Uptake of Apolipoprotein E-Containing High Density Lipoproteins by Hepatic Parenchymal Cells

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Cholesterol-enriched, apolipoprotein E-containing high density lipoproteins (apo E HDLc), which were isolated from the plasma of cholesterol-fed dogs by using agarose column chromatography or ultracentrifugation, possessed essentially identical biochemical and metabolic characteristics. Radiolabeled (125I) apo E HDLc isolated by either method gave identical rates of clearance from the plasma, i.e., >50% of the injected dose was cleared from the plasma within 5 to 10 minutes, principally by the liver. Detailed studies localizing apo E HDLc uptake to specific cell types within the liver were performed in both normal and cholesterol-fed rats. The validity of using the canine apo E HDLc in the rat was supported by observations of marked similarities in plasma clearance, i.e., a rapid acute phase of disappearance, and a near-quantitative hepatic uptake of lipoproteins in both species. Canine apo E HDLc (fluorescently labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine), which were injected into normal rats, appeared to be taken up primarily by parenchymal cells, as determined by fluorescence microscopy. Light microscopic autoradiography also revealed that the uptake of 125I apo E HDLc was principally carried out by parenchymal cells in rat liver. Likewise, the uptake of apo E HDLc by the liver of cholesterol-fed rats was extensively localized to parenchymal cells. An in situ, single-pass perfusion of a lobule of the liver of a normal dog with iodinated and fluorescently labeled apo E HDLc confirmed that the uptake of the lipoproteins was principally carried out by parenchymal cells. The plasma clearance of apo E HDLc by hepatic parenchymal cells, even in cholesterol-fed animals in which the apo B,E (LDL) receptors were markedly down-regulated (undetectable), suggests that the apo E receptor, presumably the remnant receptor, is localized in the parenchymal cells.

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uptake of apo E HDL\textsubscript{c}. Localization was accomplished using the techniques of fluorescence microscopy and autoradiography. In addition to determining which cells are involved, the studies were designed to establish whether ultracentrifugal isolation of apo E HDL\textsubscript{c} can modify their behavior in vivo. For this purpose, the plasma clearance of ultracentrifugally isolated apo E HDL\textsubscript{c} was compared to that of chromatographically isolated apo E HDL\textsubscript{c}. Previously, van't Hooft et al.\textsuperscript{12} showed that ultracentrifugation results in the accelerated plasma clearance of apo E-containing lipoproteins from rats. The present study indicates that ultracentrifugation does not alter the behavior of canine apo E HDL\textsubscript{c}.

**Methods**

**Animals**

Purebred adult foxhounds, obtained from Brink Farms (Paola, Kansas), were maintained under controlled laboratory conditions for more than 4 weeks before use. They were fed a control dog chow (Purina dog meal) ad libitum. Cholesterol-fed foxhounds received the coconut oil-cholesterol diet as previously described.\textsuperscript{5,6} They were fed their diet ad libitum for at least 6 months before the study and had plasma cholesterol levels in excess of 750 mg/dl during the course of the feeding study. Plasma was obtained after an overnight fast.

Male Sprague-Dawley rats, obtained from Simon- sen Labs (Gilroy, California) were maintained under controlled laboratory conditions and fed a standard rat chow. For a period of 3 to 6 months, cholesterol-fed rats received the same semisynthetic, coconut oil-cholesterol diet fed to the dogs. After 6 months, the rats fed normal chow weighed 200 to 400 g, whereas rats of the same age on the cholesterol diet weighed 500 to 600 g. The plasma cholesterol levels of the cholesterol-fed rats were two- to threefold higher than the values obtained for the control rats (~80 mg of cholesterol/dl). Animal studies were performed in accordance with institutional policies.

