Quantitative Studies of the Metabolism of Chylomicron Triglycerides and Cholesterol by Liver and Extrahepatic Tissues of Sheep and Dogs

E. N. BERGMAN, R. J. HAVEL, B. M. WOLFE, and T. BØHMER

From the Cardiovascular Research Institute and Department of Medicine, University of California School of Medicine, San Francisco, California 94122

ABSTRACT Unanesthetized sheep and dogs, previously fitted with indwelling catheters in the aorta, lower vena cava, mesenteric, portal, left hepatic and jugular veins, were given constant intravenous infusions of lymph in which the chylomicron lipids were variously labeled with ^{3}H or ^{14}C. Para-aminohippuric acid was infused into the mesenteric venous catheter for measurement of portal and hepatic venous blood flow. In some animals, alternately labeled free fatty acids bound to albumin were mixed with the lymph to be infused. In both species, chylomicron triglyceride fatty acids were taken up in the region drained by the lower vena cava and portal vein and free fatty acids derived from hydrolysis of these triglycerides were extensively recycled in the blood. Direct uptake of triglyceride fatty acids also occurred in liver and accounted for about 10% of the total triglyceride fatty acids removed from the blood in sheep and 22% in dogs. In sheep, 10% and, in dogs, about 40% of these triglyceride-fatty acids were released into the blood as free fatty acids. The free fatty acids recycled from various regions accounted for a substantial fraction of the chylomicron fat eventually deposited in each tissue. Uptake of chylomicron cholesterol from the blood of sheep occurred primarily in liver and to a small extent in certain tissues drained by the portal vein. The results obtained, together with other available data, demonstrate that chylomicron triglycerides are removed primarily in extrahepatic tissues of both species, while the liver removes cholesterol contained in chylomicron "skeletons" from which most of the triglycerides have been removed. The quantitative differences between transport of chylomicron lipid in sheep and dogs may be related to known differences in the structure of their hepatic sinusoids.

INTRODUCTION

Chylomicron lipids are efficiently cleared from the blood in several tissues (2). The liver is the major site of removal of chylomicron cholesterol (3–6), but its role in metabolism of triglycerides, the major constituent of chylomicrons, is uncertain. Early studies demonstrating utilization of chylomicron triglycerides in perfused rat liver (7) were confused by use of postheparin plasma containing lipoprotein lipase in the perfusion medium. Some recent investigations have suggested that the perfused liver has almost no capacity to metabolize chylomicron triglyceride (8, 9) although this has been disputed (10). In contrast, studies in intact animals have suggested that the liver accounts for direct removal of 20–30% of the chylomicron triglyceride cleared from the blood (11–13). The discrepant results with perfused liver and intact animals could be understood if the liver metabolizes chylomicron lipid mainly after it has been modified during passage through extrahepatic tissues, a possibility raised by studies performed in this laboratory several years ago. It was shown that infused chylomicron cholesterol accumulated in the blood of hepatectomized dogs, although triglycerides were still efficiently removed (6). It was suggested that the liver normally removes chylomicron cholesterol after the triglyceride has been taken up in extrahepatic tissues through the action of lipoprotein lipase. Recently, Redgrave (14)

Dr. Bergman's present address is Department of Physiology, New York State Veterinary College, Cornell University, Ithaca, N. Y.

Dr. Wolfe was a Centennial Fellow of the Medical Research Council of Canada.

Dr. Bøhmer was an International Postdoctoral Research Fellow of the United States Public Health Service.

Portions of this work were presented at the Annual Meeting of the Federation of American Societies for Experimental Biology, 13 April 1970 (1).

Received for publication 18 January 1971 and in revised form 14 April 1971.

has obtained evidence for accumulation of chylomicron "remnants" rich in cholesterol and poor in triglyceride relative to lymph chylomicrons after injecting labeled chylomicrons into functionally hepatectomized rats.

The peculiar structure of the hepatic microcirculation has been considered relevant to the ingress and egress of lipoproteins and other macromolecules in liver (15, 16). In most mammalian species, the hepatic sinusoids generally have no basement membrane and have usually been considered to be freely permeable to large macromolecules because of frequent gaps up to 1 μ diameter between endothelial cells (15). However, very recently Wisse has reported (17) that intercellular gaps are not seen in material prepared from rat livers fixed by perfusion with glutaraldehyde. In these preparations, the endothelial lining appeared to consist primarily of endothelial processes alternating with attenuated regions which contain regular oval fenestrations of about 1000 Å diameter forming sieve plates. Through these pores, the blood plasma is considered to be in direct contact with the microvillous surface of parenchymal cells via the space of Disse. Electron micrographs of liver removed shortly after injection of chylomicrons show numerous particles in the space of Disse (18). Other studies show that a substantial fraction of unhydrolyzed chylomicron triglyceride can be washed out of the liver for an appreciable period after intravenous injection of labeled chylomicrons (9, 12). These observations suggest that the sinusoidal endothelium acts as a macromolecular sieve which retards some chylomicrons in their passage through the liver. In contrast, in at least some ruminants, a well-developed basement membrane is present (19, 20) and, in the cow (19), the endothelial lining appears to be continuous, although it is frequently attenuated.

The present studies were undertaken to obtain quantitative information concerning sites of removal of chylomicron triglyceride and cholesterol under physiological conditions and to explore the possible influence of sinusoidal structure on the hepatic metabolism of chylomicrons by comparing a ruminant species, the sheep, with a nonruminant, the dog.

METHODS

Animals and surgical preparation. Both sheep and dogs were used and all were well accustomed to handling. The sheep were sheared, mature crossbred, nonpregnant, nonlactating ewes and weighed between 50 and 55 kg. The diet consisted of pelleted alfalfa hay fed *ad libitum*. Water and a salt lick were available at all times. The dogs were large adult males, of German Shepherd or Black Labrador type, and weighed between 32 and 34 kg. They were fed commercial dry dog food supplemented with meat but all experiments on dogs were performed in the postabsorptive state.

