Hepatic lipase is abundant on both hepatocyte and endothelial cell surfaces in the liver

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Abstract The cellular location of hepatic lipase was investigated in transgenic rabbits that expressed human hepatic lipase in the liver. The binding of monoclonal antibodies to human hepatic lipase, as detected by either fluorescence-tagged or gold-conjugated secondary antibodies, showed that hepatic lipase was concentrated at the surfaces of hepatic sinusoids. This distribution was the same as observed in the human liver. At the ultrastructural level, immunogold labeling of the space of Disse showed hepatic lipase on both lumenal and sublumenal surfaces of rabbit liver sinusoidal endothelial cells. An equivalent amount of hepatic lipase also was found on the external surfaces of hepatocyte microvilli in the space of Disse, as well as in the interhepatocyte spaces. The distribution suggests that a majority of the hepatic lipase produced by the liver is associated with hepatocyte surfaces, consistent with the functions of this enzyme in lipoprotein metabolism.—**Sanan, D. A., J. Fan, A. Bensadoun, and J. M. Taylor.** Hepatic lipase is abundant on both hepatocyte and endothelial cell surfaces in the liver. *J. Lipid Res.* 1997. **38:** 1002–1013.

Supplementary key words transgenic rabbit • space of Disse • sinusoid • microscopy

Hepatic lipase (HL), an M_r = 64,000 glycoprotein, is a key enzyme in plasma lipid metabolism that catalyzes the hydrolysis of lipoprotein triglycerides and phospholipids (1, 2). The absence of detectable HL activity in hepatectomized pigs (3), the production of hepatic lipase by perfused dog liver (4), the secretion of HL by cultured rat primary parenchymal cells (5), the demonstration of HL synthesis by rat liver microsomes (6), and the cloning of HL cDNA from rat liver (MK1526) demonstrated that the liver was the major (and possibly the only) source of this enzyme for the metabolism of plasma lipoproteins.

The ability of heparin to release HL from the liver into circulating blood (7) and the inactivation of HL in situ by heparinase (8) or collagenase (9) suggested that the enzyme is located on the external surfaces of liver cells. This possibility is supported by the finding that the cholesterol and phospholipid content of both low density lipoproteins (LDL, d 1.019–1.063 g/ml) and high density lipoproteins (HDL, d > 1.063 g/ml) increased after the blockade of HL by intravenous administration of HL-specific antibody (7, 10). This localization was confirmed by immunofluorescence studies in the rat liver: immunoelectron microscopy using a ferritin-labeled antibody showed HL on the lumenal sinusoidal surfaces of endothelial cells and within endothelial endocytotic vesicles (11). Hepatic lipase was absent from cell surfaces after heparin administration (11) and it was not detected on parenchymal or Küppfer cells under any conditions (11). The latter finding was supported by in vitro studies demonstrating a 100-fold greater binding of HL to nonparenchymal cells than to hepatocytes in primary cultured rat cells isolated after collagenase perfusion of the liver (12). These studies suggested that HL was synthesized in hepatocytes, and it either diffused or was transported to the surfaces of sinusoidal endothelial cells (7, 9–12). This postulated mechanism was consistent with the finding that lipoprotein lipase, a related enzyme, migrates from its site of synthesis (i.e., adipocytes or myocytes) to endothelial cell surfaces, the site of its catalytic activity on chylomicrons and very low density lipoprotein (VLDL) (reviewed in ref. 13). However, this proposed location for HL does not fit well with some of the functions of this enzyme that have emerged more recently.

Hepatic lipase may have several functions in lipoprotein metabolism. It can bind HDL (14), and it preferentially catalyzes the hydrolysis of HDL_2 phospholipids (15, 16). However, HL also can act on the phospholipids in HDL_3 (7). The action of HL on HDL surfaces

Abbreviations: BSA, bovine serum albumin; DPBS, Dulbecco's phosphate-buffered saline; HDL, high density lipoproteins; HL, hepatic lipase; LDL, low density lipoproteins: LPL, lipoprotein lipase; PBS, phosphate-buffered saline; VLDL, very low density lipoproteins.

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raises the cholesterol/phospholipid ratio: as free cholesterol is in equilibrium with the cholesterol component of lipoprotein surfaces and cell membranes, a net movement of cholesterol from HDL to the liver would be expected to occur (17, 18). Thus, HL may mediate reverse cholesterol transport by HDL, a function that would be facilitated by a close association with hepatocytes. Hepatic lipase also has been implicated in the conversion of intermediate density lipoproteins (IDL) to LDL (10, 19, 20).

Hepatic lipase facilitates the uptake of chylomicron remnants by the liver (21) and by cultured hepatoma cells (22). Studies by Ji et al. (22) and others (23) suggest that HL may serve as a ligand for remnant binding to cell surface proteoglycans and that it functions in remnant uptake via the heparan sulfate proteoglycan/LDL receptor-related protein pathway (22). Partial depletion of chylomicron remnant phospholipids by HL increases the rate of remnant uptake by the liver (24). Congenital lack of HL in humans results in an accumulation of remnant lipoproteins (25), and the inhibition of HL activity in rats impairs chylomicron removal (26). However, the normal rate of clearance of chylomicrons and VLDL from plasma requires apolipoprotein (apo) E (reviewed in ref. 27) in a receptor-mediated mechanism that is facilitated by the action of HL (28–30). Thus, high-affinity binding of chylomicrons to liver cell surfaces requires the coordinated action of HL and apoE (22, 31, 32). At the cell surface, HL appears to be bound to heparan sulfate proteoglycans (33), a heterogenous class of molecules that also binds apoE and other proteins (28, 33).

