JCI The Journal of Clinical Investigation

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J Clin Invest. 1979;64(1):162-171. https://doi.org/10.1172/JCI109435.

Research Article

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The chemical composition of this HDL fraction contains relatively more phospholipid and less cholesterol ester than normal rat HDL. Because of the difference in composition of HDL between normal rats and those given CM, we estimate that the HDL phospholipid pool increased by ≅ 25% by the infusion of ≅ 4-5 mg of CM phospholipid. Approximately 5 mg of phospholipid is secreted on CM by a fed rat in 1 h.

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ABSTRACT Small chylomicrons (CM) labeled with cholesterol, cholesterol ester, phospholipid, and, in some cases, protein, were used to study the fate of these constituents as the CM are catabolized in the circulations of the hepatectomized and intact rat. In the hepatectomized animal after ½ h, CM are greatly reduced in volume, surface area, and diameter. During this period, the CM lost >92% of the mass of their triacylglycerol, >77% of the mass of their phospholipid, and >39% of their protein. Compared to the injected CM, the chemically altered particles, called CM "remnants," have a reduction in volume of 96% and in surface area of 88%. The labeled cholesterol esters remain with the CM remnants but, strikingly, a major fraction of the labeled phospholipids and labeled soluble apoproteins leave the CM and are found in the high density lipoprotein (HDL) fraction.

The chemical composition of this HDL fraction contains relatively more phospholipid and less cholesterol ester than normal rat HDL. Because of the difference in composition of HDL between normal rats and those given CM, we estimate that the HDL phospholipid pool increased by ≅25% by the infusion of ≅4–5 mg of CM phospholipid. Approximately 5 mg of phospholipid is secreted on CM by a fed rat in 1 h.

The findings in hepatectomized rats indicate that a major fraction of the phospholipid and a minor fraction of the protein (soluble non-B apoproteins) of newly secreted CM are transferred from the CM to the HDL fraction during remnant formation. The same process probably occurs in intact rats except that the remnant particles are rapidly removed from the plasma by the liver and a smaller fraction of the surface of the CM enters the HDL fraction.

INTRODUCTION

The increase in human plasma high density lipoproteins (HDL)[1] phospholipids that accompanies absorption of a fatty meal was described as early as 1957 (2), but its metabolic basis remains unknown. In rats, as chylomicron (CM) or very low density lipoprotein (VLDL) triglycerides are hydrolyzed, cholesterol ester-rich "remnant" particles are formed (3, 4). During remnant formation in hepatectomized rats, it was shown that CM diameter decreased from ≅200 to ≅90 nm (3). This corresponds to a reduction in surface area of ≅80%. Mjøs et al. (4) also showed that triglyceride-rich particles (CM and VLDL) lose surface components. In intact rats 63% of ^{32}P-labeled CM phospholipids stayed in plasma after the CM remnants were taken up by the liver indicating loss of CM surface during CM catabolism (4). In the present experiments, CM, double labeled in vivo with [^{3}H]cholesterol and either [^{14}C]phosphatidylcholine (PC) or ^{35}S-apoproteins were injected into rats to trace the fate of CM components during CM remnant formation. From mass and radiolabeled studies, we conclude that during CM catabolism a major fraction of the surface components of the CM especially phospholipids enter the HDL fraction.

METHODS

To obtain CM, male Wistar rats weighing 180–250 g (Charles River Breeding Laboratories, North Wilmington, Mass.) were

This work was presented in part at the American Heart Association Meetings, November 1978 (1).

Received for publication 24 April 1978 and in revised form 30 January 1979.

[1] *Abbreviations used in this paper*: CM, chylomicron(s); HDL, high density lipoprotein(s); LCAT, lecithin-cholesterol-acyltransferase; PC, phosphatidylcholine; VLDL, very low density lipoprotein(s).

J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/79/07/0162/10 $1.00*
Volume 64 July 1979 162–171

prepared with thoracic duct (5) and intestinal cannulae and maintained postoperatively as previously described (6). 1 d after surgery, a sonicated lipid emulsion was injected through the intestinal cannula at 1–2 ml/h. The emulsions contained pure egg PC (Lipid Products, South Nutfield, Surrey, England) 16 mg/ml; triacylglycerol, 20 mg/ml; cholesterol, 0.2 mg/ml; [1,2-^{3}H]cholesterol, 1.6 μCi/ml (New England Nuclear, Boston, Mass.); and *N*[*methyl*-^{14}C]egg PC, 2 μCi/ml. Radioactive PC was synthesized from enzymatically prepared phosphatidic acid and [*methyl*-^{14}C]choline chloride (New England Nuclear) (7): sp act was 6.8 Ci/mol. In some experiments, L-^{35}S-methionine (New England Nuclear; sp act 573 Ci/nmol) was added to the emulsion at a concentration of 10–20 μCi/ml. Milky lymph was collected in the presence of EDTA and ethyl mercurithiosalicylate (6).

CM were isolated by centrifugation in a discontinuous salt gradient in the SW25-2 rotor of the Beckman L2-65 ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) (8). The gradient was prepared from 12-ml NaCl solutions of *d* 1.006, 1.020, 1.041, and 1.065 g/ml. After removing cells by low-speed centrifugation, the lymph was adjusted to a *d* = 1.1 g/ml with solid KBr, degassed, and then 12 ml as carefully injected under the gradient. The gradient was centrifuged first for 21.5 min at 15,000 rpm (38,460 *g*-max). The rotor was stopped with the brake on and the top 0.5 cm was removed (fraction 1). The centrifuge was then started and run for 72.5 min at 22,500 rpm (84,290 *g*-max). The rotor was again stopped with the brake and the top 0.5 cm of the gradient was removed (fraction 2). The two fractions were resuspended in 0.15 M NaCl containing EDTA 0.1 mg/ml. Fraction 1 contained large particles (>220 nm Diam) but <25% of the CM mass, whereas fraction 2 contained the smaller particles (75–220 nm Diam). The smaller CM were used within 2 d to produce remnants.

