Apolipoprotein E localization in rat hepatocytes by immunogold labeling of cryothin sections[1]

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Abstract The distribution of apolipoprotein (apo) E in rat hepatocytes was investigated with an affinity-purified polyclonal antibody raised against apoE isolated from hepatogeneous very low density lipoproteins (VLDL). The distribution of this antibody was visualized with colloidal gold complexed to anti-rabbit IgG. By epipolarization microscopy, apoE was found uniformly along the basolateral surfaces of all hepatic parenchymal cells, showing a striking intensity along the sinusoidal front. Punctate deposits of colloidal gold appeared to be randomly distributed within all hepatocytes. Widely scattered Kupffer cells also stained for apoE. Electron microscopic examination of immunogold-labeled cryothin sections showed that hepatocytic microvilli projecting into the space of Disse consistently contained clusters of immunogold. The gold particles were variably associated with evident lipoprotein particles, raising the possibility that apoE alone may bind to receptors or other macromolecules at the surface of hepatocytes. Endosomes near the sinusoidal front and multivesicular bodies in the Golgi/biliary area labeled intensely for apoE, consistent with a high content of apoE associated with triglyceride-rich lipoprotein remnants contained within these organelles. Some but not all nascent VLDL particles within putative forming Golgi secretory vesicles were labeled, but many other Golgi vesicles and cisternae that lacked evident VLDL particles were also labeled. These results suggest that at least some apoE associates with nascent VLDL in forming Golgi secretory vesicles. Unexpectedly, the matrix of all hepatocytic peroxisomes was heavily labeled. Immunoblots with the affinity-purified anti-rat apoE IgG against proteins from highly purified peroxisomes isolated from rat hepatocytes revealed a protein with an apparent molecular mass of 34.5 kDa, similar to that of rat apoE in rat blood plasma. In addition, gold was sometimes found in the area either adjacent to peroxisomes or between multivesicular bodies and the bile canaliculus not evidently associated with a membranous compartment. These observations suggest that apoE may participate in interorganellar cholesterol transport within hepatocytes. —**Hamilton, R. L., J. S. Wong, L. S. S. Guo, S. Krisans, and R. J. Havel.** Apolipoprotein E localization in rat hepatocytes by immunogold labeling of cryothin sections. *J. Lipid Res.* 1990. **31:** 1589–1603.

Supplementary key words Golgi apparatus • multivescular bodies • microvilli • peroxisomes • nascent VLDL

Apolipoprotein (apo) E, previously called the "arginine-rich peptide" (1), is a major constituent of several classes of plasma lipoproteins. It plays an important role in regulating plasma lipid concentrations as a ligand of triglyceride- and cholesteryl ester-enriched remnant particles that are taken up and degraded in hepatocytes by receptor-mediated endocytosis (2). This function is underscored by the human genetic disease, familial dysbetalipoproteinemia, in which mutant forms of apoE, defective in binding to lipoprotein receptors, lead to accumulation of chylomicron and very low density lipoprotein (VLDL) remnants and premature coronary artery disease (3, 4). Hepatocytes also secrete apoE into liver perfusates, where it is found largely in association with VLDL and discoidal high density lipoproteins (HDL) (5). Although the liver contains the highest content of apoE mRNA, many other tissues also contain the message (6, 7), and several cells in culture, including macrophages, smooth muscle cells, astrocytes (8), and human keratinocytes (9), secrete apoE into the media. By light microscopic immunocytochemistry, apoE has been found in many different cell types of the baboon, including hepatocytes and Kupffer cells (10). Because of its widely distributed cellular origins and its receptor-binding properties, it has been postulated that apoE may function broadly in cholesterol homeostasis mediating the redistribution of cholesterol among cells that require cholesterol for such uses as steroidogenesis, tissue repair, cell division, and bile acid synthesis (6–8). The function of apoE

Abbreviations: apo, apolipoprotein; MVBs, multivesicular bodies; VLDL, very low density lipoproteins.

[1]Portions of this research were presented at the Cell Biology Meetings in 1986 (50).

produced by hepatocytes is presumably closely related to the liver's dominant role in regulating the plasma concentration of lipids by the processes of plasma lipoprotein secretion and receptor-mediated endocytosis and catabolism (2).

Recent research has shown that useful new concepts may come from the subcellular localization of protein antigens in frozen thin sections. We report here the first subcellular localization of apoE in rat hepatocytes. Three compartments containing a high content of apoE were expected, but a fourth compartment, peroxisomes, was not.

MATERIALS AND METHODS

Preparation of the immunoreactive probe against rat apoE

ApoE for immunization was obtained from VLDL isolated from perfusate of rat liver (5, 11). After delipidation of perfusate VLDL with ethanol–ether 3:1, soluble proteins were separated by column chromatography and the fractions containing only apoE were identified by SDS polyacrylamide gel electrophoresis (11, 12). Rabbits were immunized by four subcutaneous injections of apoE, together with small amounts of Freund's adjuvant. Antiserum was collected 10 days after the last injection, IgG fractions were isolated, an apoE affinity column was prepared, and affinity-purified rabbit anti-apoE was isolated as described (12). The specificity of the affinity purified anti-apoE IgG was evaluated by immunoblotting the proteins of rat serum d < 1.21 g/ml lipoproteins and serum VLDL. Proteins were separated on SDS-PAGE using 3–15% gels and transferred to nitrocellulose paper for 4 h at 400 mA at 10°C as described (13), except that blocking was accomplished with 3% gelatin in 0.9% NaCl, 0.02 M Tris, pH 7.5, for 60 min at 37°C. The affinity-purified IgG fraction of antiserum against apoE (2.1 mg/ml) was diluted 1:300 with 1% gelatin in 20 mM Tris-buffered saline, pH 7.5, containing 0.05% Tween 20. The rabbit IgG was identified by goat anti-rabbit IgG labeled with ^{125}I (13). Peroxisomal proteins were separated on 9% SDS-PAGE and subjected to immunoblotting with the affinity-purified anti-apoE IgG at a concentration of 7 μg/ml. Peroxisomes, estimated at 92% purity, were isolated from livers of normal rats as described (14).

Immunocytochemical procedures for light and electron microscopy

ApoE was localized in frozen thin sections (15, 16) with affinity-purified IgG fraction of anti-apoE directed to apoE as primary label, which was visualized with goat anti-rabbit IgG colloidal gold complexes of 5 or 10 nm (Janssen Life Sciences Products, Piscataway, NJ). The same results were obtained with a Fab fraction from the parent IgG (data not shown).

For light microscopic localization of apoE, normal rats fed ad libitum were anesthetized with diethyl ether between 10–12 AM, and livers were fixed by perfusion (17). The fixative, containing 4% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, was pumped at a flow rate of 12 ml/min for 10 min. Liver slices 1 × 0.5 × 0.5 cm were fixed an additional 2 h and then washed in 7.5% sucrose in 0.1 M cacodylate buffer for 1 h. The blocks were then infiltrated overnight with 30% sucrose in 0.1 M cacodylate buffer on a rotator, embedded in OCT compound (Miles, Inc., Elkhart, IN), and frozen the next day in Freon cooled by liquid nitrogen. Tissue blocks were then sectioned at 4–6 microns on a cryostat microtome. Sections were adhered to glass slides treated with poly-L-lysine, air dried for at least 2 h, and dipped into 3% paraformaldehyde in phosphate-buffered saline (PBS) to cross-link the sections to the poly-L-lysine. Sections were washed with PBS glycine (0.02 M) to quench aldehyde groups, according to Slot (15). Immunogold staining was carried out by the silver enhancement of colloidal-gold antibody technique using the Janssen (Life Sciences Products, Piscataway, NJ) IntenSE M silver enhancement kit. Light photomicrographs were taken under both bright field and epipolarization on a Leitz Ortho Plan 2 microscope (Rockaway, NJ).