The metabolic functions and properties of HL suggest that it might be distributed widely in the space of Disse and that it may be associated with the extracellular matrix on hepatocyte surfaces. Recent refinements in immunocytochemical methods, as well as the availability of transgenic rabbits that express human HL, allowed us to investigate its location in transgenic rabbit liver. The normal rabbit liver has a relatively low content of endogenous HL activity that is associated with low levels of HL mRNA compared to other species (34). We have previously reported the production of transgenic rabbits that express human HL only in the liver (35). In these animals, transgenic human HL is not found free in plasma, but intravenous heparin administration results in its release into circulation. Expression of human HL in the transgenic rabbit, in amounts comparable to that found in humans, dramatically reduces the levels of all classes of HDL in plasma, as well as the levels of IDL and large LDL in circulation (35). Use of the transgenic rabbit permitted perfusion fixation of the liver in situ, minimizing the degradation of HL or the redistribution of HL that might occur in necropsy tissues. Our results show that hepatocyte surfaces are covered with HL, making them the major location of this enzyme in the liver.

MATERIALS AND METHODS

Transgenic rabbits

A construct containing human HL cDNA (36) in a vector that directed expression only to the liver was used to generate transgenic rabbits in the New Zealand White strain as described (35). For the current studies, F1 hemizygous female rabbits from the transgenic 4934 line (35) were used. In the absence of heparin, neither transgenic nor endogenous HL was detected in the plasma. Post-heparin plasma from this transgenic line contained ~500 ng/ml of human HL with an enzymatic activity of 11 μeq fatty acids released per ml per h from a triolein emulsion substrate (35). This level of activity was 20-fold greater than the corresponding activity in nontransgenic control rabbits. Transgenic human HL had an M_r of ~66,000, and its mRNA was detected only in the liver among 10 different tissues examined. The expression of HL in transgenic rabbits reduced HDL cholesterol to 25% of normal compared with nontransgenic controls; large HDL (HDL_1 and HDL_2) decreased markedly, and HDL_3 (small dense HDL) and IDL decreased moderately. These observations indicated that transgenic human HL was expressed in the appropriate tissue, its properties were consistent with normal human HL, and it was fully functional in lipoprotein metabolism.

Fixation and collection of tissues

Rabbits were maintained under halothane inhalation anesthesia during surgery. The portal vein was exposed and cannulated through a midline laparotomy, and the liver was perfused with phosphate-buffered saline, pH 7.4 (PBS); blood and perfusate were drained via venotomy of the inferior vena cava. The hepatic artery was clamped to prevent arterial blood from entering the liver. The liver appeared blanched within 4 min of perfusion with buffer, indicating that little blood remained in its vascular beds. The liver was perfused with fixative, ~250 ml of either 3% paraformaldehyde in PBS or periodate-lysine-paraformaldehyde (37), at room temperature via the portal cannula for 5 min. Fixation hardened the liver, permitting the cutting of 1–2-mm slices from the left lateral lobe close to the portal vein, where the fixation was reproducibly excellent. These slices were postfixed as described below and held at 4°C.

Immunocytochemistry was performed within 48 h to minimize loss of HL antigenicity due to prolonged exposure to fixative. In addition, human liver sections

were obtained from a male at 18 h post mortem, placed in 3% paraformaldehyde/PBS, and treated subsequently by the same procedures that were used to analyze rabbit liver.

Cryostat sectioning

Fixed tissues were immersed in 18% sucrose in PBS for 1 h at 4°C, drained, embedded in Tissue-Tek OCT (Miles, Elkhart, IN), and frozen in liquid nitrogen. Frozen sections 10–15 μm thick were cut with a Reichert-Jung 2800 N Frigocut cryostat microtome (Reichert Scientific Instruments, Buffalo, NY). Sections were immunostained immediately.

Vibratome sectioning

Fixed liver tissue was attached to the specimen holder with Loctite 404 Industrial Adhesive (Loctite, Newington, CT) and cut into 50-μm-thick slices on a Series 1000 vibratome (Ted Pella, Redding, CA) under ice-cold PBS. Vibratome slices were labeled immediately with immunogold.

Immunolabeling for light microscopy

All reagents were adjusted to pH 7.4, and immunolabeling was done at room temperature, unless otherwise indicated. Frozen sections of liver on glass slides were thawed for 10 min and then gently agitated in distilled water for 2 min to remove OCT. The slides were air-dried for 30 min and then immersed in three changes of 0.1% bovine serum albumin (BSA, Sigma, St. Louis, MO) with 0.02 M glycine in DPBS (Dulbecco's PBS, Gibco/BRL, Gaithersburg, MD) for 5 min each to quench reactive aldehyde groups. Sections then were incubated with 3% BSA in DPBS containing 0.02 M glycine for 30–60 min, followed by incubation with primary antibody for 1–2 h. Alternatively, primary antibody incubations were done at 4°C overnight. A mixture of two mouse anti-human HL monoclonal antibodies (#1–6 and #3–6 (38)), each at 2.5 μg/ml in DPBS containing 1.0% BSA, was used. The specificity of the monoclonal antibodies for human hepatic lipase was confirmed by Western blotting (data not shown). For controls, primary antibody was omitted. After four washes for 5 min each in DPBS containing 0.1% BSA and 0.02 M glycine, sections for immunofluorescence were incubated with goat anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC) (Zymed Laboratories, South San Francisco, CA), at 10 μg/ml in DPBS containing 1.0% BSA. Sections then were washed four times for 5 min each in DPBS containing 0.02 M glycine and 0.1% BSA, once with DPBS, rinsed briefly in water to remove salts, and mounted under coverslips in Vectashield (Vector Laboratories, Burlingame, CA).

