Nascent lipoproteins from recirculating and nonrecirculating liver perfusions and from the hepatic Golgi apparatus of African green monkeys

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Abstract Perfusate apoB-100-containing lipoproteins from the isolated, perfused livers of African green monkeys consist of significant amounts of $d > 1.006$ g/ml particles in addition to very low density lipoproteins (VLDL). Distinguishing characteristics of these perfusate lipoproteins are the relative abundance of surface lipids and deficiency of core lipids. The present studies were performed to determine the likelihood that the $d > 1.006$ g/ml perfusate lipoproteins are secretion products instead of products of post-secretory modification (e.g., lipolysis) of secreted VLDL. [^{14}C]Leucine from the perfusate became incorporated into the apoB of each of the perfusate lipoprotein classes to a similar extent in both recirculating and nonrecirculating perfusions. When endogenously radiolabeled perfusate VLDL from one liver was recirculated through a second liver, only about 15% of the radiolabeled protein appeared in the $d > 1.006$ g/ml fraction. The particle morphology and the cholesterol and apoB distribution between VLDL and $d > 1.006$ g/ml fractions were similar in recirculating and nonrecirculating perfusions. A Golgi apparatus-rich fraction was isolated from the homogenates of fresh liver samples and the isolated Golgi VLDL and $d > 1.006$ g/ml lipoproteins exhibited morphologic evidence of extra surface material analogous to that seen in perfusate. Taken together, these data support the possibility that significant amounts of $d > 1.006$ g/ml lipoproteins, many with surface-rich properties, are nascent, secretory products of the primate liver. The low level of lecithin:cholesterol acyltransferase (LCAT) in this perfusion system appears to permit detection of these secretion products and it is significant to note that the perfusate lipoprotein profile, which is unlike that of normal plasma, is similar to that of LCAT-deficient patients—**Johnson, F. L., L. L. Swift, and L. L. Rudel.** Nascent lipoproteins from recirculating and nonrecirculating liver perfusions and from the hepatic Golgi apparatus of African green monkeys. *J. Lipid Res.* 1987. **28:** 549–564.

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The strong associations that have been observed between the incidence of coronary artery atherosclerosis and the composition and concentration of plasma lipoproteins have stimulated interest in understanding the metabolic events that determine these plasma lipoprotein parameters. Much effort has been invested in studies of the structure, formation, and catabolism of lipoproteins and it is becoming increasingly apparent that the lipoproteins normally circulating in blood plasma represent the end products of extensive intravascular modifications of particles that are secreted by the liver and intestine. Nascent particles acquire apoproteins (1–3), cholesteryl esters (4), free cholesterol (5), and other lipids through protein-mediated and nonmediated exchange processes (6–8). These particles also lose neutral lipids and phospholipids via enzyme-catalyzed lipolysis (9, 10) as well as through protein-mediated transfer (11). All of this occurs rapidly relative to the residence time of the particle in the plasma. Consequently, it is difficult to determine the properties of lipoprotein particles before their entrance into the plasma, but knowledge of the structure and composition of nascent lipoproteins will be necessary for complete understanding of the extent of intravascular metabolism of lipoproteins. In turn, such information may provide insight into the role of lipoprotein secretion in determination of atherogenic lipoprotein profiles.

To this end we have conducted studies in which we have described the types and amounts of lipoproteins produced by the isolated, perfused livers of monkeys fed atherogenic diets mimicking those of North Americans (12, 13). Regardless of the fat or cholesterol content of the diet, a common feature of the lipoproteins accumulating in the recirculating perfusate is their relative deficiency of cholesteryl esters and their relative abundance of phospholipid and free cholesterol. The newly secreted LDL and HDL structurally and compositionally resem-

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; ELISA, enzyme-linked immunosorbent assay.

ble the lipoproteins found in the plasma of patients with familial lecithin:cholesterol acyltransferase (LCAT) deficiency (14–16) and the LCAT activity in the primate liver perfusates was found to be only about 2% of that in plasma (17).

Therefore, it appears that the isolated, perfused primate liver is a source of significant quantities of nascent lipoproteins relatively unmodified by post-secretory events. However, the particles accumulating in the recirculating liver perfusates may have been altered from their truly nascent state by interacting with the liver itself or with the erythrocytes of the perfusate. The present report describes our efforts to determine the extent to which newly secreted hepatic apoB-100-containing lipoproteins were altered during perfusion. We have specifically examined the possibility that, for primate livers, perfusate $d > 1.006$ g/ml apoB-100-containing lipoproteins (which includes particles rich in excess surface phospholipid and deficient in core lipids) were secreted as such instead of being derived from VLDL during recirculating perfusion. We compared the newly synthesized apolipoproteins, chemical composition, and morphology of perfusate lipoproteins from nonrecirculating and recirculating primate liver perfusions, monitored the fate of hepatic VLDL during recirculating perfusion, and examined the lipoproteins of the isolated liver Golgi apparatus. The results suggest that a variety of apoB-100-containing lipoproteins, in addition to VLDL, are hepatic secretory products.

MATERIALS AND METHODS

Animals and diets

Adult male African green monkeys *(Cercopithecus aethiops)* of the vervet subspecies were purchased from Primate Imports, Port Washington, NY, and were maintained in two different diet groups as part of other ongoing studies described previously (13). One group was maintained on semisynthetic diets containing 0.78 mg of cholesterol/kcal and 40% of calories as either butter fat or safflower oil. The other group was fed similar diets in which lard was substituted for butter fat and the source of the cholesterol was egg yolk rather than crystalline cholesterol.

Liver perfusion

Perfusion of the isolated livers by recirculation was performed for 4 hr (after a 90-min wash-out period) with 280–320 ml of lipoprotein-free medium circulated through the liver at 38°C at a rate of 0.9–1.1 ml/min per g liver as described previously (13). The medium consisted of Krebs-Henseleit original Ringer bicarbonate buffer containing glucose, amino acids, insulin, cortisol, penicillin, and streptomycin and with a 19–22% hematocrit of washed fresh human erythrocytes (13). To radiolabel newly synthesized apoproteins, 1.0 mCi L-[^{14}C(U)]leucine (New England Nuclear, Boston, MA) was included in the medium of recirculating perfusions. Perfusion of livers without recirculation was performed with 8–11 liters of medium with a 10% hematocrit. The flow rate was the same as for recirculating perfusion and required 90–120 min of perfusion to complete these experiments. One millicurie of [^{14}C]leucine was also included in the medium of nonrecirculating perfusions.

Lipoprotein isolation and characterization

For recirculating perfusions, the perfusate was collected on ice and adjusted to 0.04% 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 0.1% EDTA, 0.1% NaN_3, pH 7.4. Erythrocytes were removed by centrifugation. Isolation of VLDL by ultracentrifugation was begun immediately and the remaining perfusate lipoproteins were subsequently isolated by ultracentrifugation at d 1.225 g/ml and were fractionated by gel filtration as described previously (13). All isolated lipoprotein fractions were dialyzed to 0.01% EDTA, 0.02% NaN_3, pH 7.4, and stored at 4°C. For nonrecirculating perfusions, the perfusate was collected and erythrocytes were removed as described for recirculating perfusates. The perfusate was stored overnight at 4°C and then concentrated to approximately 250 ml using the Minitan tangential flow ultrafiltration system (Millipore, Bedford, MA). Subsequent lipoprotein isolation procedures were performed exactly as for recirculating perfusates.

The apoprotein composition of lipoproteins was characterized by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) as described previously (13) using a slab gel with a 4–30% polyacrylamide gradient. Apoproteins in the gel were stained with Coomassie Brilliant Blue R-250 and radiolabeled apoproteins were visualized by fluorography as described by Laskey and Mills (18). Measurements of the mass of lipoprotein protein, phospholipid, free cholesterol, cholesteryl ester, and triglyceride as well as negative stain electron microscopy of the intact particles were performed as described previously (13). The mass concentration of apoB in each of the perfusate lipoprotein fractions was measured by an enzyme-linked immunosorbent assay (ELISA) for apoB-100 similar to that described previously for apoA-I (19). Briefly, apoB-containing samples were diluted into the range of 0.05 to 1 μg/ml using 0.01 M phosphate-buffered saline containing 0.1% Tween and 0.1% bovine serum albumin, pH 7.0. The diluted samples were incubated at 37°C for 3 hr in the presence of the detergent. Samples containing 10 to 500 ng of apoB were then added to microtiter plates precoated with an affinity-purified goat anti-apoB polyclonal antibody prepared against cynomolgus monkey apoB. An aliquot of the same

antibody, conjugated with horseradish peroxidase, was then added and color development was with o-dianisidine. Standard curves for dilutions of purified LDL, whole serum, whole perfusate, and isolated perfusate subfractions were parallel, and the amount of apoB in each sample was calculated based on the apoB concentration of the purified LDL. Contamination of the antibody preparation with antibodies to nonapoB proteins was less than 1%. Radioactivity in apoB-100 was quantitated after slicing out the stained band separated by SDS-PAGE, solubilizing the gel in 30% H_2O_2, and measuring the radioactivity by liquid scintillation spectrometry.

Hepatic VLDL catabolism during recirculating perfusion

Radiolabeled VLDL were obtained from the medium of a recirculating perfusion by ultracentrifugation of 220–250 ml of perfusate at d 1.006 g/ml at 4°C in the 60 Ti rotor (Beckman Instruments Inc., Palo Alto, CA) at 50,000 rpm for 20 hr. After centrifugation, VLDL were isolated in the top 5 ml of each ultracentrifuge tube and were washed and concentrated by ultracentrifugation under the same conditions in a single tube. The VLDL were then dialyzed in 0.9% NaCl, pH 7.4. A second liver perfusion was performed within 6 days of the prior perfusion and within 4 days of obtaining the radiolabeled hepatic VLDL. Radiolabeled hepatic VLDL (1–4 mg of protein) containing 1 to 3 million dpm were injected into the medium of the second recirculating liver perfusion. Samples of perfusate were taken at timed intervals thereafter during 4 hr of recirculating perfusion for determination of the density distribution of radioactivity among perfusate lipoproteins. For each sample, erythrocytes were removed from the perfusate by centrifugation and perfusate lipoproteins were promptly fractionated by density gradient ultracentrifugation in the SW 40 rotor (Beckman Instruments, Inc.). Discontinuous density gradients were constructed from solutions made by adding KBr to 1.11% NaCl, 0.01% EDTA, 0.02% NaN_3, pH 7.4 (d 1.006 g/ml). The gradients consisted of 3 ml, d 1.24 g/ml solution; 3 ml, d 1.035 g/ml solution; 3 ml d 1.019 g/ml solution; and 4 ml of perfusate, d 1.006 g/ml. The gradients were centrifuged for 40 hr at 15°C in the L5-50 ultracentrifuge (Beckman Instruments, Inc.). The rotor was allowed to stop with no braking. The gradients were fractionated with a tube-piercing device (Instrumentation Specialties Co., Lincoln, NE) with which a dense solution (Fluorinert, Instrumentation Specialties Co.) was pumped into the bottom of the tube to displace the sample through the top of the tube. Equal volume fractions of approximately 0.6 ml were collected and radioactivity content was determined on aliquots by liquid scintillation. Density of fractions was determined by refractometry using standard solutions of similar composition that had known densities as determined by pycnometry.

