was observed as well. The specific activity of TGFA exceeded that of FFA in both of these animals after about 3 hours. Systolic blood pressure ranged from 70 to 110 mm Hg. Plasma glucose concentrations varied from 40 to 200 mg/100 ml, but were relatively stable in individual animals. Plasma FFA concentra-
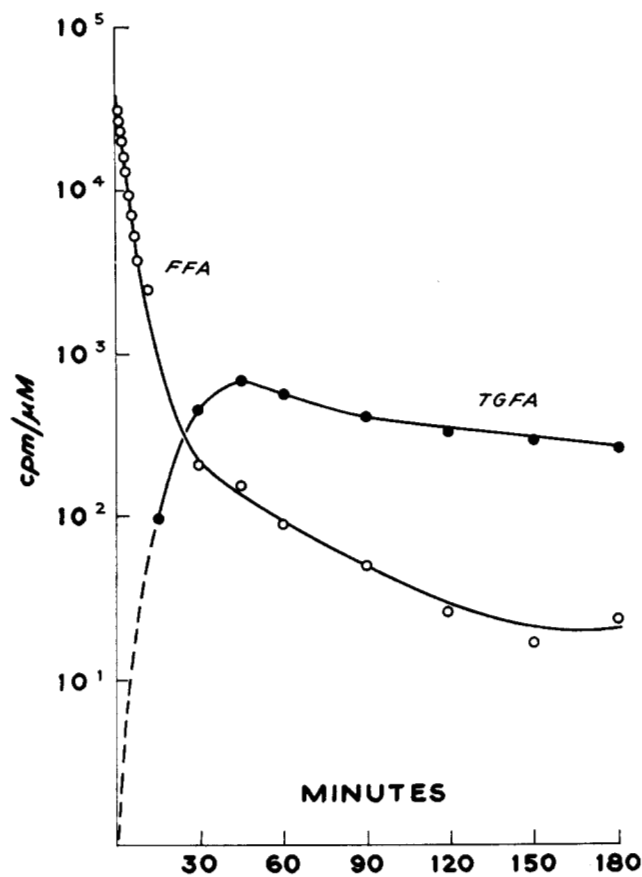
FIG. 1. Specific activities of FFA and TGFA of plasma after intravenous administration of palmitic acid-1-C^{14}, 3.8×10^7 cpm, to a fasted dog.

lymph was contained in the very low density lipoprotein-chylomicron fraction.

A single dog was eviscerated following hepatectomy. Injected palmitic acid-1-C^{14} was removed exponentially, with an initial half life of 3 minutes for 15 minutes, after which the rate slowed as described for intact dogs. Of the total dose injected, 10% appeared in expired CO_2 in 3 hours. No radioactivity was detectable in plasma TGFA in this experiment.

Chylomicron Triglyceride Metabolism. The effect of temporary exclusion of the liver on the rate of removal of chylomicron triglycerides from the circulation was studied in two dogs. In each of these experiments chylomicrons were injected after establishment of the porto-
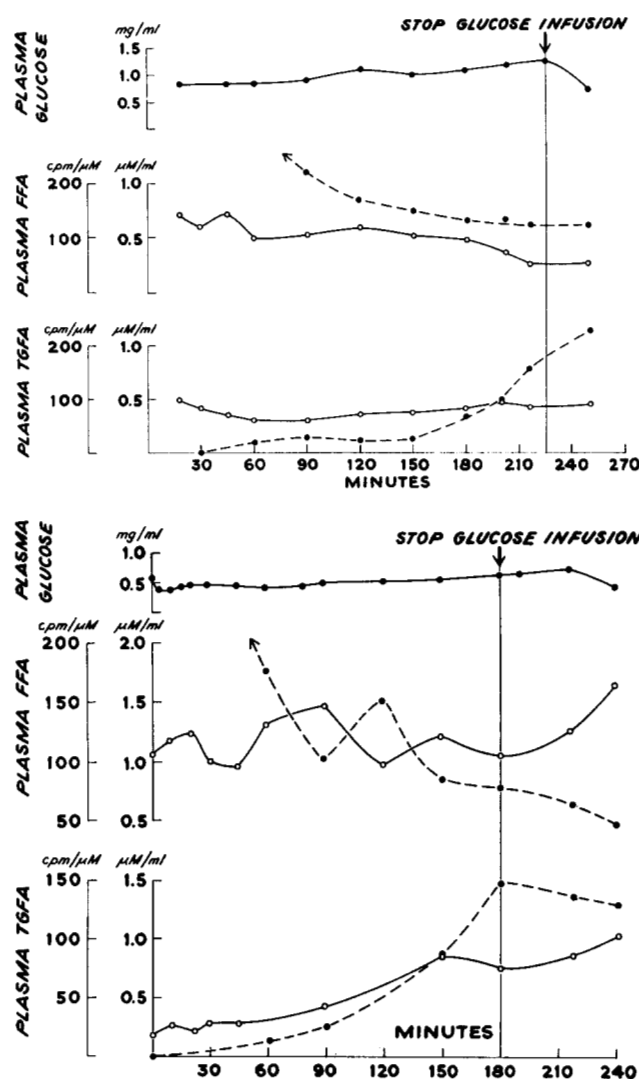