Sections for bright-field microscopy were strained with the Histogold kit (Zymed Laboratories) according to the manufacturer's instructions. Coverslips were mounted with GVA Mount (Zymed Laboratories). Epifluorescence microscopy was performed with a Bio-Rad MRC 600 laser scanning confocal system, a Nikon Optiphot microscope fitted with a BHS (blue light high sensitivity) dichroic filter (Bio-Rad Laboratories, Hercules, CA), a Nikon Optophot microscope and a Nikon planapo 601.4 NA objective. Bright-field microscopy was done with a Nikon Optiphot 2.

Immunogold labeling for electron microscopy

The livers of HL transgenic and nontransgenic rabbits were perfused via the portal vein, as described above, with ice-cold Tyrode's solution (39) until the effluent was clear. Livers were excised rapidly, and 2-mm slices were cut from the left lateral lobe close to the portal vein. Liver slices were fixed for 2 h in either 3% paraformaldehyde in DPBS or periodate-lysine-paraformaldehyde (37) and sectioned with a vibratome. Vibratome sections were immersed in immunostaining reagents in 24-well tissue culture plates and agitated gently throughout the labeling procedure. All reagents were made up in DPBS containing 0.02 M glycine (DPBS-glycine). Sections were incubated for 5 min in DPBS-glycine buffer to quench residual aldehyde groups, followed by incubation for 20 min in DPBS-glycine containing 3% BSA to block nonspecific binding sites. Then, sections were incubated for 2 h at room temperature in the monoclonal antibody mixture (#1–6/3–6 anti HL), with each antibody at 7.5 μg/ml in 1% BSA in DPBS-glycine, washed as described above for light microscopy, and incubated in secondary antibody, rabbit anti-mouse IgG (Zymed Laboratories) at 4 μg/ml in 1% BSA in DPBS-glycine buffer for 1 h. Then, sections were washed and incubated with protein A conjugated to 10-nm gold particles (provided by Jan Slot, University of Utrecht, The Netherlands) diluted 1:80 in 1% BSA in DPBS-glycine for 1 h. After washing, sections were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer, dehydrated, and embedded in Epon 812 epoxy resin. Ultrathin sections were stained with lead citrate and uranyl acetate and examined in a JEM 100 CX transmission electron microscope (JEOL, Tokyo, Japan).

Heparin infusion

Rabbits were anesthetized, given an intravenous injection of heparin (Sigma), 500 USP units/kg body weight, and then the liver was exposed. After 10 min of heparin circulation, the liver was flushed as above with Tyrode's salt solution and fixed with 3% paraformaldehyde in DPBS. Control livers were perfused with saline in a sham procedure in transgenic rabbits that had not been treated with heparin. Cryostat sections were pre-

