## Title

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# Uptake of Chemically Modified Low Density Lipoproteins In Vivo Is Mediated by Specific Endothelial Cells

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ABSTRACT Acetoacetylated (AcAc) and acetylated (Ac) low density lipoproteins (LDL) are rapidly cleared from the plasma ($t_{1/2} \simeq 1$ min). Because macrophages, Kupffer cells, and to a lesser extent, endothelial cells metabolize these modified lipoproteins in vitro, it was of interest to determine whether endothelial cells or macrophages could be responsible for the in vivo uptake of these lipoproteins. As previously reported, the liver is the predominant site of the uptake of AcAc LDL; however, we have found that the spleen, bone marrow, adrenal, and ovary also participate in this rapid clearance. A histological examination of tissue sections, undertaken after the administration of AcAc LDL or Ac LDL (labeled with either $^{125}I$ or a fluorescent probe) to rats, dogs, or guinea pigs, was used to identify the specific cells binding and internalizing these lipoproteins in vivo. With both techniques, the sinusoidal endothelial cells of the liver, spleen, bone marrow, and adrenal were labeled. Less labeling was noted in the ovarian endothelia. Uptake of AcAc LDL by endothelial cells of the liver, spleen, and bone marrow was confirmed by transmission electron microscopy. These data suggest uptake through coated pits. Uptake of AcAc LDL was not observed in the endothelia of arteries (including the coronaries and aorta), veins, or capillaries of the heart, testes, kidney, brain, adipose tissue, and duodenum. Kupffer cells accounted for a maximum of 14% of the $^{125}I$-labeled AcAc LDL taken up by the liver. Isolated sinusoidal endothelial cells from the rat liver displayed saturable, high affinity binding of AcAc LDL ($K_d = 2.5 \times 10^{-9}$ M at 4°C), and were shown to degrade AcAc LDL 10 times more effectively than aortic endothelial cells. These data indicate that specific sinusoidal endothelial cells, not the macrophages of the reticuloendothelial system, are primarily responsible for the removal of these modified lipoproteins from the circulation in vivo.

Modification of the lysine residues of the apoprotein B in low density lipoproteins (LDL)[1] by acetylation (Ac) or acetoacetylation (AcAc) results in a dramatic acceleration of the plasma clearance of these lipoproteins when they are injected into animals (1–3). When normal rat or human LDL are injected into rats, 50% of the injected dose is cleared from the plasma in ~5 h (4). In contrast, 50% of the injected dose of modified LDL is cleared in minutes (4). This clearance is not mediated via the typical LDL receptor pathway. Chemical modification inhibits the binding of these lipoproteins to LDL receptors and stimulates their uptake by the Ac LDL receptor (for review, see reference 5). This receptor has been demonstrated in vitro on two phagocytic cells of monocytic origin, cultured peritoneal macrophages and Kupffer cells (1, 4), as well as on aortic endothelial cells (6). Incubation of peritoneal macrophages with modified LDL leads to the accumulation of large amounts of cholesteryl esters in the cells, causing them to resemble the lipid-laden foam cells present in atherosclerotic lesions (7–9). While Ac or AcAc LDL are unlikely to occur in vivo, other modifications may occur that produce LDL that are recognized by this same receptor (10–13). This receptor may therefore represent one pathway that serves as

[1] *Abbreviations used in this paper:* Ac, acetylated; AcAc, acetoacetylated; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine; LDL, low density lipoproteins; PBS, phosphate-buffered saline.

THE JOURNAL OF CELL BIOLOGY · VOLUME 100 JANUARY 1985 103–117

a mechanism for lipid accumulation in these cells in vivo.

The clearance of modified LDL in vivo is primarily mediated by the liver (4). Both Mahley et al. (4) and van Berkel et al. (14) have found that the clearance of modified LDL is mediated by liver sinusoidal cells. Such cells are a mixture of endothelial cells, Ito cells, and Kupffer cells (15). Using the in vivo injection of $^{125}$I-labeled Ac LDL, followed by the isolation of hepatic cells, Nagelkerke et al. (16) found that hepatic endothelial cells were primarily responsible for the in vivo uptake of the lipoproteins. Lower levels of uptake were noted in Kupffer cells and hepatocytes. These observations, along with those showing that aortic endothelial cells in vitro metabolize low levels of Ac LDL (6), suggest that endothelia in general contribute in varying degrees to the clearance of modified lipoproteins from the blood.

To determine the specific cells responsible for the clearance of modified LDL in various organs and their relative contributions, we injected rats, dogs, and guinea pigs with Ac LDL or AcAc LDL labeled with either $^{125}$I or the lipophilic fluorescent probe, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI) (17). Tissue was then examined microscopically by two complementary techniques, fluorescence microscopy and autoradiography. The uptake of AcAc LDL, by the three most active tissues, was then confirmed by electron microscopy. These techniques avoid the problems associated with the use of cell isolation procedures and the possible metabolism of $^{125}$I-labeled Ac LDL (before and during cell isolation), as well as possible cross-contamination of the isolated cells. Using these procedures, it was found that the sinusoidal endothelial cells of the liver, spleen, marrow, and adrenal mediate the in vivo clearance of AcAc LDL. The ability of liver sinusoidal endothelial cells to metabolize AcAc LDL at a rapid rate was confirmed in in vitro studies. These results establish that specific sinusoidal endothelial cells are the major site of clearance of modified LDL from the blood.[2]

## MATERIALS AND METHODS

*Lipoproteins:* Human LDL ($d$ = 1.02–1.05) were isolated from the plasma of normal donors by sequential density ultracentrifugation (18). For certain experiments, the LDL were iodinated (19) and/or labeled with DiI[3] (17), and acetoacetylated by reaction with 80 μmol diketene/mg of lipoprotein protein (17, 20). For other experiments, acetylation was performed as described by Basu et al. (21).

*Animals:* Male and female Sprague-Dawley rats (220–250 g) and guinea pigs (~300 g) were obtained from Simonsen Laboratories (Gilroy, CA). The rats were anesthetized by intraperitoneal injection of 65 mg/kg body weight of sodium pentobarbital (Carter-Glogau Laboratories, Inc., Glendale, AZ). In some studies of ovarian uptake of AcAc LDL, human chorionic gonadotropin (Sigma Chemical Co., St. Louis, MO) was administered (50 U/d, subcutaneously) to female rats for 4 d before administration of the iodinated or DiI-labeled AcAc LDL. Guinea pigs were anesthetized by an intramuscular injection of 10 mg/kg body weight of Ace Promazine Maleate (Ayerst, New York, NY) in conjunction with 100 mg/kg body weight of Ketaset (ketamine hydrochloride) (Bristol Myers Co., Syracuse, NY). A mongrel dog (22 kg) was anesthetized with an intravenous injection of pentobarbital (25 mg/kg). The animal was maintained on a positive pressure ventilator. It was assumed that the plasma volume of the animals was 3.5% of the body weight in grams. The DiI- or $^{125}$I-labeled lipoproteins (5–100 μg of protein/ml of plasma) were injected intravenously. The dosage was calculated on the basis of the estimated plasma volume. Incubation studies with labeled lipoproteins in vitro showed no exchange of DiI between various lipoprotein classes (17).

To test for the inhibition of binding of AcAc LDL to the liver in vivo, fucoidin (ICN K&K Laboratories Inc., Plainview, NY) was administered at a 300-fold weight excess over that of the $^{125}$I-labeled AcAc LDL. The fucoidin was either mixed with the AcAc LDL before intravenous injection or was administered 15 s before the injection of the lipoproteins via a different vein. The lipoproteins were injected to produce a plasma concentration of 5 μg/ml.

*Histology:* Tissues for fluorescence microscopy were removed from the animals 15 min to 1 h after the injection of the DiI-labeled LDL. These time points were used to ensure that the lipoprotein-associated fluorescence was internalized by those cells responsible for its clearance, and not still bound to surface receptors. Specimens were washed in ice-cold phosphate-buffered saline (PBS), cut into 4-mm pieces, and immediately frozen in isobutane that had been cooled with liquid nitrogen. The tissue was stored at −70°C until processed. For cryostat sections, the samples were mounted on Tissue Tek without thawing and cut at 4 to 10 μm. Sections were picked up on gelatin-coated slides, washed in PBS, and mounted in 90% glycerol in phosphate buffer for viewing.

For autoradiography, liver perfusion was begun 8 min after the injection of the $^{125}$I-labeled AcAc LDL. This time was chosen as the time point for maximum liver incorporation before significant apoprotein degradation had occurred. At this time point, ~85% of the injected dose was present in the liver and 5% was present in the plasma. Perfusion at 110 mm Hg was initiated with ~150 ml of Eagle's minimum essential medium (4°C, pH 7.4), followed by ~250 ml of fixative containing 2% formaldehyde and 0.5% glutaraldehyde in PBS (4°C, pH 7.4). Very thin slices were cut with a razor blade, fixed for 30 min at 4°C in the formaldehyde-glutaraldehyde fixative, and then incubated with stirring for 1 h at 4°C in one-half strength glycine-buffered (0.15 M) PBS (pH 7.4).

For the histochemical demonstration of peroxidase, the tissues were next incubated at 4°C for 3 to 7 h with constant stirring in a solution containing 1 mg/ml of diaminobenzidine in 0.1 M monobasic phosphate adjusted to pH 7.0 with ammonium hydroxide. Hydrogen peroxide was added for the final hour at a final concentration of 0.005%. After an overnight wash in 0.1 M barbital buffer (pH 7.6) to remove excess diaminobenzidine, the tissue was embedded in JB-4 (Polysciences, Inc., Warrington, PA) (22). Sections of liver cut to 2-μm thickness were mounted on glass slides, hand-dipped in Ilford L4 emulsion, and then exposed for 4 d to 2 mo. Slides were developed in Microdol-X (Kodak, Rochester, NY) plus 15% sodium thiosulfate.

All photographs were taken using a Zeiss Universal microscope. Sections for fluorescence microscopy were viewed using rhodamine excitation and emission filters and epifluorescent illumination. Ilford HP5 film shot at ASA 800 was used. Bright-field images of autoradiograms were photographed with both an applegreen filter and a blue tungsten color correction filter to enhance the peroxidase reaction product and silver grains. Phase-contrast micrographs were made with the applegreen filter alone. Technical Pan shot at ASA 50 was used for bright-field photographs of the autoradiograms, and the same film was shot at ASA 100 for the phase-contrast microscopy.

For estimating the uptake of $^{125}$I-labeled AcAc LDL by Kupffer cells, silver grains associated with peroxidase-positive sinusoidal cells were counted in autoradiograms of liver sections. These results were corrected for scatter of the silver grains that resulted from the thickness of the tissue sections and the emulsion as well as from the high energy of $^{125}$I. An estimate of the scatter was determined by counting the number of grains in successive 2-mm bands from the margins of cross-cut endothelia. 75% of the grains were contained within the cells and the first 4 mm surrounding them. On this basis, the grains associated with Kupffer cells were calculated as the sum of the grains overlying peroxidase-positive cells plus those within 4 mm of them. Because this would account for only 75% of the scatter, this number was multiplied by 1.33 to give a 100% figure. This value may be an exaggerated estimation since much of the $^{125}$I was internal and not at the cell margin. In addition, neighboring endothelial cells may have contributed grains.

