Cell surface "blanket" of apolipoprotein E on rat adrenocortical cells

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Abstract Apolipoprotein (apo) E is expressed at high levels by adrenocortical cells. In the present study, an affinity-purified antibody to rat apoE was used in combination with immunogold visualization at both the light and electron microscopic levels to determine the cellular and subcellular distribution of apoE within the rat adrenal cortex. At the light microscopic level, apoE was found primarily in z. fasciculata and z. reticularis with little or none detected in z. glomerulosa and medulla. Within the z. fasciculata and z. reticularis, apoE was present in the cytoplasm of all parenchymal cells. ApoE also was found on the cell surface both on the sinusoidal front and in regions well removed from the subendothelial space. Electron microscopic examination of the z. fasciculata showed that apoE on the sinusoidal front was on the parenchymal cell surface but not the endothelial cell. Cell surface apoE was prominent on microvilli as well as non-microvillar regions of plasma membrane in the subendothelial space. ApoE was also associated with the cell surface in intercellular spaces continuous with but well removed from the subendothelial space. These findings at the light and electron microscopic levels suggest that the z. fasciculata cell is encircled or covered with apoE on all faces of the cell. These results are consistent with the idea that this cell surface "blanket" of apoE participates in the uptake of lipoprotein cholesterol by either the endocytic or selective uptake pathways.—**Williams, D. L., J. S. Wong, S. L. Wissig, and R. L. Hamilton.** Cell surface "blanket" of apolipoprotein E on rat adrenocortical cells. *J. Lipid Res.* 1995. **36:** 745–758.

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Apolipoprotein E (apoE) is a prominent component of several classes of plasma lipoproteins (1–3). A major function of apoE in the plasma is to mediate the hepatic removal of remnant lipoproteins by the LDL receptor and by mechanisms distinct from the LDL receptor (1–6). In contrast to other apolipoproteins, which are synthesized only in liver and small intestine, apoE is also made in many peripheral tissues including adrenal gland, ovary, testis, brain, adipose, skin, and lung (7–15). Studies with human and nonhuman primate tissues indicated that apoE synthetic rates and mRNA concentrations in the adrenal gland are similar to those in liver (7, 8, 10), indicating that apoE is an abundant protein product of adrenal cells. In situ hybridization analysis of rat adrenal gland showed apoE mRNA primarily in zona (z) fasciculata and z. reticularis cells, the major sites of glucocorticoid production and cholesteryl ester storage (16). The level of apoE expression is regulated in rat adrenal gland such that the mRNA concentration varies in direct proportion to cholesteryl ester stores and inversely to the level of adrenal steroidogenesis as judged by plasma corticosteroids (17). The high level expression of apoE in adrenocortical cells and its pattern of regulation led to suggestions that locally derived apoE may function in some manner to alter cholesteryl ester storage and/or modulate the availability of cholesterol for steroidogenesis (7, 17). The possibility that apoE may alter adrenocortical cholesterol metabolism is supported by a recent study in which human apoE was stably expressed in the murine Y1 adrenocortical cell. ApoE-expressing Y1 cells showed reduced levels of steroid production (18) and increased accumulation of cholesteryl ester (19).

Although apoE mRNA is present primarily in z. fasciculata and z. reticularis cells, the location of the apoE protein within the adrenal gland and within the adrenocortical cell is not well understood. Knowledge of the subcellular location of apoE is clearly important for understanding the potential function of adrenocortical apoE. Recent studies with cultured hepatoma cells, for example, suggest that cell surface apoE may participate in the acquisition of lipoprotein cholesterol via the selective uptake and endocytic pathways (20–22). In the present study we have used immunolocalization techniques at the light and electron microscopic levels to determine whether cell surface apoE occurs on adrenocortical cells in vivo. The

Abbreviations: apo, apolipoprotein; PBS, phosphate-buffered saline; PBSG, PBS-glycine; NCS, newborn calf serum; MVB, multivesicular body; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins.

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results show that the adrenocortical cell is encircled or covered with apoE on all faces of the cell, a finding consistent with the idea that locally produced apoE participates in the uptake of lipoprotein cholesterol. In addition, the localization of apoE has been compared to that of apoA-I, which is not made in adrenal cells but which is the principal protein of HDL particles that supply most of the cholesterol for steroid production and cholesteryl ester storage in rats and mice (23–25).

MATERIALS AND METHODS

Animals and tissue preparation

Male Sprague-Dawley rats (200–250 g) from Taconic Farms (Germantown, NY) were maintained on a 12-h light/12-h dark cycle and provided with standard rat chow and water ad libitum. For light microscopy, rats were anesthetized by intramuscular injection of a Ketamine (Ketaset, Aveco Co. Inc.), Xylazine (Rugby Laboratories Inc.) (4:1) cocktail and were perfused through the left ventricle with 100 ml phosphate-buffered saline followed by 150–200 ml 4% paraformaldehyde, 0.1M sodium phosphate, pH 7.5. Adrenal glands were post-fixed in the same fixative for 4 h at 4°C. Whole adrenals were infiltrated with three changes of 30% sucrose, 0.1 M sodium phosphate buffer (pH 7.5), embedded in Tissue-Tek OCT (Miles), and quick-frozen in isopentane cooled by liquid nitrogen. Tissue blocks were stored in liquid nitrogen. Eight- to 10-μm thick cryostat sections were placed on poly-l-lysine (Sigma) coated slides, air dried for 2 h, and stored in airtight containers with desiccant at −70°C.

For electron microscopic immunolocalization, the animal was perfused as above with freshly prepared 2% paraformaldehyde, 0.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. Adrenal glands were kept in fixative for 2 h, 0.3-mm slices were cut, and the middle region of the z. fasciculata was cut into cubes 0.3 mm on a side. Adrenal cubes were cryoprotected, frozen in freon cooled by liquid nitrogen, and ultrathin frozen sections were cut as described (26–28) on a Reichart Ultracut S with FCS low temperature sectioning system. For transmission electron micrographs, animals were perfused as above but the fixative contained 2% glutaraldehyde, 1% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4. To facilitate orientation during sectioning, a 1-mm disk from the center of the gland was cut into a pie-shaped wedge with the narrow end in the medulla. Adrenal wedges were postfixed with reduced osmium (1% aqueous osmium tetroxide, 1.5% potassium ferrocyanide) followed by block stain with 2% aqueous uranyl acetate. Tissue blocks were dehydrated with acetone, embedded in epon, and 70- to 90-nm sections were cut on an LKB Ultramicrotome.