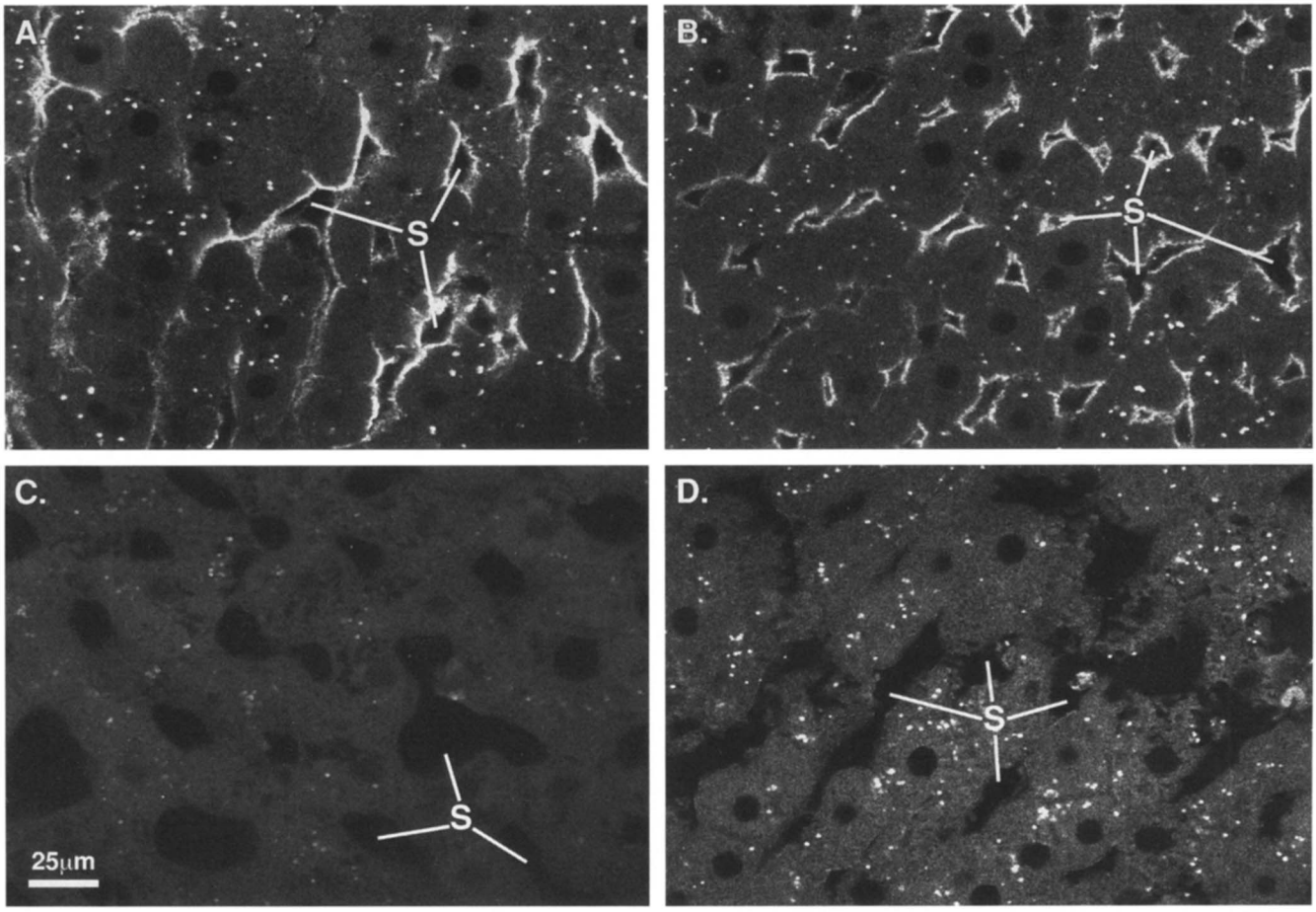

Fig. 1. Localization of HL in the liver by immunofluorescence confocal microscopy. Hepatic lipase was immunolabeled with mouse monoclonal antibodies to human HL and the bound antibody was detected with an FITC-conjugated antibody as described in Materials and Methods. Nuclear shadows and a faint background fluorescence permit visualization of hepatocyte trabeculae. The magnification is the same for all panels as indicated by the 25-μm scale bar. A: Longitudinal section of transgenic rabbit liver, showing the location of HL surrounding sinusoidal spaces (S). B: Transverse section of the same transgenic rabbit liver showing the location of HL surrounding sinusoidal spaces (S). C: Control nontransgenic rabbit liver. No immunolabeling by anti-HL is seen in the sinusoids (S) in this longitudinal section. D: Longitudinal section from a transgenic rabbit liver without any immunostaining. Dark spaces are liver sinusoids (S) between faintly autofluorescent trabeculae. Bright foci of unknown autofluorescent material are observed within hepatocytes: they do not indicate intracellular HL.

pared for immunofluorescence studies as described above.

RESULTS

Analysis of HL location at the light microscopic level

Immunostaining of frozen sections of perfusion-fixed HL transgenic rabbit livers with monoclonal antibodies against human HL revealed intense HL-specific fluorescence on all sinusoidal surfaces (**Fig. 1, A and B**). This pattern was found uniformly throughout all liver sections that were examined. Nontransgenic control rabbit liver sections did not show sinusoidal fluorescence (Fig. 1C). Bright intracellular foci caused by autofluorescence were observed within hepatocytes in all sections, even in the absence of primary and secondary antibodies (Fig. 1D). The autofluorescent foci did not represent intracellular HL, and the identity of this material remains unknown. These intracellular foci were not detected by an immunocytochemical method that did not rely on fluorescence as described.

The distribution of HL in the transgenic rabbit liver was confirmed by visualizing the primary HL antibody with a gold-conjugated secondary antibody and detection was enhanced by silver metallographic intensification. With this technique, HL was revealed as intense black borders along the sinusoidal spaces in transgenic rabbit livers (**Fig. 2, A and C**). This labeling was not seen in nontransgenic control livers (Fig. 2, B and D). Staining for HL dramatically revealed the radial pattern of sinusoids in the transversely cut liver lobule; the density of HL staining was notably increased in sinusoids