FIG. 2. Concentration of plasma glucose and concentrations and specific activities of plasma FFA and TGFA after intravenous administration of palmitic acid-1-C^{14}, 3.8×10^7 cpm, to two hepatectomized dogs. Open circles indicate concentration; closed circles indicate specific activity of FFA and TGFA.

tions were in the range of 0.2 to 0.8 μmole per milliliter in three of the four dogs. In the fourth, plasma FFA concentrations were 0.94 to 1.46 μmoles per milliliter, and plasma glucose concentrations, 0.40 to 0.55 mg per milliliter.

The thoracic ducts of two intact dogs that were fed cream 1 hour prior to operation were cannulated, and palmitic acid-1-C^{14} was injected as before. Radioactivity appeared rapidly in FFA of thoracic duct lymph, and in one of the dogs, in lymph obtained from a channel draining the head and neck (Fig. 3). The appearance of radioactivity in plasma TGFA in these animals was similar to that observed in noncannulated animals. Radioactivity of TGFA in thoracic duct lymph appeared later (Fig. 4). In both animals the specific activity of lymph TGFA exceeded that of plasma TGFA after 45 minutes. In one of the animals triglyceride concentrations in lymph and plasma were similar; in the other the concentration of lymph triglycerides was eight times that of plasma triglycerides (Fig. 4). Practically all the radioactivity in the

caval shunt and before exclusion of the liver. After a 90-minute interval the liver was excluded from the circulation and a second injection was given. After 30 minutes the liver was returned to the circulation by releasing the ligatures. As shown in Figure 5, in one experiment the rate of removal of the injected triglycerides was slower after the liver was excluded; in the other no significant difference was observed.

Chylomicrons were administered to two hepatectomized and splenectomized dogs. The removal rates were rapid until about 85% of the injected triglycerides had disappeared from the circulation; thereafter the rates slowed markedly (Fig. 6). The specific activity of chylomicron TGFA varied only 10% from the mean of all samples taken in these two experiments. Radioactivity in plasma FFA rose rapidly, reaching a maximum when approximately half the injected triglycerides had been removed from the circulation (Fig. 7). The relative specific activity of the FFA was higher and remained elevated longer in the experiment in which the greater quantity of chylomicron triglycerides had been injected. Specific radioactivity of TGFA contained in lipoproteins which sedimented at density 1.006 did not exceed 5% of that of the injected chylomicrons during the course of these experiments. In one study 1.5% of the radioactivity appeared in expired CO_2 in 2 hours, and in the other, 2.0% appeared in 3 hours. Plasma glucose concentrations were about 110 and 80 mg/100 ml, respectively, and FFA concentrations, 0.3 and 0.5 μmole per milliliter.

Serial samples of omental adipose tissue were excised during these experiments. The lipids were extracted in ethanol-acetone within 15 seconds of removal of the samples. Analyses of the radioactivity of these extracts demonstrated progressive accumulation of C^{14} (Table 1). In the two experiments specific activity of FFA in adipose tissue was much higher than that of TGFA, but much lower than that of FFA in blood plasma obtained concurrently. In both experiments samples of heart, thigh muscle, and small intestine were extracted 2 hours after administration of chylomicrons, and in one, extracts of mesenteric, renal, and subcutaneous adipose tissues were extracted as well. If it is assumed that 5% of body weight in these animals consisted of adipose tissue triglycerides, the percentage of administered C^{14} found in adipose tissue at the end of the experiments can be estimated from the observed specific activities. These values ranged from 30% when the measured specific activity of subcutaneous fat was used in this calculation to 400% when that of mesenteric fat was used. Assuming that skeletal muscle (a) had a lipid C^{14} concentration equal to that of thigh muscle, and (b) accounts for 40% of body weight,