The experimental animals for lymph infusions (recipients) were prepared as described by Katz and Bergman (21) for sheep, and by Shoemaker, Walker, van Itallie, and Moore (22), for dogs. In these preparations, polyvinyl catheters were implanted in the terminal aorta, the vena cava about 10 cm caudal to the kidneys, the portal, left hepatic, and jugular veins, and a small mesenteric vein. The spleen was removed from each dog. None of the animals were used for experiments until at least 6 days had elapsed after surgery and when they were eating normally.

Separate lymph-donor animals were prepared 2–19 days before each experiment. This was accomplished in sheep by catheterizing the main intestinal lymph duct just proximal to its junction with the cisterna chyli as described by Lascelles and Morris (23). A small polyvinyl catheter was also inserted directly through the wall of the duodenum and into its lumen about 20 cm caudal to the pylorus. This was done so that labeled fatty acids and cholesterol, which would later be administered, would bypass the rumen. Lymph-donor dogs were prepared by catheterizing the thoracic duct at its junction with the left jugular vein. Lymphatic-jugular venous shunts were established with polyvinyl tubing in both sheep and dogs in order to prevent fluid loss and so that lymph samples could be obtained as required (23, 24).

Materials. Palmitic acid-1-^{14}C (S.A. 9.9 mCi/mmole) and palmitic acid-9, 10-^{3}H (128 mCi/mmole) were obtained from New England Nuclear Corp., Boston, Mass. Generally labeled cholesterol-^{3}H (S.A. 4.5 Ci/mmole) was obtained from Amersham/Searle Corp., Des Plaines, Ill. It was completely precipitable by digitonin.

Experimental procedures. All experiments and lymph collections were performed on unanesthetized and unsedated animals. The catheters were kept filled with a heparin-saline solution between experiments but no heparin was given for 1 day before or during any experiment or lymph collection period.

Chylomicrons doubly-labeled with triglyceride-^{14}C (TGFA)[1] and cholesterol-^{3}H were prepared in two lymph-donor sheep for use in four infusion experiments. This was done by intraduodenal injection of about 300 μCi palmitate-1-^{14}C and about 2 mCi generally labeled cholesterol-^{3}H dissolved in 10 g olive oil containing 4 mg cholesterol. The lymph was collected for 6–8 hr in glass vessels, chilled, defibrinated, and filtered through glass wool. It was stored no longer than 20 hr at 3°C before use. For nine other experiments, TGFA-^{3}H-labeled chylomicrons were similarly prepared in three donor sheep and two dogs by giving about 1 mCi palmitate-9, 10-^{3}H in 10 g of olive oil. Dogs were fed the mixture together with about 500 ml of skimmed milk. In these nine latter experiments, tracer quantities of palmitate-1-^{14}C bound to human serum albumin were added to the lymph just before use (about 0.2 μCi/ml). In both species, more than 97% of the total radioactivity of the lymph was found to be present in the $d < 1.006$ lipoprotein fraction (6). In lymph from one sheep, 90% of the radioactivity was recovered in the supernatant cream layer after centrifugation for 2×10^{6} g-min. From 96 to 98% of the TGFA-^{3}H or TGFA-^{14}C in the lymph was in neutral glycerides, and 67–69% of the total cholesterol-^{3}H was present as esterified cholesterol (EC) and the remainder as free cholesterol (FC).

In the lymph-recipient animals, para-aminohippuric acid (PAH) was constantly infused into the mesenteric vein

[1] *Abbreviations used in this paper:* EC, esterified cholesterol; FC, free cholesterol; FFA, free fatty acid(s); PAH, para-aminohippuric acid; TGFA, triglyceride-fatty acid(s); VLDL, very low density lipoprotein.

catheter for simultaneous measurements of portal and hepatic venous blood flow (25). After about a 30 min equilibration period, 5 ml of lymph were injected into the jugular catheter as a priming dose and a constant infusion of lymph (0.76 ml/min) was then given for 1 hr. Six sets of blood samples were withdrawn during this hour at 10-min intervals from the arterial, portal, hepatic, and lower vena caval catheters (total of 24 samples) for measurements of the isotopes and concentrations of plasma TGFA, cholesterol, and FFA. The uptake and release of these metabolites were thus measured across three areas of the body: the portal-drained viscera, liver, and lower body (rear legs and rump). Five additional arterial samples were taken at 3, 6, 10, 20, and 30 min after the termination of lymph infusion for estimation of the turnover rate of chylomicron TGFA and cholesterol.

Analyses. Blood samples were placed in tubes containing 0.01 ml heparin (1000 U/ml) per ml. About 1 g Separaid polystyrene crystals (Uni-Tech Chemical Co.) was added and the samples were centrifuged immediately at 1600 *g* for 8 min at room temperature. The plasma was separated, mixed thoroughly and lipids in duplicate 1-ml portions were extracted in 5 ml of Dole's mixture (26). In experiments with cholesterol-labeled chylomicrons, 2 ml were also extracted in 40 ml chloroform-methanol (2:1, v/v). These operations were completed within 15 min after each set of samples was obtained. A single volumetric pipet was used for all measurements on a given set of samples. The extracted lipids in Dole's mixture were then treated as described elsewhere (27) for measurements of FFA and TGFA and for assay of isotopes in neutral lipids and FFA. After addition of an equal volume of 0.005 N H_2SO_4, lipids in portions of the chloroform phase of the chloroform-methanol extract were separated on small columns of silicic acid (28). Three fractions containing: (*a*) cholesteryl esters; (*b*) cholesterol, neutral glycerides and FFA; and (*c*) phospholipids were obtained. The first two fractions were used for assay of cholesterol-^{3}H. The infused lymph was assayed similarly. Cholesterol (29) and triglycerides were also measured in lymph. PAH was measured in extracts of whole blood (25).