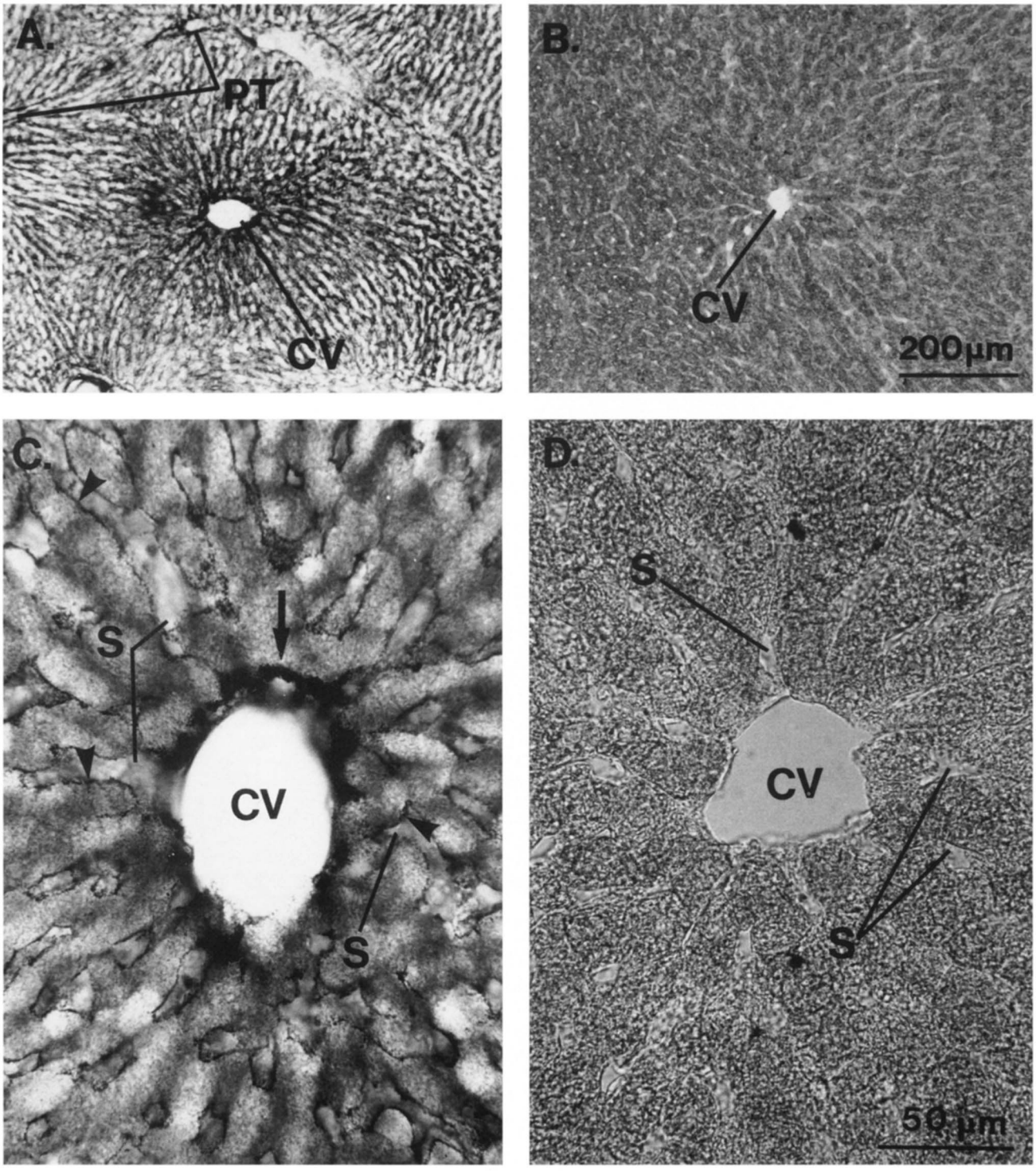

Fig. 2. Localization of HL in the liver by gold-conjugated immunolabeling and light microscopy. Sections from rabbit livers were fixed with 3% paraformaldehyde and reacted with mouse monoclonal anti-HL antibodies, which were detected with silver-intensified gold-conjugated secondary antibodies. A: Transverse section of a liver lobule from an HL transgenic rabbit. Intense black borders can be seen delineating sinusoidal spaces. An entire lobule can be seen with distended portal triads (PT) at the vertices and a central vein (CV) in the center. B: Transverse section of a nontransgenic control liver lobule showing no immunoreaction. The magnification for panels A and B is indicated by the 200-μm scale bar. C: Detailed image of the central vein (CV) region of the liver lobule from the same transgenic rabbit shown in panel A. Hepatic lipase immunolabeling is revealed as an intense black border of the sinusoidal spaces (S), indicated by arrowheads and in connective tissue around the central vein (arrow). D: The central vein (CV) region of the liver lobule from the same nontransgenic control rabbit shown in panel B. The magnification for panels C and D is indicated by the 50-μm scale bar.

located close to central veins. A higher-magnification image of the central vein region (Fig. 2C) resolved the intense black borders lining the sinusoidal spaces more clearly and showed anti-HL immunoreactivity concentrated in the region of the reticular connective tissue. The anti-human HL did not cross react with rabbit HL in nontransgenic control rabbit livers (Fig. 2, B and D).

Heparin was administered intravenously to rabbits to determine whether the anti-HL immunoreactive protein could be released from cell surfaces. Compared with the saline-perfused transgenic rabbit liver (**Fig. 3A**), heparin-perfused liver showed dramatically diminished immunofluorescence due to anti-HL immunoreactivity (Fig. 3B), even in the central vein regions, which had the greatest concentration of surface-bound HL (Fig. 2A and C). The fluorescent signal in the heparin-perfused liver was reduced nearly to the level of that in control nontransgenic rabbit livers (Fig. 1C). These data confirm that human HL in the transgenic rabbit liver is localized at cell surfaces in the sinusoids: heparin releasability indicates that HL is bound normally to the heparin sulfate proteoglycan matrix associated with the external surfaces of cells.

To determine whether the distribution of human HL in the transgenic rabbit liver reflected its location in the human liver, tissue sections from human necropsy samples were examined. The pattern of sinusoidal surface anti-HL immunofluorescence in the human liver sections was the same as that seen in the transgenic rabbits (**Fig. 4**). These results, together with the previously reported absence of HL activity in pre-heparin transgenic rabbit plasma (35), indicated that in transgenic rabbit liver, human HL was concentrated on sinusoidal surfaces and that its expression was similar to the distribution of HL in the human liver.

Ultrastructural detection of HL sites

To investigate the localization of HL in the space of Disse using electron microscopy, we used a two-stage tissue fixation protocol (described in Materials and Methods) to minimize the potential loss of HL immunoreactivity that might occur as a result of reactions with harsh denaturants, such as glutaraldehyde, solvents, or embedding compounds. Vibratome sections were collected for reaction with HL-specific monoclonal antibodies. After immunolabeling, tissue sections were immersed in glutaraldehyde and osmium tetroxide to fix proteins and lipid membranes for ultrastructural study and embedded for ultrathin sectioning. This protocol resulted in a minimal loss of ultrastructural integrity of hepatocytes and endothelial cells in the liver tissue, while permitting the maximum detection of HL by retaining antibody reactivity during the immunostaining steps. The fine structural detail of the space of Disse was preserved adequately for a clear detection of the distribution of HL within it and on cell surfaces exposed to it. Thus, the tissue handling procedures balanced a small loss of ultrastructural integrity against a large gain in immunoreactivity.