Isolation of lipoproteins from the hepatic Golgi apparatus

The hepatic Golgi apparatus from monkeys was isolated by a method described for isolation of rat liver Golgi apparatus (20). Livers were obtained from ketamine-anesthetized animals that had been exsanguinated and flushed with saline. Livers were chilled on ice and promptly minced and homogenized in batches of approximately 10 g each. The Golgi apparatus-rich fraction was isolated from the homogenate by a combination of differential pelleting and discontinuous sucrose density gradient centrifugation at 4°C. The final Golgi apparatus-rich pellets were combined and washed by pelleting in 0.9% NaCl, 0.02% NaN_3, pH 7.4. The pellet was resuspended in this solution and examined by negative stain electron microscopy as described for perfusate lipoproteins. Liver homogenates and purified Golgi apparatus fractions were assayed for galactosyltransferase activity as described previously (21).

Nascent lipoproteins were released from the isolated Golgi apparatus by passing the resuspended pellet twice through a French pressure cell (20). VLDL were then floated by ultracentrifugation in the SW 40 rotor for 18 hr at 40,000 rpm, 4°C, and were isolated in the top 1.5 ml after the tube was sliced. The density of the infranatant was raised to d 1.225 g/ml with solid KBr and lipoproteins of d 1.006–1.225 g/ml were isolated by ultracentrifugation for 40 hr under the same conditions. This fraction was further fractionated by density gradient ultracentrifugation in an SW 60 rotor (Beckman Instruments, Inc.) containing 1.5 ml sample at d 1.225 g/ml; 2.0 ml of d 1.063 g/ml solution; and 1.4 ml of d 1.006 g/ml solution. The gradient was spun for 20 hr at 50,000 rpm, 4°C, and fractionated as described for liver perfusate lipoproteins. Fractions within specific density regions were pooled and dialyzed along with the VLDL fraction against 0.01% EDTA, 0.02% NaN_3, pH 7.4, for examination by negative stain electron microscopy.

RESULTS

[^{14}C]Leucine incorporation into apoB

To determine which of the perfusate apoproteins were newly synthesized, VLDL and the d 1.006–1.2 g/ml lipoproteins were isolated from perfusates by ultracentrifugation after 4 hr of recirculating perfusion with radioactive leucine. The d 1.006–1.2 g/ml lipoproteins were separated by agarose column chromatography, as shown in **Fig. 1,** into size populations. The material in the column regions indicated by the Roman numerals was pooled and the apoprotein patterns were determined using SDS-PAGE. **Fig. 2** shows the Coomassie blue-stained apoprotein pro-

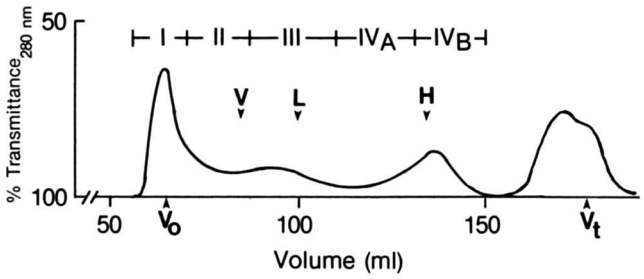

Fig. 1. Four percent agarose column chromatography of d 1.006–1.2 g/ml lipoproteins obtained from recirculating perfusate. Roman numerals indicate regions routinely pooled for analyses. The elution maxima of plasma VLDL, LDL, and HDL are indicated by V, L, and H, respectively. The material eluting at V_t included DTNB and potassium bromide.

files for all of the lipoprotein fractions from one representative perfusate and, in the lower half of the figure, the fluorogram of the same gel is shown. VLDL and column regions I, II, and III all contained apoB-100 as the major radiolabeled apoprotein. A significant amount of what we assume to be apoB breakdown products were present in the d > 1.006 g/ml column-isolated material, with relatively more being found in the smaller particles of region III. The immunologic relationship between the smaller molecular weight bands and apoB-100 was confirmed by immunoblotting with goat anti-monkey apoB-100 (data not shown). This often, but not always, was observed even when protease inhibitors, aprotinin and phenylmethylsulfonyl fluoride, were added to the perfusate at the end of perfusion. ApoE was another major radiolabeled apoprotein in each of the apoB-containing fractions and in the larger HDL of column region IV_A. ApoA-I was not found in the apoB-containing lipoproteins but was the major radiolabeled apoprotein of the region IV lipoproteins. Detectable amounts of small apoproteins were seen near the bottom of this 4–30% gradient gel. These proteins were radiolabeled although the extent of labeling of these apoproteins was low. Some non-radiolabeled proteins, slightly larger than apoE, were seen in the Coomassie blue-stained gel. Region I contained most of these proteins that were presumed to have originated from the erythrocytes in the perfusate.

In one experiment, the d > 1.006–1.2 g/ml lipoproteins were separated by density gradient ultracentrifugation instead of by column chromatography. The gradient profile is shown in **Fig. 3A** and the apoprotein staining and fluorograms for each of the gradient fractions, as determined after SDS-PAGE, are shown in **Figs. 3B** and **3C**. ApoB was the major radiolabeled protein in the material up to fraction 6 (d < 1.06 g/ml). Each of these fractions also had apoE. For apoE, progressively increasing amounts were seen in fractions 1 to 7, after which the relative amount decreased. The large amount of apoE in the most dense fraction may represent apoE that became dissociated during centrifugation. The ratio of apoB to apoE was highest in the least dense of the d > 1.006–1.06 g/ml lipoproteins. ApoA-I staining and labeling was apparent in fractions 6 to 11, and was greatest in fractions 8 and 9; the high amount of apoA-I in fraction 11 probably included some that had been dissociated during centrifugation. The smaller apoproteins were present in significant amounts in many fractions and were radiolabeled, although the extent of labeling was less than for the larger apoproteins.

Hepatic VLDL turnover during perfusion

To determine the extent of conversion of hepatic VLDL into more dense perfusate lipoproteins, five experiments were performed in which [^{14}C]leucine-labeled VLDL

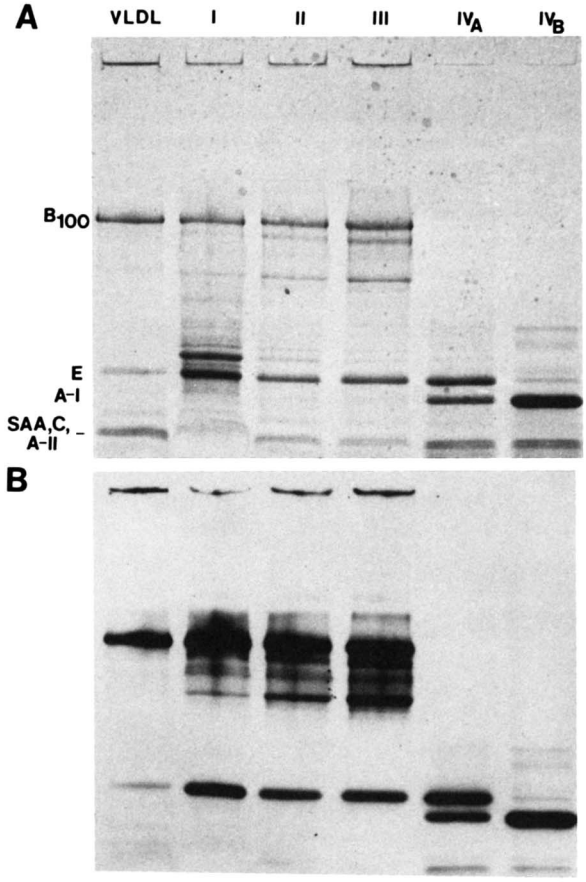

Fig. 2. SDS-polyacrylamide gradient gel electrophoretogram of recirculating perfusate lipoproteins. A liver was perfused by recirculation for 4 hr with medium containing [^{14}C]leucine. Perfusate VLDL and d 1.006–1.2 g/ml lipoproteins were isolated by differential ultracentrifugal flotation. The d 1.006–1.2 g/ml lipoproteins were fractionated by gel filtration chromatography as in Fig. 1. Panel A: Coomassie blue-stained gel. Panel B: fluorogram of gel in panel A. Roman numerals indicate lipoprotein fractions obtained by column chromatography. Apoproteins are identified on the left. Approximately 40 μg of protein was applied to each lane.