Remnant particles were prepared in functionally hepatectomized rats anesthetized lightly with ether (4). 10 U of heparin and 1 ml of a milky suspension of 75–220 nm Diam CM containing 47±18 mg (SD) of total lipid was injected into a tail vein. This dose was equivalent to ≅1 h production of lymph. After 30 min, blood was collected by cardiac puncture into EDTA and 5,5-dithionitrobenzoic acid (9) (final concentrations 5 and 2 mM, respectively) and the plasma was harvested by centrifugation at 1.5 *g* for 15–30 min. The plasma always appeared clear, the turbidity of the injected CM having disappeared. The clearing indicated that the size of the CM was reduced. The plasma was then adjusted to *d* 1.10 g/ml by the addition of solid KBr and 12 ml as loaded under the gradient described earlier and centrifuged for 20 h at 20,000 rpm (66,000 *g*-max). Eight separate fractions were removed and their density measured in an Anton Paar density meter (Graz, Austria). To obtain an HDL fraction, the bottom three fractions (*d* > 1.063) were pooled, the density readjusted to 1.21 g/ml, and then recentrifuged (10). The HDL fraction was examined for chemical composition, radioactivity, and for size in four animals by Na phosphotungstate-negative stain electron microscopy. The HDL fraction of a normal rat and two hepatectomized rats given heparin but not CM, isolated using the same procedures as CM-infused animals, were examined by negative-stain electron microscopy for comparisons to CM-infused animals.

The fate of CM constituents was traced in intact nonfasted, nonheparin-treated 250-g male rats given (by tain vein) 25 mg of a CM suspension labeled with [^{3}H]cholesterol and cholesterol ester and [^{14}C]PC. After 5, 15, or 30 min, rats were exsanguinated by cardiac puncture and the liver was removed. The livers were extracted for lipid analysis and radioactivity. Plasma was fractionated by sequential ultracentrifugation at *d* 1.006, 1.063, and 1.21 g/ml in the Spinco 40.3 rotor (Beckman Instruments, Inc., Spinco Div.) (10) and the lipoprotein fractions were analyzed for lipid and radioactivity. Rat plasma volume was calculated from the relationship 0.175 $W^{0.725}$ where W = body weight (11).

Lipids were extracted in 25 vol of chloroform-methanol (2:1 [vol/vol]), and lipid composition was analyzed by quantitative thin-layer chromatography and densitometry (12, 13). Total lipoprotein protein was estimated by the method of Lowry et al. (14) using bovine serum albumin as a standard. Lipid turbidity was extracted with $CHCl_3$ after color development. B and non-B peptides were estimated by the method of Kane (15) except that B peptides were precipitated by 2-propanol. The conditions for ultracentrifugation were calculated by applying the nomogram of Dole and Hamlin (16), assuming that the gradient retained its discontinuities of density throughout centrifugation. The mean diameter of lipoprotein particles was calculated from their chemical composition assuming spherical particles with all apolar lipids in the core and all polar lipids and protein in a 2.15-nm thick surface shell (17).

RESULTS

Recovery of labeled CM constituents. When CM were injected into hepatectomized rats, and allowed to circulate for 30 min, 91.6±2.32% (mean±SEM, *n* = 24) of total cholesterol, 91.9±1.96% (*n* = 24) of PC, and 100±4.53% (*n* = 4) of protein radioactivity were recovered in the plasma compartment. These recoveries indicate that the cholesterol, PC, and protein moieties of the injected CM remain largely in the plasma of the hepatectomized rat. Of the total PC label recovered, 96±0.3% was as diacylphosphatidylcholine and <4% was recovered as lysophosphatidylcholine. This indicates that very little of the injected PC was hydrolyzed.

The changes in CM composition, mass, and surface area during 30-min circulation. The chemical composition of the injected CM and the resultant remnant-containing fraction from the plasma are given in Table I. Compared with their parent particles, the particles in the remnant fraction were depleted in triacylglycerol and enriched in cholesterol, cholesterol esters, phospholipid, and protein. Knowing the injected dose and composition of the CM and amount of each constituent recovered in the remnant fraction, we have estimated the percent of the total CM-injected mass recovered in the remnant fraction (Table I). Because of lipolysis, only 8% of the triacylglycerol mass is recovered. Furthermore, only 23% of the phospholipid and 59% of the protein is recovered. However, the recovery of free and especially esterified cholesterol is greater than that injected with the CM. These results require some explanation. It was shown that dog CM incubated for 30 min with dog serum increase their free cholesterol by ≅10% but cholesterol esters were neither gained nor lost (8). The gain in cholesterol esters in our remnant fraction is probably explained by the fact that VLDL, which have a similar lipid composition and density compared to the CM

TABLE I
Composition and Recovery of Injected CM

	Injected CM (n = 8)	Remnant fraction (n = 8)	Percent of total CM injected mass recovered in remnant fractions* (n = 7)
		% total mass	
Overall composition			
Triacylglycerol	86.6±1.13‡	56.9±3.51	8±2
Cholesterol ester	1.3±0.18	13.4±2.30	139±30
Cholesterol	0.9±0.17	7.8±0.92	121±30
Phospholipid	9.8±0.95	16.2±2.21	23±7
Protein	0.9±0.14	5.2±0.74	59±10

* This column overestimates the mass recovery of CM components in remnants as the remnant fraction also contains some other plasma lipoproteins of similar density, for instance hepatic VLDL.
‡ Mean±SEM.

remnant, are isolated with the CM remnant and thus contaminate the remnant composition. Because VLDL also contain triacylglycerol, phospholipids, and protein, not only is the "mass recovery" of CM cholesterol ester too high, but the mass recovery of the other constituents is also too high. Therefore, the actual recovery of CM triacylglycerol is <8%, of CM phospholipid <23%, and of CM protein <59%. This indicates that there is a marked loss of both CM triacylglycerol and of surface components during remnant formation.