Immunogold labeling of transgenic rabbit liver fixed with periodate-lysine-paraformaldehyde showed that human HL was present on endothelial cell surfaces and hepatocyte microvilli in the space of Disse (**Fig. 5, A and B**). A relatively even distribution of HL was found throughout the space of Disse in all liver sections examined. This fixative enhances the preservation of the extracellular matrix material in the space of Disse: substantial portions of the endothelial basement membrane were observed. The distribution of HL in the space of Disse is consistent with its association with the extracellular matrix proteoglycans that cover hepatocyte and endothelial cell surfaces. Hepatic lipase was detected in the interhepatocyte clefts at the base of the space of Disse not in close association with endothelial cells (Fig. 5A). Almost no immunoreactivity was detected in the nontransgenic control tissues (Fig. 5, C and D), ruling out passive entrapment of gold particles. Immunoreactivity in bile canaliculi was not observed, possibly because our pre-embedding immunolabeling protocol was designed for the maximum preservation of antigens in the space of Disse and would not access the canaliculi. We also did not detect HL in bile caniculi with immunofluorescent techniques, an approach that could have detected it.

The same localization of HL was observed in the paraformaldehyde-fixed liver (**Fig. 6**). Cell structures are relatively well preserved by plain paraformaldehyde, and HL was clearly associated with both endothelial and hepatocyte cell surfaces. Hepatic lipase immunoreactivity was distributed throughout the space of Disse. However, the extracellular matrix is not well preserved by plain paraformaldehyde, and less HL was detected on tissues treated with this fixative than on those fixed with periodate-lysine-paraformaldehyde.

The association of HL with endothelial cell membranes can be seen more clearly in **Fig. 7A.** Paraformaldehyde fixation is more effective in preserving cellular ultrastructure than the extracellular matrix: relatively little of the endothelial basement membrane was detected in liver treated with this fixative. Incomplete preservation of the extracellular matrix with this fixative may account for the apparent reduction in HL detected on hepatocyte surfaces. Thus, paraformaldehyde favored the detection of HL on endothelial cells, especially on their lumenal surfaces, and to a lesser extent on hepatocyte surfaces (Fig. 7A). In nontransgenic control rabbits, the HL antibody had little immunoreactivity (Fig. 7B).