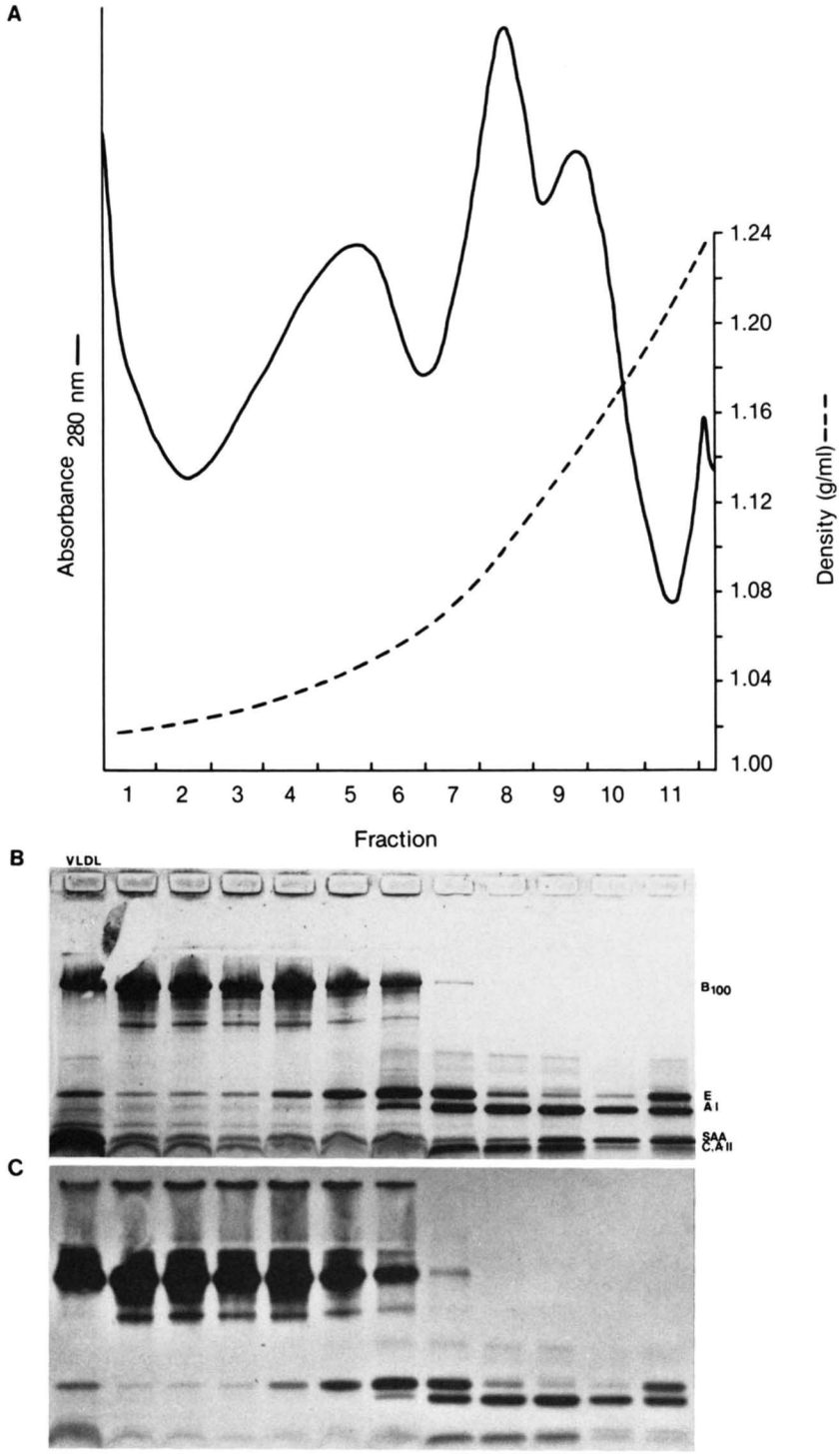

Fig. 3. Density gradient fractionation of recirculating perfusate lipoproteins. A liver was perfused with [^{14}C]leucine as described for Fig. 2. VLDL and d 1.006–1.2 g/ml perfusate lipoproteins were obtained by differential ultracentrifugal flotation. The d 1.006–1.2 g/ml lipoproteins were separated by density gradient ultracentrifugation as described in Materials and Methods. Panel A: absorbance 280 nm profile of the perfusate lipoproteins (solid line). Panel B: Coomassie blue-stained SDS-PAGE of VLDL and of fractions 1–11 obtained from the density gradient. Forty-five to 60 μg of protein containing 140–220 $\times$ 10^3 dpm were applied to each lane for VLDL and fractions 1–6. Twenty-two to 26 μg of protein containing 40–100 $\times$ 10^3 dpm were applied in fractions 7–11. Each lane of the gel is positioned roughly under the corresponding fraction from the density gradient. Apoproteins are identified on the right. Panel C: fluorogram of the gel shown in Panel A.

from one liver were recirculated through a second liver. The data in **Fig. 4** show the disappearance of the radiolabeled VLDL from the perfusate. About 20% of the injected dose disappeared from the perfusate within the first 10 min and most of this occurred during the first pass through the liver (data not shown). Little or no disappearance occurred thereafter. A significant portion of the disappearance could be accounted for by binding to the perfusion apparatus, per se, as shown by the dashed line in Fig. 4. The data in **Fig. 5** illustrate that the density distribution of the VLDL added to the perfusate shifted somewhat toward IDL, but that only about 15% of the perfusate radioactivity was associated with d 1.015–1.065 g/ml material after 4 hr of perfusion **(Table 1).** About half of this redistribution occurred merely upon addition of the radiolabeled VLDL to the unperfused medium (zero time, Table 1).

Nonrecirculating liver perfusion

To minimize the potential of the liver to modify newly secreted lipoproteins, nonrecirculating liver perfusions of 90–120 min were also performed. Perfusate lipoproteins, containing an average of 3.71 ± 1.47 mg of cholesterol, were isolated as before and the data in **Table 2** show the cholesterol distribution among nonrecirculating perfusate lipoprotein fractions compared to that for a series of recirculating perfusions. A similar percentage of cholesterol was found in the VLDL from both types of perfusion. A relatively higher percentage of cholesterol was seen in column region I of nonrecirculating liver perfusions, while a lower percentage was seen in column regions III and IV. The distribution of apoB among the lipoprotein fractions of three recirculating and three nonrecirculating liver perfusates is shown in **Table 3.** In both types of perfusion, about half of the apoB was found in the VLDL and the remainder was distributed among region I–IV lipoproteins.

The percentage composition of the lipoproteins isolated from nonrecirculating perfusates was measured in four experiments. The data **(Table 4)** are compared to similar data derived in several recirculating perfusion experiments. The percentage composition of most of the nonrecirculating perfusion lipoprotein fractions was similar to that seen for the corresponding fraction from recirculating perfusions. The one possible exception was for the region IV_A material which had a lower percentage of protein and a higher percentage of neutral lipid in the nonrecirculating perfusion. However, this fraction contained significant amounts of apoB according to SDS-PAGE, indicating that the separation of HDL and

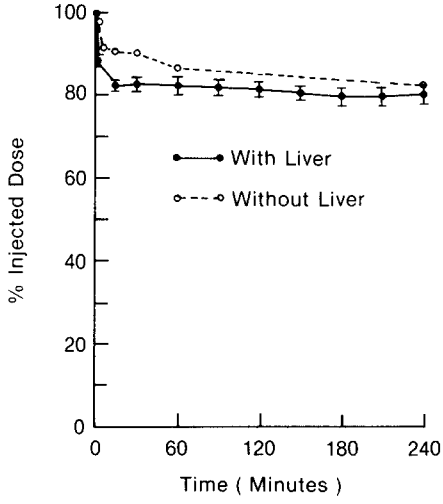

Fig. 4. Removal of hepatic VLDL by the perfused liver. In five experiments endogenously labeled hepatic VLDL obtained from the 4-hr recirculating perfusate of a liver in which [^{14}C]leucine was added to the medium was reinjected into the recirculating perfusate of another liver. Disappearance from the medium was followed by measuring perfusate radioactivity over the next 4 hr (solid line). A control experiment was performed (dashed line) in which the liver was removed from the system after 90 min of recirculating perfusion and radiolabeled hepatic VLDL was injected into the system. All time point values were corrected for samplings.

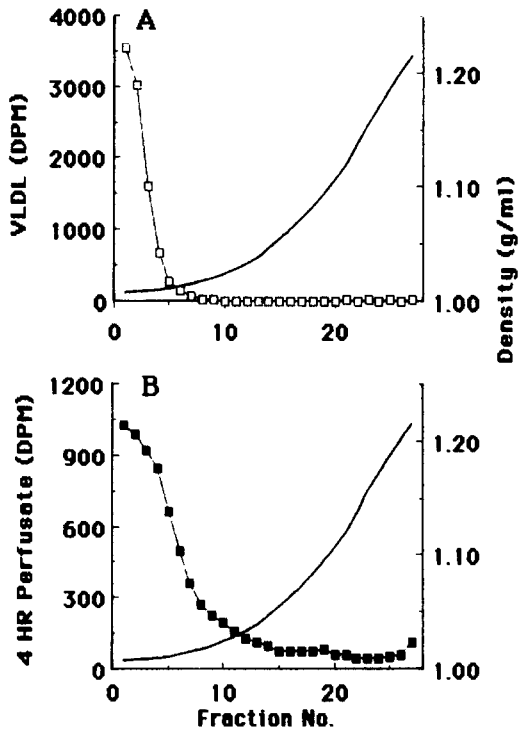

Fig. 5. Density distribution of radiolabeled protein from hepatic VLDL during recirculating perfusion. A liver was perfused for 4 hr by recirculation which endogenously radiolabeled hepatic VLDL as described for Fig. 4. Perfusate samples were obtained at timed intervals after injection of the labeled VLDL. Perfusate plasma and labeled VLDL were then fractionated by density gradient ultracentrifugation as described in Materials and Methods. The radioactivity distribution profiles are shown for the uninjected, labeled VLDL (panel A) and for the 4-hr perfusate sample (panel B). The lines without points show the density gradient.

TABLE 1. Distribution of hepatic VLDL radiolabeled protein during recirculating perfusion

Density	Injected VLDL	Duration of Perfusion (min) 0	60	120	180	240
g/ml	%			%		
< 1.015	96.2 ± 2.8	85.6 ± 4.9	74.2 ± 6.0	78.3 ± 4.6	78.7	73.9 ± 1.1
1.015-1.065	2.1 ± 2.8	7.1 ± 2.4	12.1 ± 2.2	11.2 ± 0.7	13.5	15.9 ± 1.2
1.065-1.21	1.5 ± 1.3	4.1 ± 2.0	8.4 ± 1.1	6.3 ± 2.0	4.4	6.8 ± 0.8
> 1.21	0.2 ± 0.2	3.2 ± 2.2	5.4 ± 3.7	4.2 ± 1.8	3.6	3.4 ± 0.6

In five experiments endogenously radiolabeled hepatic VLDL (containing 1-4 mg of protein) were obtained from the recirculating perfusate of one liver and were injected into the recirculating perfusate of another liver. Perfusate plasma was obtained at the times indicated and fractionated by density gradient ultracentrifugation. Radioactivity distribution was determined on the fractionated gradient as in Fig. 5 and radioactivity within the indicated density ranges was totalled for this table. The values for the zero time point were obtained by adding radiolabeled VLDL to unperfused medium before density gradient fractionation. The injected VLDL sample was loaded directly into the density gradient without mixing it with perfusion medium; n = 2 for the 180-min interval.