**Lipoprotein Isolation**

Canine low density lipoproteins (LDL) were obtained at d = 1.020 to 1.063 and purified by Pevikon (Mercer Consolidated Corporation, Yonkers, New York) block electrophoresis.\textsuperscript{13} Human LDL were prepared by ultracentrifugation at d = 1.02 to 1.05. The apo E HDL\textsubscript{c} isolated by centrifugation were prepared from the d = 1.006 to 1.02 fraction by a combination of ultracentrifugation and Pevikon block electrophoresis as described.\textsuperscript{5,6} These HDL\textsubscript{c} are referred to as ultracentrifugally isolated apo E HDL\textsubscript{c}. Cholesterol levels were determined using an enzymatic procedure (Bio Dynamics, Boehringer-Mannheim Corporation, Indianapolis, Indiana). Protein was determined by the method of Lowry et al.\textsuperscript{14} Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was carried out according to the method of Weber and Osborne.\textsuperscript{15}

**Chromatographic Separation of Apo E HDL\textsubscript{c}**

Hypercholesterolemic canine plasma (20 ml) was applied to the top of a 6% agarose column (Bio-gel A5m, Bio-Rad Laboratories, Richmond, California). The Amicon column (Amicon Corporation, Lexington, Massachusetts), measuring 3.2 x 130 cm, was equilibrated with 0.15 M phosphate buffer (pH 7.4) containing 0.1% EDTA. Chromatography was performed at 4°C using a peristaltic pump to maintain a flow rate of 60 ml/hr (5-ml fractions were collected). The fractions were pooled and then concentrated to 3 to 6 ml using an Amicon pressurized concentrator and a PM30 membrane. The apo E HDL\textsubscript{c} were purified from the mixture of lipoproteins obtained using the Pevikon block electrophoresis procedure.\textsuperscript{5,6} These HDL\textsubscript{c} are referred to as column-isolated apo E HDL\textsubscript{c}.

**Labeling of Apo E HDL\textsubscript{c}**

The column-isolated apo E HDL\textsubscript{c} were fluorescently labeled with 1,1'-dioctadecyl-3,3',3',3'-tetramethylindocarbocyanine (Dil) (Molecular Probes Incorporated, Junction City, Oregon) before their purification from the plasma by agarose chromatography. This was accomplished by adding 50 μl of Dil (3 mg of Dil/ml of DMSO) to 20 ml of plasma and incubating the plasma for 15 hours at 37°C. The Dil-labeled apo E HDL\textsubscript{c} were isolated by gel filtration as described above. The centrifugally isolated apo E HDL\textsubscript{c} were labeled with Dil using the procedure described by Pitas et al.\textsuperscript{16}

Iodination of the human LDL was performed by the modified McFarlane method.\textsuperscript{17} The apo E HDL\textsubscript{c} were labeled by the Bolton-Hunter procedure.\textsuperscript{18} Both \textsuperscript{125}I and \textsuperscript{131}I were used.

**In Vivo Experiments**

Iodinated lipoproteins (400 to 2000 cpm/ng) were injected into the cephalic vein of conscious dogs, and the catheter was flushed with 10 ml of 0.15 M NaCl. Blood samples were drawn from the jugular veins and immediately transferred into tubes containing 0.1% (wt/vol) disodium EDTA and stored on ice. The plasma was separated by centrifugation at 3,000 rpm for 25 minutes at 4°C. All data are reported as TCA-precipitable protein using the precipitation method previously described.\textsuperscript{8} Calculations of the percentage of the injected dose remaining in the plasma at the various time intervals were based on a plasma volume equalling 4.5% of the body weight.\textsuperscript{8}

Studies in rats were performed after pentobarbital anesthesia (65 mg of pentobarbital/kg body weight, i.p.). The iodinated or Dil-labeled apo E HDL\textsubscript{c} were injected into an exposed femoral vein, and blood samples (250 μl) were obtained from the opposite femoral vein. Liver samples were obtained from various lobes of the liver. Liver samples for fluorescence microscopy were frozen immediately with 2-methyl butane cooled in liquid nitrogen. Samples for autoradiography were taken after perfusion of the liver with
overnight, the tissues were embedded in JB-4 at 20° C. After rinsing the tissues in Vernal-Acetate addition of 0.005% H2O2 and continued for 1 hour at 4° C, the fixation was stopped and aldehyde in 0.08 M sodium cacodylate (pH 7.3). Perfusion of the liver was performed by infusing the solution through the portal vein following ligation of the celiac artery and cutting the abdominal vena cava.