*Electron Microscopy:* Tissues from rats injected with a large dose of AcAc LDL (50 μg of lipoprotein protein/ml of plasma) were examined by electron microscopy for the uptake of the lipoproteins. 6 min after injection of the lipoproteins, the animals were perfused at 110 mm Hg through the left ventricle of the heart, first with Medium 199 (Gibco Laboratories, Grand Island, NY) at 4°C to remove blood from the vasculature. This was followed by perfusions with fixative containing 2% paraformaldehyde and 2% glutaraldehyde in 0.066 M cacodylate buffer (4°C, pH 7.4). Tissues from both AcAc LDL-injected and nontreated (control) animals were removed and fixation was continued for 2 h at 20°C. Postfixation with 1% osmium tetroxide in 0.1 M barbital buffer (4°C, pH 7.6) was carried out for 1 h. After rinsing in water, samples were stained overnight at 4°C in 2% uranyl acetate in water, dehydrated in acetone, and embedded in Epox 812 (Polysciences, Inc.). Silver to grey sections stained with 2% uranyl acetate in water and Reynolds lead citrate (23) were photographed.

[2] Portions of this work were published previously in abstract form (*Fed. Proc.* 1983. 42:1819*a*. [Abstr.]).

[3] The manufacturer of DiI (Molecular Probes, Inc., Junction City, OR) has informed us that the chemical name 3,3′-dioctadecylindocarbocyanine used in previously published literature on DiI was incorrect. The correct name for the compound is 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine.

*Isolation and Growth of Liver Endothelial Cells:* Liver endothelial cells and hepatocytes were isolated using a modification of previously described procedures (24–26). Briefly, rat liver was dispersed by perfusion with collagenase (Sigma Chemical Co.) via the portal vein. The crude cell suspension was treated with DNase (Sigma Chemical Co.) to prevent cell aggregation and then processed by centrifugal elutriation to separate hepatocytes and nonparenchymal cells. The hepatocytes were maintained as previously described (26). The nonparenchymal cell fraction was treated with Pronase (Calbiochem-Behring Corp., San Diego, CA) and then subjected to an additional cycle of centrifugal elutriation to obtain endothelial cells. The endothelial cells were cultured in 35-mm plastic petri dishes (Lux Scientific, Newbury Park, CA) that had been coated with 10 μg of rat-tail collagen (26) and maintained in a modified Medium 199 with Hanks' salts (26). The medium was supplemented with L-ascorbate (0.3 mM), corticosterone ($10^{-6}$ M), insulin (4 mU/ml), penicillin (100 U/ml), and horse and calf serum (each 10%, vol/vol). As judged by several criteria, at least 90% of the cells in these cultures represented sinusoidal endothelial cells.[4] As seen by immunofluorescent examination, the cells contained type IV collagen but not types I or III; in normal intact rat liver, type IV collagen is localized to the perisinusoidal area, in or around endothelial cells.[4] When fixed cultures were examined by scanning electron microscopy, the cells exhibited the fenestrae typical of sinusoidal endothelium. Finally, the cells in culture lacked factor VIII-related antigen that, in the intact rat liver, is present in large vessels but absent from sinusoids (25, 27). This indicates that the cultures represent sinusoidal rather than large vessel endothelium. To remove nonadherent material, cultures were subjected to a change of medium 16 h after cell plating and were used at 24 h.

*Tissue Culture:* Unstimulated mouse peritoneal macrophages were isolated and washed as described (28). Experiments were performed 24 h after isolation. Bovine aortic endothelial cells (a gift from Dr. D. Gospodarowicz, University of California, San Francisco, CA) were grown in Dulbecco's modified Eagle's medium (Gibco Laboratories) supplemented with 10% fetal calf serum and fibroblast growth factor (29). Experiments were performed 6 d after transfer from stock cell lines to culture dishes, at which time the cells were confluent.

Human hepatocarcinoma cells, Hep G2 (30, 31) (a gift from Dr. Barbara Knowles, Wistar Institute, Philadelphia, PA) and the rat hepatoma line, Fu5AH (32) (a gift from Dr. George Rothblat, Medical College of Pennsylvania, Philadelphia, PA), were grown in Earle's minimum essential medium supplemented with glutamine (Gibco Laboratories) and 2.2 g/liter $NaHCO_3$. The Hep G2 and Fu5AH cell media contained 10% fetal calf serum and 5% calf serum, respectively. The uptake of DiI-labeled AcAc LDL (10 μg of lipoprotein protein/ml of media) by Hep G2 and Fu5AH cells was assessed 4–7 d after growth in culture dishes and by primary hepatocyte cultures 24 and 48 h after isolation.

## RESULTS

### *Modified LDL Uptake by the Liver In Vivo*

The hepatic uptake of DiI-labeled AcAc LDL was studied in the rat, dog, and guinea pig. In all species, a similar pattern of uptake was observed. Representative liver sections are shown in Fig. 1. The fluorescent labeling appeared in linear arrays, which suggested the participation of sinusoidal endothelial cells in the uptake. (If the uptake involved Ito cells or Kupffer cells, the labeling would be less frequent and would not appear in a linear pattern [15].) A gradient of fluorescence, with the highest intensity in the periportal region of the lobule decreasing toward the central vein, was often observed (not shown). There was a notable lack of fluorescence, however, in the endothelia of larger blood vessels. No detectable fluorescence was seen in hepatocytes (Fig. 1).

As a control to ensure that the DiI did not direct the uptake of the modified LDL by the endothelial cells of the liver, and that it was the modification of the LDL particle that was responsible for the uptake, DiI-labeled native LDL were injected into rats. When the rat liver was examined after 20 min, little if any fluorescence was noted, reflecting the slow catabolism of these lipoproteins in the rat and demonstrating that it was the chemical modification that directed the rapid uptake of the DiI-labeled AcAc LDL by sinusoidal cells and not the fluorescent probe.

[4] Irving, M. G., F. J. Roll, S. Huang, and D. M. Bissell. Manuscript in preparation.

Autoradiographic techniques confirmed that AcAc LDL were taken up by sinusoidal endothelial cells (Fig. 2). After injection of $^{125}$I-labeled AcAc LDL, silver grains were generally localized to endothelial cells associated with the sinusoidal lining. There was a lack of grains associated with the portal and central veins; however, silver grains accumulated over sinusoidal endothelial cells past the branch points in lobular vessels (Fig. 2). Furthermore, endothelial cells in large vessels and capillaries in the portal areas of the lobules were essentially devoid of silver grains.

In these in vivo studies, the hepatocytes did not appear to participate in the uptake of either the DiI- or $^{125}$I-labeled AcAc LDL. This is consistent with the observation that acetoacetylation of LDL prevents binding to the apolipoprotein B,E receptors (20). To assess further the ability of hepatocytes to metabolize AcAc LDL, DiI-labeled AcAc LDL were incubated with hepatocytes in culture and their uptake was monitored. When DiI-labeled AcAc LDL (10 μg of lipoprotein protein/ml of media) were incubated at 37°C for 4 h with either isolated rat hepatocytes, a rat hepatoma line (Fu5AH) or a human hepatocarcinoma line (Hep G2), no uptake of these fluorescently labeled lipoproteins was observed (data not shown). Both of these cell lines have been shown to have receptors for lipoproteins (31, 32), an observation confirmed in our laboratory (G. Friedman, T. L. Innerarity, and R. W. Mahley, unpublished observations).

Because receptors for AcAc LDL have been demonstrated on Kupffer cells and other macrophages in vitro, it was important to determine if modified lipoproteins were taken up by these cells to a significant extent in vivo. The contribution of Kupffer cells to the in vivo metabolism of iodinated AcAc LDL was assessed by autoradiography in liver sections processed to identify Kupffer cells by their peroxidatic activity (33). Relatively little labeling was seen over Kupffer cells as compared to the sinusoidal endothelial cells (Fig. 3).

To obtain a semiquantitative estimate of the extent to which the Kupffer cells participated in the uptake of AcAc LDL, areas of the autoradiogram that contained Kupffer cells were randomly photographed and grains associated with Kupffer cells ($n$ = 19, 280 grains) and those in the remainder of the field (2,248 grains) were counted. The area of the liver section containing Kupffer cells (as determined by cutting the cells from the photograph and weighing the images of the Kupffer cells and the remainder of the field) was estimated to be 1.6%. Because Kupffer cells have been reported to make up 2.1% of the parenchymal cell volume (15), the Kupffer cells could have been underrepresented in our samples. For this reason, the number of grains, associated with Kupffer cells was multiplied by 1.3 (2.1/1.6) to avoid bias. The percentage of grains associated with Kupffer cells was 10.8% before the correction and 14% after the correction. From these determinations, it was calculated that the maximum amount of AcAc LDL metabolized by Kupffer cells was 14% of the amount taken up by the liver. Because hepatocytes did not take up detectable amounts of DiI- or $^{125}$I-labeled AcAc LDL, it was assumed that the remainder of the grains were associated with sinusoidal endothelia. The sinusoidal endothelial cells therefore contained at least six times more radioactive AcAc LDL than the Kupffer cells.

The uptake of AcAc LDL by hepatic sinusoidal lining cells was confirmed by electron microscopy. We reasoned that, following the rapid uptake of a large dose of AcAc LDL by liver nonparenchymal cells, large endocytic vesicles would form in the cells mediating this uptake. Rats were therefore