All radiochemical assays were performed in a liquid scintillation spectrometer equipped with punch card readout. Quenching was found to be negligible. Content of ^{3}H and ^{14}C was calculated from triplicate 10 or 20 min counts with an IBM 360, model 50 computer after determination of discrimination ratios for the two isotopes. There were about 20% ^{14}C counts in the channel used to count ^{3}H and 0.3% ^{3}H in the ^{14}C channel.

Calculations

1. Uptake of labeled TGFA and cholesterol: (*a*) Per cent uptake of radioactivity per circulation (extraction ratio) = 100 (A-V)/A where A and V are cpm/ml of plasma entering and leaving the region (or organ); for liver, A was calculated as follows: A = FA(HA) + FP(PV) where FA and FP are the fractions of the total hepatic plasma flow contributed by the hepatic artery and portal vein and HA and PV are the cpm/ml of plasma in the hepatic artery and portal vein. (*b*) Per cent of infused metabolite taken up (or removed) in steady state = 100(A-V) × (plasma flow in ml/min) ÷ (cpm of metabolite infused/min). (*c*) Turnover rate = (cpm TGFA or cholesterol infused/min) ÷ [(cpm in TGFA or cholesterol/ml arterial plasma) × (plasma volume in ml)]; plasma volume was assumed to equal 5% of body weight.

2. Uptake of infused FFA-^{14}C and uptake and release of FFA-^{3}H derived from infused lymph TGFA-^{3}H: (*a*) extraction ratio = 100 (A-V)/A; for liver, A was calculated as noted above for labeled TGFA and cholesterol. (*b*) Per cent of infused metabolite taken up in steady state = 100 (A-V) × (plasma flow in ml/min) ÷ (cpm of metabolite infused/min). (*c*) Per cent of infused chylomicron TGFA taken up as FFA = (extraction ratio of FFA-^{14}C) × (FFA-^{3}H in A) × (plasma flow in ml/min) ÷ (TGFA-^{3}H infused/min). (*d*) Per cent of TGFA uptake released as FFA = [(extraction ratio of FFA-^{14}C) × (FFA-^{3}H in A) − (A-V of FFA-^{3}H)] ÷ (A-V of TGFA-^{3}H); in this manner, the release of FFA-^{3}H from TGFA-^{3}H taken up in a region was calculated by using the uptake of FFA-^{14}C to calculate the total uptake of FFA-^{3}H. (*e*) Transport of FFA in mmole/min = (FFA-^{14}C infused/min) ÷ (FFA-^{14}C/mmole arterial plasma FFA). (*f*) Turnover rate of FFA = (cpm FFA-^{14}C infused/min) ÷ [(cpm FFA-^{14}C/ml arterial plasma) × (plasma volume in ml)]; plasma volume was assumed to equal 5% of body weight.

3. Errors in the above estimates of uptake and release depend upon the precision of the methods in relation to the magnitude of the A-V differences observed. For uptake and release of FFA in experiments employing TGFA-^{3}H and FFA-^{14}C, these estimates also depend upon completeness of separation of TGFA and FFA and are complicated by

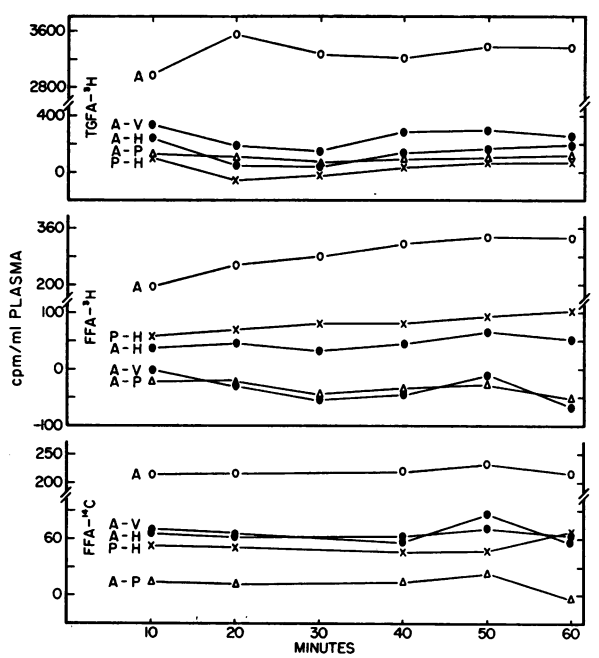

FIGURE 1 Data obtained in a sheep infused with lymph containing chylomicrons labeled with TGFA-^{3}H and palmitate-^{14}C. A, artery; V, lower vena cava; H, hepatic vein; P, portal vein. Positive A-V differences indicate uptake and negative differences indicate release. Note that chylomicron TGFA-^{3}H was taken up in all areas but this was most prominent in the lower body and that FFA-^{14}C was taken up mainly in the lower body and liver. FFA-^{3}H was produced by hydrolysis of TGFA-^{3}H in capillary beds so that arteriovenous differences of FFA-^{3}H are the result of both release (by hydrolysis) and uptake. Virtually no TGFA-^{14}C was formed from FFA-^{14}C.

TABLE I

*Plasma Concentrations of Lipids and of Lipid Radioactivity**

Species and isotopes infused	Experiment No.	Infusion rate		Arterial plasma				
				Concentration		TGFA	TC	FFA
		TG	TC	TG	FFA	^3H or ^{14}C	^3H	^{14}C
		mg/hr		*mg/dl*	*mM*		*cpm/ml*‡	
Sheep,								
TGFA-^3H + FFA-^{14}C	1				0.08 ±0.01	2148 ±70		
	2	719	86		0.20 ±0.01	4573 ±102		656 ±61
	3	595	87		0.15 ±0.02	3293 ±81		565 ±8
	6	743	41	20	0.18 ±0.01	4921 ±216		677 ±32
	7	793	42	15	0.23 ±0.01	3051 ±61		699 ±11
	Mean	713	64	17	0.17 ±0.03	3597 ±509		649 ±17
Dogs,								
TGFA-^3H + FFA-^{14}C	1	1017	35	53	0.43 ±0.02	2555 ±96		1167 ±53
	2	1131	35	52	0.26 ±0.01	2954 ±215		830 ±86
	3	506	28	35	0.56 ±0.01	2096 ±56		1207 ±19
	4	412	28	54	0.19 ±0.02	2247 ±76		705 ±29
	Mean	767	32	49	0.36 ±0.08	2463 ±189		977 ±124
Sheep,								
TGFA-^{14}C + Cholesterol-^3H	4	796	124	14	0.02 ±0.01	2489 ±55	4625 ±349	
	5	771	128	10	0.21 ±0.02	2160 ±38	3972 ±255	
	9	492	53	11	0.31 ±0.02	2412 ±58	5519 ±151	
	10	424	52	14	0.05 ±0.01	2751 ±62	4333 ±178	
	Mean	621	89	12	0.15 ±0.07	2453 ±122	4612 ±331	