For electron microscopic localization of apoE, livers of both untreated and ethinyl estradiol-treated (18) male Sprague-Dawley rats (250–300 g) fed ad libitum were also perfusion-fixed. (No differences in apoE localization related to hormone treatment were observed). The best results for electron microscopic localization of apoE were reproducibly obtained with fixative containing freshly prepared 2% paraformaldehyde (Baker Chemical Co., Phillipsburg, NJ) and 0.5% glutaraldehyde (Ladd Research Industries, Burlington, VT) in 0.1 M cacodylate buffer, pH 7.4. When glutaraldehyde at 0.2% was used, organelles were inadequately preserved and when 1% glutaraldehyde was used, detection of apoE was greatly reduced.

Ultracryomicrotomy and localization of apoE at the electron microscopic level was performed as described by Slot (15) and Tokuyasu (16). Cryosections for electron microscopy were prepared on a Sorvall MT-6000 ultramicrotome equipped with the FS-1000 cryosectioning unit. Controls for colloidal gold labeling were: *a*) gold control (i.e., no exposure of the section to our primary antibody followed by exposure of the cryosection to goat anti-rabbit IgG complexes with 5 or 10 nm gold); and *b*) IgG control (i.e., nonimmune rabbit IgG at 5- to 10-fold the protein concentration used for anti-apoE IgG, followed by gold complexes). We found that the most reproducible labeling that appeared to be specific for apoE was obtained with 0.5–2.0 μg IgG per ml.

RESULTS

Specificity of antibody against apoE

The affinity-purified IgG against apoE was evaluated rat serum d < 1.21 g/ml apolipoproteins and serum VLDL apolipoproteins by immunoblotting. As shown in **Fig. 1**, only apoE was detected.

Light microscopy

A striking distribution of apoE in frozen sections of rat liver, particularly at low magnification, was seen with the silver-enhanced colloidal-gold antibody technique (**Fig. 2**). All hepatocytes were labeled. The most intense localization appeared to be at the sinusoidal front, which was uniformly labeled across the hepatic lobule from the portal tracts to the central veins. Gold was also widely distributed in a punctate pattern in the cytoplasm of every hepatocyte with no apparent pericanalicular localization (Fig. 2 and **Fig. 3**). Imaging with epipolarization light source dramatically enhanced the visualization of gold particles (Figs. 2 and 3). Under oil immersion, the space of Disse appeared to be filled with gold (Fig. 3). At this magnification, intense labeling of some Kupffer cells was also seen. Other Kupffer cells were also labeled, but with much less intensity (Fig. 3). Kupffer cells lacking apoE label were not identified. Slight localization was also observed on the apical membrane of bile duct epithelium and along the endothelium of larger blood vessels (data not shown).

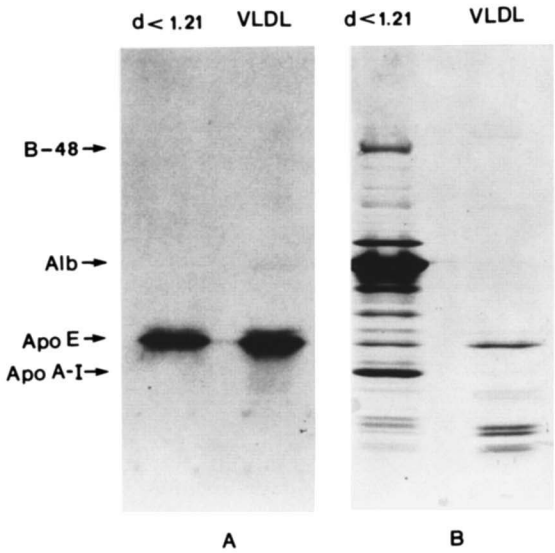

Fig. 1. Specificity of affinity-purified anti-rat apoE IgG. Proteins of d < 1.21 g/ml rat serum lipoproteins (100 μg) and rat serum VLDL (30 μg) were separated by SDS-PAGE (3–15% gradient). A, immunoblots; B, equivalent gels stained with Coomassie Blue. Note the large amount of albumin in the d < 1.21 g/ml fraction obtained by a single ultracentrifugation.

Electron microscopy

Gold particles were concentrated in hepatocytic Golgi areas and appeared to be widespread within many of the cisternae and vesicles of the Golgi apparatus (**Fig. 4** and **Fig. 5**). However, the dilated cisternae containing electron-lucent nascent VLDL particles within putative forming secretory vesicles were not uniformly associated with gold. In contrast, flat cisternae and smaller profiles of Golgi vesicles, which lacked evident nascent VLDL particles, frequently contained gold particles. In these experiments, 1 μg/ml of rabbit IgG anti-apoE was incubated with sections for 1 h at room temperature. With either the gold control or the IgG control (5 μg/ml IgG from normal rabbit serum), virtually no gold adhered to the tissue sections incubated under identical conditions. Thus, the small amount of colloidal gold seen in mitochondria and rough endoplasmic reticulum may reflect the presence of apoE.

Surprisingly, in every experiment except in controls with nonspecific IgG, the peroxisomal matrix was intensely labeled (Figs. 4, 5, and **Fig. 6**). Although it is not possible to make reliable quantitative measurments of antigen in frozen thin sections of tissue by this technique (19, 20), virtually all peroxisomes contained many gold particles, usually in clusters in the matrix. In about one-half of the peroxisomes, gold particles also appeared to be associated with the peroxisomal limiting membrane (Fig. 6). In addition, the cytoplasmic area adjacent to peroxisomes sometimes contained clusters of gold particles unassociated with an evident organelle or vesicle (Fig. 6). To determine whether the immunogold labeling of peroxisomes represents apoE or an immunologically related protein of quite different molecular weight (see ref. 13), we immunoblotted the proteins of highly purified peroxisomal fractions isolated from normal rat hepatocytes. Whereas serum VLDL contained two major components with apparent molecular masses of 35 and 37 kDa, peroxisomes contained a single prominent band of 34.5 kDa (**Fig. 7**). A second much less prominent band, with an apparent molecular weight of ~63 kDa, was also seen.

MVBs, found largely in the Golgi-bile canalicular zone of hepatocytes, invariably contained large numbers of gold particles, although the contents were not uniformly labeled (**Fig. 8**). The cytoplasmic area of hepatocytes adjacent to heavily stained MVBs often contained clusters of gold particles indicative of a high concentration of apoE, particularly between the MVBs and the adjacent bile canaliculus (Fig. 8).