TABLE 1.   Content and Specific Activities of Lipids of Omental Adipose Tissue and Blood Plasma During Removal of Chylomicron Triglycerides from the Circulation of Hepatectomized Dogs

Minutes After Injection	FFA as Per Cent of TGFA	TGFA	FFA	
		Adipose Tissue	Adipose Tissue	Blood Plasma
		cpm/μmole	cpm/μmole	
Exp. 1				
11	0.08	0.8	16	260
24	0.07	1.2	22	300
43	0.06	1.4	7	230
67	0.05	2.2	22	
92	0.05	2.7	43	160
120	0.06	3.6	25	180
Exp. 2				
10	0.07	0.3	4	970
22	0.05	0.6	10	1420
60	0.05	1.3	31	800
120	0.05	1.3	28	260

19% and 27% of the total radioactivity was present in this tissue. The concentration of radioactivity in heart (cpm per gram wet weight tissue) was approximately ten times that of skeletal muscle or intestinal tissue in both studies.

DISCUSSION

The results of these experiments provide further information concerning the pathways involved in the transport and metabolism of plasma FFA and triglycerides, but they do not permit quantification of the roles of the liver and other tissues under physiologic conditions.

The rate of removal of FFA from the circulation of hepatectomized or eviscerated dogs was about two-thirds that found in intact dogs. Although this observation is compatible with previous evidence that the liver removes a large portion of FFA from the blood, decreased tissue perfusion may have been of equal or greater importance in producing slower removal rates in these experiments. The reduced oxidation of FFA in hepatectomized dogs probably resulted from a variety of factors which cannot be separated, such as decreased cardiac output and altered regional blood flow, absence of the liver, and infusion of glucose. Rapid passage of FFA across the capillary bed in a peripheral area was demonstrated in the experiment in which a cervical lymphatic vessel was cannulated. The mechanism of this rapid transcapillary transport of FFA is not known. The fact that maximal specific activity of lymph FFA
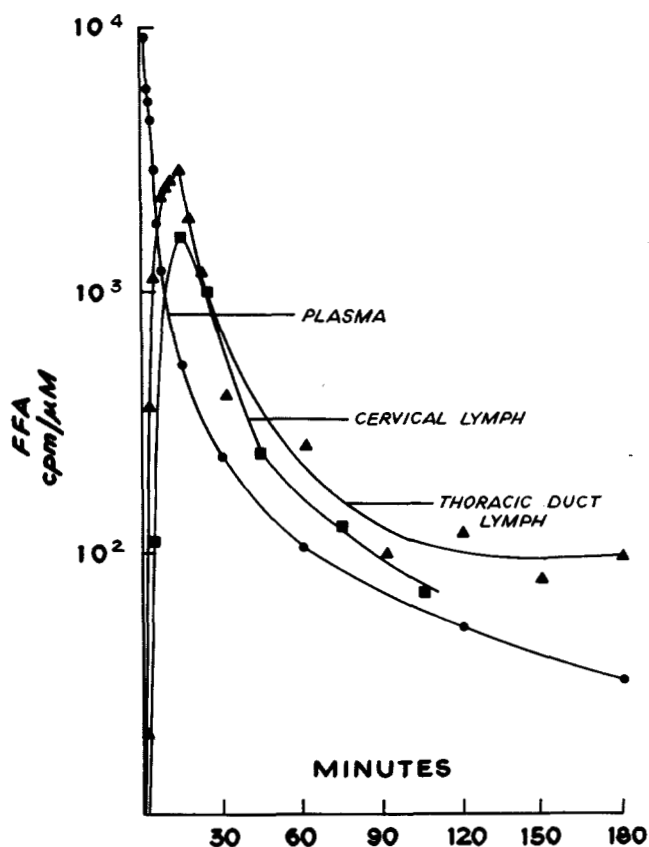
these triglycerides enter the blood stream primarily by way of the hepatic sinusoids rather than through the lymphatic system. Results of our experiments in hepatectomized dogs are similar to those of Harper *et al.* (15), who showed that plasma triglycerides do not become labeled in hepatectomized dogs given C^{14}-labeled acetate. It appears, therefore, that fatty acids synthesized in the liver or FFA delivered to the liver from adipose tissue are sources of plasma triglycerides. It is also likely that triglycerides which enter the liver from chylomicrons can re-enter the plasma. Thus the extent of endogenous triglyceride transport must be a function of hepatic lipogenesis, of the rate of delivery of FFA to the liver from adipose tissue, and possibly of the uptake of chylomicron triglycerides by the liver.