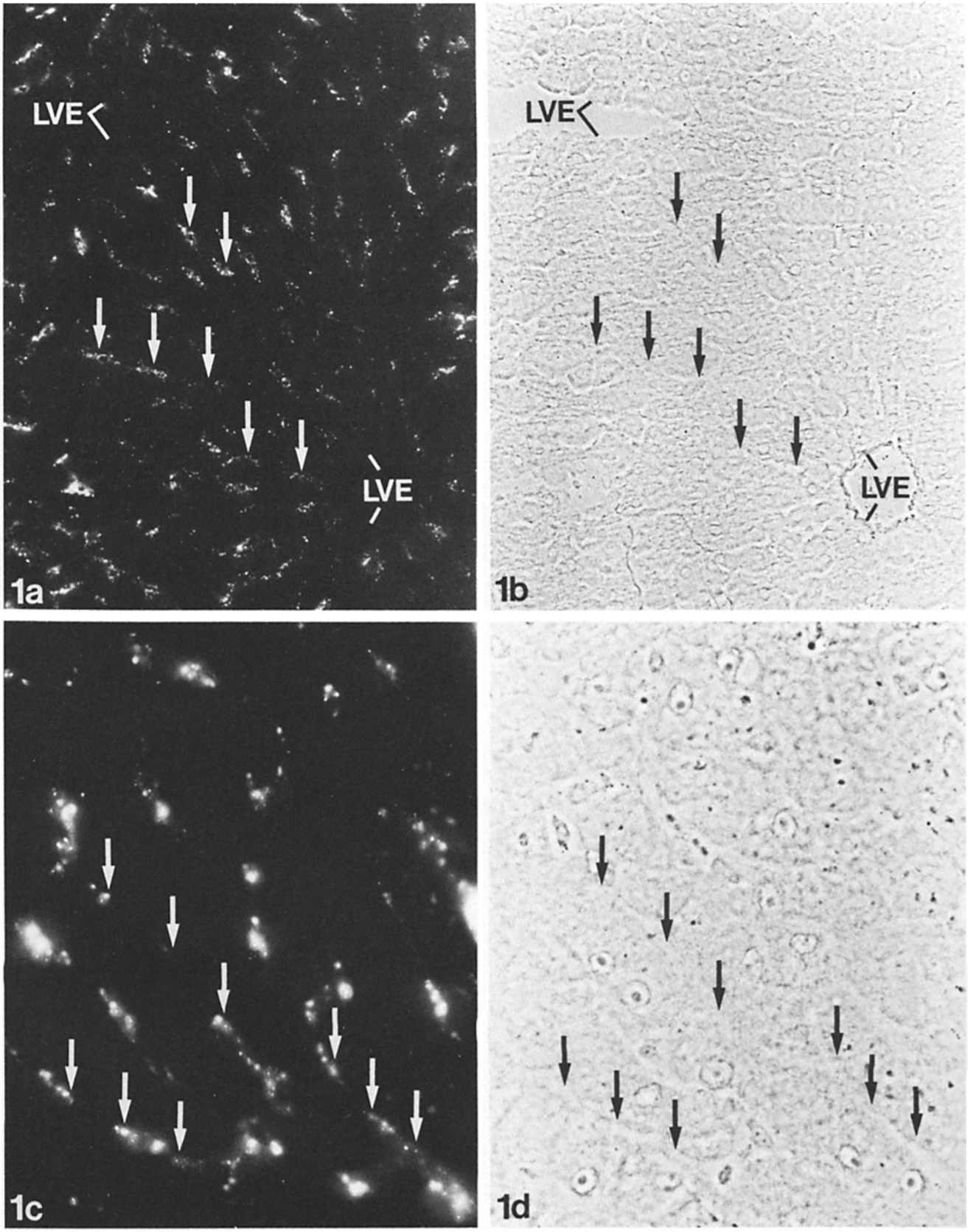



FIGURE 1 Photomicrographs of fresh frozen liver sections showing the uptake of DiI-labeled, AcAc LDL at low magnification in the rat (*a* and *b*, × 185) and at higher magnification in the dog (*c* and *d*, × 750). The same fields have been photographed under fluorescence (*a* and *c*) and phase-contrast (*b* and *d*) illumination. (*a* and *b*) At low magnification, the linear pattern of fluorescence (↓) is suggestive of binding to sinusoidal cells. Large vessel endothelia (*LVE*) lack fluorescence. (*c* and *d*) At higher magnification, the linear pattern of fluorescence (↓) is clearly associated with the sinusoids of the liver as seen by comparing the phase and fluorescent images. Labeled lipoproteins were injected intravenously to yield a plasma concentration of 10 μg of lipoprotein protein/ml. Liver samples were taken 15 min after the injection of lipoproteins into the dog and 20 min after the injection of lipoproteins into the rat.

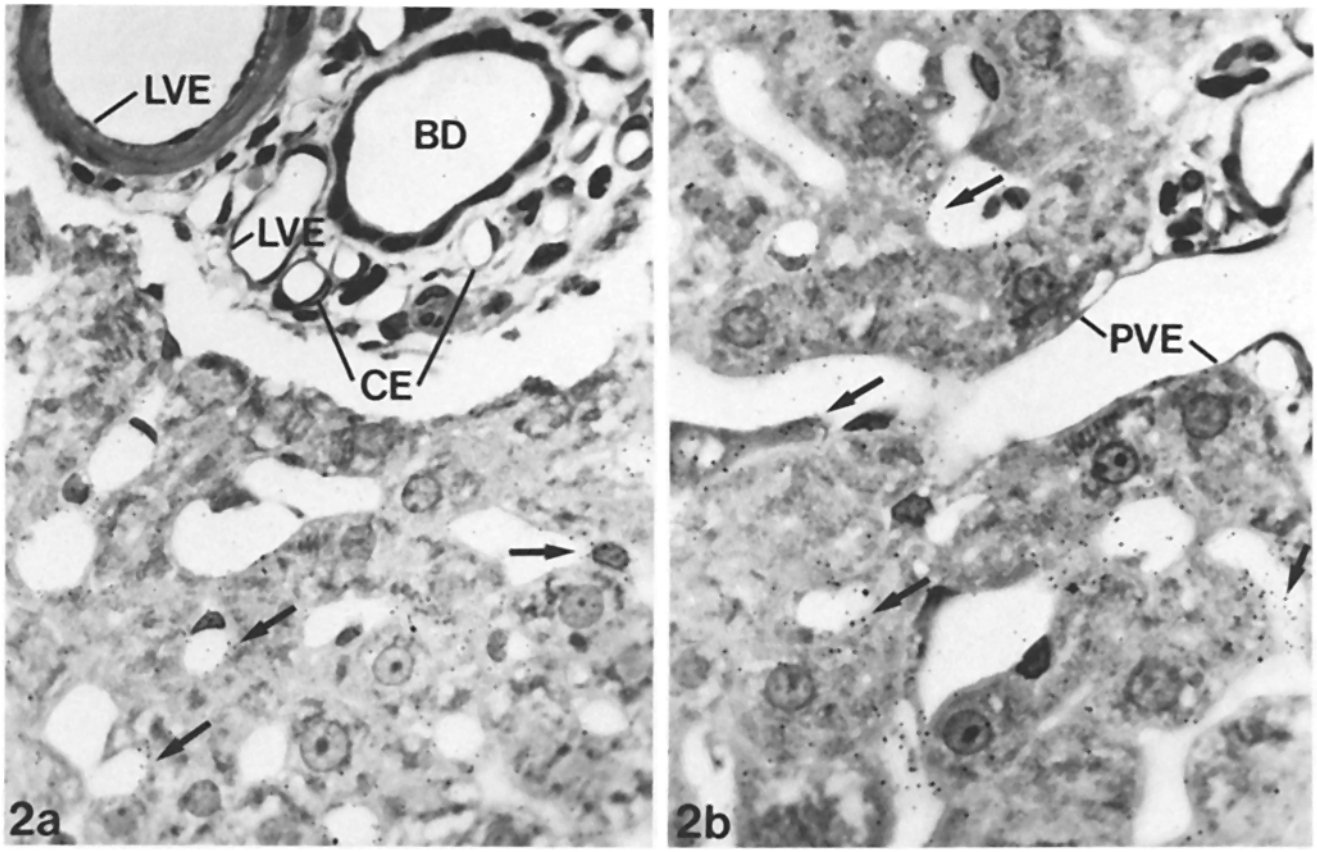



FIGURE 2 Photomicrographs of 2-μm, toluidine-stained, plastic-embedded rat liver sections processed for autoradiographic demonstration of the sites of $^{125}$I-labeled AcAc LDL uptake. (*a*) Silver grains appear predominantly over sinusodial endothelial cells (→) and not over large vessel endothelia (*LVE*) or the endothelia of capillaries (*CE*) in the stroma surrounding the bile duct (*BD*) and vasculature. (*b*) Silver grains are not only absent over the portal vessel endothelia but also over its small branches (*PVE*). They appear only over the sinusoidal endothelia (→) served by these vessels. The liver was perfusion-fixed 8 min after the injection of $^{125}$I-labeled AcAc LDL into the rat (320 cpm/ng; 10 μg of lipoprotein protein/ml of plasma). × 950.

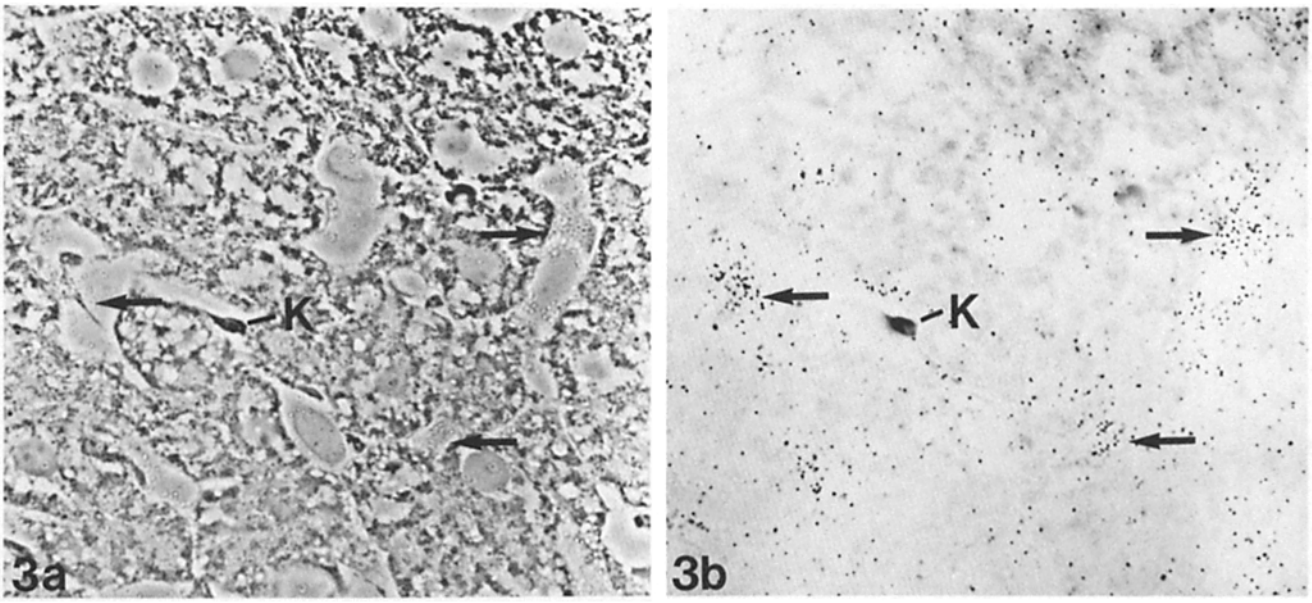
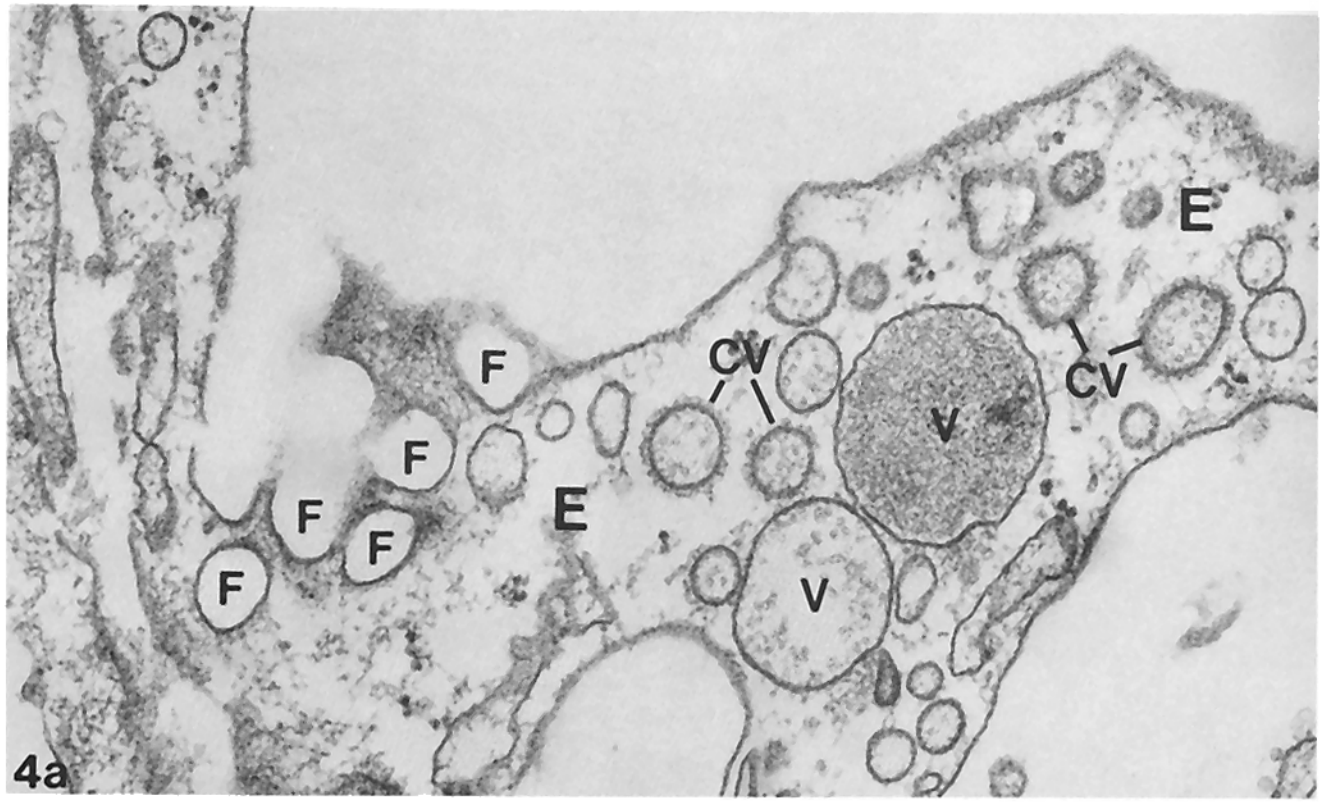
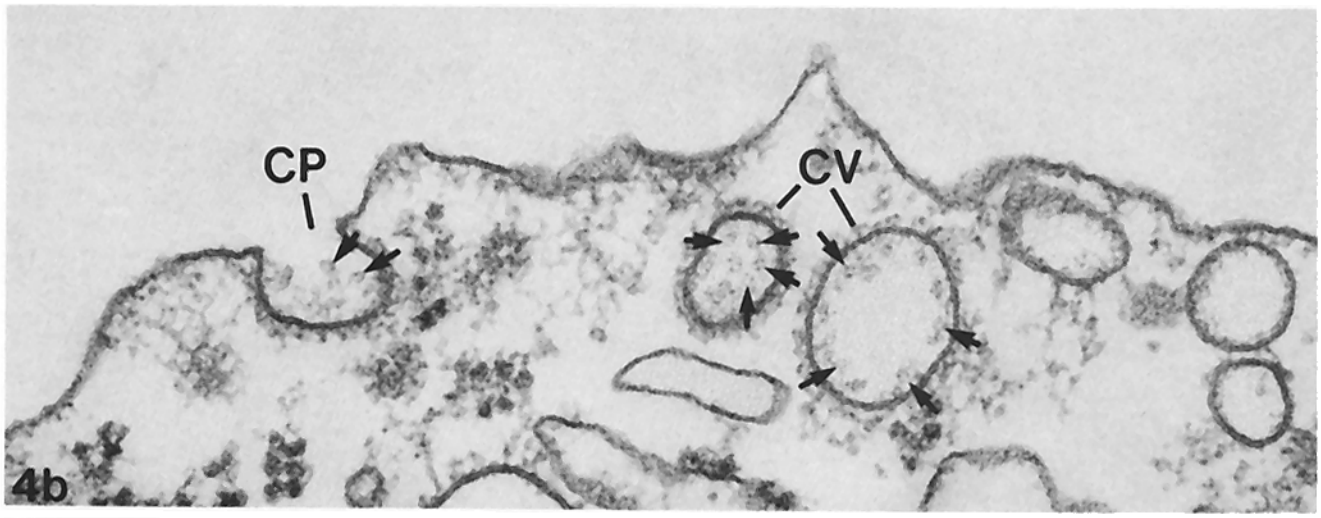