The microvillous plasma membranes of the hepatocyte in the space of Disse was consistently associated with clusters of gold particles (**Fig. 9** and **Fig. 10**), whereas the microvilli of bile canaliculi contained virtually none (Figs. 5 and 8). Although the concentration of gold particles on the sinusoidal microvilli varied somewhat between differ-

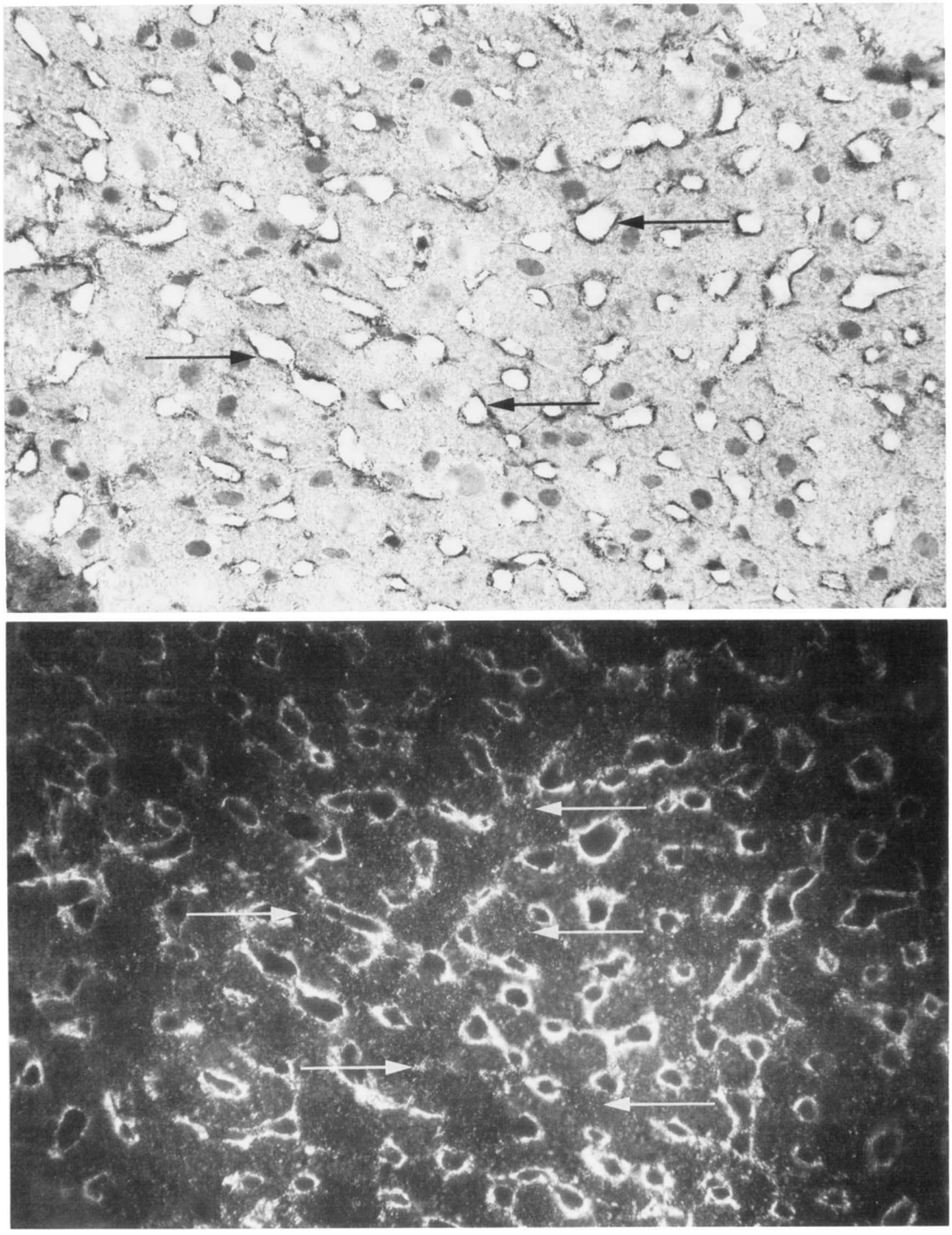

Fig. 2. Light microscopic localization of apoE in frozen sections (4 μm) of rat liver. Top, brightfield illumination; bottom, epipolarization illumination. In the brightfield image the silver-enhanced colloidal gold antibody can be seen largely as black deposits in the space of Disse facing the sinusoidal spaces (arrows). This localization is greatly enhanced by epipolarization of the same field (bottom), with gold appearing as bright white outlines of the space of Disse. In addition, punctate deposits of gold (white arrows) are revealed by this illumination within the cytoplasm of all hepatocytes (× 350). Untreated animal.

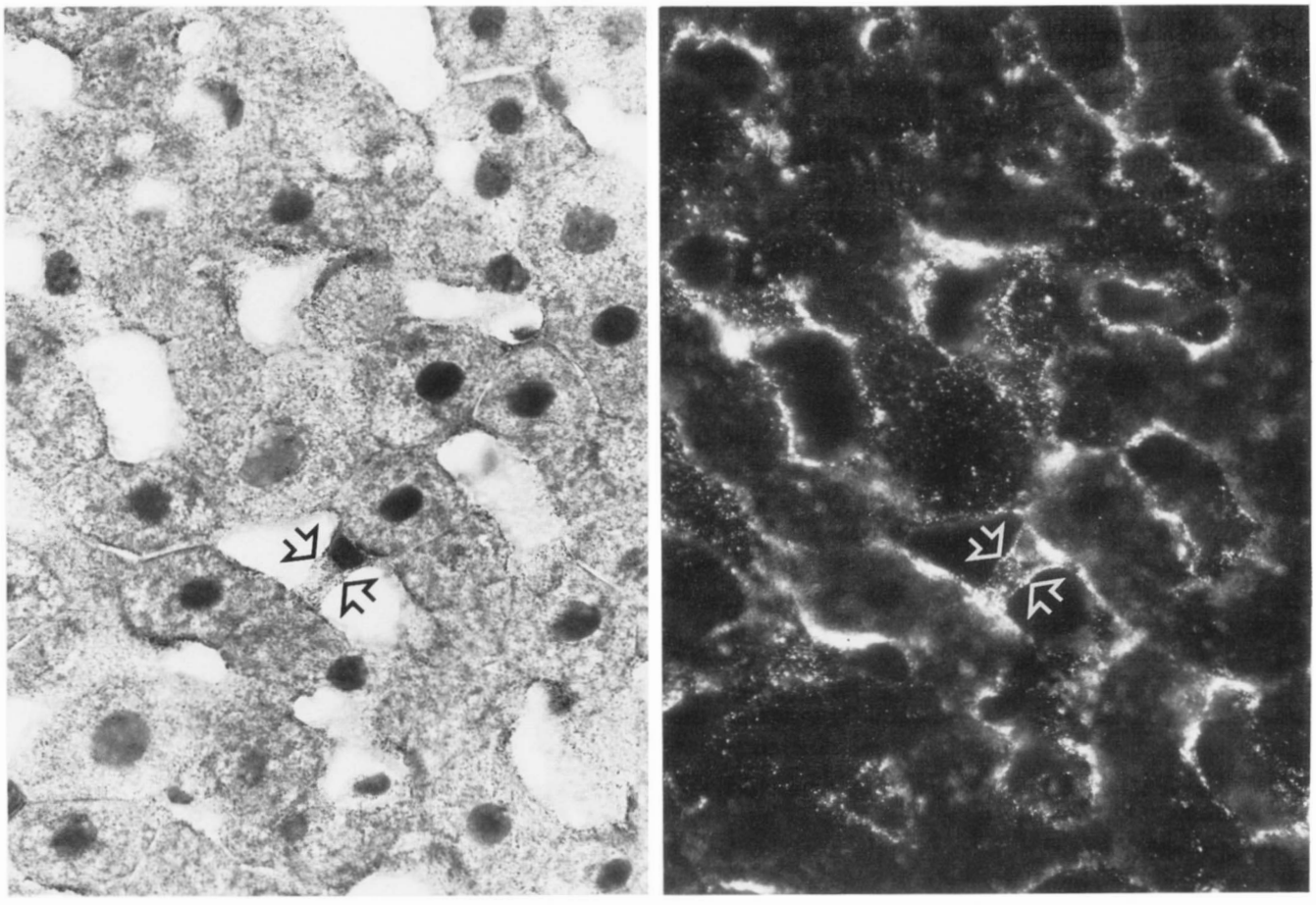

Fig. 3. Light microscopic localization of apoE in frozen sections of rat liver showing the same distribution as in Fig. 2 at higher magnification. Left, brightfield; right, epipolariztion. This figure also shows apoE in a Kupffer cell spanning the sinusoidal lumen (arrows). (× 550). Untreated animal.

ent hepatocytic surfaces, the pattern of labeling was always consistent; clusters of gold particles were found in distinct patches on individual microvilli (Figs. 9 and 10). In some instances, the outline of a putative lipoprotein particle, which appeared to be adherent to a microvillus (Fig. 9), was observed associated with multiple gold particles; most of the gold, however, was not associated with evident lipoprotein particles.