**Tissue Culture Assay**

Normal human fibroblasts were grown in Dulbecco’s modified Eagle’s medium (GIBCO) with 10% fetal calf serum (Sterile Systems, Logan, Utah) at 37° C in a 7.5% CO2 concentration. One week before the experiment, the fibroblasts were plated; the medium was switched to a 10% lipoprotein-deficient serum 48 hours before the 4° C binding experiment was performed. The assays were performed using 2 μg of human 125I-LDL/ml as described previously.19

**Liver Membrane Binding Assay**

Membranes from rat livers were prepared according to the procedure of Kovanan et al.20 Binding assays were performed at 4° C in 100 μl of incubation buffer containing 50 mM Tris-HCl (pH 7.5), 25 mM NaCl, 1.0 mM CaCl2, and 20 mg/ml of bovine serum albumin, as described by Basu et al.21 Nonspecific, calcium-independent binding was determined by inclusion of 20 mM EDTA in the assay tube.

**Microscopy**

Negative stains of apo E HDL were performed by using freshly prepared carbon films deposited on mica. Staining was accomplished with 1% phosphotungstic acid in a KOH-neutralized solution, and the diameters of 100 particles were measured.

Light microscopic autoradiography was performed on the perfusion-fixed tissue samples. Kupffer cells were identified by their endogenous peroxidase activity using diaminobenzidine (Sigma Chemical Company, St. Louis, Missouri) as a substrate. After 30 minutes of fixation in 2% formaldehyde (freshly prepared from paraformaldehyde) and 0.5% glutaraldehyde in 0.08 M sodium cacodylate buffer (pH 7.4) at 4° C, the fixation was stopped and aldehyde groups were quenched by immersing thin slices of liver in phosphate-buffered saline diluted 1:1 with 0.3 M glycine-HCl (pH 7.4) for 1 hour at 20° C. The liver slices were preincubated for 3 to 6 hours in diaminobenzidine (1 mg/ml) in 0.1 M NH4PO4 buffer (pH 7.0) at 4° C, with stirring to allow penetration of the tissues. The peroxidase reaction was initiated by the addition of 0.005% H2O2 and continued for 1 hour at 20° C. After rinsing the tissues in Vernal-Acetate buffer (Fisher Scientific, Santa Clara, California) overnight, the tissues were embedded in JB-4 (Polysciences Incorporated, Warrington, Pennsylvania). Two-micron sections were cut, placed on glass slides, and dipped in Ilford L-4 emulsion (Polysciences Incorporated). Slides were exposed for 2 days to 9 weeks before their development. Kupffer cells were identified by their morphology and by the presence of a yellow or brown peroxidase reaction product. Silver grains were positively identified by polarized light (silver grains can be confused with cytoplasmic granules by bright field or phase contrast microscopy). Identification of the cell type associated with silver grains was assessed by phase microscopy.

Identification of the cells responsible for the uptake of the Dil-labeled lipoproteins was performed on 4- to 12-μm-thick frozen sections of freshly frozen tissue rinsed in phosphate-buffered saline and mounted in glycerol. Fluorescence microscopy was accomplished using a Zeiss (Universal) microscope equipped for epifluorescence. A standard green excitation filter coupled with a barrier filter excluding wavelengths below 590 nm was used for Dil localization.

**Results**

It has been shown previously that cholesteryl ester-rich apo E HDL can be isolated from the fraction of hypercholesterolemic canine plasma by ultracentrifugation and Pevikon block electrophoresis. These lipoproteins are ~200 to 250 Å in diameter and contain apo E as their exclusive or predominant apoprotein constituent. As an alternative method for the isolation of apo E HDL, we examined the feasibility of using a combination of gel filtration and Pevikon block electrophoresis without ultracentrifugation. Fractionation of plasma from hypercholesterolemic dogs using a Bio-gel A5m column resulted in a 280 nm profile with four major peaks (Figure 1). Cholesterol and apo E determinations on the individual fractions revealed that the majority of the cholesterol and apo E were in fractions 100 to 130. Triglyceride was principally recovered in fractions 80 to 120.