The size and density of the injected CM are compared to the remnant fraction particles in Table II. The mean diameter of the CM is decreased to 35%, the surface area to 12%, and the mass to 4% of the original. If each CM produced one remnant particle, then not only has the mass of the particle been decreased greatly by the removal of triacylglycerol but the surface of the particle has also been diminished. Thus, both mass recovery and size changes indicate that during circulation of the CM in the hepatectomized rat not only is triacylglyceride removed by lipolysis but also a major portion of the surface is lost from the CM.

The distribution of radiolabeled CM components 30 min after CM injection. 30 min after injection of CM labeled with [^{3}H]cholesterol, and either [^{14}C]PC or ^{35}S-apoproteins, the plasma was isolated and centrifuged on the density gradient. Fig. 1 shows the percentage of recovered label found in fractions of different density. The remnants are contained in fractions 1 and 2 ($d < 1.020$). Most of the plasma cholesterol radioactivity floated with the remnant-containing fractions, but, strikingly, PC and non-B-protein radioactivities were found in the more dense fractions. The majority of the cholesterol label in the remnant fraction was found as cholesterol ester, but free cholesterol accounted for most of the label in the more dense fractions.

The data for all experiments are given in Table III. The data are expressed as three density cuts: a remnant fraction ($d < 1.020$); pooled middle fraction (d 1.025–1.050); and the bottom fractions ($d > 1.063$). About 80% of the recovered [^{3}H]cholesterol floated with the remnant fraction (Table III). Because the total recovery of injected CM [^{3}H]cholesterol in plasma was 91.6% we calculate that ≅73% of the total injected [^{3}H]cholesterol

TABLE II
Calculated Density and Size of CM and the Particles Recovered in the Remnant Fraction

	Injected CM	Remnant fraction particles	CM
			%
Density, *g/ml**	0.93	0.96	
Diameter, *nm*‡	131±12.8	46±3.62	35
Surface area, nm^2‡	53,900±10,500	6,650±1,050	12.3
Molecular weight, $\times 10^8$‡	8.2±2.2	0.34±0.08	4.1

* The density is estimated from the composition given in Table I and the partial specific volumes of the components as given in Sata et al. (17).
‡ The diameter, surface area, and molecular weight are calculated by the method of Sata et al. (17) with the assumptions: (*a*) that the particles are spherical and not widely heterogeneous in size; (*b*) that all the protein, phospholipid, and free cholesterol form a 2.15-mm thick surface shell around a core of triacylglycerol and cholesterol ester; and (*c*) that the apparent partial specific volumes of the individual components are similar to their partial specific volume in the intact lipoproteins.

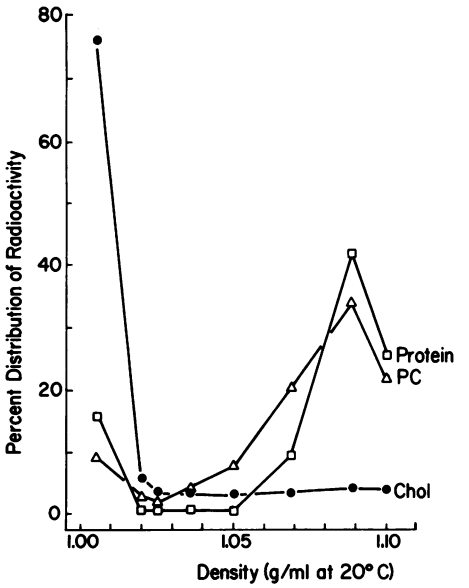

FIGURE 1 Percent distribution at different densities of labeled CM components after injection into hepatectomized rats. Plasma, adjusted to d = 1.10 g/ml, was loaded under a discontinuous density gradient (d 1.065–1.006 g/ml) and centrifuged. Separate density fractions were collected and their densities were measured in an Anton Paar density meter. The least dense fraction was nearly clear and contained most of the remnants. Most of the cholesterol was associated with this fraction and a very high percentage of this cholesterol was esterified. The cholesterol in the more dense fractions was largely unesterified. Labeled PC was present to a small extent in the remnant fraction. Most was found in the more dense fractions. The labeled apoproteins followed a similar pattern. However, the remnant fraction contained both B protein and soluble non-B apoprotein whereas only non-B apoproteins were found in the more dense fractions. Each point is the mean of three separate experiments for PC and cholesterol (Chol), and two for protein. The two least dense fractions were called the remnant fraction. Table III gives the distribution of the remnant fraction (d < 1.020), the pooled middle fractions (d 1.025–1.050), and the bottom fractions (d > 1.063).

remained with the CM remnant. Most of the [^{3}H]cholesterol in the remnant fraction was esterified (79±2.2%). The rest of the [^{3}H]cholesterol was divided between the middle and bottom fractions. The bottom fraction contained 10% of the recovered label or ≅9.2% of the injected label and it was mostly unesterified (17±3.8% esterified). Only 8.6% of the recovered [^{14}C]PC and 15.9% of the recovered ^{35}S-soluble non-B apoprotein was recovered in the remnant fraction. Most of these labels were found in the bottom fractions. We estimate, based on the total recovery of injected radioactivity, that the bottom fraction contained ≅71.4% of the injected CM [^{14}C]PC and 68% of the injected CM ^{35}S-soluble apoproteins.