Clusters of gold particles sometimes were seen just beneath the plasma membrane facing the sinusoidal front (Figs. 9 and 10), usually unassociated with an obvious organelle. Occassionally, a larger endosome filled with clusters of gold was seen near the sinusoidal front (Fig. 10).

DISCUSSION

The light microscopic localization of apoE observed in this research demonstrates that all rat hepatocytes contain this apolipoprotein, distributed in a consistent pattern. By far the most intense staining was seen at the sinusoidal front and over the adjacent space of Disse. This pattern of apoE localization at the light microscopic level was similar to that which we found previously for apoB (17). In agreement with the report that about 0.7% of rat liver apoE mRNA is in Kupffer cells (21), we observed specific labeling for apoE in widely scattered Kupffer cells. Endothelial lining cells of the sinusoids apparently contained very little apoE. In a light microscopic study of apoE in baboon tissues, Kupffer cells reportedly also contained apoE (10).

At the electron microscopic level, colloidal gold particles were widely dispersed in all parts of the Golgi apparatus. Although the putative forming secretory vesicles containing nascent VLDL particles sometimes contained immunoreactive apoE, there was no consistent or strong reactivity associated with this portion of this organelle. On the other hand, many cisternae and vesicles of the

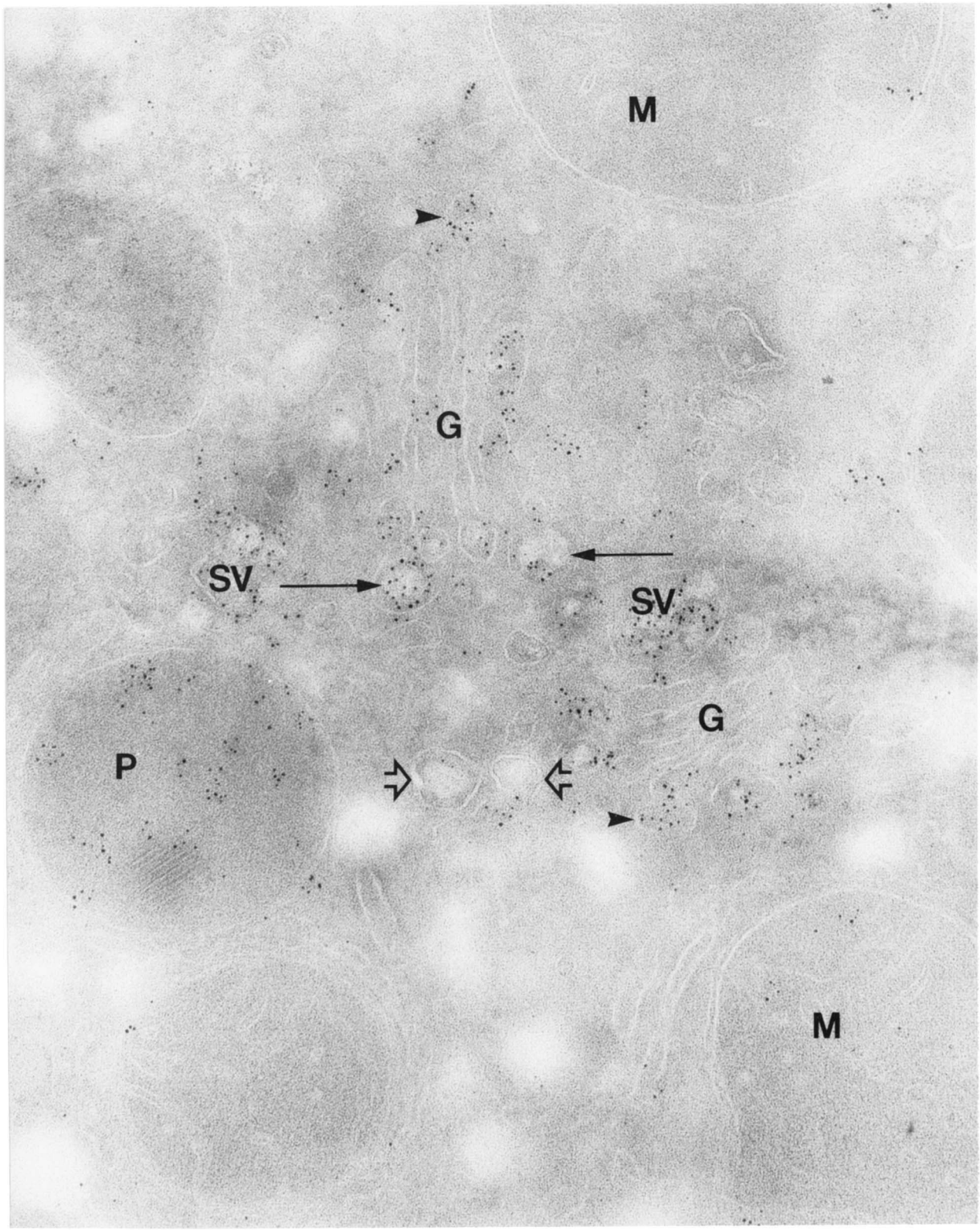

Fig. 4. Electron microscopic localization of apoE in cryothin sections. The peroxisome (P) contains many gold particles (5 nm) with clustering, as do most of the cisternae and vesicles (SV) of the Golgi apparatus (G), which fills most of this field. ApoE occurs in Golgi vesicles in the apparent absence of nascent VLDL particles (arrowheads) and in apparent association with some nascent VLDL (arrows), which are electron lucent. Some nascent VLDL, however, are not associated with gold particles (open arrows). Mitochondria (M) often show small amounts of gold labeling (× 90,000). Estradiol-treated animal.