* In this and subsequent Tables, values are means ±SEM of four to six samples obtained from 10–60 min after starting constant infusions. TG, triglyceride; TC, total cholesterol; FFA, free fatty acids. Body weights were 50–55 kg for sheep and 32–34 kg for dogs.

‡ Values adjusted to an infusion rate of 10^6 cpm/min for ease of comparison between experiments.

the added errors in estimating A-V differences of FFA-^{14}C and FFA-^3H and by the correction for "spill" of ^{14}C in the channel used to count ^3H. When tripalmitin-^3H was added to 5 ml of Dole's mixture containing 1 ml unlabeled plasma, less than 0.2% of the ^3H appeared in the fraction (27) used to assay radioactivity in FFA. Four to six samples were analyzed in duplicate in all experiments. Coefficients of variation of such duplicate analyses for the typical experiment shown in Fig. 1 were 1.9% for TGFA-^3H, 4.1% for FFA-^3H, and 4.6% for FFA-^{14}C. Mean values obtained from these samples and their standard errors are provided for each experiment.

RESULTS

Experimental conditions. Table I summarizes data on amount of isotopes infused and plasma concentrations of lipids and lipid radioactivity. The quantities infused (FFA, tracer amounts; triglyceride, <1200 mg/hr; and cholesterol, <130 mg/hr) were kept small so that their plasma concentrations and metabolism would be within physiological limits. The concentrations of these lipids did not vary systematically during the experiments. All were within the accepted normal range but concentrations of triglycerides were generally lower in sheep than in dogs even though sheep were studied during *ad libitum* feeding and dogs in the postabsorptive state.

Values for rates of splanchnic plasma flow (Table II) also are in agreement with previously published values for both animal species (25, 30). The portal plasma flow varied from 88 to 95% of total hepatic plasma flow in sheep and 70–80% in dogs. Lower body (rear legs and rump) plasma flow rates were assumed to be 1000 ml/min for sheep and 500 ml/min for dogs for purposes of calculating total uptakes of metabolites. This represents roughly one-fifth of the cardiac output in each species (31).

Results representative of a dual experiment using chylomicrons labeled with TGFA-^3H, and FFA labeled with ^{14}C are presented in Fig. 1. Characteristic variability of the data thus is illustrated, but nearly constant values were obtained, at least during the latter two-thirds of the 1 hr infusion period. The gradual increase in plasma content of FFA-^3H during the first part of the study shown here was observed in most experiments,

TABLE II
Splanchnic Plasma Flow

Experiment No.	Portal vein*	Hepatic vein*	Hepatic artery*	Portal as fraction of hepatic venous flow
		liter per min		
Sheep				
1	2.04 ±0.12			
2	1.20 ±0.08	1.27 ±0.08	0.07 ±0.02	0.94
3	1.58 ±0.13	1.69 ±0.08	0.12 ±0.09	0.93
6	1.33 ±0.05			
7	2.28 ±0.18	2.39 ±0.18	0.11 ±0.11	0.95
4	2.26 ±0.05			
5	1.56 ±0.03	1.72 ±0.06	0.16 ±0.06	0.91
9	1.30 ±0.15	1.47 ±0.14	0.17 ±0.08	0.88
10	1.80 ±0.11	1.93 ±0.11	0.13 ±0.08	0.93
Dogs				
1	0.50‡	0.62 .	0.12 .	0.80
2	0.51 ±0.05			
3	0.53 ±0.11	0.66 ±0.03	0.13 ±0.11	0.80
4	0.70 ±0.03	1.01 ±0.05	0.31 ±0.07	0.70

* Mean value ±SEM.
‡ Only one sample in this experiment.

while that of FFA-^{14}C remained constant. This may be the result of delayed release of FFA back into the blood after chylomicron TGFA have been removed. No such time dependence was found for extraction ratios of FFA-^{3}H or FFA-^{14}C. Very little TGFA-^{14}C was formed when FFA-^{14}C was infused.

Results of an experiment using chylomicrons labeled with cholesterol-^{3}H are presented in Fig. 2. In the experiment shown, arterial-portal and arterial-vena caval differences were negative for ester cholesterol indicating release rather than uptake at 20 min, while that of total cholesterol was nil or slightly positive. Such differences were seen sporadically in other experiments in these two regions but no systematic time-dependent changes in pattern of uptake were observed. Variability among samples was usually greater for ester cholesterol than for total cholesterol (see Table VI), presumably reflecting larger technical error related to chromatographic separation of cholesteryl esters.

FFA metabolism (Table III). Results were similar in sheep and dogs. Turnover rates of FFA were rapid in both species and the uptake of FFA was greater in the lower body and liver than in the organs drained by the portal vein. The lower body removed about one-fourth of the FFA presented per circulation. Extraction ratios were somewhat lower in sheep in portal region and liver, perhaps reflecting the larger visceral blood flow in the ruminant. In both species, the organs drained by the portal vein accounted for about 8% and the liver about 25% of the total FFA removed from the blood. Using the assumed values for lower vena-caval flow, the three areas together accounted for 40–50% of the total FFA transport.