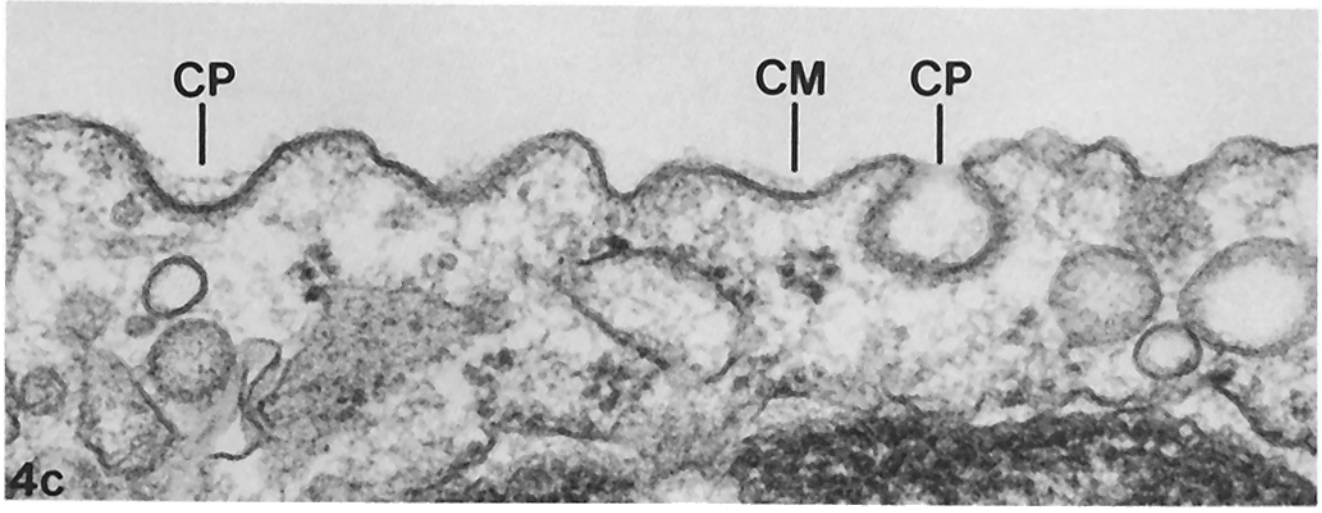

FIGURE 3 Micrographs of 2-μm, unstained, plastic-embedded rat liver sections processed for autoradiographic demonstration of $^{125}$I-labeled AcAc LDL and the endogenous peroxidase of Kupffer cells. (*a*) Phase-contrast micrograph taken for orientation at the focal plane of the tissue. (*b*) Bright-field image taken at the focal plane of the silver grains, showing their distribution, as well as the dark diaminobenzidine reaction product of peroxidase localized in a Kupffer cell (*K*). Silver grains are primarily localized over sinusoidal endothelial cells (→). Those grains over the remaining cells of the liver probably represent scatter due to the limited resolution of this technique. Fluorescence labeling experiments demonstrated no uptake by other cells. Peroxidase-labeled Kupffer cells are notably free of silver grains. The liver was perfusion-fixed 8 min after the intravenous injection of $^{125}$I-labeled AcAc LDL (370 cpm/ng of protein; 10 μg of lipoprotein protein/ml of plasma). × 750.




4a




4b




4c

injected with an amount of AcAc LDL capable of delivering ~1 mg of cholesterol to the liver, and the livers from control and treated animals were examined by electron microscopy. The sinusoidal endothelial cells from animals injected with AcAc LDL contained numerous large vesicles with particulate contents in various stages of condensation (Fig. 4), while only small or apparently empty vesicles were seen in the endothelial cells of control animals that did not receive the AcAc LDL. In addition to large and small endocytic vesicles, many coated pits and coated vesicles in the endothelial cells from rats receiving the AcAc LDL contained spherical objects that were homogeneous in size (~20 nm in diameter) (Fig. 4). Only rarely were particles seen over uncoated areas of membrane. These spherical particles, which were the approximate size of LDL and which were not present in controls, probably represented AcAc LDL. No such particles were seen over coated areas of Kupffer cells. The electron microscopy studies therefore confirmed that the uptake of AcAc LDL was carried out predominantly by hepatic sinusoidal endothelial cells and suggested that this uptake was mediated by coated pits and vesicles—a pathway generally associated with receptor-mediated endocytosis.

Because both AcAc LDL and Ac LDL are bound to the same receptor on macrophages in vitro and have similar rates of plasma clearance in vivo, it was important to determine if sinusoidal endothelial cells were also capable of taking up Ac LDL in vivo. The hepatic uptake of labeled Ac LDL was studied using autoradiography and fluorescence microscopy. In both rats and guinea pigs, the results using Ac LDL were indistinguishable from those using AcAc LDL. Uptake was predominantly carried out by sinusoidal endothelial cells, with little contribution by Kupffer cells and large vessel endothelia (data not shown).

It has been shown that the binding and uptake of Ac LDL and AcAc LDL by macrophages in vitro is blocked by the sulfated polysaccharide fucoidin (34, 35). To determine if fucoidin could prevent AcAc LDL uptake in vivo, AcAc LDL, dual-labeled with $^{125}I$ and DiI, were injected into rats along with a 300-fold excess of fucoidin. A substantial reduction in the rate of disappearance of $^{125}I$ from the plasma was noted. After 1.5 min, only 20–30% of the injected dose was cleared, as compared with 75–78% in the absence of fucoidin. Similar results were obtained when the fucoidin was injected 15 s before the injection of AcAc LDL via another vein. The decrease in the plasma clearance of AcAc LDL caused by fucoidin was associated with a decreased uptake of $^{125}I$-labeled AcAc LDL by the liver. The fluorescence observed in the livers of fucoidin-treated animals was much less intense than in control animals but remained associated with the sinusoidal cells. These data suggest that the receptors on the sinusoidal endothelial cells and the receptors on macrophages share the ability of fucoidin to prevent AcAc LDL uptake in vivo.

## *In Vitro Studies*

The explanations for the uptake of modified LDL by the sinusoidal endothelial cells and the lack of uptake (or very low levels of uptake) by other vessel endothelia were explored by comparing the uptake and degradation of $^{125}I$-labeled AcAc LDL by mouse peritoneal macrophages, bovine aortic endothelial cells, and hepatic endothelial cells in culture. The liver endothelial cells isolated for the in vitro studies were known to be the same as those sequestering AcAc LDL in vivo because liver endothelial cells prepared from animals receiving DiI-labeled AcAc LDL intravenously 5 min before liver perfusion were brilliantly and uniformly fluorescent.

A comparison of the degradation of iodinated, AcAc LDL by rat liver sinusoidal endothelial cells and mouse peritoneal macrophages demonstrated that the endothelial cells degraded 40% as much modified LDL as the macrophages (Fig. 5). In similar studies, the ability of bovine aortic endothelial cells and mouse peritoneal macrophages to metabolize AcAc LDL (10 μg AcAc LDL/ml of media) was compared. In 2 h at 37°C, the bovine endothelial cells degraded only 40 ng of the modified LDL/mg of cell protein while the macrophages degraded 1,200 ng/mg. Therefore, the bovine aortic endothelial cells degraded only 3.5% as much AcAc LDL as the macrophages. These results are comparable to those obtained by Stein and Stein (6). Thus, the liver sinusoidal endothelial cells appear to be 10 times more active than bovine aortic endothelial cells in the metabolism of these lipoproteins.