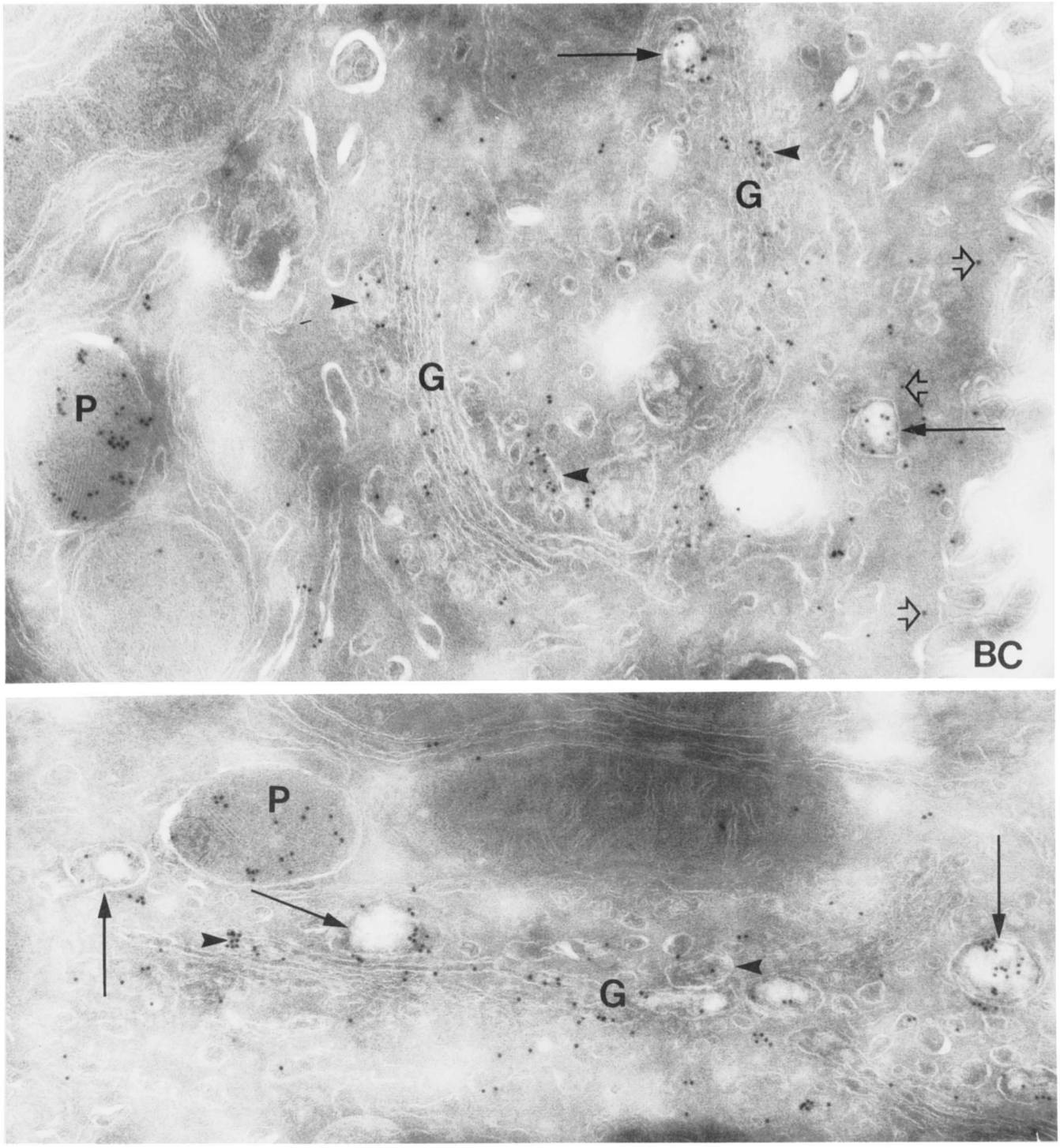

Fig. 5. Localization of apoE in hepatocyte peroxisomes (P) and adjacent Golgi (G) apparatus cisternae and vesicles with 10 nm gold complexes. Note the clustering of gold particles in the peroxisomes (P). As in Fig. 4, Golgi cisternae and vesicles contain gold (arrowheads) in the absence of evident VLDL particles, which are electron lucent and are best seen as clusters in putative forming secretory vesicles (arrows). In the top image, a few gold particles appear to be free in the cytoplasm (open arrows) close to the bile canaliculus (BC). (× 60,000). Estradiol-treated animal.

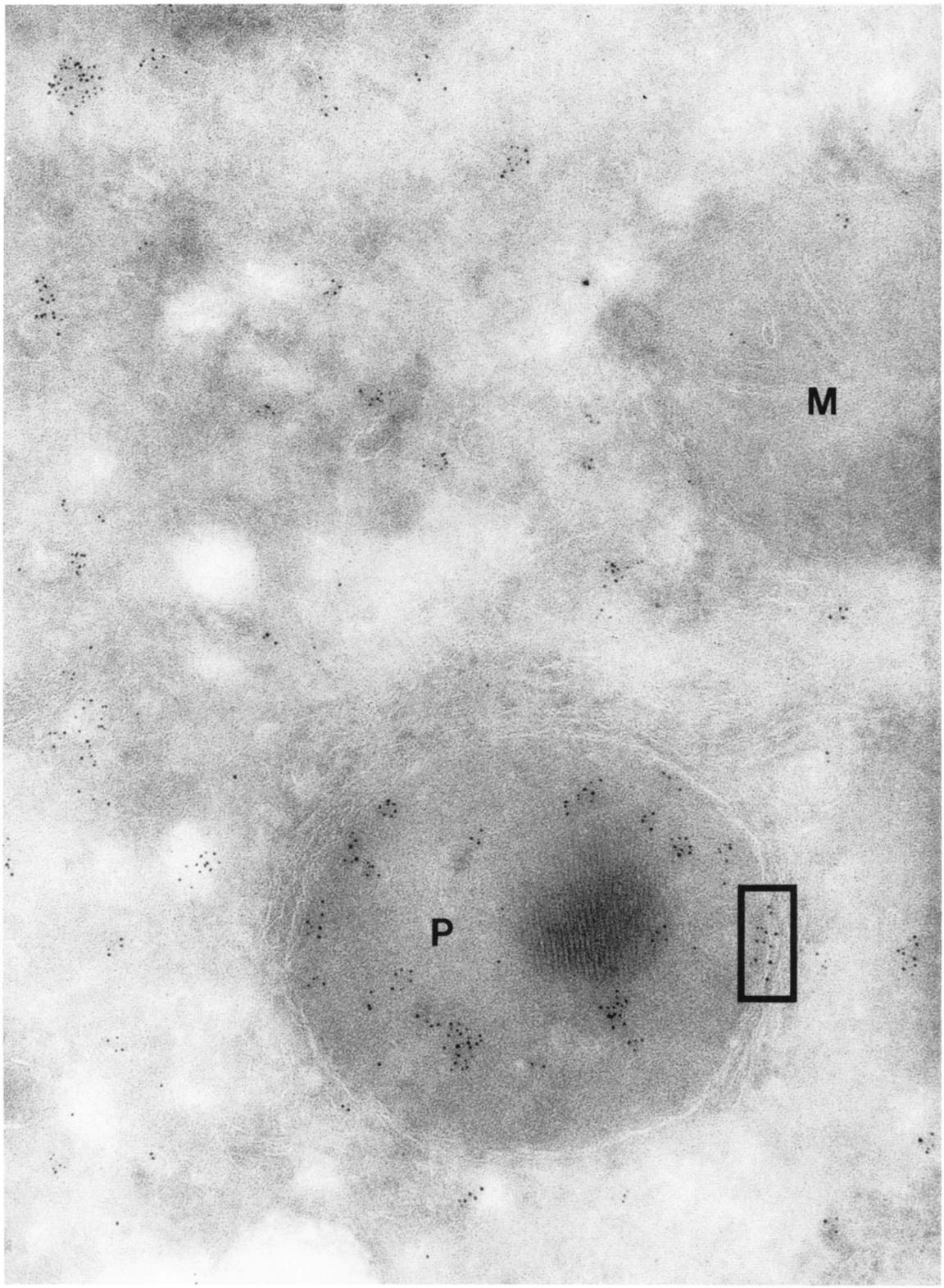

Fig. 6. Localization of apoE in peroxosomal (P) matrix and adjacent cytoplasm of rat hepatocyte (5 nm gold). The gold particles in the peroxisome matrix characteristically occur in clusters, as shown in this image. Note the apparent gold labeling of the peroxisomal limiting membrane (enclosed area). Clusters of gold particles are also seen in the cytoplasm, unassociated with evident organelles. No such localization of gold clusters was ever seen in control experiments, even when a fivefold higher concentration of IgG was used. (× 90,000). Untreated animal.