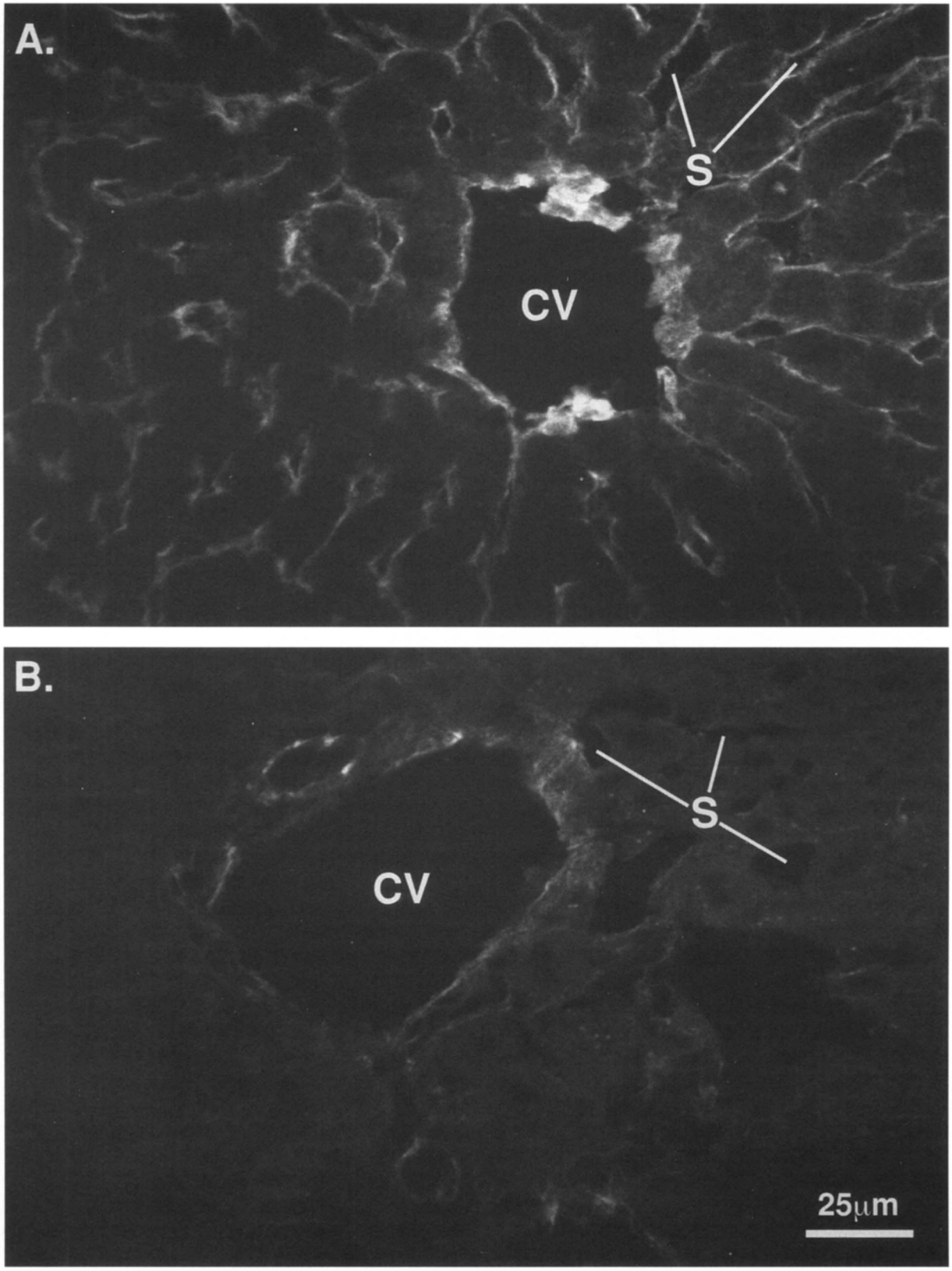

Fig. 3. Release of HL from the liver cell surfaces of transgenic rabbits by heparin. A: Immunofluorescence detects HL on the surfaces of sinusoids in a central vein (CV) region. The hepatic portal vein was injected with saline as a control sham procedure before fixation with 3% paraformaldehyde, tissue collection, and sectioning. Greater intensity of fluorescence is detected on central vein surfaces than on the surfaces of sinusoids (S). B: Heparin was administered via portal vein injection and allowed to circulate for 10 min. Liver sections were collected and stained by immunofluorescence as above. Longitudinal sections are shown in both panels; they have the same magnification.

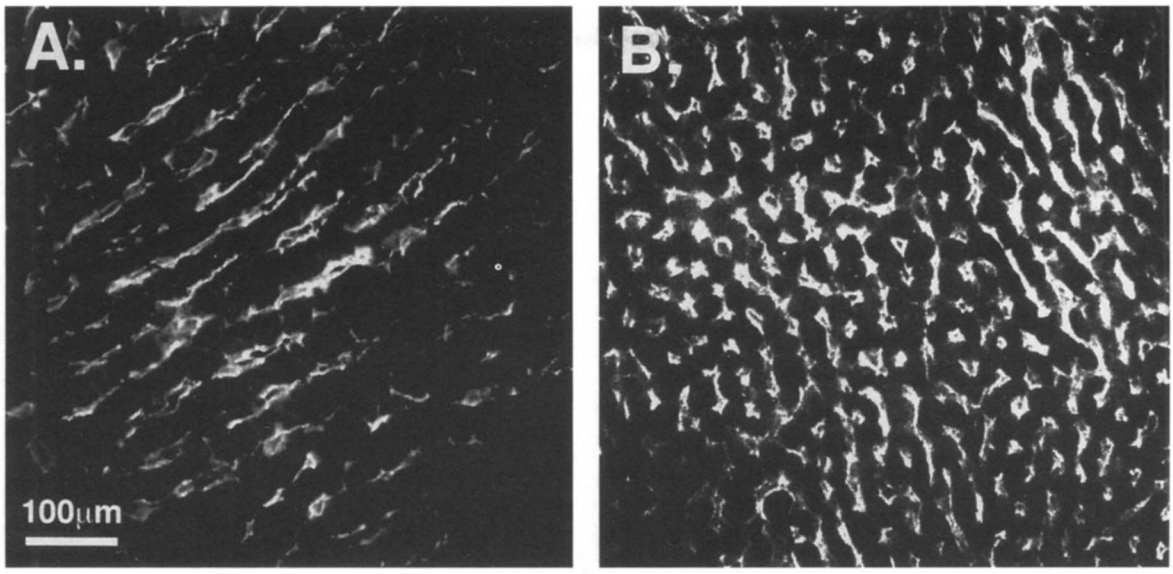

Fig. 4. Localization of HL in human liver by immunofluorescence. A: Longitudinal representative section of a human liver lobule showing hepatocyte trabeculae separated by sinusoidal spaces. Fluorescence immunolabeling shows HL lining the sinusoids. B: Transverse representative section of a human liver lobule immunostained as in panel A. The magnifications for panels A and B are indicated by the 100-μm scale bar.

DISCUSSION

We have demonstrated that HL is more extensively distributed on liver cell surfaces than was previously reported (11). Our studies confirm the presence of HL on lumenal sinusoidal endothelial cell surfaces. In addition, we find HL on the sublumenal surfaces of endothelial cells. However, a substantial amount of HL is bound to the surfaces of hepatocyte microvilli in the space of Disse, with additional HL found in interhepatocyte spaces. Our results suggest that the external surfaces of the hepatocyte are the major site of HL localization in the liver, and consistent with an association with the extracellular matrix.

Our ability to detect HL on hepatocytes is likely to be the result of improved tissue fixation and immunolabeling procedures than were available for an earlier study (11). In this earlier study (11), rats were injected with a rabbit antibody to rat HL and the livers were perfused with a ferritin-conjugated sheep anti-rabbit immunoglobulin before fixation with glutaraldehyde and detection by electron microscopy. Ferritin particles were observed in patches on endothelial cell sinusoidal surfaces, on the surfaces of nascent endocytotic vesicles, and in endocytotic vesicles within endothelial cells. Ferritin particles were not found on the subendothelial surfaces of endothelial cells in the space of Disse and were not associated with the surfaces of any other cell type. The inability to detect ferritin conjugates in the space of Disse may reflect incomplete fixation of the liver, leading to a breakdown in cell surface components, or an initial antibody concentration that was insufficient to overcome lumenal binding sites on the endothelium. The ferritin conjugates of the second antibody may have had a reduced ability to penetrate into the space of Disse and interhepatocyte spaces.

The earlier proposed localization of HL to the lumenal surfaces of sinusoidal endothelium (11) was supported by an apparent binding preference of HL for endothelial cells compared to hepatocytes (12). However, the primary liver cells were prepared by a procedure that involved collagenase perfusion of the liver, which is likely to have damaged cell surface proteoglycans that normally would bind HL, thereby complicating the interpretation of the results. These uncertainties regarding the earlier methods used to investigate the localization of HL, together with the more recent understanding of its functions in remnant lipoprotein and cholesterol clearance (summarized in the Introduction), led us to re-examine the localization of HL in the liver.