Immunolocalization

Affinity-purified rabbit polyclonal antibody specific for rat apoE has been described previously (28). Affinity-purified rabbit antibody against rat apoA-I was prepared in the same manner. Western blot analysis showed a prominent single band of immunoreactivity coincident with apoA-I when tested against rat serum HDL with trace reactivity to unidentified high molecular weight components. No reactivity was detected toward apoE (data not shown). Except where indicated, all immunostaining procedures were carried out at room temperature. For light microscopic studies, sections were treated with 3% paraformaldehyde in phosphate-buffered saline (PBS) and quenched with PBS-glycine (PBSG) (0.02 M). Sections were blocked sequentially for 10 min with 1% newborn calf serum (NCS) and 10% NCS in PBSG, rinsed in PBSG, incubated for 60 min with primary antibody in PBS–1% NCS, and rinsed 4 times over a 15-min period with PBSG. Anti-apoE (0.1 μg/ml), anti-apoA-I (1 μg/ml), and preimmune IgG (0.5 to 4 μg/ml) were cleared by centrifugation immediately before use. Secondary antibody (anti-rabbit IgG colloidal gold (5 nm), Amersham Corp., Arlington Heights, IL) (1:50 dilution) in PBS-1% NCS was applied for 30 min followed by 4 washes with PBSG over a 15-min period, incubation for 5 min with 2% glutaraldehyde in PBS, and 3 washes with water over a 10-min period. Immunogold staining was carried out by the silver enhancement technique using the Amersham IntenSE M silver enhancement kit. Slides were counterstained with hematoxylin. Light micrographs were taken under both bright field and epipolarization illumination on a Leitz Ortho Plan 2 microscope.

For electron microscopic studies, anti-apoE (1–2 μg/ml), anti-apoA-I (2 μg/ml), and preimmune antibody (4–10 μg/ml), and secondary antibody (1:20 dilution) were diluted in PBS-1% NCS and cleared by centrifugation. Sections were blocked as above, incubated with primary antibody for 40 min, rinsed 4 times in PBSG, incubated 20 min with secondary antibody, rinsed 5 times with PBSG, and 4 times with water. Grids were stained for 10 min with 2% uranyl acetate in 0.15 M oxalic acid, pH 7, washed 3 times in water, stained for 10 min with 0.5% uranyl acetate in 2% methyl cellulose followed by 5 min in the same solution on ice. Grids were blotted, air dried, and examined immediately in a Siemens Elmiskop 101 microscope.

RESULTS

ApoE–light microscopy

Using the silver-enhanced immunogold technique at the light microscopic level, staining for apoE was seen almost exclusively in the z. fasciculata and z. reticularis

(**Fig. 1a**) with no detectable staining in the z. glomerulosa or the medulla. Parallel sections reacted with 0.5 μg/ml of preimmune IgG (5-fold excess relative to anti-apoE) showed no stain detectable by epipolarization illumination (Fig. 1b). In other experiments, preimmune IgG at up to 4 μg/ml showed no staining (data not shown). At higher magnification (**Fig. 2b**) apoE stain was seen within the cytoplasm of all parenchymal cells of the z. fasciculata in a punctate pattern. ApoE stain also was seen prominently bordering the sinusoid suggesting a cell surface location for apoE on either the parenchymal cell or the sinusoidal endothelial cell (Fig. 2b, arrows). In addition to the apparent cell surface location on the sinusoidal face, apoE stain often was seen encircling the z. fasciculata cell; that is, stain was prominent between adjacent cells in areas well removed from the subendothelial space. This pattern of encirclement suggests that apoE may be present on all faces of the parenchymal cell and not just on the sinusoidal face.

ApoE–electron microscopy

ApoE localization in z. fasciculata cells at the electron microscopic level in ultrathin cryosections shows a number of interesting features. Gold particles were abundant with anti-apoE, but rarely were seen with preimmune IgG at 2- to 5-fold higher concentrations (data not

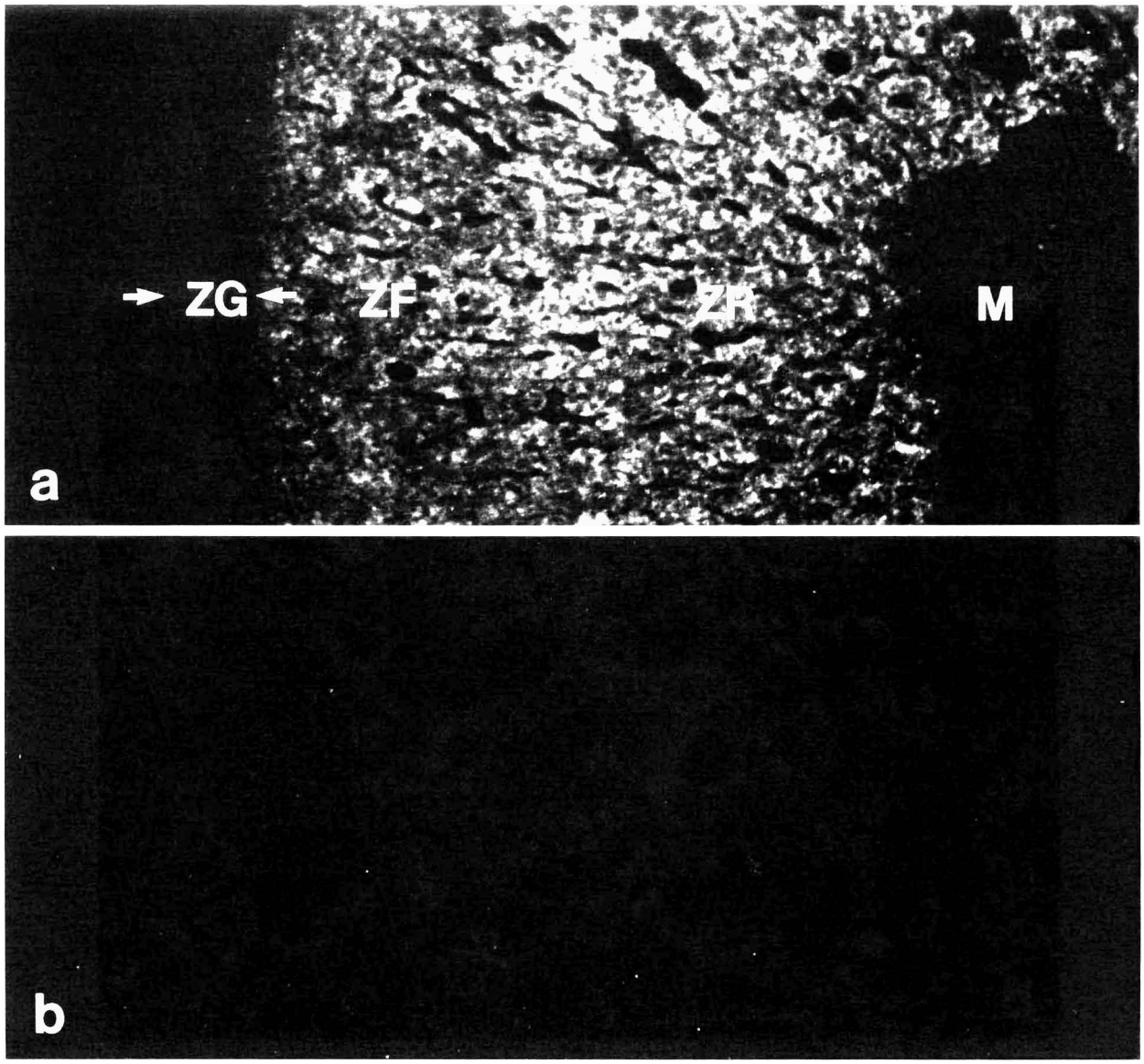

Fig. 1. Light microscopic localization of apoE in frozen sections of rat adrenal gland. (a) Epipolarization illumination of silver-enhanced immunogold reaction with anti-apoE (0.1 μg/ml) shows reactivity in z. fasciculata (ZF) and z. reticularis (ZR) but not in medulla (M) or z. glomerulosa (ZG). (b) Parallel tissue section reacted with preimmune IgG (0.5 μg/ml) shows no reactivity under epipolarization illumination; (×130).