Recovery of HDL from bottom fractions. By raising the density of the bottom fractions of the density gradient (d > 1.063) to 1.21 g/ml and recentrifuging, an HDL fraction was obtained, composed of 25.8% protein (±2.87), 1.6% triacylglycerol (±1.64), 40.5% phospholipid (±3.48), 24.4% cholesterol ester (±2.89) and 6.8% free cholesterol (±0.87, n = 5). Most of the radioactivity present in the pooled bottom fractions of the density gradient was recovered with this HDL fraction. The recoveries given as a percent of counts in the bottom fraction were: [^{3}H]cholesterol 83.1±2.57% (mean±SEM, n = 5); [^{14}C]PC 71.1±2.20%; ^{35}S-soluble apoprotein 71.0%. The small amount of total cholesterol radioactivity in the HDL fraction was 83% unesterified. Phospholipid radioactivity remained as diacylphosphatide in the HDL fraction with <4% lysophosphatide present. The HDL fraction of four separate animals was negatively stained with Na phosphotungstate and examined by electron microscopy. Fig. 2 shows that even in a fairly dense preparation no rouloux are seen. Most of the particles appear round. A rare collapsed vesicle and a very rare flattened particle resembling a 5.0-nm thick disk on edge was seen in some preparations. These particles represented <1 in 200 particles. Occasional large particles were also seen. The distribution of particle size for each animal are given in Fig. 3. The mean in each animal varied from 17.7 to 24.1 nm. The distributions are rather wide but with the exception of a very few large particles, the distributions are not bimodal. With the same electron microscopy techniques a normal rat HDL had a mean diameter of 13.3±2.3 nm (n = 245) and two control hepatectomized animals given heparin but not CM had a mean diameter of 13.3±3.8 nm (n = 146) and 13.7±4.8 nm (n = 117). These values compare reasonably to diameters of rat HDL estimated by negatively stained electron microscopy by others (18, 19). Thus rats given CM have larger particles in their HDL fraction.

The lack of an appreciable discoidal or vesicle fraction and the increased size of the HDL suggests that surface components of the CM might fuse with normal HDL to produce the larger chemically altered particle.

Injection of labeled CM into intact rats. Because the preceding observations were made in hepatectomized rats, it was necessary to show that the catabolic events described also occurred in the intact animal. Table IV shows the disappearance of labeled cholesterol and PC from the plasma and the appearance of label in the liver after intravenous injection of CM. In accordance with previous reports (3, 4), CM cholesterol disappeared quickly from the plasma. At 5 min, 54–71% of the injected [^{3}H]cholesterol was recovered in plasma but only 5–6% remained 30 min after injection. Cholesterol label removed from the plasma was quantitatively recovered in the liver. Removal of [^{14}C]PC from the plasma was slower, so that 40–44% remained 30 min after injection. Recovery of cleared

TABLE III

*Distribution of CM Radioactivity in Plasma Fractions of Different Density after 30-min Circulation in Hepatectomized Rats**

	[^{3}H]cholesterol			
	% Total	% Esterified‡	[^{14}C]PC	^{35}S-Soluble non-B apoprotein
Top remnant fractions ($d < 1.020$)	79.8±3.6§ (73.1±5.1)	79±2.2	8.6±1.5 (7.9±1.5)	15.9 (15.9)
Middle fractions (d 1.025–1.050)	10.2±3.8 (9.3±3.7)		13.7±2.0 (12.6±2.1)	16.1 (16.1)
Bottom fractions ($d > 1.063$)	10.0±2.5 (9.2±2.5)	17±3.8	77.7±2.5 (71.4±3.9)	68.0 (68.0)

* The values are given as percent of total recovered counts. The values in parentheses give calculated recovery as percent of injected dose. This value was obtained by multiplying the total plasma recovery of each CM label injected ([^{3}H]cholesterol = 91.6%; [^{14}C]PC = 91.9%; ^{35}S-soluble non-B apoprotein = 100%) by the percent total recovered counts in each fraction. The individual values were used to calculate the SEM.

‡ The percent esterified refers to the percent of label of [^{3}H]cholesterol which was esterified. The [^{3}H]cholesterol in the injected CM was 64±2.6% esterified. Thus, the remnant fractions are enriched in labeled cholesterol ester and the bottom fractions which contain HDL are very poor in labeled cholesterol esters.

§ The value for [^{3}H]cholesterol and [^{14}C]PC are mean±SEM of 24 experiments. The values for ^{35}S-soluble apoprotein represent the mean of four separate experiments.

PC in the liver was much less than for cholesterol, but 27–30% of the injected dose was found in this organ after 30 min.