Chylomicron TGFA metabolism (Table IV). Turnover rates of TGFA, estimated in the steady state, were also rapid in both species (mean, 0.14 min^{-1} in sheep and 0.24 min^{-1} in dogs. Turnover rates, estimated from the initial exponential rate of disappearance of labeled TGFA from plasma after termination of lymph infusions, were closely similar to these values in sheep and about two-thirds as great in dogs. The lower body directly removed large quantities of TGFA (11–15% per circulation) and the portal region removed about 6%. The liver of sheep directly removed only small amounts (2% per circulation), significantly less ($P < 0.05$) than that of dogs (12%). In all regions, 40–50% of the TGFA taken up was promptly hydrolyzed and released back into the blood as FFA except in liver of sheep where only about 10% was released as FFA.

The total uptake of TGFA as percentage of the infused dose is expressed as a direct removal (as TGFA) and also as an indirect removal (as FFA which previously had been released into the blood after hydrolysis of TGFA). Extrahepatic tissues accounted for most of the direct TGFA removal in both species but this extrahepatic removal was significantly greater in sheep than in dogs ($P < 0.05$). About 10% of the TGFA was directly

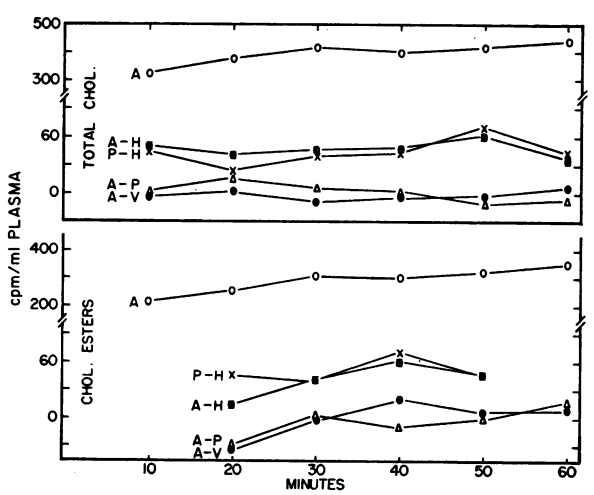

FIGURE 2 Data obtained in a sheep infused with lymph containing chylomicrons labeled with cholesterol-^{3}H. The chylomicrons were also labeled with TGFA-^{14}C but the results are not presented here since other data for TGFA are given in Fig. 1. A, artery; V, lower vena cava; H, hepatic vein; P, portal vein. Note that chylomicron cholesterol-^{3}H was taken up mainly in liver.

TABLE III

Metabolism of Free Fatty Acids

Experiment No.	Turnover rate	Transport*	Extraction ratio*			Infused FFA taken up		
			Lower body	Portal	Hepatic	Lower body‡	Portal	Hepatic
	min^{-1}	*mmoles/hr*		%			%	
Sheep								
2	0.61	18 ±1		10 ±2	22 ±2		8	16
3	0.70	16 ±2	27 ±3	5 ±2	25 ±2	15	4	22
6	0.59	16 ±1	26 ±6	10 ±4		18	10	
7	0.57	20 ±1	26 ±1	6 ±3	25 ±3	18	9	40
Mean	0.62 ±0.03	17 ±1	26 ±1	8 ±1	24 ±1	17 ±1	8 ±1	26 ±7
Dogs								
1	0.50	22 ±1		14 ±2	33 ±1		8	22
2	0.71	19 ±1		10 ±2			4	
3	0.48	28 ±1	11 ±1	9 ±1	29 ±1	7	6	19
4	0.83	16 ±1	31 ±5	18 ±4	44 ±3	11	9	28
Mean	0.63 ±0.08	21 ±3	21 ±10	13 ±2	35 ±5	9 ±2	7 ±1	23 ±3

* Mean value ±SEM.

‡ In this and later Tables, the lower vena caval plasma flow was assumed to be 1000 ml/min in sheep and 500 ml/min in dogs.

taken up in sheep liver as compared with about 22% in dog liver. All regions of the body also removed TGFA indirectly, i.e., after uptake of TGFA and its hydrolysis to form FFA which was returned to the blood (Table IV). This was particularly prominent in liver and no species differences were evident in this respect. TGFA directly taken up in the three regions, when corrected for release as FFA, accounted for a net tissue storage or

TABLE IV

Metabolism of Chylomicron Triglyceride Fatty Acids

Experiment number	Turnover rate	Extraction ratio*‡			Uptake released as FFA*			Infused TGFA taken up					
								As TGFA			As FFA		
		Lower body	Portal	Hepatic	Lower body	Portal	Hepatic	Lower body	Portal	Hepatic	Lower body	Portal	Hepatic
	min^{-1}		%			%				%			
Sheep													
1	0.19	15 ±2	2 ±1					32	9				
2	0.09		4 ±1	3 ±1		49 ±10	10 ±6		20	15		4	11
3	0.12	8 ±1	3 ±1	1 ±1	48 ±6	47 ±6	1 ±9	25	17	7	8	2	13
6	0.08	10 ±1	3 ±1		30 ±2	32 ±11		51	19		7	3	
7	0.13	10 ±1	3 ±1	3 ±1	46 ±3	49 ±5	18 ±9	30	19	18	11	6	27
4	0.16	15 ±2	6 ±1					38	31				
5	0.19	8 ±1	6 ±1	0 ±1				18	19	0			
9	0.17	15 ±1	6 ±1	6 ±1				35	17	18			
10	0.15	8 ±1	5 ±1	1 ±1				21	23	3			
Mean	0.14 ±0.013	11 ±1	5 ±1	2§ ±1	41 ±6	44 ±4	10§ ±5	31§ ±4	19§ ±2	10 ±3	9 ±1	4 ±1	17 ±5
Dogs													
1	0.23		13 ±2	12 ±2		26 ±10	57 ±10		16	18		6	15
2	0.20		3 ±1			61 ±10			6			2	
3	0.28	12 ±1	4 ±1	6 ±1	57 ±4	90 ±15	37 ±7	13	4	8	4	4	15
4	0.26	17 ±1	7 ±2	18 ±2	33 ±3	15 ±8	23 ±2	19	11	39	7	6	19
Mean	0.24 ±0.018	15 ±3	7 ±2	12 ±3	45 ±12	48 ±17	39 ±10	16 ±3	9 ±3	22 ±9	6 ±2	4 ±1	16 ±1

* Mean value ±SEM.