The properties of the receptors on the isolated liver endothelial cells were determined in direct binding studies con-

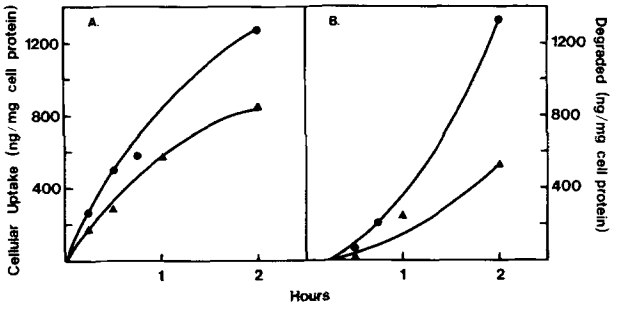



FIGURE 5 Comparison of the cellular uptake (*A*) and degradation (*B*) of $^{125}I$-labeled AcAc LDL by mouse peritoneal macrophages (●) and liver endothelial cells (▲). Unstimulated mouse peritoneal macrophages and liver endothelial cells were prepared as described in Materials and Methods, and the binding study was performed 24 h after isolation. The experiment was conducted at 37°C using $^{125}I$-labeled AcAc LDL at 10 μg of protein/ml of medium containing 10% fetal calf serum. Data points are the mean of duplicate determinations. The mean cellular protein was 86 μg per dish for the mouse peritoneal macrophages and 70 μg per dish for the liver endothelial cells.

FIGURE 4 Electron micrographs of liver sinusoidal endothelial (*E*) cells from a rat injected with AcAc LDL (*a* and *b*) and from a control animal (*c*). (*a*) This cell is identified as an endothelial cell by the fenestrated (*F*) sieve plate at the left and numerous small vesicles within the cell's cytoplasm. Within the cell are numerous coated (*CV*) and uncoated (*V*) vesicles with a particulate content. (*b*) In an endothelial cell at higher magnification, particles (→) ~20 nm in diameter are seen within coated pits (*CP*) and coated vesicles (*CV*). The AcAc LDL were injected to yield a plasma concentration of 50 μg of lipoprotein/ml. 6 min after injection of the AcAc LDL, the animal was perfusion-fixed and tissue was processed for electron microscopy. (*c*) In an endothelial cell from a control animal, the external surfaces of coated pits and coated areas of the membrane (*CM*) have some fuzzy material associated with them, but particulate matter is not seen. (*a*) × 62,000; (*b* and *c*) × 108,000.

ducted at 4°C. The liver endothelial cells demonstrated saturable, high affinity binding of iodinated AcAc LDL (Fig. 6). An equilibrium dissociation constant ($K_d$) of $2.5 \times 10^{-9}$ M was calculated for this binding. The addition of nonlabeled AcAc LDL, but not native LDL, competitively inhibited the binding of the $^{125}$I-labeled AcAc LDL to the sinusoidal endothelial cells.

## Tissue Distribution of $^{125}$I-labeled AcAc LDL in Vivo

Because sinusoidal endothelial cells are not unique to the liver, the contribution of other organs to the metabolism of AcAc LDL in vivo was assessed by following the uptake of $^{125}$I-labeled AcAc LDL by various tissues of rats 6 min after the injection of the lipoproteins. As previously reported, the liver was the predominant site of uptake (4). The liver accumulated 40-fold more $^{125}$I than the next most active organ, the spleen. All other tissues studied showed a relatively insignificant contribution to the metabolism of the lipoproteins when compared with the liver (Table I). However, when the data were expressed per gram of organ weight, it was apparent that the spleen, bone marrow, and adrenal were 25–30% as active in the uptake of AcAc LDL as the liver (Table I) and the ovary was 3.9% as active. The relatively high level of $^{125}$I that accumulated in the kidney appeared to represent the uptake of free $^{125}$I or iodotyrosine, as will be shown. All of the other tissues studied, including lung, aorta, heart, brain, small intestine, adipose tissue, skeletal muscle, lymph node, and skin, demonstrated <1.6% of the activity of the liver. In experiments with male rats, all of the values observed were comparable to those reported in Table I. The testes, however, showed the lowest binding of any tissue studied. On a per gram basis, the testes were only 0.2% as active in the uptake of AcAc LDL as the liver.

## Identification of Specific Uptake Sites in Extrahepatic Tissues

The specific cells in extrahepatic tissues responsible for the uptake of AcAc LDL were assessed histologically by their uptake of fluorescently labeled and iodinated AcAc LDL. When DiI-labeled AcAc LDL were injected into rats at a concentration of ~10 μg/ml of plasma and frozen sections of various tissues were screened for uptake, intense fluorescence was noted in the spleen and bone marrow. Weaker but significant fluorescence was seen in the adrenal cortex and ovary.

In the red marrow of the rat femur, the uptake of DiI-labeled AcAc LDL was also noted to occur in linear arrays (Fig. 7*a*), indicative of binding to sinusoidal endothelial cells. Silver grains, observed by autoradiography after injection of $^{125}$I-labeled AcAc LDL, were clearly associated with endothelial cells lining all the sinusoids (Fig. 7*b*).

Uptake of AcAc LDL by sinusoidal endothelial cells of bone marrow was confirmed by electron microscopy. Large endocytic vesicles were observed in sinusoidal endothelial cells of marrow (Fig. 8) from the animals receiving an injection of AcAc LDL but not in the endothelial cells of control animals. It was possible to visualize spherical particles (~20 nm in diameter) resembling LDL within coated pits and endocytic vesicles.

In the spleen, linear arrays of a discrete, punctate fluorescent labeling pattern, typical of that observed for AcAc LDL internalized by liver sinusoidal cells, were observed in the red pulp (Fig. 9*a*). In addition to this discrete fluorescence, the spleen contained a high level of diffuse autofluorescence, demonstrated in both control and experimental animals. No uptake of the fluorescent lipoproteins was noted in the central artery, and only low levels of uptake were noted in some small vessels of the white pulp and marginal zone. (It is of interest that low levels of uptake were also noted in the high venular endothelia of the lymph nodes, a tissue analogous to the white pulp of the spleen.)

These observations concerning the spleen were confirmed by autoradiography. Essentially no silver grains were observed in the central arteries. Low levels of uptake were observed in some vessels of the white pulp and marginal zone, while intense labeling occurred in association with the sinusoidal endothelial cells of the red pulp (Fig. 9*b*). The macrophages of the spleen did not appear to take up significant amounts

TABLE I

*The Uptake of $^{125}$I-labeled Acetoacetylated Low Density Lipoproteins In Vivo by Selected Tissues in the Rat**

| Tissue | Relative uptake‡ | |
|---|---|---|
| | Per organ | Per gram |
| Liver | 100 | 100 |
| Spleen | 2.7 ± 0.50 | 34.4 ± 4.5 |
| Bone marrow | nd§ | 26.0 ± 10.6 |
| Adrenal | 0.15 ± 0.06 | 24.4 ± 6.1 |
| Kidney | 1.47 ± 0.20 | 5.8 ± 0.5 |
| Ovary | 0.04 ± 0.02 | 3.9 ± 1.6 |
| Lung | 0.27 ± 0.11 | 1.6 ± 0.5 |
| Aorta | 0.01 ± 0.00 | 1.0 ± 0.4 |
| Heart | 0.08 ± 0.05 | 0.8 ± 0.3 |
| Small intestine | 0.32 ± 0.07 | 0.4 ± 0.04 |

*The $^{125}$I-labeled AcAc LDL were injected intravenously into 220-g female rats to yield a plasma concentration of 10 μg/ml. 6 min after the injection, the rats were killed and their organs were taken for determination of radioactivity.

‡ Data are given relative to uptake by the liver, which was arbitrarily assigned a value of 100 cpm/g or 100 cpm/organ. The data reported are the means ± the standard deviations for five animals.

§ *nd*, not determined.

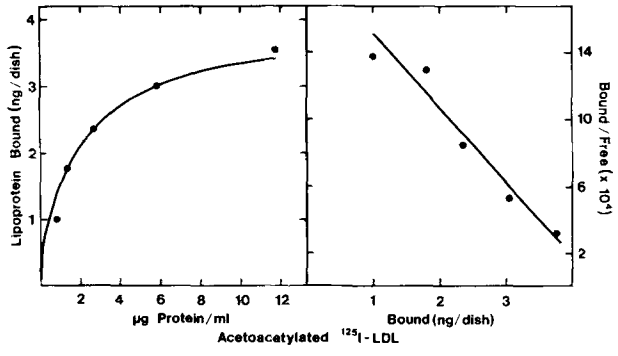



FIGURE 6 Concentration-dependent binding of $^{125}$I-labeled AcAc LDL to liver endothelial cells in vitro (*left panel*) and Scatchard plot (*right panel*) derived from this data. Liver endothelial cells were isolated and cultured (see Materials and Methods), and the experiment was performed 24 h later. Binding was for 3 h at 4°C in a medium containing 10% fetal calf serum. Nonspecific binding observed in the presence of noniodinated AcAc LDL (150 μg of protein/ml) has been subtracted. Data points are the mean of duplicate determinations. The mean cellular protein was 85 μg per dish.

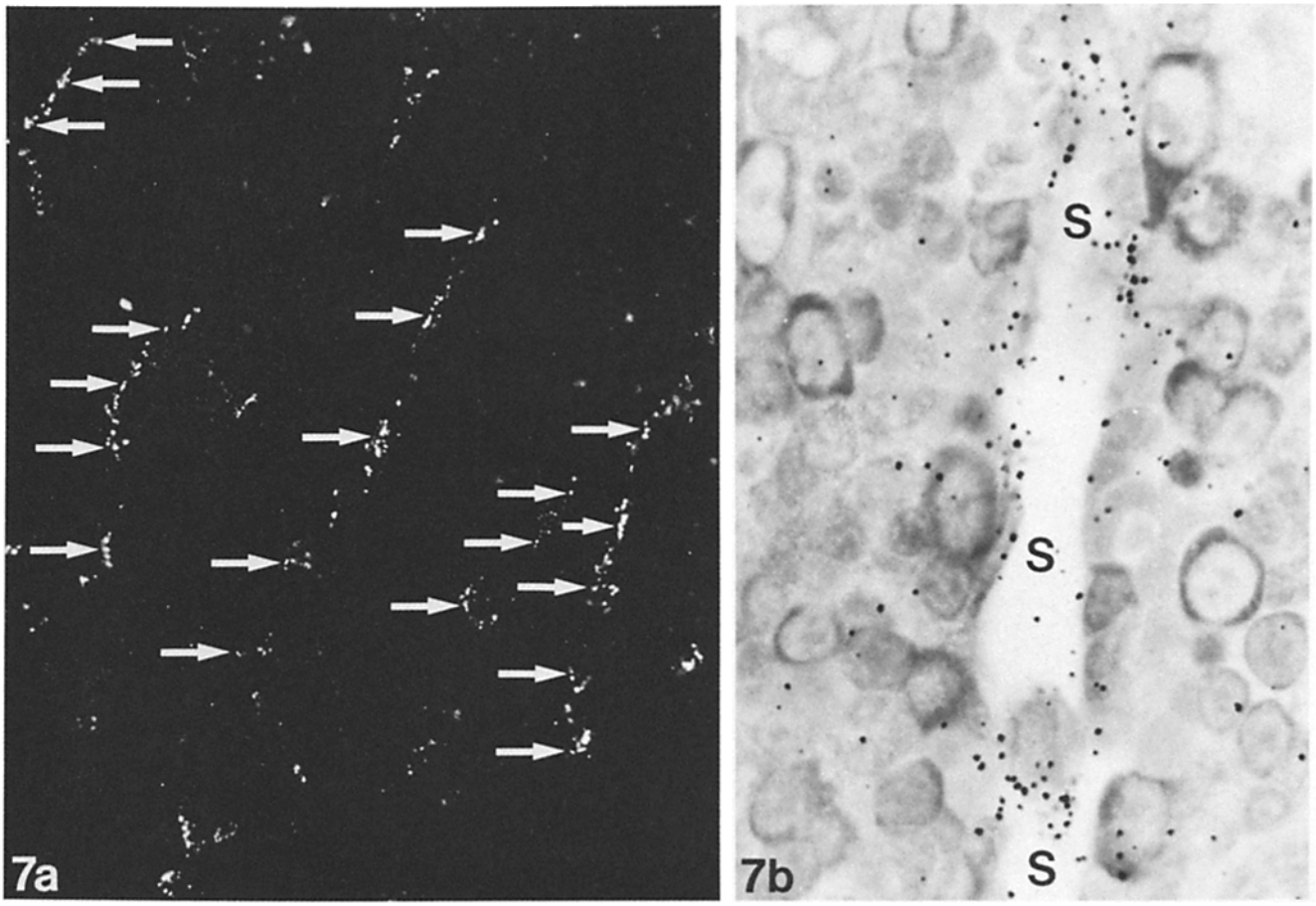



FIGURE 7 Photomicrograph of a frozen section of rat femur bone marrow showing a linear pattern of fluorescence (→) due to the uptake of DiI-labeled AcAc LDL (*a*). This same pattern is seen in plastic-embedded tissue processed for autoradiography (*b*). The silver grains are associated with the lining of the sinusoids (*S*). Both $^{125}$I-labeled AcAc LDL and DiI-labeled AcAc LDL were injected at 20 μg of lipoprotein protein/ml of plasma. Tissue was processed as described in the legend to Fig. 9. (*a*) × 300; (*b*) × 1,500.