Fractionation of plasma radioactivity after CM injection into the major lipoprotein classes by ultracentrifugation (Table V) showed that [^{3}H]cholesterol ester radioactivity was associated with the lightest fraction ($d < 1.006$ g/ml). However, after 30 min very little cholesterol ester radioactivity was present in any fraction. Cholesterol radioactivity in the HDL fraction (d 1.063–1.21 g/ml) was due mainly to free cholesterol; cholesterol ester was never >2% of the injected dose. In marked contrast, 23–32% of the injected CM [^{14}C]PC radioactivity appeared in the HDL fraction as early as 5 min after injection of CM. Only 14–22% of the injected [^{14}C]PC radioactivity remained in the simultaneously recovered fraction containing the remnants ($d < 1.006$ g/ml). Radioactivity in HDL as a result of [^{14}C]PC remained fairly constant during the 30 min whereas at this time <2% of the injected dose was found at 30 min in $d > 1.006$ g/ml. Because of some loss of some lipoproteins during the process of ultracentrifugal separation of the plasma into lipoprotein fractions, we recovered only 61 and 70% of the counts originally found in plasma. Therefore, the percent of injected CM radioactivity reported in Table V is low by 30–39%. Assuming that the loss is evenly distributed over the three lipoprotein fractions we estimate that the true percent of the injected CM label appearing in each fraction should be ≅30–39% higher. Thus, we estimate that 38–48% of the injected [^{14}C]PC goes to HDL in 5 min, and at 30 min 32–53% is still in the HDL fraction. In contrast, we estimate that 21–36% of the injected [^{14}C]PC remained with the remnant at 5 min but no >3% was present at 30 min. Up to 8% of radioactivity of both isotopes was recovered with the 1.006- to 1.063-g/ml fractions; like the $d < 1.006$-g/ml fraction, this activity declined rapidly for [^{3}H]cholesterol, but, like HDL, it persisted for [^{14}C]PC. This suggests that the 1.006- to 1.063-g/ml fraction may contain at least two populations, one of CM remnants rich in cholesterol ester which are rapidly taken up by the liver and one of PC-rich particles of low protein content allowing flotation at 1.063 g/ml.

Exchange and net loss of phospholipids has been observed when CM are incubated with serum. Up to 20% of dog CM phospholipid mass and somewhat more CM phospholipid radioactivity could be lost in 30-min incubation with dog serum (8). To test for net loss of lipid and loss of radioactive CM phospholipid we incubated 25 mg of fresh rat CM labeled with PC with 10 ml of saline or rat plasma. (A 275-g rat has ≅10 ml of plasma.) The CM were incubated for 0, 5, 15, and 30 min and then isolated on a density gradient. There was no net loss of rat CM triacylglycerol or phospholipid but radioactive phospholipid was lost. In saline, the percent of original phospholipid radioactivity remaining on the CM was 84, 87, and 87% after 5, 15, and 30 min, respectively. In plasma more radioactive phospholipid was lost. 66, 60, and 55% of the original radioactivity remained with the CM after 5, 15, and 30 min, respectively. Thus, since there is no net loss of

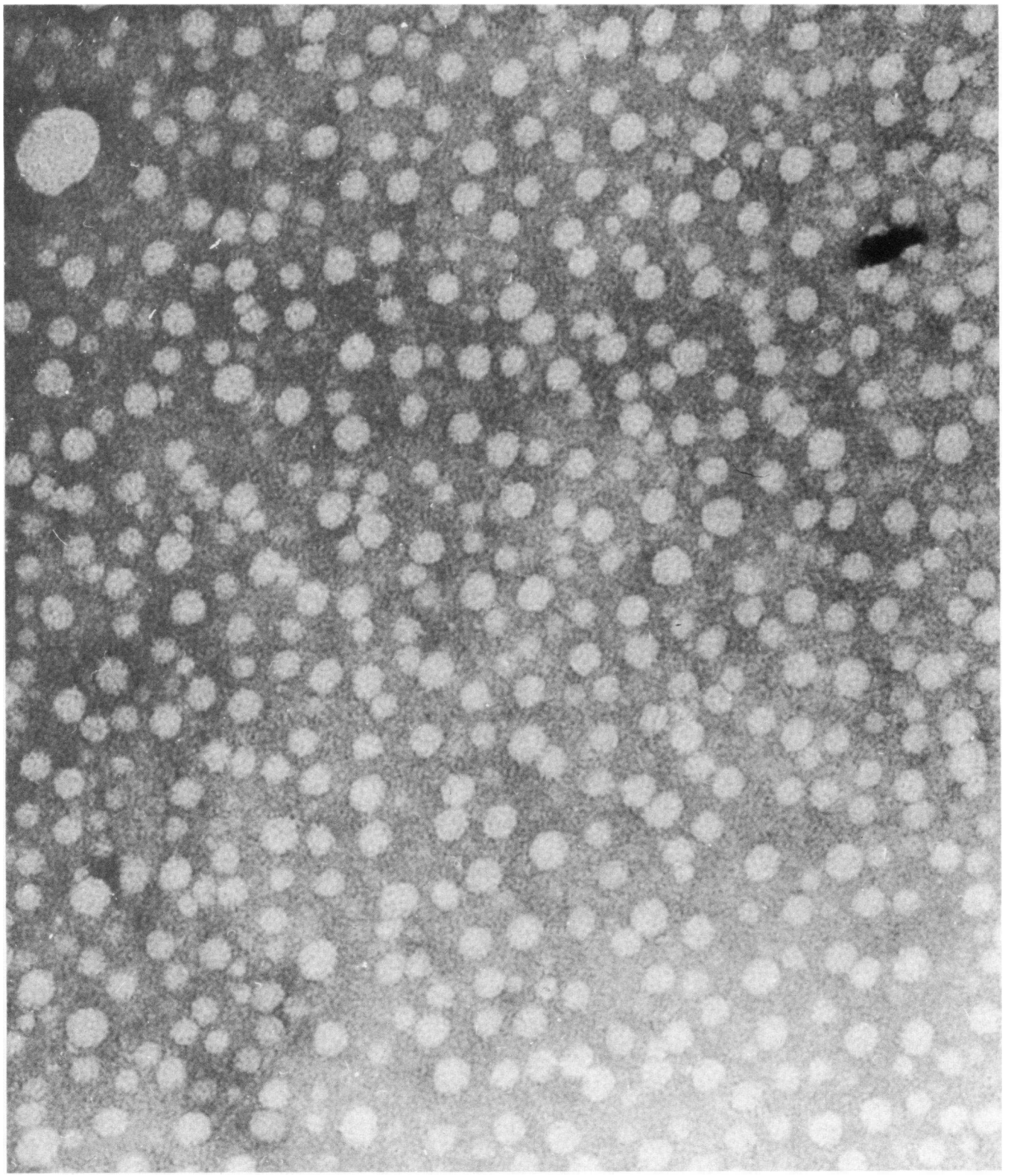

FIGURE 2 Morphology of HDL fraction isolated from CM-injected hepatectomized rat. After the HDL were isolated by ultracentrifugation, they were placed on a Formvar-coated grid and negatively stained with Na phosphotungstate. ×318,000.