‡ Uptake as TGFA or direct removal.

§ Significant differences between sheep and dogs ($P < 0.05$).

utilization of about 38% of that infused in sheep and 27% in dogs (Table V). Total net uptake (as TGFA + FFA) in the three regions was 68% of that infused in sheep and 53% in dogs.

Chylomicron cholesterol metabolism in sheep (Table VI). Of the total chylomicron cholesterol-^{3}H infused, 68 ±1% was present as cholesteryl esters and the remainder as free cholesterol. The metabolism of chylomicron cholesterol differed greatly from that of chylomicron TGFA. The turnover rates of total cholesterol were about two-thirds of those for TGFA (0.09 and 0.14 min^{-1}, respectively). Estimates of turnover rates of cholesterol derived from the initial exponential rate of disappearance from plasma after termination of lymph infusions were about 20% lower than those estimated from plasma content of cholesterol-^{3}H in the steady state. In addition, liver, rather than extrahepatic tissues removed most of the cholesterol (10% per circulation) and this accounted for an average of 76% of the total cholesterol infused. The lower body removed no detectable total cholesterol and the portal-drained viscera removed about 12% of that infused.

TABLE V

Net Percentage of Infused Chylomicron Triglyceride Fatty Acids Taken up in Various Regions

	Sheep*			Dogs*		
	As TGFA‡	As FFA	Total	As TGFA‡	As FFA	Total
		%			%	
Lower body	18 ±4	9 ±1	27	9 ±4	6 ±2	15
Portal	11 ±2	4 ±1	15	5 ±3	4 ±1	9
Hepatic	9 ±3	17 ±5	26	13 ±9	16 ±1	29
Total	38	30	68	27	26	53

* Mean value ±SEM.

‡ Per cent of infused TGFA taken up directly as TGFA minus the per cent of TGFA taken up which was promptly released as FFA.

DISCUSSION

In this study, removal of chylomicron lipids in various regions was quantified without first separating chylomicrons from plasma. Rather, we measured total radioactivity in lipid extracts in order to minimize technical error in estimating arteriovenous differences. Thus, the results obtained provide reliable information concerning the sites at which chylomicron TGFA and cholesterol leave the blood but they do not demonstrate directly the nature of any intermediates formed during the process of removal. It has been shown that the rate at which chylomicron TGFA is removed from the blood is a function of particle size (32). The turnover rates we obtained during continuous infusions presumably reflect the weighted mean of a continuous distribution of size-dependent rates and in addition include rates of transport of any intermediate species formed. Hepatogenous VLDL-TGFA derived from chylomicron TGFA may also enter the blood during the period of study. In both sheep and dogs, concentration of VLDL-TGFA is low (see Table I) and very little TGFA-^{14}C was found in either species when FFA-^{14}C was infused, suggesting that the influence of such recycling on the estimated turnover rates of chylomicron TGFA was negligible.

Our results show that in the sheep, as in the rat (3–5), and dog (6), removal of chylomicron cholesterol from the blood is largely a function of the liver. They show further that in the sheep certain tissues drained by the portal vein also take up a small fraction while little or none is removed in other extrahepatic tissues. From the results obtained for tissues drained by the lower vena cava in both sheep and dogs, it is evident that this region selectively removes triglycerides from chylomicrons with no measurable uptake of cholesteryl esters. This observation indicates that, as in the dog (6) and rat (14), skeletons or remnants of chylomicrons are produced in these tissues. These partially degraded particles are then taken up in the splanchnic region, especially the liver. The liver also removes some chylomicron tri-

TABLE VI

Metabolism of Chylomicron Total Cholesterol and Ester Cholesterol in Sheep

Experiment number	Turnover rate	Extraction ratio*						Infused TC or EC taken up					
		Total cholesterol			Ester cholesterol			Total cholesterol			Ester cholesterol		
		Lower body	Portal	Hepatic	Lower body	Portal	Hepatic	Lower body	Portal	Hepatic	Lower body	Portal	Hepatic
	min^{-1}	%			%			%					
4	0.086	0.4 ±1.1	0.8 ±1.4		−0.6 ±0.7	2.4 ±0.6		1.6			−3.0	25	
5	0.100	−0.8 ±1.2	2.3 ±1.2	12 ±2	−1.8 ±1.6	3.1 ±1.3	9 ±2	−4.4	15	74	−7.2	30	75
9	0.073	0.7 ±0.7	2.3 ±0.7	8 ±1	−1.0 ±0.7	1.2 ±1.3	10 ±1	4.1	17	61	−5.4	8	77
10	0.093	−0.3 ±0.5	0.6 ±0.9	11 ±1	−1.1 ±3.0	1.4 ±4.2	10 ±7	−1.3	4	93	−1.8	8	103
Mean	0.088 ±0.006	0.0 ±0.3	1.5 ±0.5	10 ±1	−1.1 ±0.3	2.0 ±0.4	10 ±1	0.0 ±2.0	12 ±4	76 ±9	−4.4 ±1.2	18 ±4	85 ±9

* Mean value ±SEM.

glycerides. The value of 13% for net direct uptake of TGFA for the dog (Table V), is somewhat lower than estimated previously by Nestel, Havel, and Bezman (6). Even less (9%) was removed directly in sheep. Thus, the liver is a minor site of direct incorporation of chylomicron triglyceride in these species. Whether the liver removes any triglycerides from undegraded chylomicrons remains uncertain, since it is quite possible that the partially degraded particles still contain a small fraction of the triglyceride originally present in addition to cholesterol. Our results are, therefore, consistent with the interpretation that hepatic removal of intact chylomicrons (as in perfused rat liver) is limited. From the evidence presented by Wisse (17) on the size of the pores in the sieve plates of the hepatic endothelium in rats, it seems possible that in this species many chylomicrons may effectively be excluded from direct contact with the parenchymal cells.