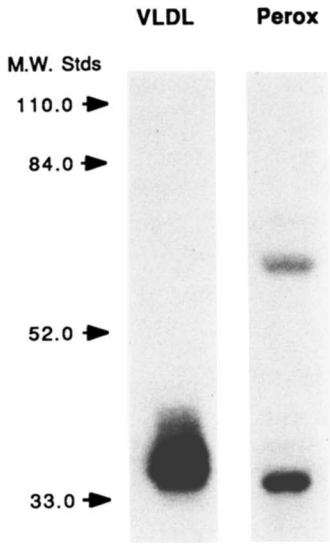

Fig. 7. Immunoblot of proteins of rat serum VLDL (20 μg) and highly purified hepatocytic peroxisomes (100 μg) separated by SDS-PAGE (9% gel). Affinity-purified anti-rat apoE IgG (20 μg/ml) was used as primary antibody, and the immunoblot was visualized with the reagents specified in the Bio-Rad Western blot protocol (14).

Golgi appartus that apparently lacked nascent VLDL particles consistently contained apoE. In addition, many nascent VLDL particles in Golgi vesicles were not associated with gold particles. The apparent lack of apoE on nascent VLDL particles could have three explanations. First, some nascent VLDL may indeed lack apoE; second, if the number of apoE molecules on a nascent VLDL particle is low, the plane of section could fail to detect the protein; third, penetration of the antibody probes into the frozen thin sections may be too limited to detect apoE (19). Numerous Golgi compartments, both cisternal and vesicular, contained apoE in the absence of a visible nascent VLDL particle. This observation strongly suggests that at least some apoE is transported to the Golgi independently of nascent VLDL particles and becomes associated with nascent VLDL particles in the forming secretory vesicles. This interpretation is supported by the observation that gold particles were frequently present in flat Golgi cisternae where nascent VLDL normally are rarely seen (22, 23). For many years, it has been recognized that putative nascent VLDL particles appear to accumulate as clusters within the dilated ends of Golgi cisternae (24), which have been called secretory vesicles (22, 23, 25, 26). These vesicles contain albumin together with nascent VLDL (25) and probably many other secreted proteins. These clusters of nascent VLDL particles, together with albumin and other plasma proteins, appear to be transported to the sinusoidal front where vesicular fusion with the plasma membrane releases nascent VLDL particles and other proteins by exocytosis into the space of Disse (23–26). Although morphological data can only suggest that nascent VLDL particles reside for prolonged periods in these forming secretory vesicles of the Golgi apparatus, recent biochemical data support this concept. In pulse-chase studies of chick hepatocytes in culture, apoB was estimated to have a residence time in the Golgi compartment of ~45 min (27). ApoA-I, which moved rapidly from the endoplasmic reticulum (6 min), also remained much longer (~28 min) in the Golgi (27). These observations are consistent with accumulation of apoB-containing particles as clusters in the forming secretory vesicles. If the non-B apolipoproteins of VLDL are bound to apoB-containing VLDL at the time of exocytosis, they must in part be synthesized and transported to and through Golgi compartments independently, but become associated with VLDL particles in forming secretory vesicles prior to release from the cell. We have found that apoE, proapoA-I, and the C apoproteins are all bound to nascent VLDL released from intact Golgi fractions (R. L. Hamilton and R. J. Havel, unpublished observations). As reported by others, nascent VLDL from rat liver Golgi fractions also contain apoE (28, 29). In human plasma, an apoE-deficient and phosphatidylethanolamine (PE)-enriched VLDL is a precursor of PE-depleted apoE-containing VLDL particles, suggesting that the apoE-deficient particle is a nascent form of VLDL from human liver (30, 31). We have recently reported that nascent VLDL from intact Golgi fractions (which are virtually free of contaminating endosomal organelles) contain four times more PE than plasma VLDL (32). Recent studies have shown that isolated hepatocytic Golgi fractions contain many of the enzymes of phospholipid synthesis, particularly those of PE, suggesting that some of these phospholipids are synthesized in the Golgi specifically for lipoprotein assembly (33–35). Taken together, these observations suggest that certain apolipoproteins and phospholipids associate with nascent VLDL particles late in the secretory pathway, possibly in the forming secretory vesicles of the Golgi apparatus.

The microvilli in the space of Disse showed intense labeling for apoE, distributed as clusters of gold particles associated with the microvillous plasma membrane. In some instances, a vague electron density was evident, suggesting that the clusters of gold were associated with a lipoprotein bound to the microvillous membrane. This scattered patchy distribution of gold label for apoE is very similar to that we have found for the asialoglycoprotein receptor (R. L. Hamilton and R. J. Havel, unpublished observations). Thus, apoE, associated or not with lipoprotein particles, may be widely distributed on microvilli, bound to lipoprotein receptors, heparan sulfate (36), or possibly hepatic lipase. Remnants of chylomicrons or VLDL could, at such sites, become associated with addi-