of AcAc LDL. These results were confirmed by electron microscopy (data not shown). The ability to differentiate the uptake of the AcAc LDL by endothelial cells and macrophages was confirmed by a second observation. Canine Fraction I β-VLDL, extensively modified by cyclohexanedione treatment, are taken up by Kupffer cells and not by liver sinusoidal endothelial cells. In the spleen, these particles are removed from the circulation by cells primarily in the marginal zone, apparently by the macrophages, and not by the sinusoidal lining cells of the red pulp.[5]

In the adrenal, significant uptake of DiI-labeled AcAc LDL was seen in the glomerular region of the adrenal cortex where the incoming arteries divide to form the sinusoids of this organ (Fig. 10, *a* and *b*). The fluorescent labeling increased in intensity and regularity near the middle of the cortex where it appeared in a linear punctate pattern (Fig. 10, *c* and *d*). The fluorescence intensity in the adrenal never equaled that seen in the bone marrow or spleen. Very low uptake of the fluorescently labeled lipoproteins was observed in the sinusoids of the adrenal medulla, but no uptake was observed in the larger veins. These results were clearly demonstrated by autoradiography (Fig. 11). The appearance of silver grains was associated with sinusoidal lining cells in the glomerular region of the cortex and seen in increased numbers in endothelial cells of the fasiculata.

[5] Boyles, J., H. Funke, and R. W. Mahley. Unpublished observations.

The uptake of DiI-labeled AcAc LDL was found in capillaries and small veins of the ovarian interstitia. In addition, luteal uptake was observed in rats administered human chorionic gonadotropin, a treatment that increases sterol synthesis (36) and causes the formation of corpora lutea. This fluorescence, similar to that observed in the adrenal cortex, occurred in a pattern suggestive of that seen in sinusoidal lining cells. These observations were confirmed by autoradiography. In the ovarian interstitia (Fig. 12, *a* and *b*) and corpora lutea (Fig. 12*c*), silver grains were observed lining the capillaries and small veins or sinuses. No concentration of grains was observed over ovarian cells.

Although the kidney appeared to contain significant radioiodine after the injection of $^{125}$I-labeled AcAc LDL (Table I), screening of the kidney for fluorescence after injection of DiI-labeled AcAc LDL revealed essentially no fluorescence associated with the endothelial, glomerular, or tubular cells. Autoradiography revealed that almost all of the silver grains were associated with kidney cells of the proximal tubules and not with the sinusoidal lining cells (data not shown). These results suggested that the radioactivity in the kidney (Table I) was due to the presence of $^{125}$I-labeled apoprotein B, free $^{125}$I, or [$^{125}$I]iodotyrosine. The uptake of intact AcAc LDL by the kidney therefore appears to be insignificant. The kidney was the only organ containing significant $^{125}$I in which the radioiodine was not associated with capillary or sinusoidal endothelia. All other tissues, including the aorta, the heart,

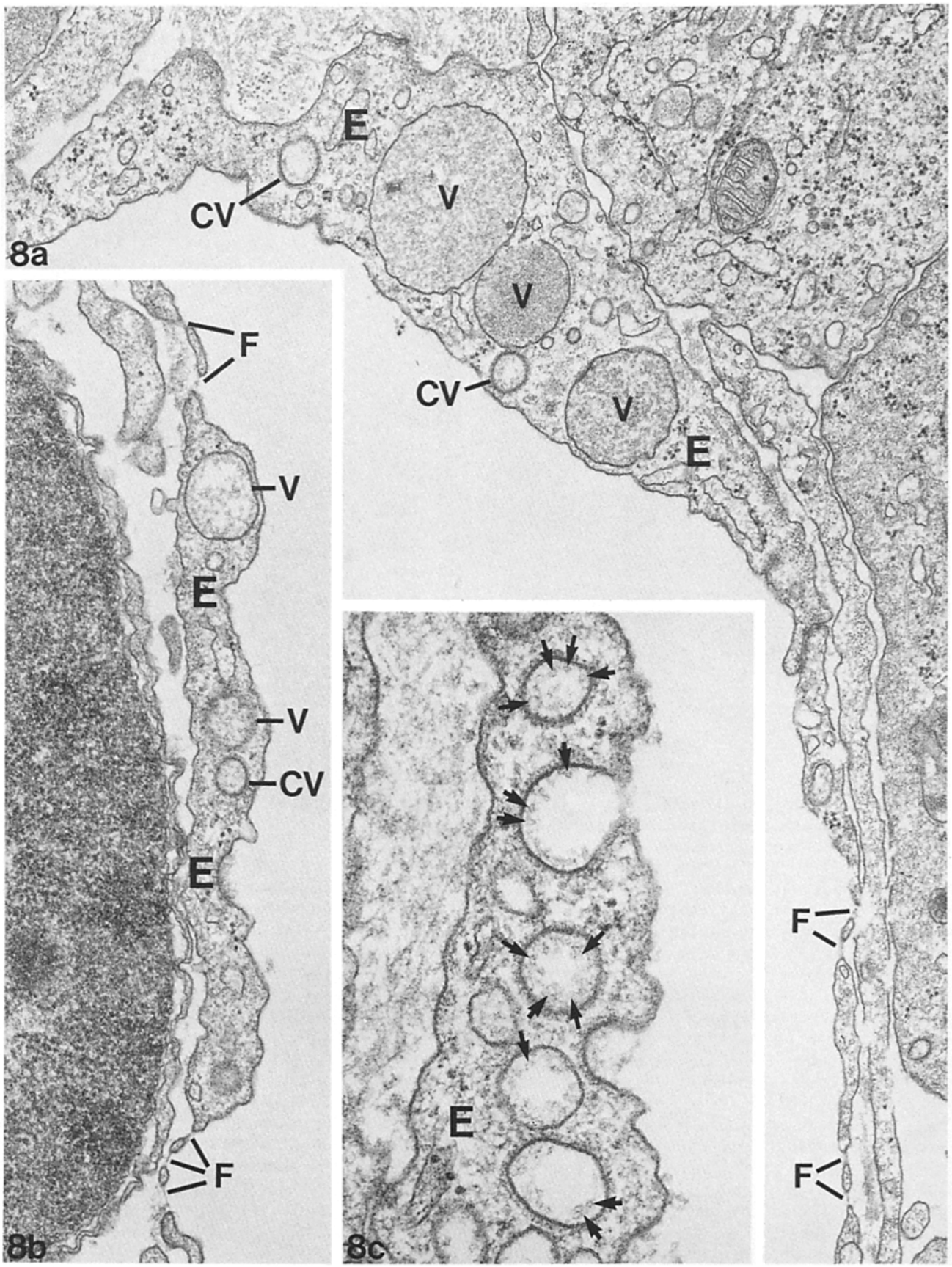



FIGURE 8 Electron micrographs of rat bone marrow following the in vivo injection of AcAc LDL. (*a* and *b*) Endothelial cells (*E*), identified by the presence of fenestrae (*F*), contained coated (*CV*) and uncoated (*V*) vesicles with a particulate content. (*c*) At higher magnification, particles (→) ~20 nm in diameter are observed in coated pits and coated vesicles of the endothelial cells. (*a*) × 40,000; (*b*) × 43,000; (*c*) × 80,000. For further details, see the legend to Fig. 4.