The generally comparable results obtained for removal of chylomicron triglyceride and cholesterol in dogs and sheep raise new questions about the mechanism by which chylomicrons or chylomicron skeletons are taken up in the liver. The electron microscopic radioautographic studies of Stein and Stein (18) provide no support for endocytosis of chylomicrons by parenchymal cells as shown by the intravenous injection of glycerol-^{3}H-labeled chylomicrons into rats, although labeled particles were observed in the space of Disse. Similar studies with cholesterol-^{3}H-labeled chylomicrons (33) indicate that chylomicron cholesteryl esters are concentrated at the plasma membrane of parenchymal cells and are hydrolyzed as the labeled cholesterol appears in intracellular organelles. In ruminants, a well-developed basement membrane is present (19, 20) and sinusoidal pores may not be found (19). Electron microscopic studies of the livers of some of the sheep used in the present investigation (34) have confirmed the presence of a basement membrane and have shown a continuous endothelial lining with attenuated regions containing fenestrae about 700 Å in diameter which are uniformly closed by diaphragms. The ready uptake of chylomicron-cholesteryl esters in the sheep suggests, therefore, that removal of chylomicron skeletons may not depend upon the porous structure of the sinusoidal membrane characteristic of nonruminants (17). Whether the mechanism of uptake in the sheep is similar to that of rats is unknown. The site of uptake of chylomicron-cholesteryl esters in the extrahepatic splanchnic region of sheep is also uncertain, but it is of interest to note that the capillary structure of intestinal mucosa in several species is similar to that observed in hepatic sinusoids of sheep (15). Further studies are required to determine whether and how macromolecules pass across hepatic sinusoids of ruminants.

Deposition of chylomicron TGFA in tissues depends not only upon incorporation of those fatty acids which are hydrolyzed in a given capillary bed, but also upon uptake of fatty acids which rapidly recirculate in the blood. In both sheep and dogs, about half of the TGFA hydrolyzed in the lower body and extrahepatic splanchnic region is recycled as FFA (Table IV); the remainder presumably enters and is oxidized or incorporated into lipids in parenchymal cells adjacent to the site of hydrolysis in the capillary endothelium. These results confirm earlier studies on the metabolism of chylomicron triglycerides in the dog (2) and show that the bulk of hydrolysis to form FFA is extrahepatic. However, in dogs, about 40% of the TGFA entering the liver also were rapidly returned to the blood as FFA. Since virtually no FFA enter the blood from liver of dogs in the usual postabsorptive state (35), it is likely that this hydrolysis of chylomicron triglycerides occurs before incorporation into intracellular pools. Isolated liver cells from rats can bind chylomicrons, hydrolyze their constituent TGFA, and release the FFA into the incubation medium (36). Rat liver contains a lipoprotein lipase which has been considered to be inactive (37, 38). Dog liver contains a lipase that can be released into the blood by injection of heparin into the portal vein (39–41). Our studies are consistent with the presence of an active lipoprotein lipase at a cell surface in dog liver. According to Hamilton (41), the enzyme which is released during a single passage of heparin through the hepatic vascular bed, hydrolyzes TG of lymph chylomicrons much more slowly than TG in an artificial fat emulsion. This observation could be relevant to the utilization of TG in chylomicron skeletons.

In sheep, recycling in the blood of FFA derived from direct hepatic uptake of TGFA, was much more limited (about 10%) than in dogs (about 40%). Because of the variability of this value in individual sheep (Table IV), it is uncertain whether any such FFA are indeed released from the liver in this species. Further studies are needed to determine whether the difference in hepatic metabolism of chylomicron TGFA in sheep and dogs is related to the distinct sinusoidal structure or to dissimilar enzymatic mechanisms.

ACKNOWLEDGMENTS

We thank B. Carlander, C. Benjamin, and L. Jones for expert technical assistance and Doctors R. L. Hamilton and A. L. Jones for their critical reading of the manuscript.

These studies were supported by United States Public Health Service Grants HE-06285 and AM-05976.