LDL-like material may not have been complete in nonrecirculating perfusates and the composition may reflect this contamination.

Fig. 6 illustrates the morphology of the lipoprotein fractions isolated from nonrecirculating liver perfusion. VLDL were seen as round, electron-lucent particles (indicating a spherical shape) of varying size within the range of 250 to 500 Å in diameter. The presence of excess surface appeared (to varying degrees depending on the animal) as an attached tab on the particles. Region I material consisted mostly of large amorphous structures, reminiscent of cellular membrane isolates. The region II material was heterogeneous in appearance. It consisted of round, electron-lucent (presumably spherical) particles, some with attached tabs, and darker particles (300-700 Å in diameter) that appeared flat (~100 Å thick) when seen on edge. The particles of region III were of similar morphology to those of region II but were generally smaller (200-250 Å in diameter) and many showed an elliptical or angular appearance. The region IV material was also heterogeneous, containing discoidal particles of 85-185 Å in diameter that tended to form rouleaux upon negative staining in addition to smaller round and irregularly shaped particles averaging about 75 Å in diameter.

The data in **Table 5** show the specific activities for the apoB of the VLDL and lipoproteins of column regions I-III from three nonrecirculating liver perfusions. The apoB mass was measured by ELISA and the radioactivity of apoB was determined after slicing and counting the gel. The values shown are normalized to the VLDL apoB specific activity, and they indicate that the degree of labeling of the apoB from each lipoprotein subfraction is approximately equivalent.

In one experiment the effect of exogenous free fatty acids on lipoprotein secretion was examined. A liver was perfused for 2 hr with 300 ml of medium containing 3% fatty acid-free human serum albumin. The medium was then changed and the liver was perfused for an additional 2 hr with 300 ml of fresh medium containing 3% human serum albumin with 5 moles of sodium oleate per mole of albumin and into which 912 μmoles of sodium oleate was infused over the 2-hr perfusion. The cholesterol secretion rates in the period without fatty acids versus the period with fatty acids were 3.72 and 3.06 mg/hr per 100 g liver, respectively, and the distributions of cholesterol in these two periods were 24% versus 31% in VLDL; 2% versus 4% in column region I, 12% versus 10% in column region II, 48% versus 42% in column region III, 4% versus 5% in column region IV_A, and 9% versus 8% in column region IV_B.

In one experiment, a 90-min nonrecirculating perfusion was performed after a 4-hr recirculating perfusion of the same liver. The data for the size distribution of the d 1.006-1.2 g/ml lipoproteins for both perfusions are

TABLE 2. Cholesterol distribution among perfusate lipoproteins

	n	VLDL	I	II	III	IV_A	IV_B
				%			
Recirculated	16	29.7 ± 3.8	11.1 ± 1.9	14.4 ± 1.9	28.4 ± 3.8	8.4 ± 1.1	8.0 ± 1.2
Nonrecirculated	5	37.2 ± 7.5	26.9 ± 5.1	11.5 ± 2.5	14.9 ± 1.6	4.8 ± 0.3	4.7 ± 0.6

Cholesterol mass was measured in perfusate VLDL and d 1.006-1.2 g/ml lipoproteins fractionated by 4% agarose column chromatography. Four-hour recirculating perfusates contained an average of 11.4 ± 0.34 mg of lipoprotein cholesterol. An average of 3.71 ± 1.47 mg of lipoprotein cholesterol was isolated from the 90-120-min nonrecirculating perfusates. Roman numerals indicate column fractions as shown in Fig. 1.

TABLE 3. ApoB distribution among perfusate lipoproteins

Animal	Perfusion	VLDL	d > 1.006	I	II	III	IV
				%			
T-241	Recirculated	54.0	46.0	1.2	5.9	35.8	3.1
354	Recirculated	50.7	49.3				
365	Recirculated	25.9	74.1				
Mean ± SEM		43.5 ± 8.9	56.5 ± 8.9				
201	Nonrecirculated	53.8	46.2	7.7	20.2	16.6	1.8
306	Nonrecirculated	51.7	48.3	1.3	15.7	29.3	2.0
233	Nonrecirculated	33.9	66.1	17.5	17.6	25.7	5.4
Mean ± SEM		46.5 ± 6.3	53.5 ± 6.3	8.8 ± 4.7	17.8 ± 1.3	23.9 ± 3.8	3.1 ± 1.2

ApoB mass was measured by ELISA in perfusate VLDL, d > 1.006 g/ml perfusate, and d 1.006–1.2 g/ml perfusate lipoproteins separated by 4% agarose column chromatography in recirculating and nonrecirculating perfusions. Total perfusate apoB mass was 3.87 ± 0.81 mg for 4-hr recirculating perfusates and 1.08 ± 0.50 mg for 90–120-min nonrecirculating perfusates. In two of the recirculating perfusions, apoB mass was measured in the d < 1.006 g/ml and d > 1.006 g/ml fractions only. Roman numerals indicate column fractions of the d > 1.006 material as shown in Fig. 1 and the percent distributions among these fractions are indicated.

shown in **Fig. 7.** The distribution of material absorbing at 280 nm was similar for both periods, although more region I absorbance was present in the nonrecirculating perfusate. The distribution of [^{14}C]leucine radioactivity, that had been added to the nonrecirculating perfusion medium, was also similar to the absorbance pattern except for the relatively reduced proportion of labeled material in regions I and II. **Fig. 8** shows the apoprotein patterns by SDS-PAGE for the lipoprotein fractions isolated from the nonrecirculating perfusate and the fluorogram for this same gel. It is clear that the major radiolabeled apoprotein of VLDL was apoB-100, and that the apoB-100 of region II and III was labeled as well. Little of the protein present in region I was labeled, suggesting that this material was not liver-derived. ApoE, when present, was also labeled as was apoA-I. The distribution of mass and label of these apoproteins among nonrecirculating perfusate lipoprotein fractions appeared similar to that for recirculating perfusate lipoprotein fractions (Fig. 2). In the limited number of nonrecirculating perfusions performed, no diet-related effects were observed in either the relative apoB specific activities or distribution of label among the various perfusate particles.

Hepatic Golgi apparatus lipoproteins

To obtain hepatic lipoproteins in a nascent condition for comparison to perfusate lipoproteins, livers from some animals were homogenized and the Golgi apparatus-enriched fraction was isolated. **Fig. 9** illustrates the morphology of the isolated Golgi apparatus secretory vescicles. Several vesicles were seen that contained numerous lipoproteins of various sizes, although some vesicles. Several vesicles were seen that contained numerous lipoproteins of various sizes, although some particles with redundant surface material that resembled a phospholipid bilayer on the edge of the particle, as shown in the insert in Fig. 9. In livers from six monkeys, galactosyl transferase activities were measured in homogenates and in Golgi pellets, and the fold-enrichment in Golgi pellets was 35.2 ± 11.3 (mean ± SEM). A comparable value in hepatic Golgi pellets in rats using this same procedure was a 60.8-fold enrichment.

After disruption of the Golgi apparatus, VLDL were isolated. The VLDL particles appeared as round, electron-lucent particles with only an occasional tab being apparent **(Fig. 10).** The Golgi apparatus-derived lipoproteins of d 1.006–1.2 g/ml were then isolated and separated using density gradient centrifugation. **Fig. 11** shows the gradient elution profile for these lipoproteins with a peak of material at the top of the tube (d < 1.02 g/ml), a second peak centered on a density of 1.035 g/ml, and a third peak at a density of 1.07 g/ml. The morphology of these particles is shown in **Fig. 12.** Particles with the same characteristics as those seen in the lipoproteins of region II and III of Fig. 6 are present, in both the d < 1.02 g/ml and the d 1.035 g/ml material isolated from the Golgi apparatus. Numerous particles were observed to possess extra surface material as evidenced by their having *1*) an attached tab, *2*) an elliptical or angular shape, or *3*) a flat or sheet-like shape. The material isolated from the d 1.07 g/ml peak contained large, amorphous sheets of material. In addition, smaller particles 75–150 Å in diameter were seen, many of which appeared as single discs on edge.

DISCUSSION

Evidence presented in this study and in previous studies (12, 13) demonstrates that the apoB-containing lipoprotein classes isolated from African green monkey liver perfusates contain numerous d > 1.006 g/ml surface-rich

TABLE 4. Chemical composition of liver perfusate lipoproteins

	n	PR		PL		FC		TG		CE	
		%	μg	%	μg	%	μg	%	μg	%	μg
VLDL											
Recirc.	19	10.6 ± 0.6		23.4 ± 0.9		6.1 ± 0.4		48.5 ± 1.6		11.5 ± 1.6	
Nonrecirc.	4	9.4 ± 1.1	1155 ± 561	22.4 ± 2.1	2363 ± 932	7.4 ± 1.0	776 ± 297	51.0 ± 1.5	6043 ± 2630	9.9 ± 2.5	1382 ± 750
I											
Recirc.	16	13.0 ± 1.1		49.3 ± 2.2		18.4 ± 1.4		11.6 ± 2.3		7.7 ± 1.6	
Nonrecirc.	4	24.3 ± 4.8	1310 ± 780	43.8 ± 3.4	1970 ± 734	18.6 ± 2.1	774 ± 243	8.8 ± 4.4	318 ± 140	4.5 ± 2.0	229 ± 112
II											
Recirc.	11	15.5 ± 0.9		38.4 ± 2.2		13.5 ± 1.0		21.6 ± 2.1		11.1 ± 2.0	
Nonrecirc.	3	17.9 ± 4.9	934 ± 722	39.8 ± 2.4	1581 ± 910	12.7 ± 0.7	557 ± 345	18.0 ± 4.7	529 ± 180	11.5 ± 3.8	523 ± 281
III											
Recirc.	10	18.1 ± 1.4		30.6 ± 1.4		10.0 ± 0.8		24.3 ± 2.2		17.1 ± 2.8	
Nonrecirc.	4	19.2 ± 3.3	601 ± 237	29.9 ± 3.0	937 ± 319	10.3 ± 0.5	306 ± 93	27.6 ± 7.0	714 ± 148	13.1 ± 2.9	477 ± 208
IV_A											
Recirc.	11	33.1 ± 1.4		49.8 ± 1.1		11.8 ± 0.9		3.3 ± 0.9		2.0 ± 0.3	
Nonrecirc.	3	22.1 ± 3.4	219 ± 105	54.8 ± 9.2	499 ± 150	9.9 ± 2.5	83 ± 27	8.6 ± 3.6	105 ± 75	4.7 ± 1.8	551 ± 335
IV_B											
Recirc.	12	45.0 ± 1.9		43.0 ± 1.2		5.5 ± 0.5		3.9 ± 1.2		2.4 ± 0.4	
Nonrecirc.	4	38.2 ± 2.6	744 ± 423	45.6 ± 2.7	784 ± 386	7.3 ± 1.2	118 ± 61	2.9 ± 0.7	64 ± 45	6.2 ± 2.4	167 ± 112