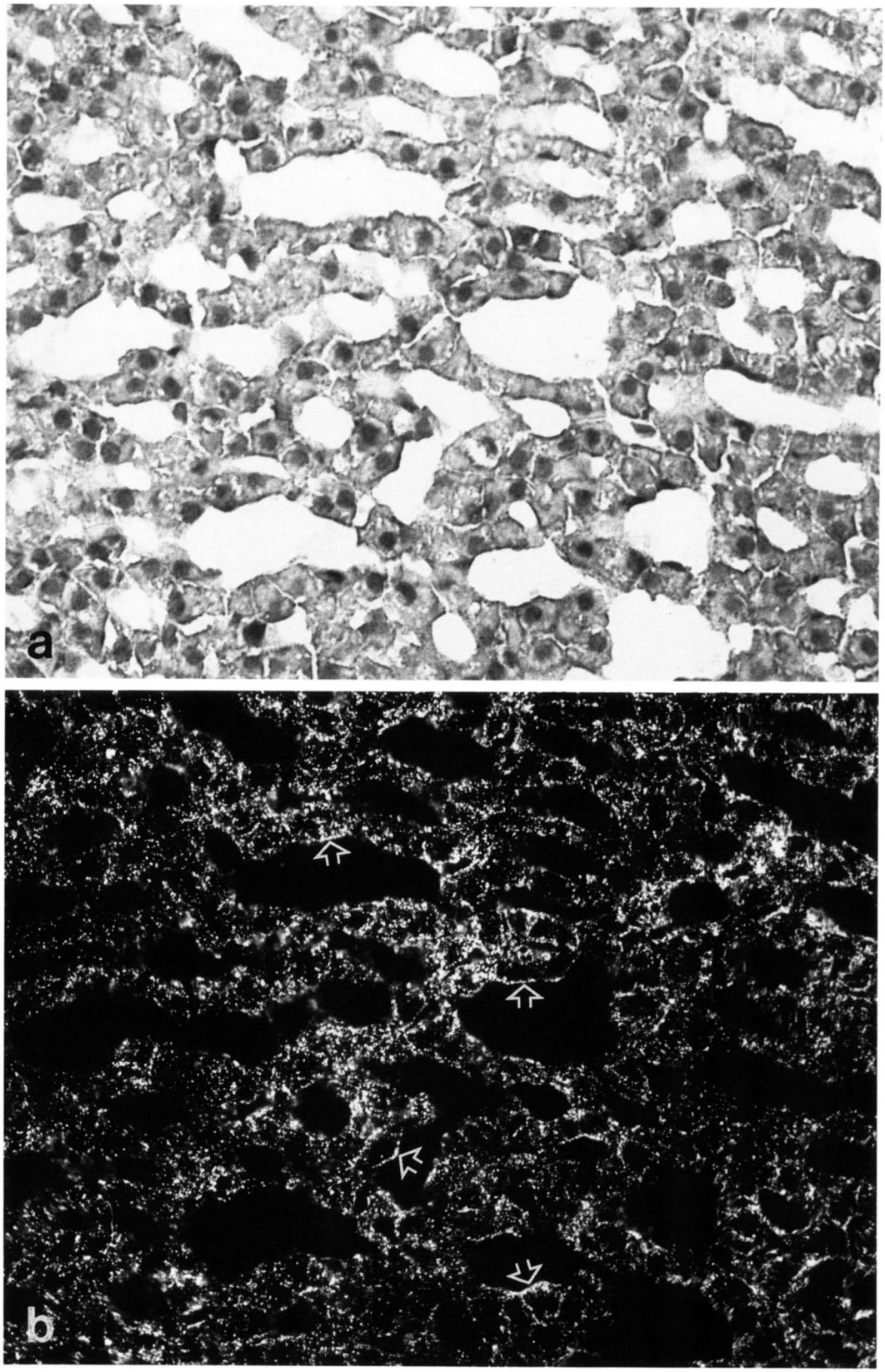

Fig. 2. Light microscopic localization of apoE in z. fasciculata at higher magnification. (a) Brightfield micrograph of z. fasciculata stained for apoE. (b) Same section under epipolarization illumination shows immunoreactive product in punctate spots throughout the cytoplasm of parenchymal cells and on the cell surface (arrows). Note that gold is present not only on the sinusoidal face of the z. fasciculata cell but also on the plasma membrane in regions well removed from the sinusoid; (×400).