The fractions were pooled (Figure 1), and the lipoproteins within these pools were characterized by paper electrophoresis. A mixture of α2- and β-migrating lipoproteins were present in pools 6 through 9. The α2- and β-migrating lipoproteins in these pools were separated by Pevikon block electrophoresis and further characterized. The α2-migrating lipoproteins in pools 8 and 9 represented HDL and possessed both apo E and apo A-I (data not shown), similar to the HDL isolated at d = 1.02 to 1.063 by ultracentrifugation. The apo E HDL, possessing almost exclusively apo E, were present in pools 6 and 7 (Figure 2). Therefore, these two pools were combined and the apo E HDL were isolated from the pools (Figure 2) and used in the studies to be described.

As shown in Figure 2, the column-isolated apo E HDL contained predominantly apo E. Furthermore,
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Figure 1. Gel filtration chromatography of plasma from a cholesterol-fed dog. Plasma (20 ml) was applied to the 6% agarose A5m column. The column was operated at 4°C with a constant flow rate of 15 ml/hr. Fractions were collected at 15-minute time intervals. Absorbance at 280 nm was continuously measured (- - -); cholesterol (▲ ▲ ▲) and apo E (• • •) were measured for each fraction. Tubes were pooled (1 to 14) as indicated. CF-LDL indicates the LDL obtained from a cholesterol-fed dog.

![Paper Electrophoretogram Gel](image)

Figure 2. A paper electrophoretogram of pools 6 and 7 reveals the presence of a major β band (LDL) and a minor α2 band (apo E HDL_c). These two lipoproteins were resolved by Pevikon block electrophoresis, which allows for the separation of the LDL (β) from the apo E HDL_c. The apo E HDL_c possess apo E as their exclusive protein constituent, as shown on 11% sodium dodecyl sulfate-polyacrylamide gel electrophoretograms. The paper electrophoretograms were stained with Oil red O, and the acrylamide gel was stained with Coomassie blue.

These apo E HDL_c were spherical particles with a diameter of ~220 to 250 Å. Analysis of two different preparations of column-isolated apo E HDL_c revealed the following composition: 4.4% triglyceride, 41.2% total cholesterol, 33.4% phospholipid, and 21.2% protein. The size and composition of these column-isolated apo E HDL_c closely resembled that of centrifugally isolated apo E HDL_c.

An additional characteristic of apo E HDL_c is the enhanced binding activity of these lipoproteins to the apo B,E (LDL) receptors of cultured human fibroblasts. A 50% displacement of human 125I-LDL was shown to occur at a concentration of 0.05 μg of column-isolated apo E HDL_c protein, a value essentially identical to that previously reported for centrifugally isolated apo E HDL_c.

Previously, it was shown that the rapid clearance of apo E HDL_c is due predominantly to the uptake of these lipoproteins by the liver. In the present study in which this was documented in a dog, the apo E HDL_c were almost quantitatively removed from the plasma by the liver during a 20-minute period. As shown in Figure 3, ~80% of the injected apo E HDL_c were cleared from the plasma during the first 20 minutes, and 75% of the injected dose could be accounted for in the liver.

To determine which cells within the liver were responsible for the uptake of the apo E HDL_c, two labeling procedures were used to localize the uptake of the lipoproteins. Apoprotein E was labeled by iodination for localization by autoradiography. In addition, the fluorescent probe Dil was inserted into the lipoproteins and used to visualize directly the cellular uptake of apo E HDL_c by fluorescence microscopy. Previously, it was shown that the use of Dil does
Figure 3. Plasma clearance and liver uptake of ultracentrifugally isolated apo E HDLc. An adult, male foxhound was anesthetized, and an abdominal incision was made to expose the liver and to allow cannulation of the ureters. The ultracentrifugally isolated 125I-apo E HDLc (0.5 mg of protein, 44 x 10^6 cpm) were injected intravenously into the jugular vein, and blood samples were collected at the indicated times. Two liver biopsies (100 to 200 mg net weight) were taken from different lobes at 5, 20, 60, and 120 minutes after the injection, and the radioactivity was determined by gamma counting. Activity remaining in the plasma was reported as TCA-precipitable counts and calculations were based on a blood volume of 4.5% of the body weight. Approximately 7 ml of urine was collected during the 120-minute study, and 12 ml of bile was obtained from the gall bladder following the study.