Values for percentage composition of 4-hr recirculating perfusate lipoproteins were obtained from previously published data (13). Values for nonrecirculating perfusate include the average total amount of material isolated from the 90–120-min perfusate and were determined from measurements of cholesterol distribution among the various fractions as shown in Table 2.

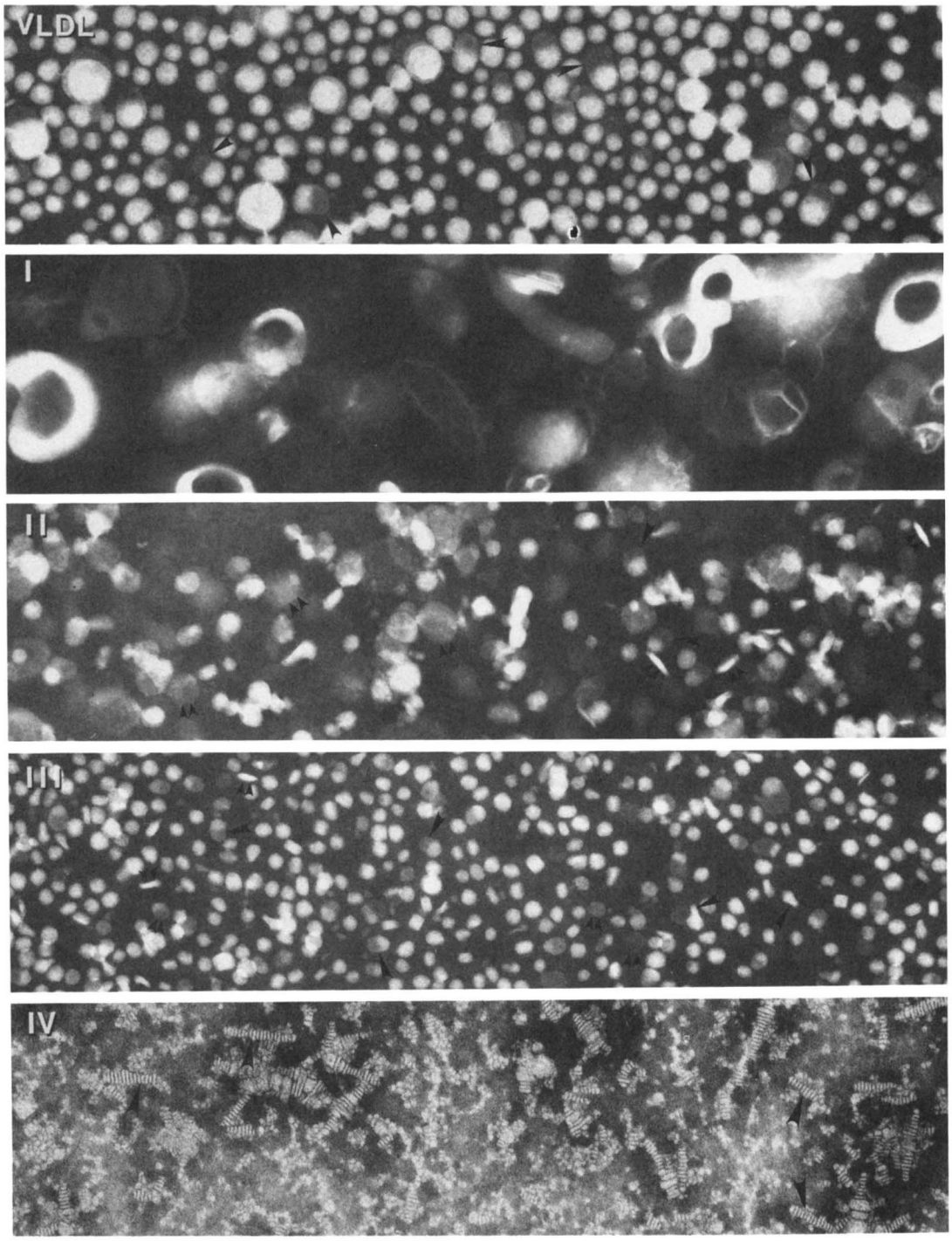

Fig. 6. Negative stain electron micrographs of nonrecirculating perfusate lipoproteins. A liver was perfused without recirculation for 90 min. Perfusate VLDL and d 1.006–1.2 g/ml lipoproteins were isolated and fractionated as described in Fig. 7 except that column regions IV_A and IV_B were taken as a combined fraction. Negative stain electron microscopy was performed with 2% potassium phosphotungstate, pH 6.5. VLDL and column fractions of d 1.006–1.2 g/ml perfusate lipoproteins are labeled in each panel. VLDL generally consisted of round, electron-lucent (spherical) particles but frequently (in this example) demonstrated evidence of carrying excess surface material in the form of an attached, more electron-dense tab (arrows). Column region I consisted largely of very large, amorphous structures resembling membrane isolates of cells that probably represent perfusate erythrocyte fragments. Column regions II and III consisted of a large variety of particle structures including *1*) generally round, electron-lucent particles that sometimes showed evidence of carrying excess surface material in the form of an attached tab (arrows) *2*) round, more electron-dense particles that could be seen to be flat sheets when viewed on edge (double arrows), and *3*) elliptical or angular, electron-lucent particles. Column region IV consisted of discoidal particles that tended to form rouleaux upon negative staining (arrows) and also contained very small particles which tend to aggregate upon negative staining. The actual structure of these was difficult to determine because of their small size. The bar marker in the lower right corner indicates 1000 Å.

TABLE 5. Specific acitivity of apoB in nonrecirculating perfusate lipoproteins

Lipoprotein	Animal 306		201		233		Mean	
	dpm/μg							
VLDL	1133	(1.00)	806	(1.00)	361	(1.00)	767	(1.00)
I	840	(0.74)	780	(0.97)	327	(0.91)	649	(0.87)
II	1190	(1.05)	423	(0.52)	484	(1.34)	699	(0.97)
III	1869	(1.65)	782	(0.97)	600	(1.66)	1089	(1.42)

ApoB mass measured by ELISA in nonrecirculating perfusate VLDL and d 1.006–1.2 g/ml lipoproteins fractionated by 4% agarose column chromatography. Roman numerals indicate column fractions as shown in Fig.1. Apoproteins were separated in each lipoprotein fraction by SDS-PAGE. ApoB radioactivity was measured by counting apoB in solubilized gel slices. Values in parentheses are normalized in each animal for VLDL-apoB specific activity.

structures that do not resemble the spherical lipoproteins of plasma. The surface-rich characteristic may derive from the fact that these lipoproteins are relatively nascent particles that have not been significantly modified by LCAT during recirculation perfusion. Alternatively, the particles secreted from the liver may have been modified (by hepatic lipase for example) and may represent core lipid-poor, surface lipid-rich remnants created during perfusion. Since our studies with African green monkeys represent the only liver perfusion studies in which significant amounts of d>1.006 g/ml surface-rich, apoB-containing lipoproteins have been reported (contrast

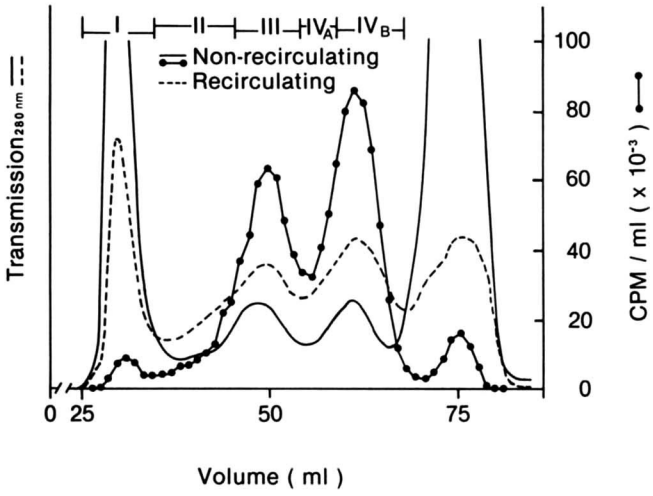

Fig. 7. Agarose column chromatography of d 1.006–1.2 g/ml perfusate lipoproteins. A single liver was perfused for 4 hr by recirculation and was then perfused for 90 min without recirculation with medium containing [^{14}C]leucine. Perfusate d 1.006–1.2 g/ml lipoproteins were isolated from both perfusates by differential ultracentrifugal flotation and were fractionated by 4% agarose column chromatography. The transmittance 280 nm column profiles are shown for the recirculating (dashed line) and nonrecirculating (solid line) perfusate lipoproteins. The radioactivity distribution of the nonrecirculating perfusate lipoproteins is also shown (●—●). The material eluting at Vo + Vi (75 ml) includes KBr, DTNB, and [^{14}C]leucine.

those of Marsh (22) and Hornick et al. (23), for example), the origin of these particles is of importance. The goal of this study was to determine which of the two possibilities for the origin of the surface-rich particle is more likely to be the case.