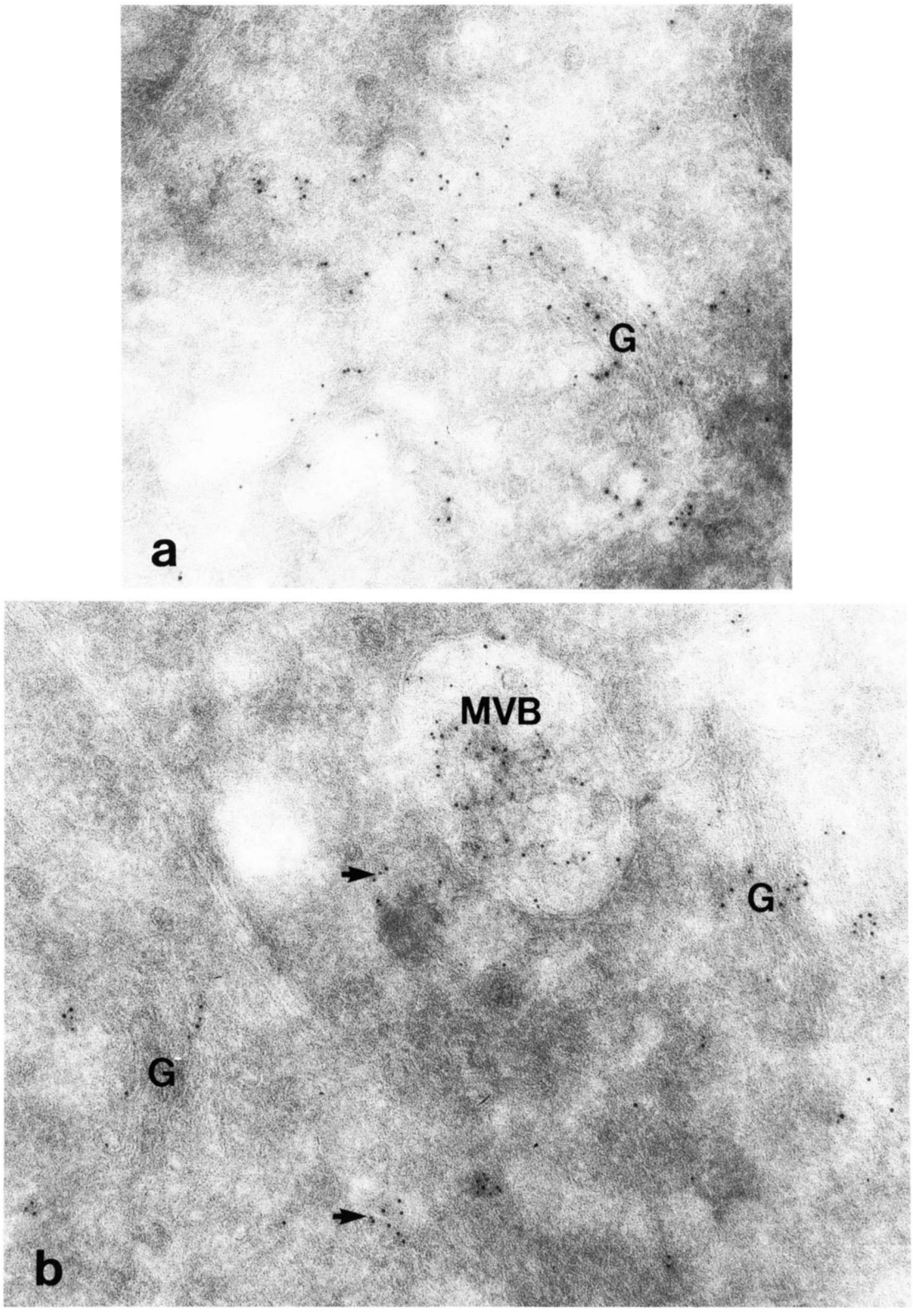

Fig. 3. Electron microscopic localization of apoE in ultrathin cryosections. (a and b) Gold particles are abundant in Golgi (G) regions that contain putative secretory vesicles. (b) A multivesicular body (MVB) is heavily labeled with gold. Gold particles were sometimes seen over cytoplasmic areas (arrows) free of apparent membrane bound organelles; (a and b, ×90,000).

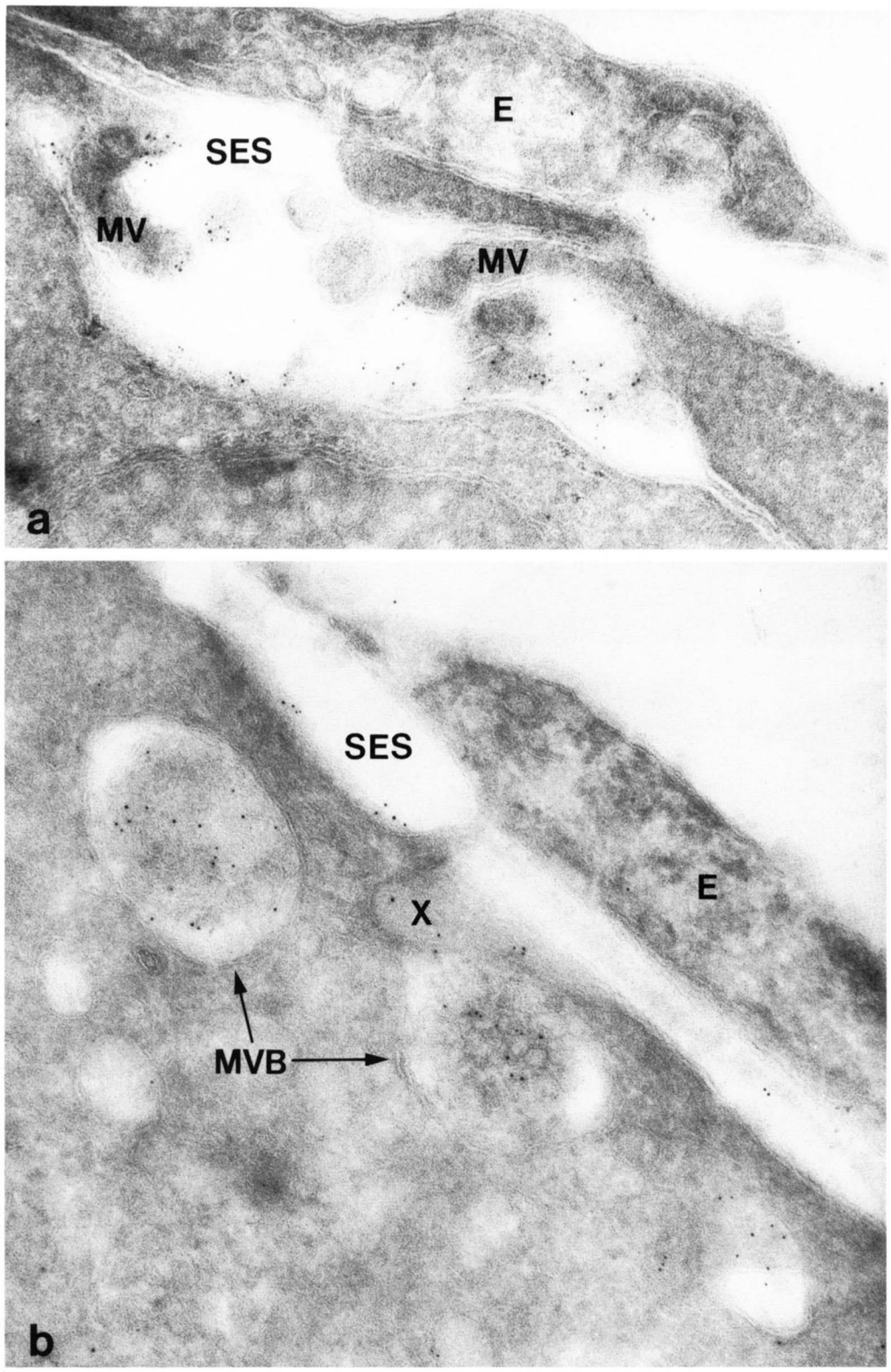

Fig. 4. Cell surface location of apoE on z. fasciculata cells in the subendothelial space. (a) After staining with anti-apoE, gold particles are seen on the parenchymal cell plasma membrane and on microvilli (MV) in the subendothelial space (SES). The endothelial cell (E) is not labeled. (B) Gold particles are seen in multivesicular bodies (MVB), in a putative coated pit (X), and along the parenchymal cell surface after staining with anti-apoE; (a and b, ×76,500).