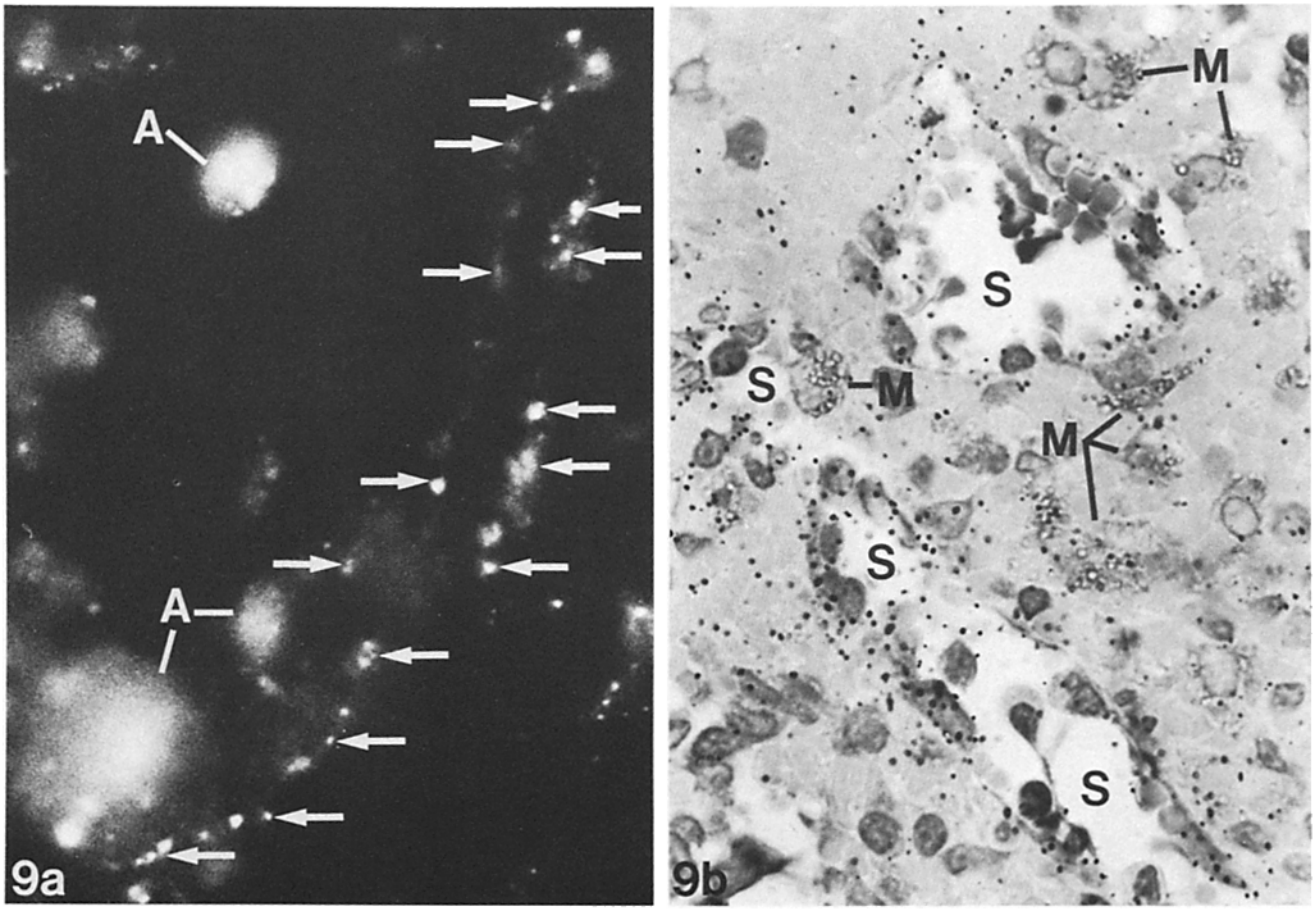



FIGURE 9 Photomicrographs taken in the red pulp of the spleen showing the uptake of DiI-labeled (*a*) or $^{125}$I-labeled AcAc LDL (*b*). (*a*) A fluorescent photomicrograph of a frozen section showing a linear pattern of fluorescence suggestive of DiI-labeled AcAc LDL uptake by sinusoidal lining cells (→). The bright globular fluorescence and some of the punctate fluorescence is autofluorescence (*A*) present in controls. The autofluorescence is never observed in linear arrays. (*b*) A photomicrograph of a 2-μm, toluidine-stained, plastic-embedded spleen processed for autoradiography. Note the predominance of silver grains along the sinusoids (*S*). A small number of silver grains are also associated with macrophages (*M*). The DiI- and $^{125}$I-labeled AcAc LDL were injected intravenously to yield a plasma concentration of 20 μg of lipoprotein protein/ml. Tissues were removed 20 min after injection of the DiI-labeled AcAc LDL and frozen. 6 min after the injection of the $^{125}$I-labeled AcAc LDL, the animal was perfusion-fixed through the heart and then processed for autoradiography. (*b*) Bright field. (*a* and *b*) × 1,200.

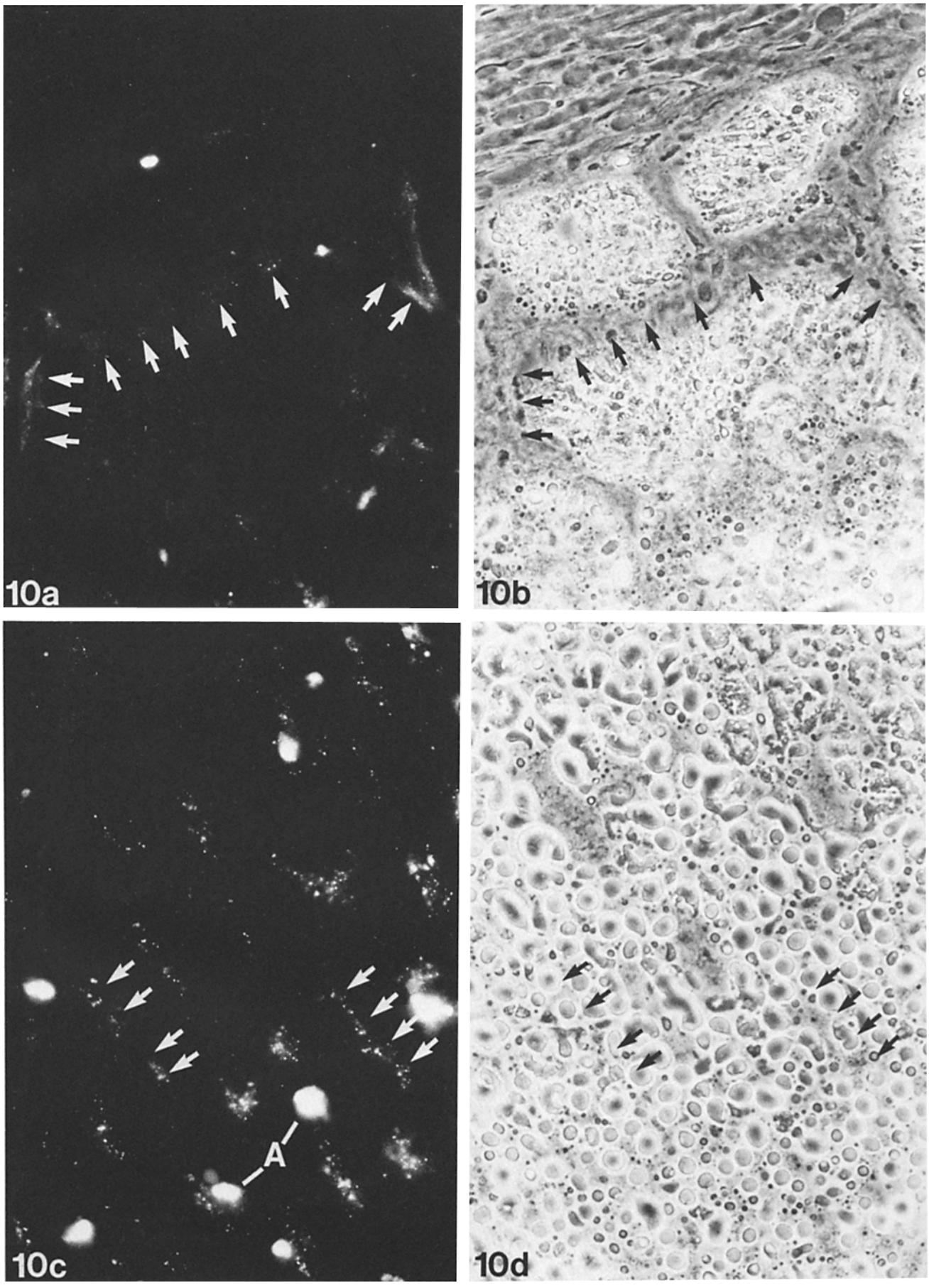
the small intestine, the brain, adipose tissue, and the testes, were found to be negative for the uptake of AcAc LDL, as assessed either by fluorescence microscopy or autoradiography.

For these in vivo studies, lipoproteins were injected to a plasma level of 10 to 20 μg of lipoprotein protein/ml. At this level, they are removed so rapidly by the liver as to preclude their binding and uptake by tissues with a relatively low number of receptors. In an additional study, lipoproteins were administered to a plasma concentration of 50 to 100 μg/ml. Even at these high levels, no uptake of lipoproteins was noted in the capillaries of the testes, large vessels of the adrenal medulla, or vessels of the heart. However, low levels of uptake by the large vessel endothelia of the liver and the aorta were observed.

## DISCUSSION

In this report, we have demonstrated that the in vivo uptake of AcAc LDL is mediated by the sinusoidal endothelial cells of the liver, spleen, marrow, adrenal, and to a lesser extent, by the endothelia of the ovary. It was determined that the particles are internalized via coated pits and vesicles, suggesting a receptor-mediated mechanism. Although a receptor for acetyl LDL was first described on peritoneal macrophages in in vitro experiments (1), macrophages of the spleen and liver that have free access to the lipoproteins circulating in vivo do not metabolize highly significant amounts of modified LDL. Hepatic sinusoidal endothelial cells were shown to take up at least sixfold more $^{125}$I-labeled AcAc LDL than Kupffer cells.

The specific cells responsible for the uptake of AcAc LDL in vivo were determined by two complementary means, autoradiography and fluorescence microscopy. Fluorescence microscopy of cells undertaken after the in vivo administration of DiI-labeled AcAc LDL provided a rapid technique for assessing the uptake of lipoproteins and an excellent method for localizing uptake in frozen sections. In this study, we noted no difference in the cellular distribution of DiI if tissue samples were taken 15 min or 1 h after the injection of the labeled lipoprotein when catabolism of the lipoprotein is likely to have occurred. Since DiI was retained by specific endothelial cells after the catabolism of the lipoprotein, these studies suggest that sinusoidal endothelial cells are the sites of catabolism of AcAc LDL and are not simply functioning to transfer these particles from the plasma to the tissues. Previously, DiI


10a
10b
A
10c
10d

has been shown to be quantitatively retained within the lysosomes of cells for several days while the lipoproteins themselves are degraded (35). Autoradiography, on the other hand, provides a marker for lipoproteins that are internalized or at the cell surface and not substantially degraded since the degradation product [$^{125}$I]iodotyrosine is expected to be lost from cells in vivo as well as during processing for microscopy. Therefore, for these studies, short time points where degradation would be minimal (6–8 min after injection) were used. Autoradiography of perfusion-fixed tissue after the in vivo administration of $^{125}$I-labeled AcAc LDL therefore complemented and confirmed the results of the fluorescence studies and provided positive identification of Kupffer cells through the localization of endogenous peroxidase. The uptake of AcAc LDL by specific cells could be established directly, and a semiquantitative estimate of the uptake determined by counting silver grains.

This is the first report of the in vivo uptake of modified lipoproteins by sinusoidal endothelia of organs other than the liver, and the first report to establish clearly that this hepatic uptake is mediated by sinusoidal endothelia and not a combination of sinusoidal and large vessel endothelia. In agreement with our data, Nagelkerke et al. (16) determined that hepatic endothelial cells, isolated after the in vivo injection of $^{125}$I-labeled Ac LDL, contained five times more Ac LDL per milligram of cell protein than the Kupffer cells and 31 times more than hepatocytes. Our data do not demonstrate uptake by hepatocytes either in vivo or in vitro.