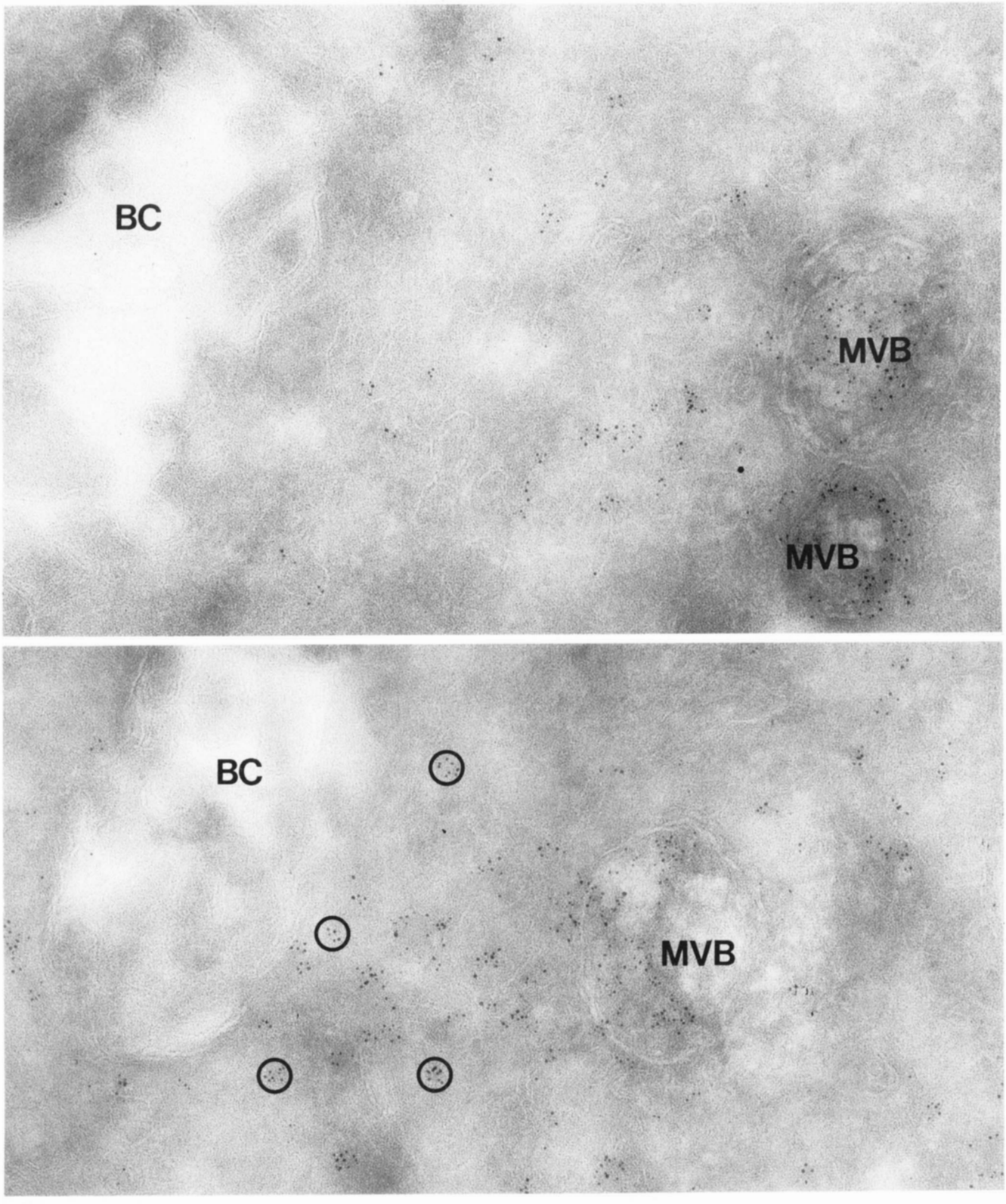

Fig. 8. Localization of apoE with 5 nm gold in multivesicular bodies (MVB) and in the adjacent cytoplasm near bile canaliculi (BC). In the top image, gold appears more concentrated in two MVBs than in the cytoplasm, whereas in the bottom image the number of clusters in the cytoplasm (circles) is similar to that within the MVB. Note absence of gold on bile canalicular microvilli, (× 90,000). Estradiol-treated animal.

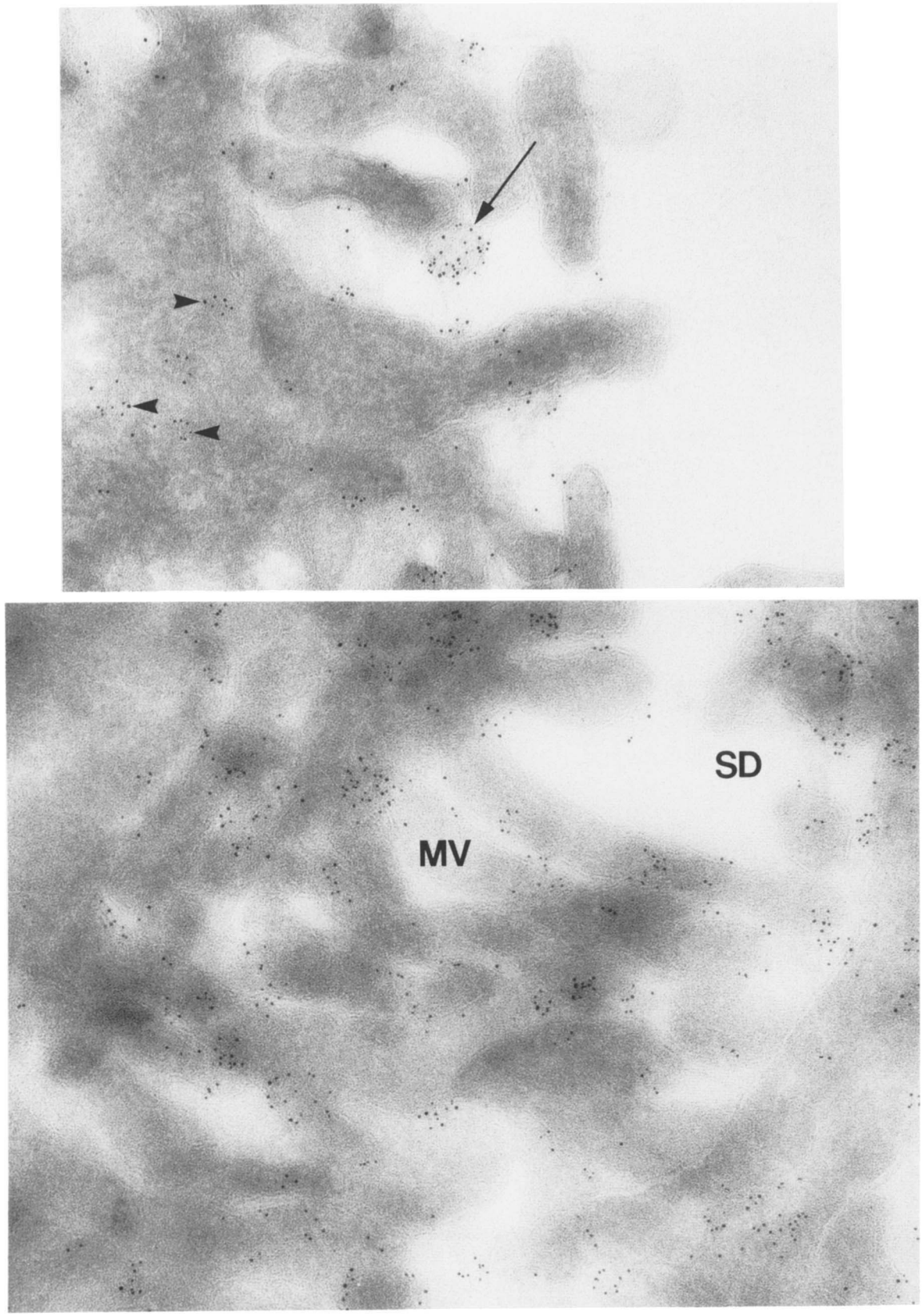

Fig. 9. Localization of apoE on sinusoidal microvilli in the space of Disse. The top image shows intense gold label on a putative large (~1,000 Å) remnant particle, apparently attached to a microvillus (arrow) and some clusters of gold in the cytoplasm (arrowheads) in the region of early endosomes. The bottom image shows scattered clusters of gold particles, largely associated with microvilli (MV) in the space of Disse (SD). (× 90,000). Untreated animal.