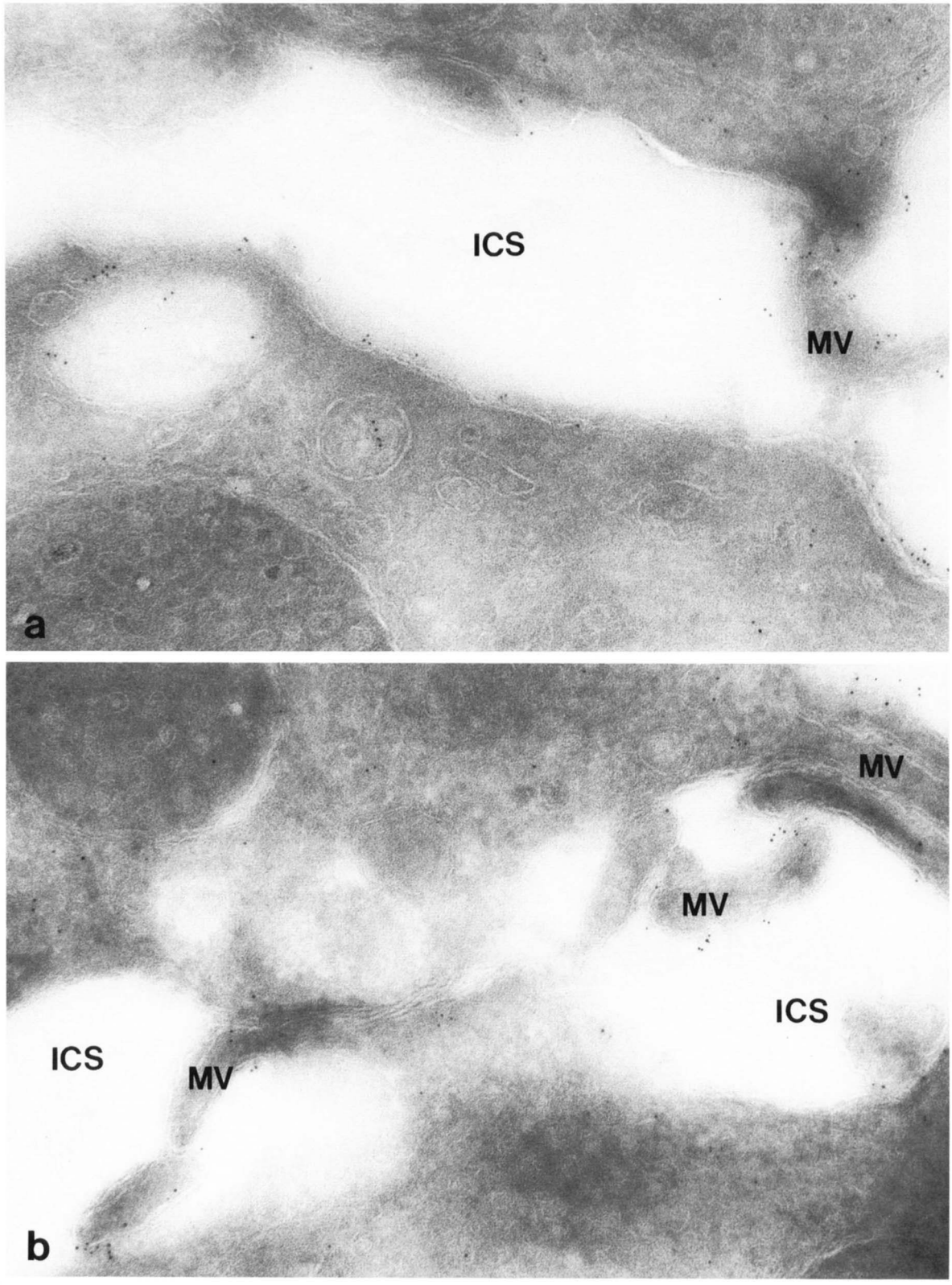

Fig. 5. Cell surface location of apoE on adjacent z. fasciculata cells bordering an intercellular space. (a and b) After staining with anti-apoE, gold particles are seen on the parenchymal cell plasma membrane and microvilli (MV) bordering apparently expanded intercellular spaces (ICS); (a and b, ×76,500).

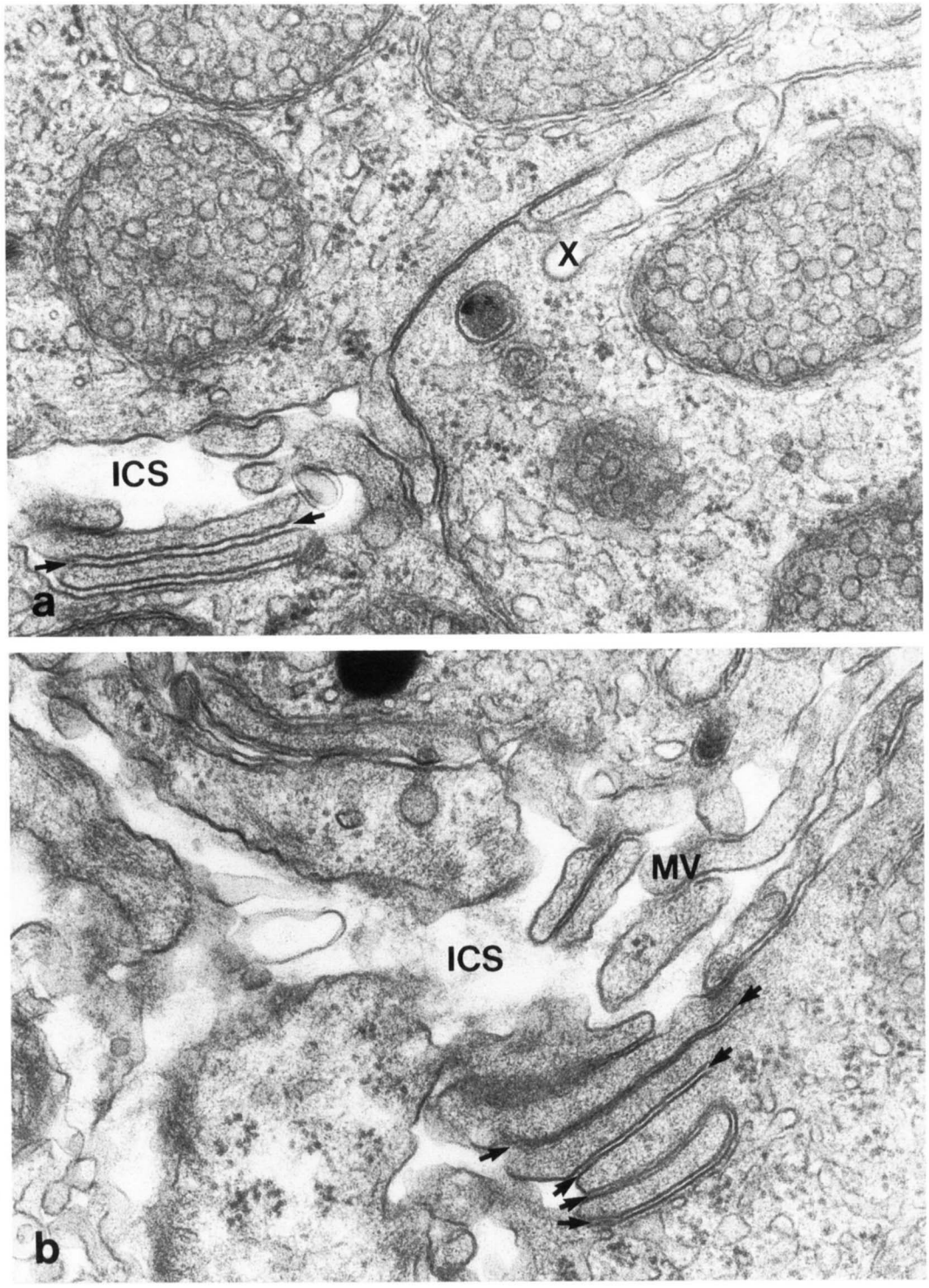

Fig. 6. Microvilli and microvillar channels are found in the intercellular space between adjacent z. fasciculata cells. (a and b) Transmission electron micrographs of adrenal tissue fixed and embedded by standard procedures shows numerous microvilli (MV) in intercellular spaces (ICS). Microvillar channels (arrows) are seen between stacked microvilli. A coated pit (X) is shown in panel (a) apparently forming from a microvillar channel; (×51,000).