not alter the physical or chemical properties of lipoproteins or the ability of labeled LDL and HDLc to interact with cell surface receptors. In addition, as shown in Figure 4, Dil-labeled apo E HDLc were cleared from the plasma of a normal dog at a rate essentially identical to apo E HDLc that had not been fluorescently labeled. Furthermore, ultracentrifugally isolated apo E HDLc were cleared at a rate essentially identical to that of the column-isolated apo E HDLc (Figure 4).

Because of the difficulty in obtaining the large quantities of apo E HDLc necessary for localization of the lipoproteins within specific cells in the dog liver, numerous studies were performed in the rat (and then confirmed in the dog). The canine apo E HDLc, isolated by either column or centrifugation, were cleared from the plasma of normal and cholesterol-fed rats (Figure 5) with kinetics similar to those observed in dogs, i.e., a rapid acute phase of clearance (the initial 10 minutes after injection) followed by a slower terminal phase. Within 5 to 10 minutes following the intravenous injection of the iodinated apo E HDLc, 60% to 65% of the injected dose was cleared from the plasma. At least one-half of the injected dose was present in the liver (data not shown).

Localization of the uptake of the canine apo E HDLc to specific cells within the rat liver was accomplished using autoradiography and fluorescence microscopy. The Dil-labeled apo E HDLc were injected into the femoral vein of normal rats and allowed to circulate for 5, 20, or 60 minutes. At the end of these time periods, small slices of liver were taken from the sacrificed rats and immediately frozen. Figure 6 is a micrograph of a liver section as seen by fluorescence microscopy. As shown in this figure, the parenchymal cells were heavily labeled, whereas there was no evidence that sinusoidal lining cells (Kupffer cells or endothelial cells) accumulated extensive fluorescence. Faint labeling of Kupffer cells in the perportal region of a liver lobule was observed in some instances. Intense punctate fluorescence, due to the accumulation of the Dil label, was observed near the cell nucleus of parenchymal cells. Uptake of the 125I-apo E HDLc was confirmed by autoradiography. As shown in Figure 7, the sinusoidal cells, including the clearly observable Kupffer cells identified by cytochemistry, were essentially free of silver grains.
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Figure 5. Plasma clearance of ultracentrifugally isolated (•,•) or column-isolated (o) 125I-apo E HDL by normal or cholesterol-fed rats. Blood was obtained from the femoral vein at the indicated times. All data are expressed as a percentage of the injected dose that remained in the plasma. Calculations were based on a blood volume of 4.0% of the total body weight and the data represent TCA-precipitable counts. Each rat received ~5 μg of apo E HDL protein.

whereas the parenchymal cells were diffusely labeled. Again, occasional sinusoidal cells in the periportal region of each lobule showed low levels of labeling; however, there was never a significant concentration of grains associated with the Kupffer or endothelial cells.

The uptake of apo E HDL by the liver of cholesterol-fed rats had to be monitored by autoradiography because of high levels of autofluorescence within the liver following cholesterol feeding. As shown in Figure 8, autoradiograms likewise localized the apo E HDL uptake in these livers predominantly to the parenchymal cells. A similar distribution of silver grains was observed at both 5 and 20 minutes following the injection of 125I-apo E HDL. The similarity in the distribution of the uptake of apo E HDL by both normal and cholesterol-fed rats is noteworthy, since cholesterol feeding has been shown to down-regulate the expression of apo B,E (LDL) receptors, but does not significantly decrease the expression of the hepatic apo E receptors. In the present study, no binding of canine or rat 125I-LDL to the liver membranes obtained from cholesterol-fed rats could be detected; (there may be apo B,E receptors present at levels that are too low to be detected by the mem-