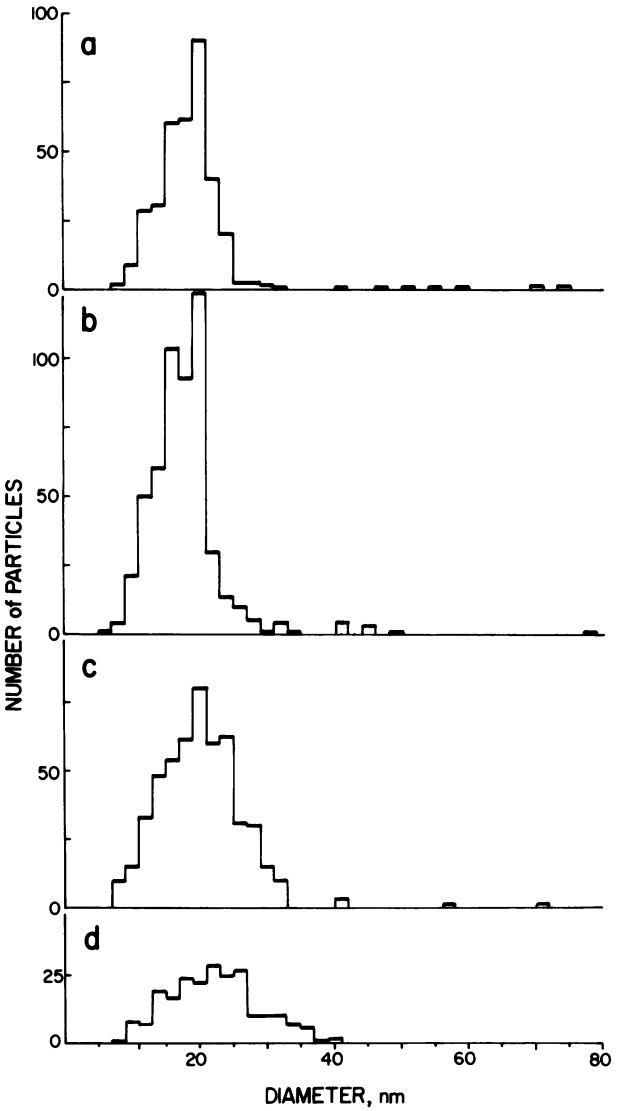

FIGURE 3 Size distribution of HDL fraction isolated from hepatectomized animals infused with CM. The HDL fraction from four animals (*a–d*) were isolated by centrifugation and studied by Na phosphotungstate negative-staining electron microscopy. The particles were measured on random grids and the distribution plotted. The mean, SD, and number of particles counted (*n*) are given for each animal. (*a*) 18.4±3.9 nm, *n* = 364; (*b*) 17.7±4.2 nm, *n* = 553; (*c*) 20.1±5.6 nm, *n* = 527; (*d*) 24.1±6.2 nm, *n* = 215. These mean diameters are considerably larger than the mean values for a normal rat HDL (13.3±2.3 nm, *n* = 245) or for two hepatectomized rats given heparin but not CM (13.3±3.8 nm, *n* = 146 and 13.7±4.8 nm, *n* = 117).

phospholipid, the decrease in CM radioactivity with time indicates that phospholipid exchange occurs.

When CM labeled with [^{3}H]lysine were incubated with rat plasma ≅30% of the labeled protein was lost in 30 min. However, the total mass of CM protein increased by about fourfold. Thus, CM exposure to plasma results in some loss of ^{3}H-labeled protein and a net adsorption of nonlabeled apoproteins. Since only 59% of the total CM-injected protein mass is recovered with the CM remnant (Table I) the movements of soluble apoproteins are complex. We infer that as CM enter plasma there is a rapid increase in apoproteins due to adsorption to the CM but as catabolism to CM remnants occur a larger fraction of apoproteins are released back into plasma.

TABLE IV

Percentages of Injected CM Radioactivities Found in Liver and Whole Plasma 5, 15, and 30 min after CM Injection in Two Groups of Intact Rats

	PC			Total cholesterol		
Rats (*a*)						
Time, *min*	5	15	30	5	15	30
Liver	11	15	30	23	48	108
Plasma	84	72	44	71	46	5
Total recovery	95	88	74	93	94	113
Rats (*b*)						
Time, *min*	5	15	30	5	15	30
Liver	12	17	27	32	63	87
Plasma	76	71	40	54	34	6
Total recovery	98	87	67	86	97	93

DISCUSSION

The changes in CM mass and composition during 30-min circulation in the hepatectomized rat show that as the mass of the CM triacylglycerol is reduced to <8% of the original CM, phospholipid mass is decreased to <23%, and protein to <59%. Triacylglycerol is the major constituent of the core of the CM (20), so its loss results in a shrunken remnant particle, which we estimate contains only ≅4% of the original mass of the CM.