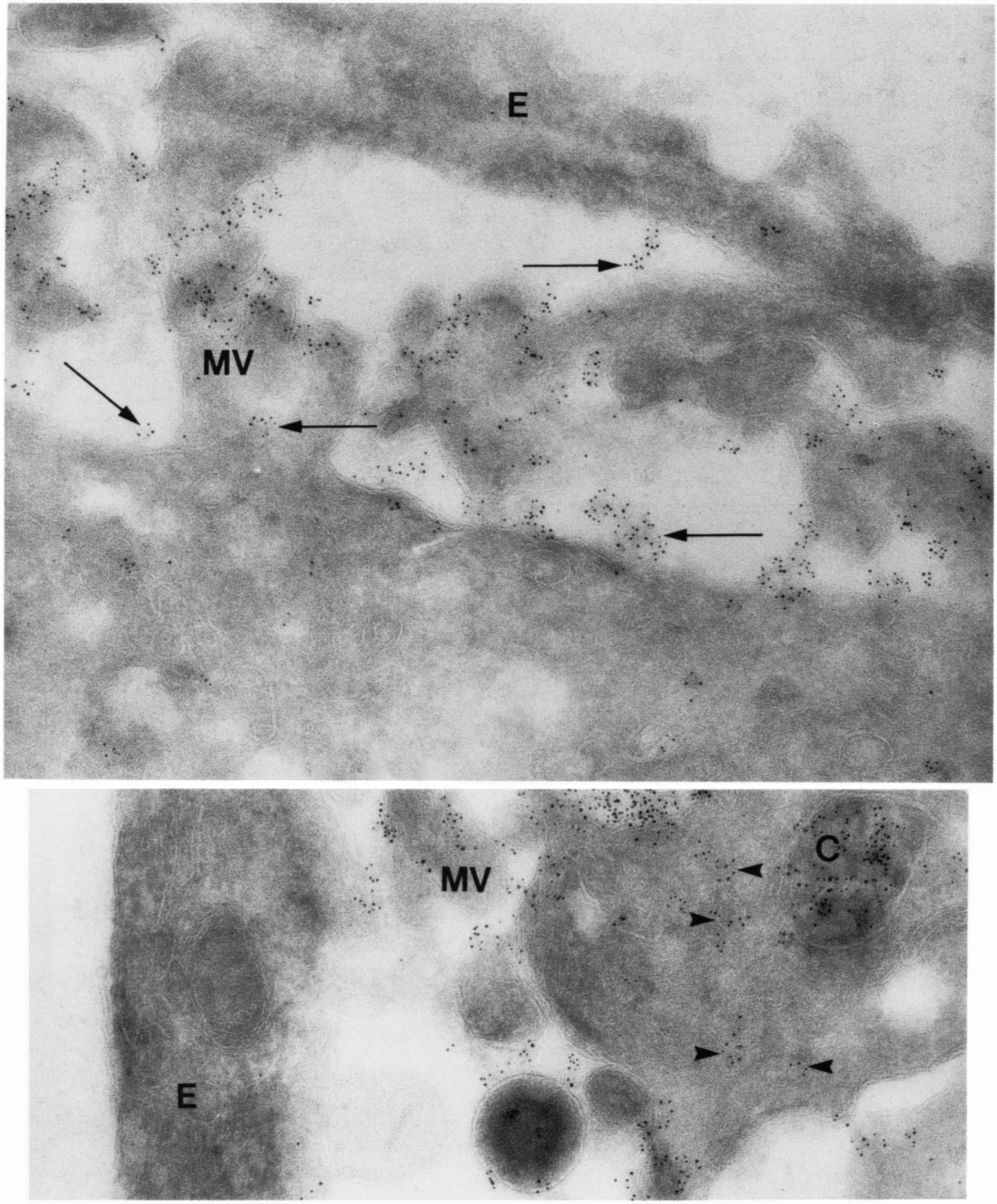

Fig. 10. Top, localization of apoE in clusters (arrows) associated with the hepatocytic microvillous (MV) surface, suggestive of lipoprotein remnants adherent to receptors or other molecules. The endothelial lining cell (E) is virtually free of gold label. Bottom, the endothelial lining cell (E) remains free of gold, whereas the microvilli stain intensely for apoE. An endosome, presumably CURL (C) filled with remnants, is intensely labeled. Some clusters of adjacent gold may represent coated pits and vesicles containing apoE (arrowheads). (× 90,000). Estradiol-treated animal.

tional apoE, facilitating their binding to hepatic lipase or to receptors such as the LDL receptor (37) or the LDL receptor-related protein (LRP) (13, 38) and subsequent lipolytic processing or endocytosis. Since coated pits occur only at the base of the microvilli, such bound remnant lipoproteins would have to pass downward along the microvillous membrane, a process that may require some time. Large numbers of remnant lipoproteins could thus be effectively removed from the blood plasma compartment into the space of Disse where they become concentrated and bind to receptors or other molecules on microvilli prior to endocytosis. Such bound remnants might also be modified at this site prior to endocytosis or to release into the blood as LDL.

The labeling of MVBs for apoE is most likely explained by the high content of triglyceride-rich remnants that are concentrated in this organelle during receptor-mediated endocytosis (2). Remnants of both chylomicrons and VLDL contain substantial amounts of apoE (3, 4). This presumably increases their affinity for the LDL (B-100,E) receptor or the other hepatocytic receptors (2–4). The small clusters of immunogold just beneth the microvillus plasma membrane most probably represent individual coated pits, coated vesicles, small endosomes, and CURL (compartment of uncoupling of receptor and ligand) vesicles (Fig. 10) containing apoE-enriched remnants in the endocytic pathway of hepatocytes, as shown previously in electron microscopic studies of the uptake of LDL-colloidal gold conjugates (39, 40). This interpretation is supported by independent studies (R. L. Hamilton and R. J. Havel, unpublished data), that have shown a high concentration of apoE, by immunogold labeling of frozen thin sections, in CURL and MVB fractions isolated from rat hepatocytes (2, 37, 41).

Why do peroxisomes consistently stain for apoE and why does the cytoplasmic area between MVBs and the bile canaliculus and that surrounding some peroxisomes sometimes also stain intensely for apoE? This unexpected reactivity was thoroughly evaluated in control experiments in which frozen thin sections were exposed to high concentrations of nonspecific rabbit IgG followed by goat anti-rabbit IgG–colloidal gold complexes. In these control experiments, no gold particles were found adsorbed to the tissue. Thus, peroxisomal and cytoplasmic labeling do not appear to be nonspecific. ApoE is widely thought to function in cholesterol transport and homeostasis (7, 8). Recently, peroxisomes have been strongly implicated in cholesterol metabolism in hepatocytes by their content of HMG-CoA reductase (42), acetoacetyl CoA thiolase (43), several of the enzymes that convert cholesterol to bile acids (44), and sterol carrier protein-2 (14, 45). Moreover, rat liver peroxisomes can convert mevalonic acid to cholesterol (46).

The presence of apoE immunoreactivity in high concentration in the area adjacent to MVBs, between MVBs and the bile canaliculus, and in the area surrounding perioxisomes, in both cases unassociated with evident organelles, suggests that apoE may be involved in interorganelle transport of cholesterol (and perhaps other sterols). The immunoreactive protein in peroxisomes has an apparent molecular weight slightly smaller than that of VLDL apoE (Fig. 7), and it will be of great interest to determine whether this apoE contains the targetting signal sequence for peroxisomes (47, 48). This signal is typically the carboxy-terminal tripeptide ser-lys-leu, but certain substitutions are known to be consistent with targetting to peroxisomes. Preliminary data (not shown) indicate that the peroxisomal targetting tripeptide is not present on rat serum VLDL, but a ~35-kDa protein in hepatocytic peroxisomes immunoblots with the targetting antibody against ser-lys-leu. Although the resolution obtained with frozen thin sections is limited and no firm conclusions are possible, our immunocytochemical data raise the possibility that some apoE may exist in the hepatocyte cytoplasmic area adjacent to MVBs and peroxisomes in a form not enclosed by a cell membrane. The density of gold particles near the bile canaliculus (Fig. 5, top) and in the cytoplasmic area between the bile canaliculus and adjacent MVBs (Fig. 8, bottom) appears to exceed the amount of membrane-bound vesicles that occur in this area of hepatocytes. More important to the issue of cytoplasmic apoE is that peroxisomes do not obtain matrix proteins, which are synthesized by free cytosolic polysomes, by vesicular transport mechanisms (49). This suggests that apoE present in the matrix may be transported to and across the peroxisomal membrane by non-vesicular mechanisms used by other peroxisomal proteins (49). Our observation that about one-half of the peroxisomes photographed appeared to have gold label on the peroxisomal limiting membrane (Fig. 6) is consistent with ongoing transport of apoE between the hepatocyte cytoplasm and the peroxisomal matrix.

We know a great deal about the role of apoE in receptor-mediated endocytosis of lipoproteins in hepatocytes leading to lysosomal degradation of cholesteryl esters by acid lipase, but much less is known about transport of the released unesterified cholesterol and how it is carried to the appropriate organelles for conversion to bile acids or excretion into the bile canaliculus. The observations reported here suggest that apoE may be a carrier for cholesterol transported between subcellular compartments within hepatocytes and perhaps in other cells that express apoE.

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