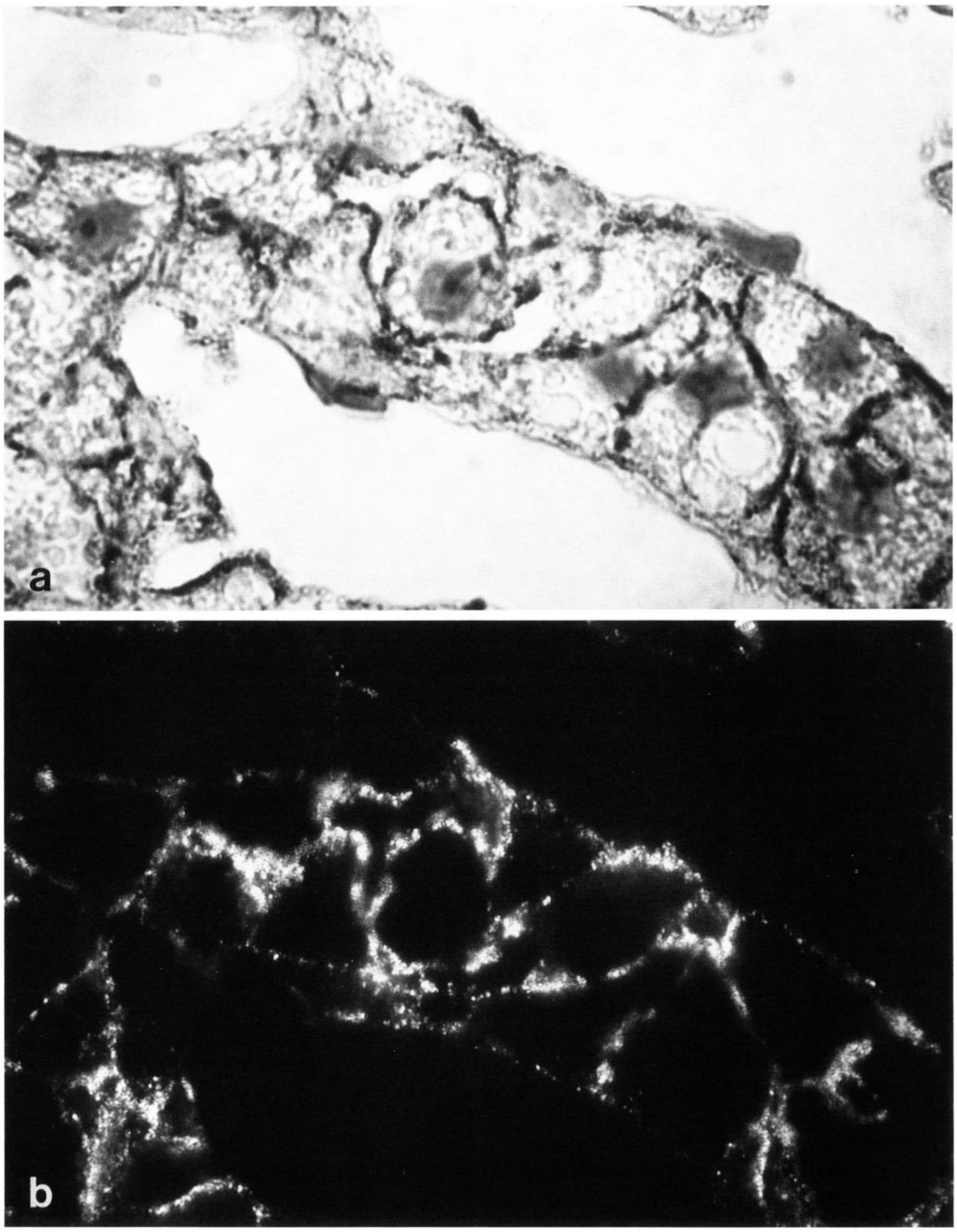

Fig. 7. Light microscopic localization of apoAI in z. fasciculata. (a) Brightfield micrograph of a cord of z. fasciculata cells stained with anti-apoA-I (1 μg/ml). Note dark silver-enhanced gold reaction product on the cell surface but not in the cytoplasm. (b) Epipolarization illumination illustrates more clearly the cell surface localization of apoA-I; (×1530).