Labeled triglycerides were also found to enter the circulation by way of the thoracic duct. The specific activity of lymph TGFA was found to exceed that of plasma TGFA, even when TGFA concentration was far greater in lymph than in plasma. This observation suggests strongly that some FFA entering intesti-
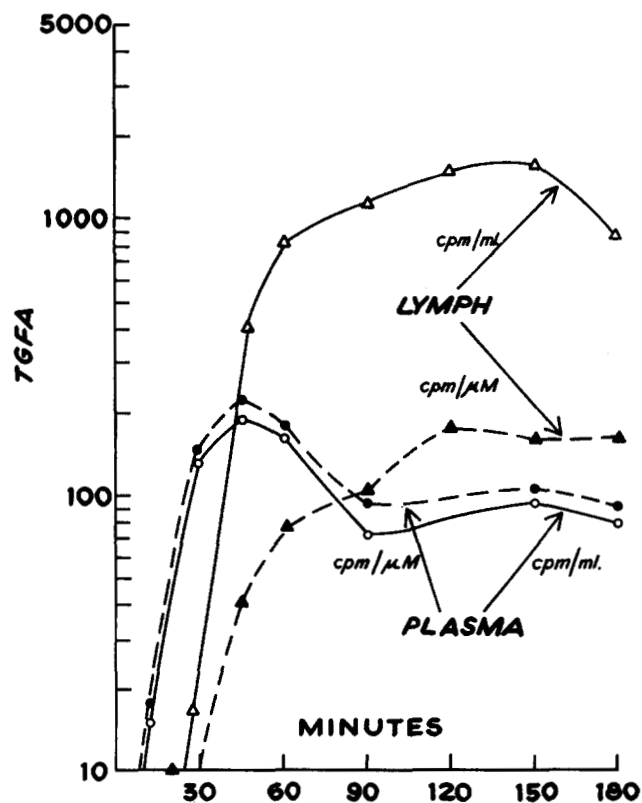

Fig. 3. Specific activities of FFA of plasma and of cervical and thoracic duct lymph after intravenous administration of palmitic acid-1-C^{14} to an intact dog.

was not reached until 15 minutes after injection probably reflects delay in transport from extravascular fluid to the sites of collection.

It is apparent from these studies that the liver is the major site of esterification of plasma FFA to form triglycerides which re-enter the circulation. Laurell (13) recently reached the same conclusion from experiments in rats, in which he compared the specific activities of triglycerides of liver and plasma after injection of palmitic acid-1-C^{14}. Stein and Shapiro (14) perfused rat livers at various intervals after injection of palmitic acid-1-C^{14} and showed that 5% to 20% of the liver triglycerides appeared in the perfusate, in agreement with our studies in intact animals.

Since FFA entering the liver are esterified almost instantaneously,[4] the delay in appearance of labeled triglycerides in the circulation must reflect the time required for transport of triglycerides in liver to the blood. Our studies in dogs with thoracic duct fistulae show that

[4] Unpublished data.