The evidence generated by the experiments with radioactive leucine incorporation into the perfusate lipoprotein apoproteins showed that the apoB, apoE, and apoA-I of each of the perfusate lipoproteins was radiolabeled in both recirculating and nonrecirculating perfusions, indicating the potential for the liver to have synthesized each of these lipoprotein fractions. Since the specific activity of the apoB of VLDL and of each of the region I–III lipoproteins from nonrecirculating perfusion were similar (Table 5), it appears that little if any of the d>1.006 g/ml lipoproteins was derived from resecretion

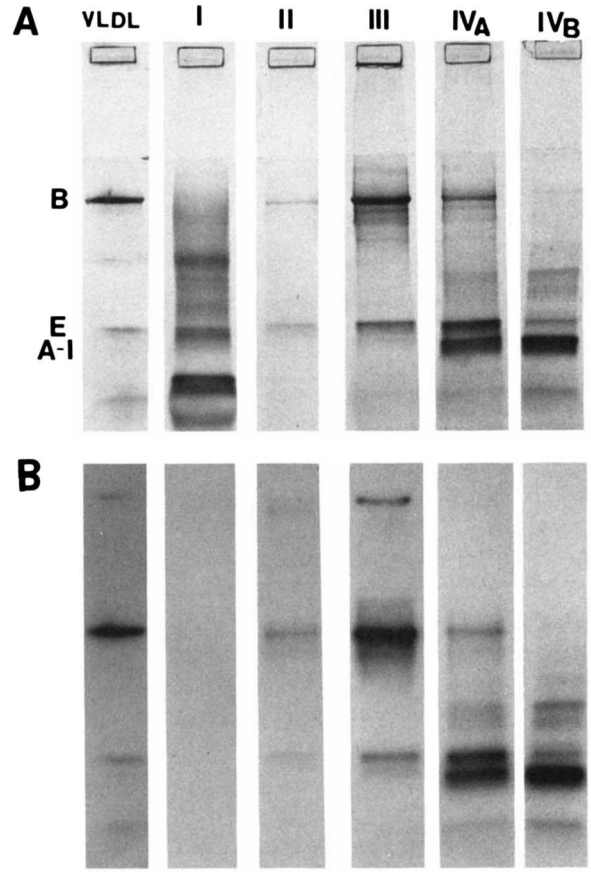

Fig. 8. SDS-polyacrylamide gel electrophoretogram of nonrecirculating perfusate lipoproteins. A liver was perfused with [^{14}C]leucine without recirculation of the medium. Perfusate VLDL and d 1.006–1.2 g/ml lipoproteins were isolated by differential ultracentrifugal flotation and the d 1.006–1.2 g/ml lipoproteins were fractionated by gel filtration chromatography as in Fig. 6. VLDL and column fractions are indicated above each lane. Apoproteins are indicated on the left. Protein was applied to an SDS-polyacrylamide gel as follows: VLDL, 28 μg, 2,014 cpm; I, 86 μg, 721 cpm; II, 15 μg, 1,233 cpm; III, 58 μg, 10,572 cpm; IV_A, 39 μg, 10,556 cpm; IV_B, 20 μg, 8,526 cpm. Panel A: Coomassie blue-stained gel. Panel B: fluorogram of gel in panel A.

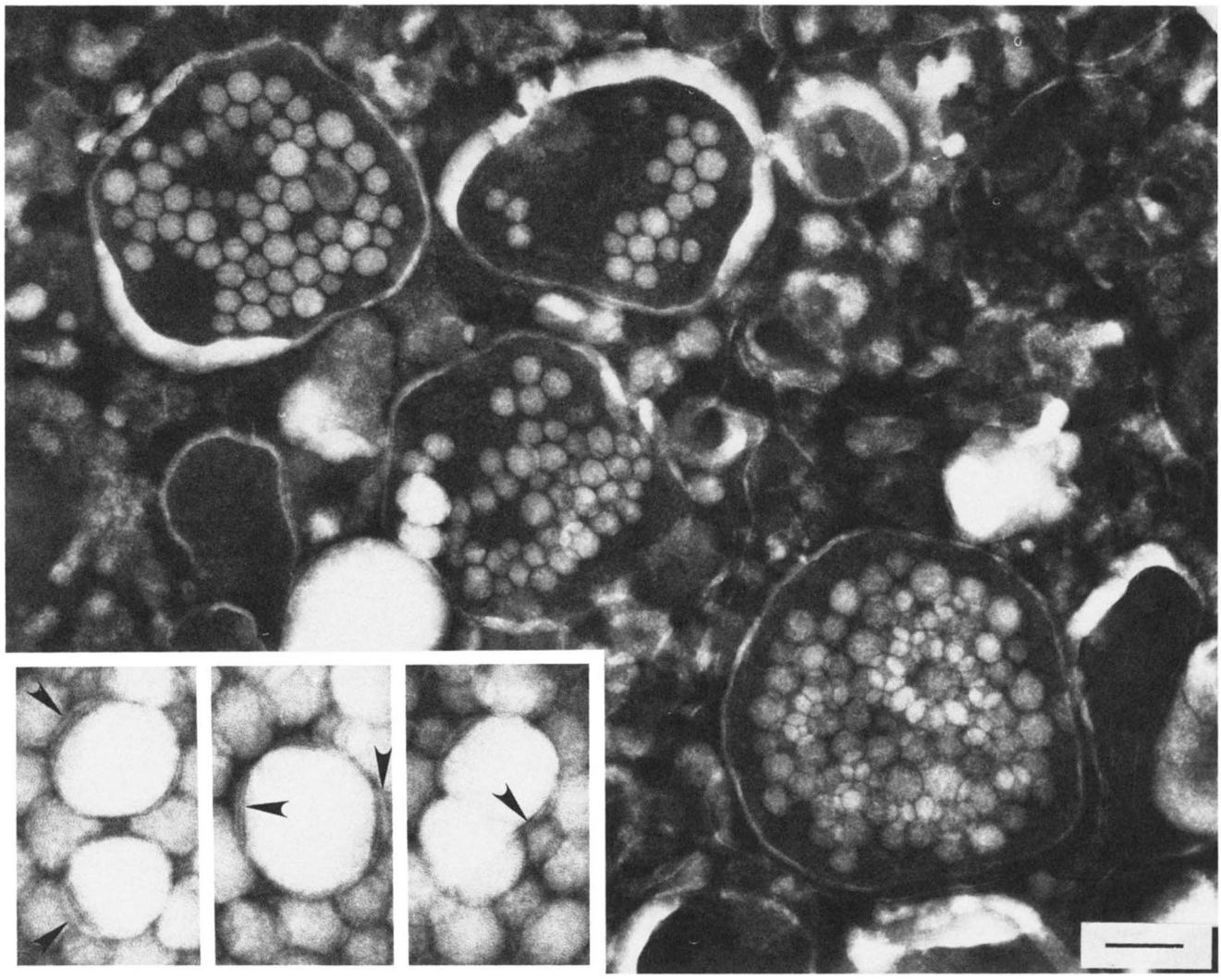

Fig. 9. Negative stain electron micrograph of vesicles of the isolated Golgi apparatus. In contrast to other vesicles which contained particles with a wide range of diameters, the vesicles in this example contained a relatively homogeneous population of VLDL-like particles. Occasionally nonspherical particles were found within the secretory vesicles. Insert (2 × magnification): some vesicles contained VLDL-like particles that appeared to carry excess surface material in the form of a lamellar or myelin structure at the edge of the particle (arrows) that may represent an attached but folded phospholipid tab. The bar marker at the lower right represents 1000 Å for the main micrograph.

or washout of unlabeled lipoproteins as has been suggested by others (24). The possibility that newly secreted VLDL were rapidly converted (during a single pass through the liver) into particles of LDL density has not been eliminated. However, if such were the case, one would expect to find significant differences in the distribution of apoB-100 among the perfusate lipoproteins for the two perfusion systems unless the conversion mechanism was extremely efficient, i.e., essentially complete conversion in one pass.

To test the potential of the liver to modify newly secreted VLDL, endogenously labeled hepatic VLDL were recirculated through a second liver, and the relative amount and density distribution of the material remaining in the perfusate after 4 hr of perfusion were measured. Only about 20% of the labeled hepatic VLDL was removed and 75% of the radioactivity remaining in perfusate was still in VLDL after 4 hr of perfusion although a slight shift in the density gradient was observed. Since the density distribution of radiolabeled VLDL apoproteins after recirculation did not closely approximate that in the recirculating perfusions, these results suggest that many of the $d > 1.006$ g/ml lipoproteins were secreted rather than being derived from VLDL during perfusion.

Further evidence that hepatic secretion products included $d > 1.006$ g/ml lipoproteins was obtained from the experiments in which the cholesterol and apoB distribution among the perfusate lipoproteins was compared between recirculating and nonrecirculating perfusions (Tables 2 and 3). The percentage of apoB and cholesterol

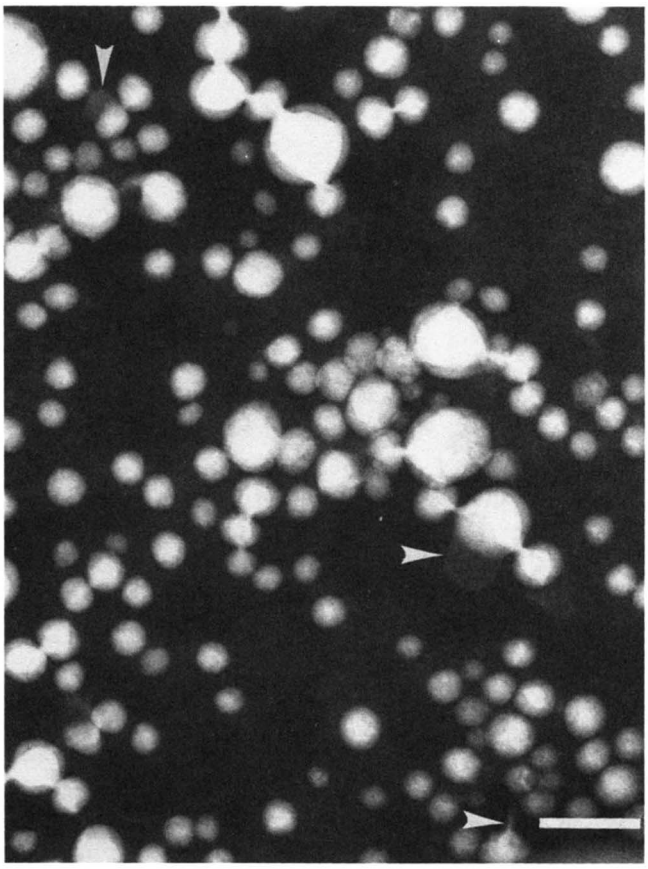

Fig. 10. Negative stain electron micrograph of VLDL obtained from the isolated Golgi. The isolated Golgi apparatus was disrupted with a French pressure cell and VLDL were isolated from the mixture by ultracentrifugal flotation and examined by negative stain electron microscopy. Particles were generally electron-lucent, round (spherical) in appearance, and had a wide range of diameters (250–800 Å), but occasionally appeared to carry excess surface material as an attached tab (arrows).