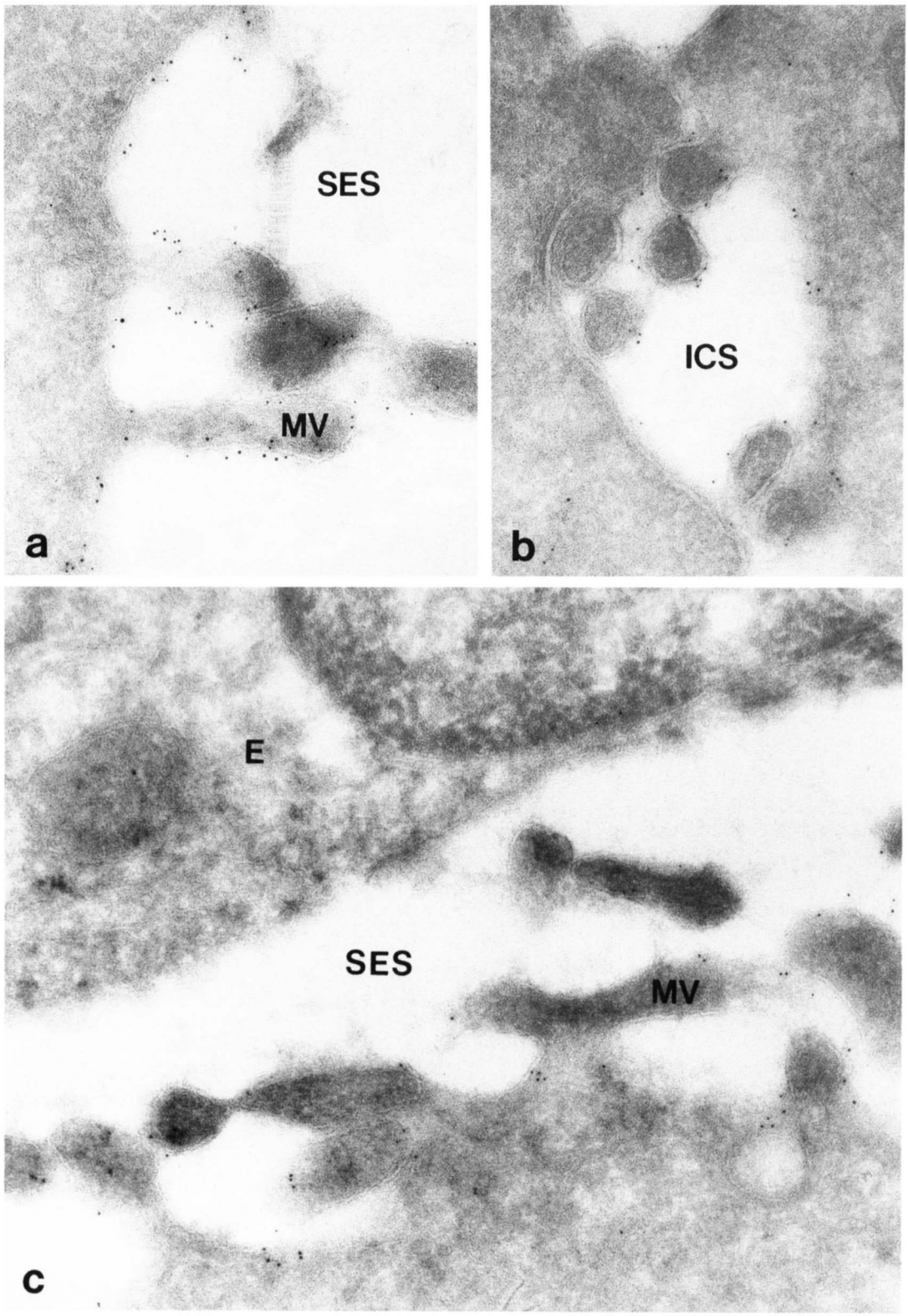

Fig. 8. Cell surface location of apoA-I on z. fasciculata cells. (a and c) Electron micrographs of ultrathin cryosections after immunogold staining for apoA-I shows gold particles on the plasma membrane and microvilli of the parenchymal cell in the subendothelial space (SES). The endothelial cell (E) in panel c is not labeled. (b) Microvilli are also stained for apoA-I in the intercellular space (ICS) between adjacent parenchymal cells; (a, b, and c, ×76,500).

shown). As would be expected for cells making apoE, gold particles were found in Golgi areas and in putative secretory vesicles (**Figs. 3a and 3b**). ApoE also was found in the endocytic pathway as judged by numerous gold particles in multivesicular bodies (MVB) (Figs. 3b and 4b). About half of the MVBs stained strongly for apoE suggesting an active endocytic traffic of apoE. Mitochondria and nuclei were uniformly negative for apoE. Gold particles also were seen over cytoplasmic areas in the apparent absence of organelles although it was not possible to determine whether such particles reflect apoE that is actually free in the cytoplasm or apoE associated with poorly resolved membranes (see arrows, Fig. 3b).

Interestingly, the parenchymal cell surface in the subendothelial space was strongly positive for apoE while gold particles were not found on either the sinusoidal or subendothelial surface of endothelial cells (**Figs. 4a and 4b**). On the parenchymal cell surface gold particles were associated with the numerous microvilli as well as nonmicrovillar domains of plasma membrane. In addition to apoE localization on the subendothelial face of the parenchymal cell, we also observed gold particles associated with the plasma membrane in intercellular spaces between adjacent parenchymal cells (**Figs. 5a and 5b**). Such spaces appear to be continuous with but well removed from the subendothelial space. The density of labeling in these areas was similar to that in the subendothelial space; gold particles were seen on cell surfaces, on isolated microvilli, and on stacks of microvilli that probably represent the microvillar channels described by Reaven, Spicher, and Azhar (29). Our impression is that the intercellular space is distorted by the cryosectioning procedure, artifactually expanding this space and disrupting the ordered layering of microvillar channels. Transmission micrographs of rat adrenal gland after standard fixation and embedding techniques show clearly that stacked microvilli and microvillar channels (arrows) occur in these intercellular regions between adjacent parenchymal cells (**Figs. 6a and 6b**).

ApoA-I–light microscopy

At the light microscopic level, the pattern of apoA-I staining was confined almost exclusively to the z. fasciculata and z. reticularis, and almost all gold particles localizing apoA-I were confined to the cell surface. As shown in **Fig. 7**, apoA-I immunostaining was seen clearly at the cell surface but not in the cytoplasm of z. fasciculata cells. ApoA-I stain was prominent on the sinusoidal face but also was seen on those surfaces of the cell adjacent to other adrenal parenchymal cells facing the intercellular space. This pattern of encirclement with apoA-I stain is similar to that seen with apoE (Fig. 2b).

ApoA-I–electron microscopy

At the electron microscopic level, staining with anti-apoA-I showed numerous gold particles associated with parenchymal cell microvilli as well as non-microvillar domains of plasma membrane. This is illustrated in **Figs. 8a and 8c** which show the z. fasciculata cell surface in the subendothelial space. Endothelial cells were negative for apoA-I staining (Fig. 8c). As was the case with apoE, apoA-I also was found on the microvilli of the cell surface in intercellular spaces between parenchymal cells well removed from the subendothelial space (Fig. 8b). This confirms the impression from the light microscopic analysis that plasma membrane-bound apoA-I encircles the adrenocortical parenchymal cell. In contrast to observations with anti-apoE, when staining for apoA-I, gold particles were rarely seen intracellularly. Gold particles were seen in only an occasional MVB but the frequency was far less than for apoE.

DISCUSSION

The light microscopic localization of apoE showed that essentially all parenchymal cells within the z. fasciculata and z. reticularis stain for apoE while apoE staining was not seen in the z. glomerulosa or the medulla. This distribution of apoE protein is similar to the distribution of apoE mRNA in rat adrenal gland determined by in situ hybridization (16), suggesting that much of the adrenocortical apoE is due to the local synthesis of this apolipoprotein. The finding that apoE protein and mRNA (16) are very low in z. glomerlosa as compared to z. fasciculata cells may reflect a greater demand for cholesterol for steroid production or cholesteryl ester storage in z. fasciculata cells. Z. fasciculata cells typically have larger stores of cholesteryl esters as judged by the size and number of cytoplasmic lipid droplets (30) or the intensity of oil red O staining (16). The lack of significant staining for apoE in z. glomerulosa or medulla is in contrast to the results of Lin et al. (31) who observed in baboon adrenal gland staining for apoE in the z. glomerulosa which they interpreted to be extracellular, and moderate staining in the medulla which appeared to be cytoplasmic. These differences with the present results could reflect a species difference. Alternatively, this difference could be due to the fact that we have used vascular perfusion to remove blood from the sinusoids prior to perfusion fixation of the tissue whereas Lin et al. (31) fixed unperfused tissues by immersion, a procedure that would be expected to trap blood lipoproteins in the sinusoids.