The low level of uptake of Ac and AcAc LDL by Kupffer cells in vivo contrasts with the substantial capacity of Kupffer cells to degrade Ac LDL in vitro (1, 4). A possible explanation for this is that in vivo Kupffer cells may express fewer Ac LDL receptors than sinusoidal endothelial cells. In addition, it has been determined from morphometric studies that liver sinusoidal endothelial cells have 3.6 times more surface area than Kupffer cells (15). As a result, circulating AcAc LDL may be removed more effectively by endothelial cells of the liver than by Kupffer cells. However, in the spleen it appears that AcAc LDL are in contact with macrophages of the marginal zone before entering the red pulp but are not taken

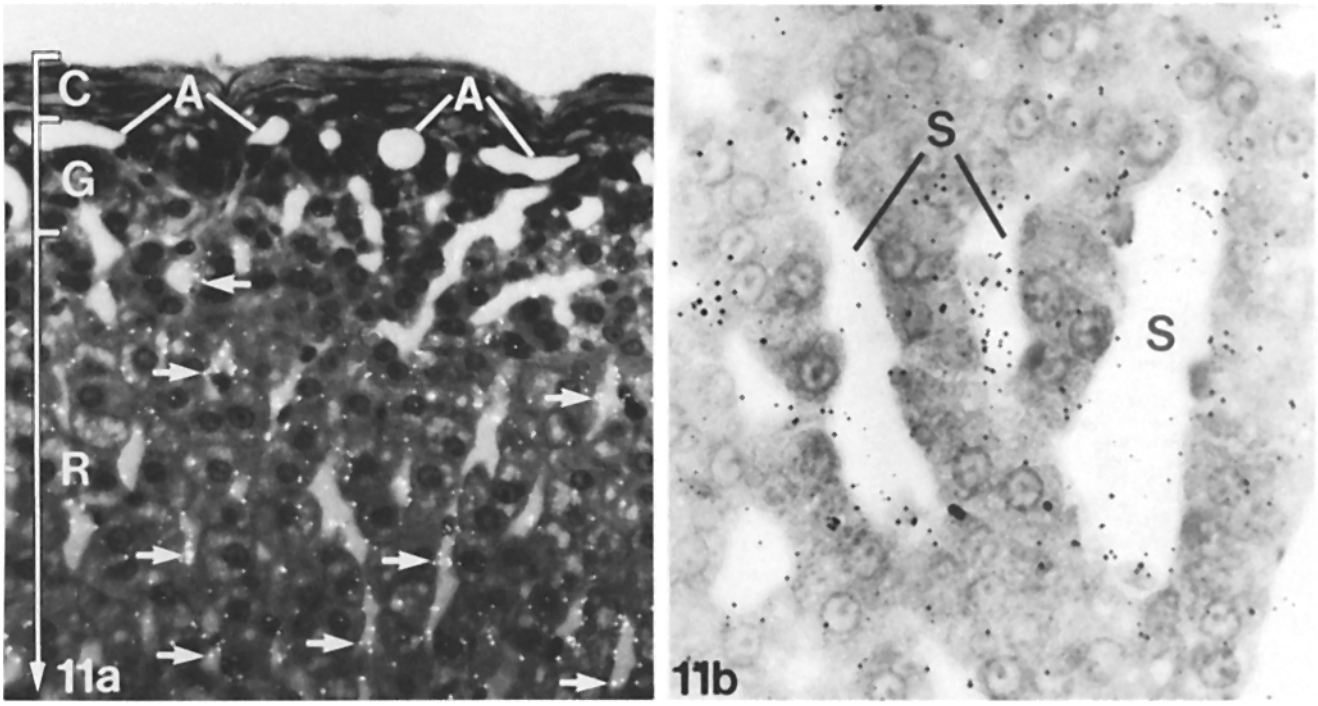



FIGURE 11 Photomicrograph of adrenal processed for autoradiography showing the uptake of $^{125}$I-labeled AcAc LDL. (*a*) Low magnification (× 490) view photographed in bright field using crossed polarizing filters. Under these conditions, silver grains appear white. Arteries (*A*) beneath the capsule (*C*) are unlabeled. With the formation of sinusoids in the glomerular region (*G*), silver grains appear. The sinusoidal linings of the reticulata (*R*) of the adrenal cortex are most heavily labeled (→). (*b*) Higher magnification image (× 950), again showing uptake by the sinusoidal lining of the adrenal cortex (*S*). The animals were injected with 20 μg of lipoprotein protein/ml of plasma, perfusion-fixed, and processed for autoradiography as described in the legend to Fig. 9.

FIGURE 10 Photomicrographs of frozen dog adrenal sections showing the uptake of DiI-labeled AcAc LDL. The same field has been photographed under fluorescent (*a* and *c*) and phase-contrast (*b* and *d*) illumination. (*a* and *b*) Uptake of fluorescent label (→) is observed in the glomerula region of the adrenal cortex where the arteries divide to form the sinusoids. The cells of the capsule in the upper left portion of the micrographs as well as the nest of the adrenal cortical cells between the vessels are negative. (*c* and *d*) Deeper in the adrenal cortex, a linear punctate pattern of fluorescence is observed (→). This pattern is suggestive of uptake by sinusoidal cells rather than adrenocortical cells. The bright globular fluorescence is autofluorescence (*A*) also present in controls. The phase-contrast image is obscured by lipid droplets. Labeled lipoprotein was injected to yield a plasma concentration of 10 μg of lipoprotein/ml. The adrenals were removed 1 h after the injection of the labeled lipoprotein into the dog. × 700.

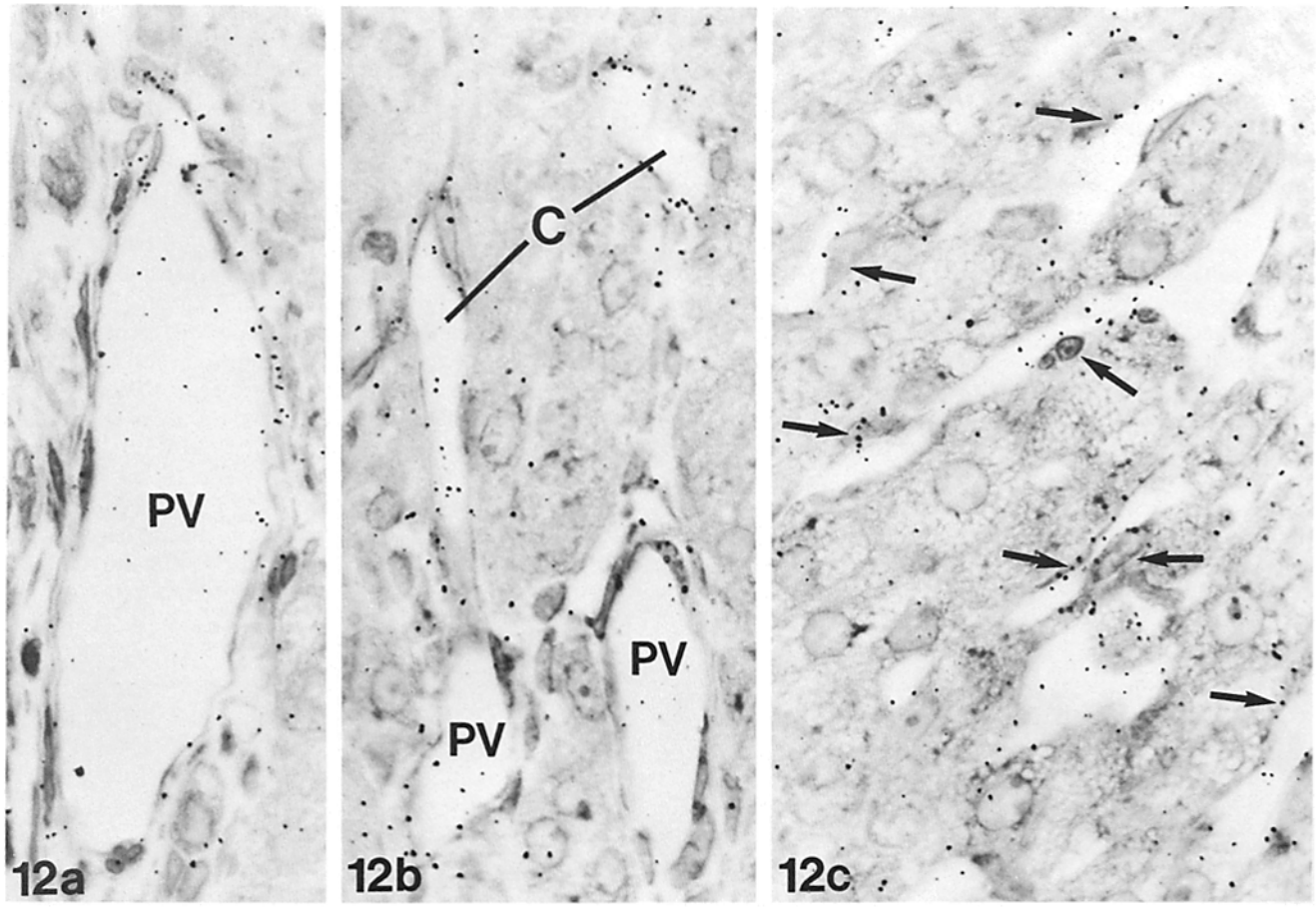



FIGURE 12 Photomicrographs showing the uptake of $^{125}$I-labeled AcAc LDL by rat ovary. *a* and *b* show uptake by postcapillary venules (*PV*) and capillaries (C) present among the steroid-secreting cells of the ovarian interstitia. *c* demonstrates uptake by sinusoidal lining cells (→) of the corpora lutea. The animal received human chorionic gonadotropin (see Materials and Methods) for 4 d before the experiment. The lipoproteins were injected to yield a plasma concentration of 20 μg of lipoprotein protein/ml; the animal was perfusion-fixed 8 min after injection, and the tissues were processed for autoradiography as described in the legend to Fig. 9. × 1,000.

up. These data suggest that macrophages in vivo express fewer receptors for AcAc LDL than do the sinusoidal endothelial cells.

The ability to specifically label sinusoidal endothelial cells in vivo by injection of DiI-labeled AcAc LDL may be useful for the isolation of these cells from the liver, spleen, marrow, and adrenal. Using these techniques, we have easily detected low levels of contamination of hepatocytes with endothelial cells by fluorescence microscopy. This fluorescently labeled ligand may therefore be useful in isolating and identifying cells by conventional techniques, as well as offering the potential for cellular isolation using the fluorescence-activated cell sorter following enzymatic digestion of the tissue.

The physiological function of, as well as the physiological ligand or ligands recognized by, AcAc LDL receptors on these specific sinusoidal endothelia is unknown. The specific uptake of the AcAc LDL by these tissues could reflect their requirement for cholesterol: in the liver for bile acid production, in the ovary and adrenal for hormone production, and in the spleen and bone marrow for blood cell membrane formation. It is not known whether the sinusoidal endothelia of all of these organs have any other unique properties in common. However, the liver, adrenal, and ovary do possess hepatic lipase, in contrast to the other endothelia that possess lipoprotein lipase (37). It is not known whether the spleen and bone marrow endothelia possess hepatic lipase. In addition, the liver, spleen, and marrow are recognized as components of the reticuloendothelial system.

In our in vitro studies, the liver sinusoidal endothelial cells demonstrated high affinity, saturable binding of AcAc LDL, and had a 10-fold greater capacity to metabolize AcAc LDL compared with aortic endothelial cells. These results are consistent with the observation that the uptake of AcAc LDL by aortic endothelium and large vessels of the liver in vivo could only be demonstrated when high levels of AcAc LDL were injected. The selectivity of uptake of AcAc LDL by the sinusoidal endothelia probably reflects a relatively high number of receptors on these cells and not a receptor unique to them.

Although modified LDL have not been demonstrated in plasma, it is possible that they are produced and rapidly cleared. Fogelman et al. (10) have demonstrated that LDL can be modified in the presence of malondialdehyde released by aggregating platelets as a by-product of prostaglandin synthesis. These modified LDL bind to the same receptor on macrophages as Ac LDL (38). Recently, Henriksen et al. (11, 39) showed that LDL incubated with endothelial cells are modified so that they bind, at least in part, to the same receptor that recognizes the Ac LDL. Although not yet demonstrated in vivo, these mechanisms for the production of

modified LDL could play an important role in lipoprotein metabolism. Modified lipoproteins that stimulate cholesteryl ester synthesis in macrophages (13, 40) have been reported in human atherosclerotic lesions, and negatively charged LDL have been demonstrated in interstitial inflammatory fluid (12). Modified LDL might contribute to lipid accumulation in macrophages of atherosclerotic lesions because macrophage-like foam cells from atherosclerotic lesions have been shown to possess receptors for AcAc LDL (35). Our data suggest that certain sinusoidal endothelial cells are the primary cell type responsible for the clearance of modified LDL found in the circulation. Tissue macrophages, however, may be an important site for the clearance of modified LDL formed outside of the circulation.

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