in VLDL was similar for both types of perfusions. This outcome would only be expected if the pattern of secretion is reflected in these distributions. If there was significant conversion of VLDL into more dense lipoproteins during recirculating perfusion, the percentage of cholesterol and apoB in VLDL would be expected to be higher in the nonrecirculating perfusions. Contaminating membrane fragments (presumably from broken erythrocytes) were seen in region I material and this occurred to a much greater extent in nonrecirculating than in recirculating perfusates, presumably because the Kupffer cells cleared them from the perfusate during recirculation perfusion. These membranes undoubtedly increase the percentage of region I cholesterol in Table 2, therefore the apoB distribution of Table 3 may be the more appropriate comparison of the distribution of newly secreted lipoproteins. In any case, the finding that the distribution, morphology, and chemical composition of each of the lipoprotein classes of nonrecirculating perfusions were similar to those of recirculating perfusions supports the idea that the various phospholipid-rich, $d > 1.006$ g/ml lipoproteins were secreted instead of being derived from VLDL during recirculation perfusion.

Given the outcome of the nonrecirculating liver perfusion experiments, only one case can be foreseen in which these results could be explained by conversion of lipoproteins after secretion. This would be the situation in which, for example, hepatic lipase was able to bind to and modify newly secreted lipoproteins before their exit from the liver. To provide evidence for the likelihood of this possibility, an antibody directed against the active site of hepatic lipase would be needed. Alternatively, the nature of the apoB-containing particles within the cells prior to secretion would have to be determined. In the absence of an antibody to the active site of hepatic lipase, we characterized the lipoproteins within the hepatic Golgi apparatus. In order to do this, it was necessary to isolate a Golgi apparatus-rich fraction that was as free as possible of multivesicular bodies described by Hornick et al. (25) in rat liver. Accordingly, we have used a Golgi apparatus isolation procedure patterned after the one described by these workers. Our results indicated that we obtained a fraction enriched in intact Golgi apparatus containing lipoprotein particles. The lipoproteins harvested from the Golgi apparatus consisted of particles morphologically similar to perfusate lipoproteins particles. Some surface-rich particles within the intact Golgi apparatus were also apparent, although the wide variety of particle types obtained from the disrupted Golgi apparatus was not identified easily in the intact organelle. This may be due partly

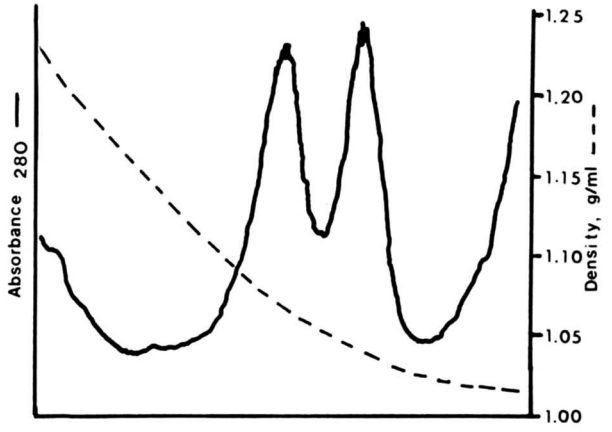

Fig. 11. Density gradient fractionation of lipoproteins obtained from the isolated Golgi apparatus. D 1.006–1.2 g/ml lipoproteins were obtained from the disrupted Golgi apparatus by ultracentrifugation and fractionated by density gradient ultracentrifugation as described in Materials and Methods. The absorbance 280 nm profile of the density gradient is shown by the solid line. The density gradient is shown by the dashed line. Material obtained at $d < 1.02$ g/ml, d 1.02–1.06 g/ml, and d 1.06–1.2 g/ml from the gradient was pooled separately for examination by electron microscopy.

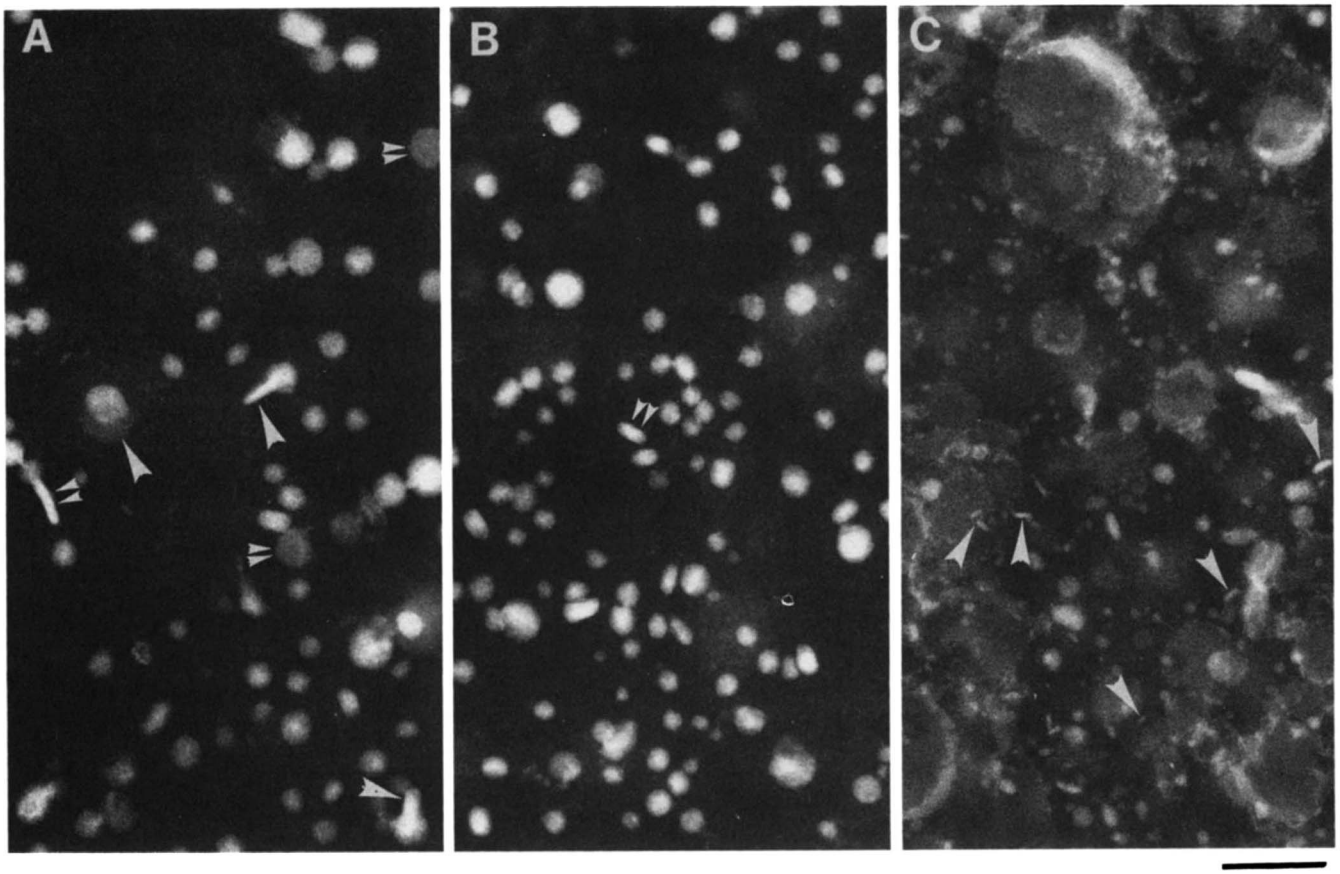

Fig. 12. Negative stain electron micrographs of Golgi apparatus lipoproteins obtained by density gradient ultracentrifugation. The d 1.006–1.2 g/ml lipoproteins obtained from the isolated Golgi apparatus were fractionated by density gradient ultracentrifugation as described for Fig. 11 and material obtained at d 1.006–1.02 g/ml (panel A), d 1.02–1.06 g/ml (panel B), and d 1.06–1.2 g/ml (panel C) were pooled separately and examined by negative stain electron microscopy. Both the 1.006–1.02 g/ml and the 1.02–1.06 g/ml fractions consisted of *1*) generally round, electron-lucent (spherical) particles which occasionally exhibited evidence of carrying excess surface material in the form of an attached tab (arrows), *2*) round, electron-dense particles (double arrows) which were observed as sheets when seen on edge, and *3*) elliptical or angular, electron-lucent particles. The HDL fraction consisted of *1*) large, irregularly shaped sheet-like material that probably represents membranous fragments from the disrupted Golgi apparatus and *2*) small discoidal particles (arrows) 85–200 Å in diameter. The bar marker at the lower right represents 1000 Å.

to the fact that the particles within the secretory vesicles were not separated from one another. Also, it may be that some surface-rich particles form spontaneously immediately upon release from the environment of the secretory vesicle both in vitro and in vivo.