TABLE V

Percentages of Injected CM Radioactivities Found in Isolated Lipoprotein Fractions 5, 15, and 30 min after CM Injection in Two Groups of Intact Rats

Lipoprotein fraction	PC			Cholesterol			Cholesterol ester		
Rats (*a*)									
Time, *min*	5	15	30	5	15	30	5	15	30
d < 1.006 g/ml	22	13	2	20	11	0	56	37	1
d 1.006–1.063 g/ml	6	6	6	7	6	1	5	7	1
d 1.063–1.21 g/ml	23	30	21	9	6	2	1	1	0
Rats (*b*)									
Time, *min*	5	15	30	5	15	30	5	15	30
d < 1.006 g/ml	14	4	1	15	4	1	42	18	1
d 1.006–1.063 g/ml	5	11	6	6	14	2	5	8	2
d 1.063–1.21 g/ml	32	33	21	9	6	2	1	2	1

When the core of a spherical particle shrinks, surface must also be lost if the particle is to remain spherical. With the assumptions (17, 20) that all the protein, phospholipid, and most of the free cholesterol form a 2.15-nm thick surface around a core of triacylglycerol and cholesterol esters, the mean composition of both the CM and the remnant fractions (Table I) are consistent with spherical emulsion particles (Table II). We calculate that the remnant particle has lost ≅88% of its original surface, which compares well with the 77% net loss of phospholipid, the major surface component. During in vitro incubation of CM with plasma triacylglycerol and phospholipids are not lost and proteins are actually increased so the loss of phospholipid and protein occurring during formation of the CM remnant must be linked to that process.

The composition of the HDL fraction isolated 30 min after CM injection in the hepatectomized rat is quite different from normal rat HDL (18, 19). For instance, the relative weight proportions of the three major lipids of normal rat HDL (phospholipids, cholesterol esters, and cholesterol), expressed as weight percent of the sum of the weight of three lipids are: 48% phospholipids, 43% cholesterol esters, and 9% cholesterol. Expressed in the same way the HDL fraction isolated from our rats has 56% phospholipid, 33% cholesterol ester, and 9% cholesterol. Thus, our HDL contain relatively more phospholipid and less cholesterol ester. These changes are illustrated on triangular coordinates (21) in Fig. 4, which shows that the direct addition of about one part CM surface lipids to four parts normal HDL can account for the lipid composition of our CM-injected rat HDL. In absolute terms, we have given these animals ≅47 mg of CM containing ≅4.5 mg of phospholipid. During CM catabolism to remnants ≅77% or ≅3.5 mg of the CM phospholipid was lost. The HDL phospholipid pool in normal rats is between ≅3.4 and 7.3 mg (10, 23, 24). If al the phospholipid lost from the CM were transferred to HDL the preexisting HDL phospholipid pool would be appreciably expanded.

Exchange of PC between CM and HDL (8) is insufficient to account for our observations. About 45% of the PC radioactivity exchanged when 25 mg of CM was incubated with rat plasma for 30 min. However, when a larger amount of similar CM was injected into hepatectomized rats, ≅92% of the injected radioactive PC left the CM by 30 min, and most of it was found in HDL. Because this enormous loss of labeled PC in vivo cannot be accounted for solely by exchange, net loss of both labeled and unlabeled PC must have occurred, consistent with the loss of mass already described.

The CM also loses some of its protein during CM remnant formation (4, 25). Mjøs et al. (4) and Schaefer et al. (25) showed that the proteins lost are almost entirely soluble non-B apoproteins. In our experiments

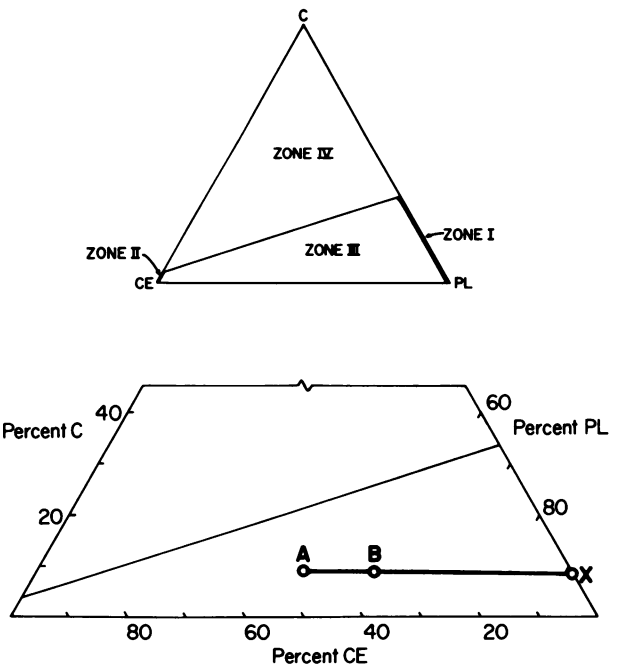

FIGURE 4 Lipid composition of normal HDL and the HDL fraction isolated from CM-injected hepatectomized rats plotted on triangular coordinates. Triangle above represents simplified phase diagram (21) of the major lipid classes of HDL. Zone I contains a single phospholipid lamellar liquid-crystal phase, into which 33% cholesterol (C) and 2% cholesterol ester (CE) can be incorporated. Zone II also contains a single phase, composed of liquid or liquid-crystalline cholesterol ester. Zone III contains both the cholesterol ester and phospholipid (PL) phases. In zone IV, a third phase, cholesterol-monohydrate crystals, is also present. Below, the CM-injected HDL fraction from hepatectomized rats (B) contained more phospholipid and less cholesterol ester than normal rat HDL (A). A line connecting the compositions A and B extended to the cholesterol-phospholipid axis gives the composition of the material which must be added to normal HDL (A) to produce the composition at point B. The composition is given by the point X which is precisely the composition of the CM surface in respect to phospholipid and cholesterol. (From Table I, the CM phospholipid:cholesterol ratio is 90:10.) This indicates that CM surface lipids were added to circulating HDL (A) to produce the HDL fraction of composition B. The ratio of the mass of X to A is given by the law of levers (22). Thus, the ratio of X to A to produce B is (X)/A = (AB)/(BX) = 0.25. Thus, about one part of surface composition X added to four parts of normal HDL will result in composition B.