Fig. 4. Radioactivity in TGFA of plasma and thoracic duct lymph after intravenous administration of palmitic acid-1-C^{14}, 3.4 × 10⁷ cpm, to a dog fed cream prior to cannulation of the thoracic duct.
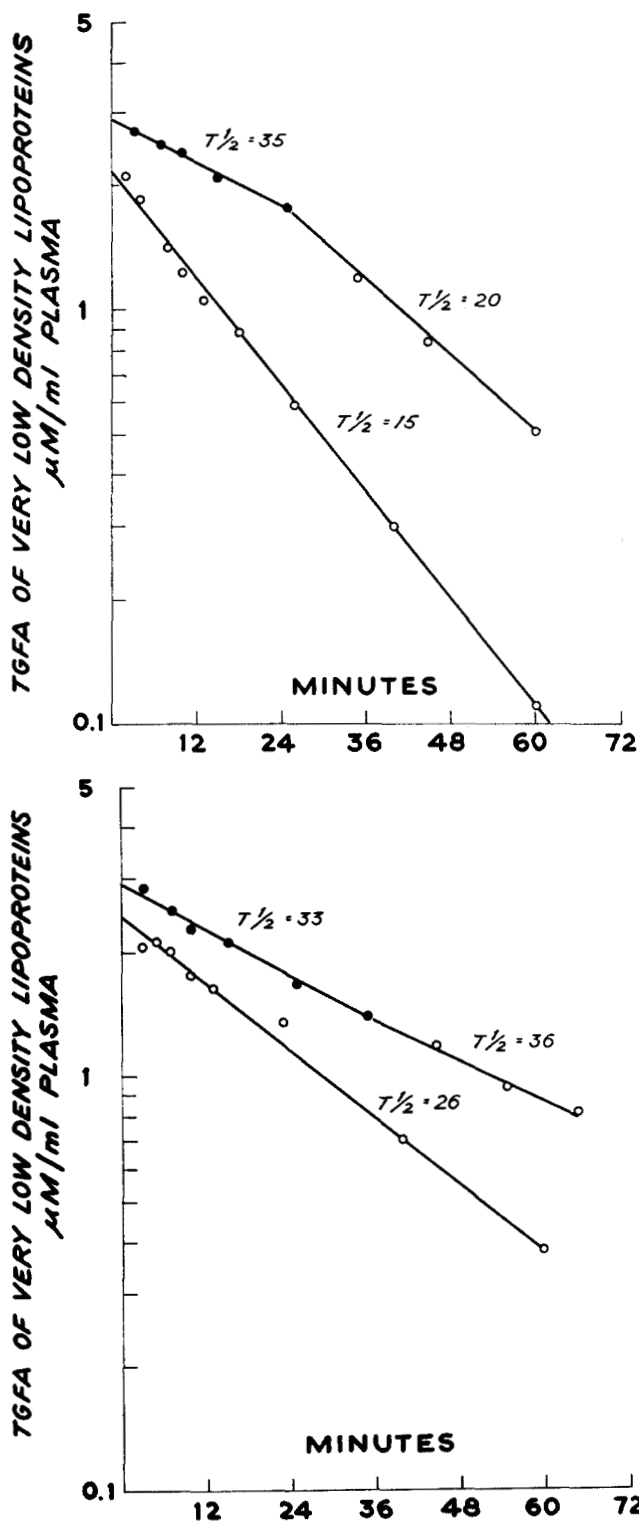
nal mucosal cells are esterified to form triglycerides which appear in chylomicrons of thoracic duct lymph. Therefore these fatty acids appear to mix, at least in part, with newly absorbed fatty acids at this site, although it is possible that a highly active fraction of plasma lipoprotein triglyceride might pass into the intestinal or hepatic lymph. No evidence was found to support the concept that triglycerides can enter the circulation from adipose tissue as such, although the possibility was not ruled out, since the extent to which the injected palmitic acid-1-C^{14} entered adipose tissue was not determined. Furthermore, it was observed that the concentration of triglycerides failed to fall progressively in hepatectomized animals.

The experiments with labeled chylomicrons demonstrate that the liver is not the only site of removal and oxidation of chylomicron triglycerides, and that hydrolysis of these triglycerides to form circulating FFA occurs in the absence of the liver. This finding is in accord with the reported presence of lipoprotein lipase in

FIG. 5. Disappearance of TGFA from the very low density lipoprotein-chylomicron fraction of plasma after intravenous administration of labeled chylomicrons containing 2.0 mmoles of TGFA to two dogs with portocaval anastomoses. Rate of disappearance of radioactivity from this fraction was practically identical. Open circles indicate hepatic circulation intact; closed circles indicate hepatic circulation occluded.
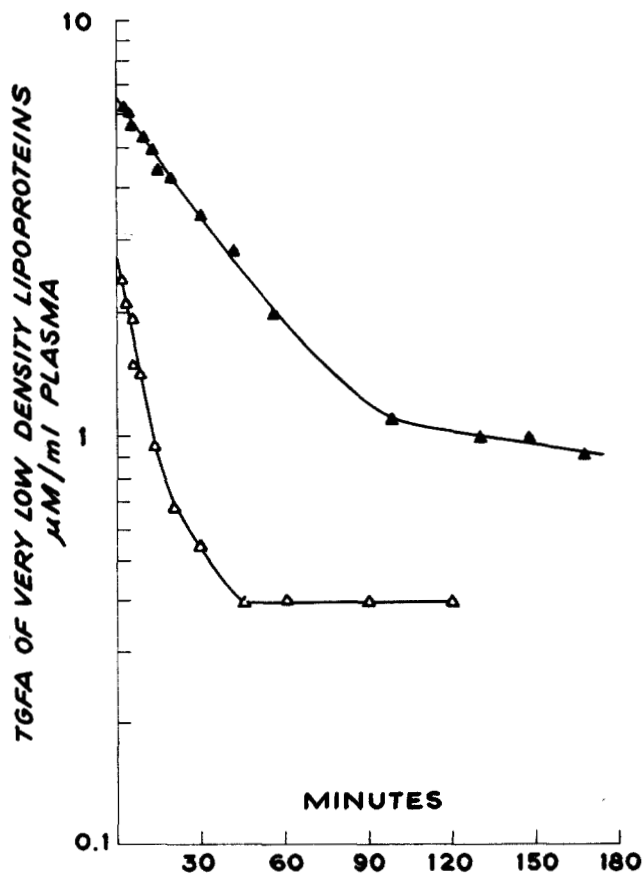