Figure 6. Distribution of fluorescence within the normal rat liver 60 minutes following the injection of Dil-labeled apo E HDL. A. This phase contrast micrograph is of a freshly frozen section of liver near a venula (V) in the periportal region. Cords of parenchymal cells are visible between open sinusoids. Sinusoidal cells (S) (either Kupffer or endothelial cells) are identified. The endothelial cells (E) of the venule are also indicated (× 500). B. This fluorescence micrograph is of the same image shown in A. The parenchymal cells are heavily but unevenly labeled. Fluorescence is visible in a punctate pattern, tending to cluster at one side of the cell nucleus. The majority of the sinusoidal cells (S) and the endothelial cells (E) of the venule (V) do not show an accumulation of the fluorescence (× 500).
brane assay). However, substantial binding of canine $^{125}$I-apo E HDL was observed ($n = 2; K_d = 10$ and $11 \times 10^{-10} M; B_{max} = 62$ and $68$ ng/mg, respectively).

The apo E HDL$_c$ isolated by ultracentrifugation gave results identical to those just reported for column-isolated HDL$_c$ (data not shown). That is, parenchymal cells were shown to be responsible for almost all of the HDL$_c$ uptake in both normal and cholesterol-fed animals in experiments using either Dil fluorescence or $^{125}$I autoradiography. However, even less Kupffer cell uptake was seen in these preparations.

To establish that parenchymal cells were responsible for most (if not all) of the uptake of canine apo E HDL by the dog liver, an adult, male foxhound was anesthetized with pentobarbital, and a laparotomy was performed. A catheter was inserted into the portal vein and advanced into a small branch of the portal vein within a liver lobe. Column-isolated, $^{125}$I-apo E HDL$_c$ ($6.9 \times 10^8$ cpm) were infused into this segment of the liver. Nine minutes after the injection, the liver was removed, and the lobe was perfused with 1 liter of phosphate-buffered saline to remove labeled lipoproteins from the sinusoidal spaces. A Geiger counter was used to quickly determine the region of the liver lobe that received the infusion, and slices of this area were placed in the fixative. The entire procedure, from infusion to fixation, required less than 30 minutes. As shown in Figure 9, the silver grains were diffusely scattered over the parenchymal cells of the liver. There was only an occasional concentration of grains over any sinusoidal cells, and labeled Kupffer cells were near the portal region, not near the central vein. Autofluorescence within the adult dog liver prevented the use of Dil-labeled apo E HDL$_c$. Thus, the results obtained using rat liver were in agreement with those obtained using canine liver.

Discussion

Canine apo E HDL$_c$ can be isolated from the plasma of hypercholesterolemic dogs by a combination of gel filtration chromatography and Pevikon block
Figure 8. Distribution of silver grains within the cholesterol-fed rat liver 20 minutes after the injection of \( ^{125}\text{I}-\text{apo E HDL}_c \). A. This phase contrast micrograph of an unstained, plastic-embedded section of liver reveals two peroxidase-positive Kupffer (K) cells (\( \times 700 \)). B. This bright field micrograph is of the same image shown in A. The parenchymal cells are diffusely labeled, whereas the Kupffer cells do not display a concentration of silver grains (\( \times 700 \)).

Electrophoresis, without the use of ultracentrifugation. The characteristics of the apo E HDL\(_c\) isolated by this procedure appear to be identical to those of apo E HDL\(_c\) isolated by a combination of ultracentrifugation and Pevikon block electrophoresis. The apo E HDL\(_c\) isolated by either procedure were cleared from the plasma with apparently identical kinetics, i.e., >50% cleared in 5 to 10 minutes. Furthermore, the physical and chemical properties of the apo E HDL\(_c\) and the high affinity reactivity to apo B,E receptors shown by the apo E HDL\(_c\) isolated by the two procedures were essentially identical.