The bulk of our evidence supports the likelihood that many of the apoB-containing d > 1.006 g/ml lipoprotein particles of the African green monkey liver perfusates were secreted directly by the liver. Given our findings that hepatic VLDL shifted slightly in density during recirculating perfusion, that apoB breakdown is more apparent in more dense subfractions, and that the d 1.006–1.06 g/ml perfusate particles are morphologically heterogeneous, it remains possible that some subpopulation(s) of the perfusate particles is derived from hepatic VLDL during recirculation perfusion. For example, the most surface-rich structures may be derived from hepatic VLDL and contain only a small proportion of apoB, whereas other subpopulations, less enriched in surface material and in apoB, may be secreted by the liver. More thorough fractionation and characterization of radiolabeled d > 1.006 g/ml perfusate particles would be required to resolve this issue.

Why surface-rich lipoproteins accumulate in the perfusate may be related to the relative level of LCAT accumulating in the perfusate. LCAT accumulates in the monkey liver perfusate to a level of less than 2% of that in plasma (17). This permits excess surface material to remain on the perfusate particles. In vitro incubation of perfusate with exogenous purified LCAT causes the extra surface phospholipid and cholesterol to be removed and perfusate LDL and HDL become more spherical and enriched in cholesteryl ester (26). The reason for secretion of more surface material than required for the available core lipid is also unknown, but may be related to the availability of core lipid. The livers are taken from the animals about 16 hr after their last meal and we have not added fatty acids to the perfusate in most experiments. It

is possible that the secretion of apoB with a complement of surface lipid occurs at a relatively constant rate even when triglyceride secretion is limited. Since addition of fatty acids to the perfusate did not appear to prevent the appearance of d 1.006–1.06 g/ml perfusate lipoproteins, we assume that these particles may occur in vivo and are not just a result of our in vitro conditions. Many, if not all, of the unusual particles described in primate liver perfusates would appear to have a compositional and morphological counterpart among the lipoprotein fractions from the plasma of patients with the familial deficiency of LCAT (16). Therefore, our operating hypothesis is that many of the plasma lipoproteins from these patients are particles of hepatic origin. Support for this hypothesis comes from our own results as well as from the work of Hamilton et al. (27) who demonstrated that significant amounts of discoidal HDL accumulated in rat liver perfusates, but only when perfusate LCAT activity was chemically inhibited during the perfusion.

That the primate liver produces such a heterogeneity of particles containing apoB-100 may be significant in that each of these particles may have the potential to be converted into cholesteryl ester-rich, plasma LDL after entering the circulation in vivo. The heterogeneity of these hepatic particles may also relate to the unexplained heterogeneity of the plasma LDL in monkeys (28, 29) and in human beings (30). The hepatic secretion of LDL-density particles would also help to explain the observations of others that the total production rate of plasma LDL-apoB in pigs (31), monkeys (32), and in human beings (33) cannot be completely accounted for by the conversion of VLDL-apoB to LDL-apoB. The potential of each of these hepatic lipoproteins to become plasma LDL has yet to be fully determined. However, preliminary studies have indicated that a substantial portion of radiolabeled apoB-100 on the hepatic surface-rich, LDL-density particles remains in the circulation after their reinjection in vivo and becomes indistinguishable from the animal's own plasma LDL (Marzetta, C. A., F. L. Johnson, and L. L. Rudel, unpublished observations).

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REFERENCES

1. Hamilton, R. L., and H. J. Kayden. 1974. The liver and the formation of normal and abnormal plasma lipoproteins. *In* The Liver: Normal and Abnormal Functions. Part A. F. F. Becker, editor. Marcel Decker, Inc. New York. 531–572.
2. Windler, E., Y-s. Chao, and R. J. Havel. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat. Opposing effects of homologous apolipoprotein E and individual C apoproteins. *J. Biol. Chem.* **255:** 8303–8307.
3. Dolphin, P. J., S. J. Forsythe, and E. S. Krul. 1986. Post-secretory acquisition of apolipoprotein E by nascent rat hepatic very-low-density lipoproteins in the absence of cholesterol ester transfer. *Biochim. Biophys. Acta.* **875:** 21–30.
4. Nöel, S-P., R. Dupras, C. Vezina, and Y. L. Marcel. 1984. Comparison of very-low-density lipoproteins isolated from rat liver perfusate, rat serum, and human plasma as acceptors for cholesteryl ester transfer. *Biochim Biophys. Acta.* **796:** 277–284.
5. Minari, O., and D. B. Zilversmit. 1963. Behavior of dog lymph chylomicron lipid constituents during incubation with serum. *J. Lipid Res.* **4:** 424–436.
6. Bell, P. 1978. Lipid exchange and transfer between biological lipid-protein structures. *Prog. Lipid Res.* **17:** 207–243.
7. Zilversmit, D. B., L. B. Hughes, and J. Balmer. 1975. Stimulation of cholesterol ester exchange by lipoprotein-free rabbit plasma. *Biochim. Biophys. Acta.* **409:** 393–398.
8. Ha, Y. C., and P. J. Barter. 1982. Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. *Comp. Biochem. Physiol.* **71B:** 265–269.
9. Pierce, F. T. 1954. The interconversion of serum lipoproteins in vivo. *Metabolism.* **3:** 142–153.
10. Jansen, H., A. van Tol, and W. C. Hulsmen. 1980. On the metabolic function of heparin-releasable liver lipase. *Biochem. Biophys. Res. Commun.* **92:** 53–59.
11. Morton, R. E., and D. B. Zilversmit. 1983. Interrelationships of lipids transferred by the lipid transfer protein isolated from human lipoprotein-deficient plasma. *J. Biol. Chem.* **258:** 11751–11757.
12. Johnson, F. L., R. W. St. Clair, and L. L. Rudel. 1983. Studies on the production of low density lipoproteins by perfused livers from nonhuman primates. Effect of dietary cholesterol. *J. Clin. Invest.* **72:** 221–236.
13. Johnson, F. L., R. W. St.Clair, and L. L. Rudel. 1985. Effects of the degree of saturation of dietary fat on the hepatic production of lipoproteins in the African green monkey. *J. Lipid Res.* **26:** 403–417.
14. Torsvik, H. M., H. Solaas, and E. Gjone. 1970. Serum lipoproteins in plasma lecithin:cholesterol acyltransferase deficiency studied by electron microscopy. *Clin. Genet.* **1:** 139–150.
15. Forte, T., K. R. Norum, J. A. Glomset, and A. V. Nichols. 1971. Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: structure of low and high density lipoproteins as revealed by electron microscopy. *J. Clin. Invest.* **50:** 1141–1148.
16. Glomset, J. A., and K. R. Norum. 1973. The metabolic role of lecithin:cholesterol acyltransferase: perspective from pathology. *Adv. Lipid Res.* **11:** 1–65.
17. Johnson, F. L., L. Babiak, and L. L. Rudel. 1986. High density lipoprotein accumulation in perfusates of isolated livers of African green monkeys. Effects of saturated versus polyunsaturated dietary fat. *J. Lipid Res.* **27:** 537–548.
18. Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of H-3 and C-14 in polyacrylamide gels by fluorography. *Eur. J. Biochem.* **56:** 335–341.
19. Koritnik, D. L., and L. L. Rudel. 1983. Measurement of apolipoprotein A-I concentration in nonhuman primate serum by enzyme-linked immunosorbent assay (ELISA). *J. Lipid Res.* **24:** 1639–1645.

20. Swift, L. L., N. R. Manowitz, G. D. Dunn, and V. S. LeQuire. 1980. Isolation and characterization of hepatic Golgi lipoproteins from hypercholesterolemic rats. *J. Clin. Invest.* **66:** 415–425.
21. Morre, D. J., L. M. Merlin, and T. W. Keenan. 1969. Localization of glycosyltransferase activities in a Golgi apparatus-rich fraction isolated from rat liver. *Biochem. Biophys. Res. Commun.* **37:** 813–819.
22. Marsh, J. B. 1974. Lipoproteins in a nonrecirculating perfusate of rat liver. *J. Lipid Res.* **15:** 544–550.
23. Hornick, C. A., T. Kita, R. L. Hamilton, J. P. Kane, and R. J. Havel. 1983. Secretion of lipoproteins from the liver of normal and Watanabe heritable hyperlipidemic rabbits. *Proc. Natl. Acad. Sci. USA.* **80:** 6096–6100.
24. Jones, L. A., T. Teramoto, D. J. Juhn, R. B. Goldberg, A. H. Rubenstein, and G. S. Getz. 1984. Characterization of lipoprotein produced by the perfused rhesus monkey liver. *J. Lipid Res.* **25:** 319–335.
25. Hornick, C. A., R. L. Hamilton, E. Spaziani, F. H. Enders, and R. J. Havel. 1985. Isolation and characterization of multivesicular bodies from rat hepatocytes—an organelle distinct from secretory vesicles of the Golgi apparatus. *J. Cell Biol.* **100:** 1558–1569.
26. Babiak, J., H. Tamachi, F. L. Johnson, J. S. Parks, and L. L. Rudel. 1986. LCAT-induced modifications of liver perfusate discoidal HDL from African green monkeys. *J. Lipid Res.* **27:** 1304–1317.
27. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667–680.
28. Rudel, L. L., L. L. Pitts, and C. A. Nelson. 1977. Characterization of plasma low density lipoproteins of nonhuman primates fed dietary cholesterol. *J. Lipid Res.* **18:** 211–222.
29. Rudel, L. L., M. G. Bond, and B. C. Bullock. 1985. LDL heterogeneity and atherosclerosis in nonhuman primates. *Ann. NY Acad. Sci.* **454:** 248–253.
30. Krauss, R. M., and D. J. Burke. 1982. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J. Lipid Res.* **23:** 97–104.
31. Huff, M. W., D. E. Telford, K. Woodcroft, and W. L. P. Strong. 1985. Mevinolin and cholestyramine inhibit the direct synthesis of low density lipoprotein apolipoprotein B in miniature pigs. *J. Lipid Res.* **26:** 1175–1186.
32. Goldberg, I. J., N-A. Le, H. N. Ginsberg, J. R. Paterniti, Jr., and W. V. Brown. 1983. Metabolism of apoprotein B in cynomolgus monkey: evidence for independent production of low density lipoprotein apoprotein B. *Am. J. Physiol.* **244:** E196–E201.
33. Howard, B. V., G. Egusa, W. F. Beltz, Y. A. Kesäniemi, and S. M. Grundy. 1986. Compensatory mechanisms governing the concentration of plasma low density lipoprotein. *J. Lipid Res.* **27:** 11–20.