FIG. 6. Disappearance of TGFA from the very low density lipoprotein-chylomicron fraction of plasma of two hepatectomized dogs after intravenous administration of labeled chylomicrons containing 5.8 and 3.6 mmoles of TGFA, respectively.

several peripheral tissues (16). The observation that a large portion of labeled fatty acids derived from chylomicron triglycerides was deposited in adipose tissue is in general agreement with the findings of Bragdon and Gordon in fed rats (2). The large differences in specific activity among different adipose tissues probably reflect several factors, particularly variations in blood flow and enzymatic activities. Since the specific activity of FFA was much lower in adipose tissue than in plasma, it is unlikely that labeled plasma FFA were
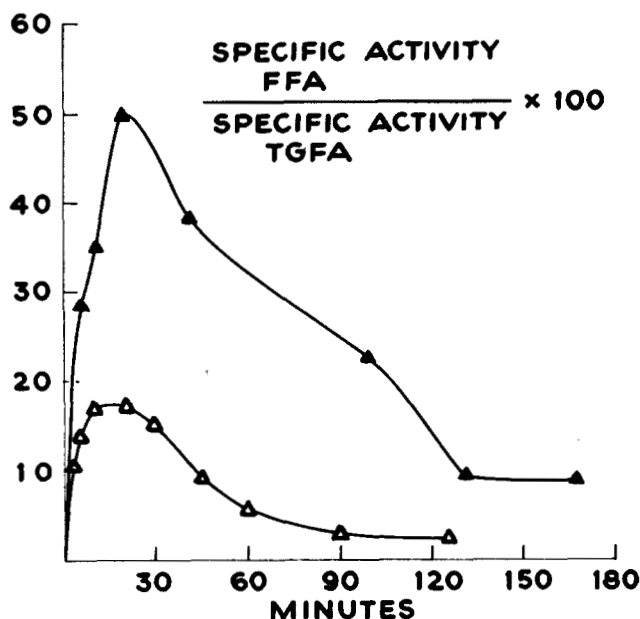

FIG. 7. Specific activities of plasma FFA of two hepatectomized dogs after intravenous administration of labeled chylomicrons. The specific activities are expressed as per cent of that of the TGFA in the administered chylomicrons.

derived from a single pool of FFA in adipose tissue. Although other mechanisms are not excluded, the results suggest that the adipose tissue FFA were derived in part from plasma and in part from adipose tissue triglycerides of very low specific activity. This finding is consistent with the concept (5) that extrahepatic hydrolysis of chylomicron triglycerides occurs prior to entrance of their fatty acids into body tissues, and that localization of fatty acids derived from chylomicron triglycerides in extrahepatic tissues is a function of the local activity of lipoprotein lipase. Such a mechanism

is also supported by the recent demonstration by Borgström and Jordan (17) that after injection of chylomicrons labeled in both the glycerol and fatty moieties into rats, the ratio of glycerol to fatty acid radioactivity was much lower in adipose tissue than in plasma.

Our studies raise the question of the fate of circulating triglycerides which are derived from the liver. If their metabolic fate is similar to that of chylomicron triglycerides, they would be expected to enter peripheral tissues containing lipoprotein lipase, and thus provide a mechanism whereby liver triglycerides can be transported to peripheral tissues for utilization or storage. Current studies in rabbits strongly support this concept (18).

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