REFERENCES

1. Bergman, E. N., R. J. Havel, B. M. Wolfe, and T. Bøhmer. 1970. Metabolism of chylomicron triglyceride

(TG) and cholesterol (C) by liver and extrahepatic (EH) tissues in sheep. *Fed. Proc.* **29**: 327.
2. Havel, R. J. 1965. Metabolism of lipids in chylomicrons and very low-density lipoproteins. *In* Handbook of Physiology-Adipose Tissue. A. E. Renold, and G. F. Cahill, Jr., editors. Williams & Wilkins Co., Baltimore, Md. 499.
3. Biggs, M. W. 1957. Studies on exogenous cholesterol metabolism in human atherosclerosis with the aid of isotopes. *Advan. Biol. Med. Phys.* **5**: 357.
4. Goodman, DeW. S. 1962. The metabolism of chylomicron cholesterol ester in the rat. *J. Clin. Invest.* **41**: 1886.
5. Lossow, W. J., N. Brot, and I. L. Chaikoff. 1962. Disposition of the cholesterol moiety of a chylomicron-containing lipoprotein fraction of chyle in the rat. *J. Lipid. Res.* **3**: 207.
6. Nestel, P. J., R. J. Havel, and A. Bezman. 1963. Metabolism of constituent lipids of dog chylomicrons. *J. Clin. Invest.* **42**: 1313.
7. Morris, B. 1963. The metabolism of free fatty acids and chylomicron triglycerides by the isolated perfused liver of the rat. *J. Physiol. (London).* **168**: 564.
8. Felts, J. M., and P. A. Mayes. 1965. Lack of uptake and oxidation of chylomicron triglyceride to carbon dioxide and ketone bodies by the perfused rat liver. *Nature (London).* **206**: 195.
9. Felts, J. M. 1965. The metabolism of chylomicron triglyceride fatty acids by perfused rat livers and by intact rats. *Ann. N. Y. Acad. Sci.* **131**: 24.
10. Ontko, J. A., and D. B. Zilversmit. 1967. Metabolism of chylomicrons by the isolated rat liver. *J. Lipid Res.* **8**: 90.
11. Nestel, P. J., R. J. Havel, and A. Bezman. 1962. Sites of initial removal of chylomicron triglyceride fatty acids from the blood. *J. Clin. Invest.* **41**: 1915.
12. Belfrage, P. 1966. Metabolism of chyle triglycerides in the liver. I. Studies on the mechanisms for liver uptake of intravenously injected, glycerol- and fatty acid-labeled chyle in the carbohydrate-fed rat. *Biochim. Biophys. Acta.* **125**: 474.
13. Schotz, M. C., B. Arnesjö, and T. Olivecrona. 1966. The role of the liver in the uptake of plasma and chyle triglycerides in the rat. *Biochim. Biophys. Acta.* **125**: 485.
14. Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J. Clin. Invest.* **49**: 465.
15. Fawcett, D. W. 1963. Comparative observations on the fine structure of blood capillaries. *In* The Peripheral Blood Vessels. J. L. Orbison and D. E. Smith, editors. Williams & Wilkins Co., Baltimore, Md. 17.
16. Havel, R. J. 1958. Transport and metabolism of chylomicra. *Am. J. Clin. Nutr.* **6**: 662.
17. Wisse, E. 1970. An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *J. Ultrastruct. Res.* **31**: 125.
18. Stein, O., and Y. Stein. 1967. The role of the liver in the metabolism of chylomicrons, studied by electron microscopic autoradiography. *Lab. Invest.* **17**: 436.
19. Wood, R. L. 1963. Evidence of species differences in the ultrastructure of the hepatic sinusoid. *Z. Zellforsch.* **58**: 679.
20. Kuhn, N. O., and M. L. Olivier. 1965. Ultrastructure of the hepatic sinusoid of the goat Capra hircus. *J. Cell. Biol.* **26**: 977.
21. Katz, M. L., and E. N. Bergman. 1969. A method for simultaneous cannulation of the major splanchnic blood vessels of the sheep. *Amer. J. Vet. Res.* **30**: 655.
22. Shoemaker, W. C., W. F. Walker, T. B. van Itallie, and F. D. Moore. 1959. A method for simultaneous catheterization of major hepatic vessels in a chronic canine preparation. *Amer. J. Physiol.* **196**: 311.
23. Lascelles, A. K., and B. Morris. 1961. Surgical techniques for the collection of lymph from unanesthetized sheep. *Quart. J. Exp. Physiol.* **46**: 199.
24. Doemling, D. B., and F. R. Steggerda. 1960. Chronic thoracic duct-venous shunt preparation in dogs. *J. Appl. Physiol.* **15**: 745.
25. Katz, M. L., and E. N. Bergman. 1969. Simultaneous measurements of hepatic and portal venous blood flow in the sheep and dog. *Amer. J. Physiol.* **216**: 946.
26. Dole, V. P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35**: 150.
27. Havel, R. J., J. P. Kane, E. O. Balasse, N. Segel, and L. V. Basso. 1970. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J. Clin. Invest.* **49**: 2017.
28. Havel, R. J., J. M. Felts, and C. M. Van Duyne. 1962. Formation and fate of endogenous triglycerides in blood plasma of rabbits. *J. Lipid Res.* **3**: 297.
29. Sperry, W. M., and M. Webb. 1950. A revision of the Schoenheimer-Sperry method for cholesterol determination. *J. Biol. Chem.* **187**: 97.
30. Bergman, E. N., M. L. Katz, and C. F. Kaufman. 1970. Quantitative aspects of hepatic and portal glucose metabolism and turnover in sheep. *Amer. J. Physiol.* **219**: 785.
31. Brockway, J. M., and E. H. McEwan. 1969. Oxygen uptake and cardiac performance in the sheep. *J. Physiol. (London).* **202**: 661.
32. Quarfordt, S. H., and DeW. S. Goodman. 1966. Heterogeneity in the rate of plasma clearance of chylomicrons of different size. *Biochim. Biophys. Acta.* **116**: 382.
33. Stein, O., Y. Stein, DeW. S. Goodman, and N. H. Fidge. 1969. The metabolism of chylomicron cholesteryl ester in rat liver. A combined radioautographic-electron microscopic and biochemical study. *J. Cell Biol.* **43**: 410.
34. Grubb, D. J., and A. L. Jones. 1971. Ultrastructure of hepatic sinusoids in sheep. *Anat. Rec.* **170**: 75.
35. Basso, L. V., and R. J. Havel. 1970. Hepatic metabolism of free fatty acids in normal and diabetic dogs. *J. Clin. Invest.* **49**: 537.
36. Green, C., and J. A. Webb. 1964. The uptake of chylomicron fatty acids by isolated liver cells. *Biochim. Biophys. Acta.* **84**: 404.
37. Felts, J. M., and P. A. Mayes. 1967. Release of lipoprotein lipase from the perfused liver of the rat. *Nature (London).* **214**: 620
38. Mayes, P. A., and J. M. Felts. 1968. The functional status of lipoprotein lipase in rat liver. *Biochem. J.* **108**: 483.
39. LeQuire, V. S., R. L. Hamilton, R. Adams, and J. M. Merrill. 1963. Lipase activity in blood from the hepatic and peripheral vascular beds following heparin. *Proc. Soc. Exp. Biol. Med.* **114**: 104.
40. Boberg, J., L. A. Carlson, and L. Normell. 1964. Production of lipolytic activity by the isolated, perfused dog liver in response to heparin. *Life Sci.* **3**: 1011.
41. Hamilton, R. L., Jr. 1964. *In* Postheparin Plasma Lipase from the Hepatic Circulation. University Microfilms, Inc., Ann Arbor, Michigan.