The hepatic uptake of apo E HDL\(_c\) was localized predominantly to the parenchymal cells. This observation was confirmed using iodinated and fluorescently labeled canine apo E HDL\(_c\) injected into a normal dog, as well as into normal and cholesterol-fed rats. The smaller rat model was used because of our inability to obtain quantities of the labeled apo E HDL\(_c\) sufficient to produce high levels of accumulation of labeled apo E HDL\(_c\) within the dog liver. The rat appears to be a reasonable model in which to study the hepatic uptake of canine apo E HDL\(_c\) in detail (results using rats were identical to those obtained with the dog). Previously, we have shown\(^6\) that canine apo E HDL\(_c\) behave similarly to rat chylomicron remnants in the perfused rat liver. Furthermore, the in vivo plasma clearance of the canine apo E HDL\(_c\) was similar in the dog and rat. It is noteworthy that these results, which showed the apo E HDL\(_c\) clearance from the plasma of cholesterol-fed rats and its localization within parenchymal cells, resembled those results observed in the normal, chow-fed rat. Moreover, in the cholesterol-fed rats, it was shown that the expression of LDL binding to the hepatic membrane receptors (apo B,E receptors) was reduced to undetectable levels, whereas apo E HDL\(_c\) binding (apparently via the apo E receptor) was readily demonstrable. These results were consistent with previous reports.\(^{10,11,24}\) It is reasonable to speculate that apo E HDL\(_c\) uptake was receptor-mediated in the cholesterol-fed animals, that the receptors involved were probably apo E receptors, and that these receptors may be located on parenchymal
cells. Additional studies will be required to prove this contention.

Localization of the uptake of apo E HDL\(_c\) to the parenchymal cells was accomplished by autoradiography using \(^{125}\text{I}\)-labeling and by fluorescence microscopy using Dil labeling. Autoradiographic localization of various lipoproteins within specific cells in the liver has been widely used.\(^{25,26}\) In addition, radiolabeled lipoproteins labeled with cholesteryl ether\(^{27}\) or sucrose\(^{28}\) have been used because these substances, delivered to cells by specific lipoproteins, accumulate in the lysosomes. Likewise, fluorescent probes have been used to study receptor binding of LDL to fibroblasts.\(^{29,30}\) Pitas et al.\(^{16}\) introduced Dil as a fluorescent probe that can be inserted into various lipoproteins and used to study lipoprotein interactions with fibroblasts, smooth muscle cells, and macrophages in vitro.\(^{16,31}\) This probe has several useful properties. In addition to being fluorescent, Dil is retained by the specific lipoprotein into which it has been inserted; it has been shown\(^{16}\) that Dil is not transferred to other lipoproteins during a 4-hour incubation at 37\(^{\circ}\)C. Furthermore, once the Dil is taken up by smooth muscle cells or macrophages, it is retained within the cells for up to 5.5 days without any detectable loss of fluorescence.\(^{16,31}\) In addition, the Dil-labeling procedure does not alter the physical or chemical properties of LDL and apo E HDL\(_c\) and does not interfere with the receptor-binding characteristics of these lipoproteins, as previously reported.\(^{16}\) In the present study, it was shown that Dil labeling did not alter the in vivo plasma clearance of apo E HDL\(_c\). Thus, the fluorescent labeling procedure using Dil should have wide applicability in both in vitro and in vivo studies.

It was possible to follow the uptake of the Dil-labeled apo E HDL\(_c\) at various time intervals after injection using fluorescence microscopy. The Dil rapidly accumulated in the hepatocytes in the region surrounding the nucleus. This observation is consis-

Figure 9. Distribution of silver grains within the normal dog liver 9 minutes after the injection of \(^{125}\text{I}\)-apo E HDL\(_c\). A. This bright-field micrograph illustrates that silver grains are distributed diffusely over the liver cells. Occasionally, sinusoidal lining cells do have grains associated with them, but there is no obvious concentration of grains over these cells (x 500). B. This higher magnification, bright-field micrograph demonstrates that silver grains are not concentrated in the region of Kupffer cells (K) or Ito (fat) cells (F) but are diffusely distributed over the parenchymal cells (x 1200).
tent with the idea that apo E HDLc are probably degraded within the lysosomes. There was a virtual absence of fluorescence (as well as significant concentrations of silver grains) associated with endothelial cells or Kupffer cells at all time intervals. The occasional Kupffer cells that were labeled were predominantly localized to the periperital portions of the liver lobule (not near the central veins). This localization may suggest that those HDLc taken up by the Kupffer cells may have been altered during the isolation procedure. The first Kupffer cells to be encountered would be in this region of the lobule.

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References


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