ApoE was found both within the cytoplasm and on the surface of z. fasciculata cells. At the electron microscopic

level, approximately 50% of the MVBs were labeled with anti-apoE suggesting an active endocytic uptake of apoE. Interestingly, few MVBs were labeled with anti-apoA-I indicating that the apoE within the endocytic pathway was not present on apoA-I-containing HDL particles. The internalized apoE could represent apoE on non-apoA-I-containing lipoprotein particles internalized by the B/E receptor or lipoprotein-free apoE that is endocytosed by virtue of its cell surface location. Although the adrenal gland of rats and mice derives most of its cholesterol from HDL particles (23, 32) via the selective uptake mechanism (25, 33), adrenocortical cells also express the B/E receptor and use this mechanism to internalize lipoproteins (32, 34, 35).

ApoE was present in the Golgi apparatus and in putative secretory vesicles as expected in cells making this protein. Gold particles also were seen in regions of cytoplasm that contained no apparent membrane-bound organelles although the morphology with frozen thin sections is not always adequate to conclude that small membrane-bound organelles are absent. A similar result was seen in rat liver where apoE was present in peroxisomes as well as over regions of cytoplasm lacking obvious organelles, suggesting that some apoE may be present in the cytoplasm outside the secretory and endocytic pathways (28). Because of the morphological limitations of frozen thin sections, the smaller size of adrenal peroxisomes as compared to liver, and the absence of the characteristic urate oxidase crystal in adrenal peroxisomes, it was not possible to identify adrenal peroxisomes in frozen thin sections.

ApoE was prominent on the surface of z. fasciculata cells on the sinusoidal face as well as in intercellular spaces away from the subendothelial space. At the electron microscopic level, apoE on the sinusoidal face was shown to be on the parenchymal cell and not the endothelial cell. This is similar to the result seen in rat liver where the hepatocyte cell surface along the space of Disse was strongly positive for apoE but the endothelial cell was not (28). Also as with the hepatocyte, apoE was prominent on microvilli and on non-microvillar regions of membrane on the subendothelial face of the z. fasciculata cell. In contrast to the hepatocyte, however, apoE was found on microvilli and on non-microvillar regions of z. fasciculata membrane in intercellular spaces well removed from but apparently continuous with the subendothelial space. These findings suggest that the z. fasciculata cell in vivo is encircled or covered with a "blanket" of apoE on all faces of the cell. These findings are consistent with studies of HepG2 cells showing cell surface apoE (22) and the finding that newly synthesized apoE is associated with the cell surface by interaction with heparin sulfate proteoglycan (36). Cell surface apoE on z. fasciculata cells could arise from systemic apoE or locally synthesized apoE that is either lipoprotein-free or lipoprotein-associated. Although the present data do not distinguish among these possibilities, the high concentration of apoE mRNA and the high rate of apoE synthesis in adrenal cells suggest that much of the cell surface apoE arises from the z. fasciculata cell.

What is the function of cell surface apoE in adrenocortial cells? While we can only speculate on this at present, two possibilities are suggested by studies of apoE in other situations. First, it is known that apoE mediates the endocytic uptake of remnant particles by the hepatocyte (1). This is due to the activity of apoE as a ligand for cellular receptors but may also involve the association of apoE with cell surface proteoglycan, a process that may facilitate the binding of lipoproteins to the cell surface and the subsequent delivery to receptors (21). Recent studies with cultured cells expressing transfected apoE genes have shown increased binding and uptake of VLDL and β-VLDL (20, 21). The apoE-mediated increase in β-VLDL uptake by rat hepatoma cells was blocked by the treatment of cells with heparinase, suggesting that the effect of apoE may involve cell surface proteoglycans (21). Cell surface apoE on adrenocortical cells could provide a similar enhancement of lipoprotein binding and uptake and is consistent with the presence of apoE within the endocytic pathway of z. fasciculata cells. However, this would not be expected to have a major influence on cholesterol uptake as rat adrenocortical cells obtain most of their lipoprotein cholesterol by nonendocytic mechanisms.

The second way that apoE could influence the uptake of lipoprotein cholesterol in adrenal cells is via the selective uptake mechanism in which cholesterol and cholesteryl ester are transferred from HDL particles into the cell without the uptake and degradation of the particle. Gwynne and Hess (25) demonstrated that the HDL-mediated increase in corticosteroid production in rat adrenocortical cells greatly exceeded what could be accounted for by HDL particle uptake. Subsequent studies in cell culture and in vivo showed directly that HDL cholesteryl ester uptake into adrenal cells occurred without equivalent particle uptake and degradation (24, 33, 37–39). Furthermore, Gwynne and Mahaffee (40, 41) showed that HDL free cholesterol and cholesteryl ester could serve as substrate for steroidogenesis with HDL free cholesterol being the preferred substrate, at least for acute stimulation of steroid production. Selective uptake of HDL cholesterol by adrenal cells is believed to occur in specialized membrane structures that are formed by the juxtaposition of adjacent microvilli to form microvillar channels that fill with HDL particles (29). In a manner not understood, cholesterol and cholesteryl ester are transferred into the cell to be used for steroid production and cholesteryl ester storage. How might apoE influence this selective uptake process in adrenal cells? Two possibilities may be suggested. First, apoE could facilitate the

localization of HDL particles to the microvillar membrane surface either by associating with HDL particles to enhance binding to the cell surface or by first being bound to cell surface proteoglycan to provide additional low affinity binding sites for HDL particles. Support for a role of cell surface apoE in selective uptake is provided by Leblond and Marcel (22) who showed that hepatoma cells have non-lipoprotein apoE bound to the cell surface. In addition, treatment of cells with a monoclonal antibody to apoE prior to the addition of HDL reduced the selective uptake of HDL cholesteryl ester. A second possibility is suggested by the recent studies of Thuren et al. (42–44) who have shown that apoE can activate the hepatic lipase-mediated hydrolysis of phospholipids including HDL phospholipids. This is of interest because previous studies showed that hepatic lipase treatment of HDL increases the selective uptake of both free and esterified cholesterol into hepatoma cells (45, 46). Furthermore, hepatic lipase is present in the adrenal gland, a finding that led to the proposal that hepatic lipase plays a role in the delivery of HDL cholesterol to adrenal cells (47–49). Thus, a potential role for adrenal apoE is to facilitate the selective uptake of HDL cholesterol through local activation of hepatic lipase. Potential actions of apoE to localize HDL particles to the microvillar membrane and to activate hepatic lipase are not mutually exclusive.

The finding in the present study that apoE is present on all faces of the z. fasciculata cell and not just on the membrane in the subendothelial space is in contrast to the observation in the liver where cell surface apoE appears to be restricted to the hepatocyte surface in the space of Disse (28). This result may indicate that HDL cholesterol uptake into the adrenocortical cell occurs on all surfaces of the cell and not just in the subendothelial space. This possibility is supported by observations that apoA-I also encircles the z. fasciculata cell in association with the cell membrane and microvilli in intercellular spaces well removed from the subendothelial space. Furthermore, stacks of microvilli and microvillar channels were seen in intercellular spaces in standard transmission electron micrographs. These findings suggest that HDL particles percolate over the entire z. fasciculata cell surface. Such an arrangement could serve to maximize the membrane area available for HDL cholesterol and cholesteryl ester uptake. This may facilitate the rapid acquisition of lipoprotein cholesterol for acute steroidogenic responses.

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