the CM proteins labeled during ^{35}S-methionine infusion were both insoluble B apoproteins and soluble non-B apoproteins. We presume that most of the soluble apoproteins labeled on our CM were of the A species,which are synthesized in the gut and secreted on CM (26, 27). After infusion the CM loses ≅84% of its labeled soluble apoproteins and most of these are found in the HDL fraction probably because of a net movement of A peptides to HDL during CM remnant forma-

tion. The same mechanism probably operates in man (28). Normal human lymph CM contain a large amount of A-1 peptide (29) and CM isolated from chylous plural effusion (25) or from urine of humans having fistulae between intestinal lymphatics and the urinary tract contain an abundance of A peptides especially A-1 and an A-4-like peptide (30). CM obtained from plasma lack A-1 (31). Thus, once the CM has circulated in plasma, the A-1 is lost. Further, when CM labeled with ^{125}I-A-1 are injected into normal humans, >90% of the labeled peptide appears in HDL in 1 h (32). Finally, both HDL phospholipids (2) and total protein (33) increase during fat feeding.

In the intact rats a similar transfer of phospholipid to HDL appears to occur. 5 min after CM injection only 14–22% of the injected CM [^{14}C]PC remains within the CM remnant formation, whereas in vitro incubation of similar quantities of CM with 10 ml of plasma for 5 min show that much more of the radioactive PC (≅66%) is retained by the CM. This suggests that there is also a net loss of phospholipid from CM to HDL in the intact animal. Most of the CM [^{14}C]PC lost can be recovered in the HDL fraction and at this time only a very small percent of the injected dose (≅11%) is found in the liver. However, because remnants are rapidly taken up by the liver, some of the [^{14}C]PC which otherwise goes to HDL in hepatectomized animals enters the liver at 15 and 30 min. In the intact animal, CM remnants larger than those produced by 30 min catabolism in the hepatectomized rat will be taken up by the liver.

Both the perfused rat liver (18) and rat intestine (19) secrete discoidal "nascent" HDL particles which contain mainly surface components (phospholipids, free cholesterol, apoproteins) but very little cholesterol ester. The importance of lecithin-cholesterol-acyltransferase (LCAT) in the conversion of discoidal HDL particles to spherical HDL has been shown in vitro (18) and implicated in LCAT deficiencies. Familial LCAT deficiency (34) and the LCAT deficiency in alcoholic hepatitis (35) are both associated with the presence of discoidal HDL in the plasma. Compared to these discoidal nascent particles, HDL isolated from normal plasma are spherical (19, 34) and contain relatively more cholesterol ester. Discoidal particles are not readily found in the normal HDL fraction and it is likely that after their formation, they are acted on by LCAT which leads to increased cholesterol esters, decreased PC, and the subsequent structural change from disk to sphere (18, 36).

In our experiments the composition and size of the HDL fraction isolated from CM-injected, hepatectomized animals are different from normal rat HDL. It contains more phospholipid, less cholesterol ester, and the particles are larger than normal rat HDL. The LCAT reaction would form new cholesterol esters and simultaneously decrease phospholipid. Thus, the particle would subsequently increase core components (cholesterol esters) and decrease surface components (phospholipids). However, it would gain an absolute amount of cholesterol ester so its mass and diameter would be bigger than the original circulating HDL. Such a mechanism might explain the rather specific increase in the larger HDL (HDL_2) found after fat feeding (33).

Significant formation of lysophosphatide was shown by Eisenberg and Schurr (37) when rat plasma VLDL were degraded in vitro by postheparin plasma, leading to the conclusion that hydrolysis of PC was a major mechanism for the removal of phospholipids from VLDL during their degradation. Our data in vivo suggest that very little PC is converted to lysophosphatide during CM catabolism, so we must conclude either that the surface phospholipids of CM and plasma VLDL are metabolized by different mechanisms or that studies in vitro must be applied with caution to physiological events in vivo.

Fresh CM produced by the intestine have a very low cholesterol-phospholipid ratio (0.18), whereas remnant has a very high ratio (0.95) (Table I). Therefore, the surface lipids of the CM can rapidly pick up cholesterol. The HDL fraction isolated after CM injection also has a low cholesterol-phospholipid ratio and may accept cholesterol from other tissues (38). Thus, the addition of large amounts of phospholipid to the HDL fraction not only increases one of the substrates for LCAT but also permits the other to enter the HDL fraction from other lipoproteins and cell membranes. Such a mechanism might protect against tissue cholesterol accumulation and atherosclerosis. Man has a fasting pool of HDL phospholipid of ≅1.5 g (39). After a fatty meal, man can absorb 50 g of triacylglycerol which would be secreted into lymph as CM containing ≅3 g phospholipid. If only 25% of the CM phospholipid entered the HDL fraction it would account for half the pool of HDL phospholipid, and would be important in the formation of HDL and cholesterol homeostasis (38).

ACKNOWLEDGMENTS

We wish to thank Dr. Barry Sears for help in preparing labeled phospholipid and Dr. David Waugh, Dr. Susanne Bennett Clark, and Ms. Vicki Rosenberger for help with the experiments.

This investigation was supported in part by a Public Health International Research Fellowship FO5 TW2407-01, and U. S. Public Health Service grants HL18623 and TG 17291.

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