In the current investigation, a brief initial exposure of the tissue to gentle fixatives was used to preserve the cellular location of HL, thereby minimizing its extraction during subsequent treatments and preventing loss of its immunoreactivity. Two alternative fixatives were used to seek the most effective preservation of extracellular components. As previous biochemical studies suggested that HL might be bound to the extracellular matrix, periodate-lysine-paraformaldehyde fixation was used. The periodic acid cross-links carbohydrate moieties in glycoproteins and proteoglycans that are abun-

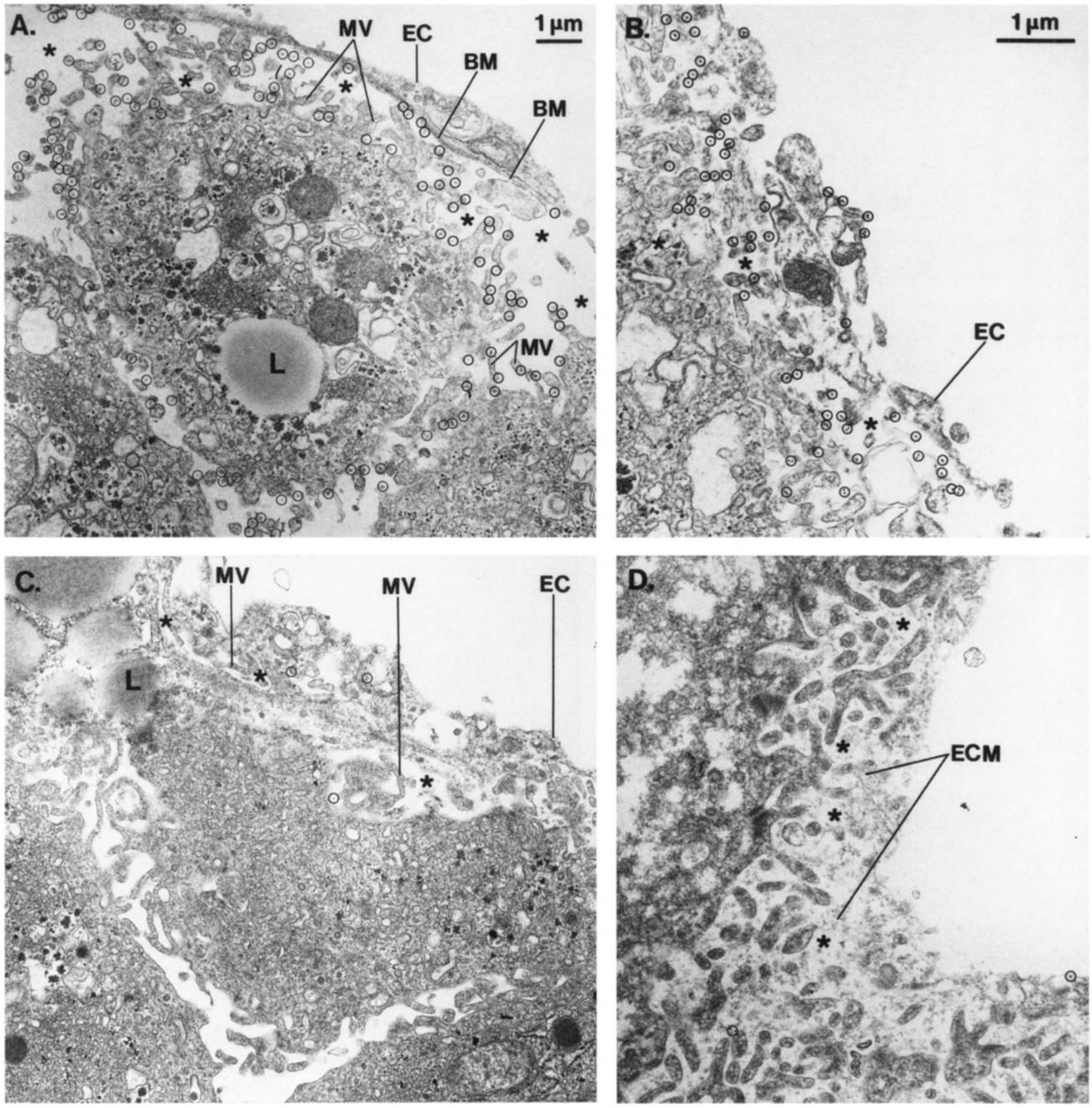

Fig. 5. Electron micrographs show HL on the microvilli (MV) of hepatocytes, on endothelial cell (EC) surfaces, and on the extracellular matrix (ECM) within the space of Disse. Livers were prefixed with periodate-lysine-paraformaldehyde, collected, sectioned, and stained with immunogold for electron microscopy as described in Materials and Methods. Gold particles are outlined by small circles. Lower-power micrographs that show a tangential section of the apical face of a hepatocyte surrounded by interhepatocyte clefts in the space of Disse (panels A and C) and higher-power micrographs detailing the space of Disse (*) (panels B and D) are shown for transgenic (panels A and B) and nontransgenic (panels C and D) rabbits. Lipid droplets (L) can be seen within hepatocytes, and microvilli are observed on all hepatocyte surfaces. In addition, endothelial cells, basement membrane (BM) fragments, and extracellular matrix material are observed. The magnification for each panel is indicated by the 1-µm scale bars.

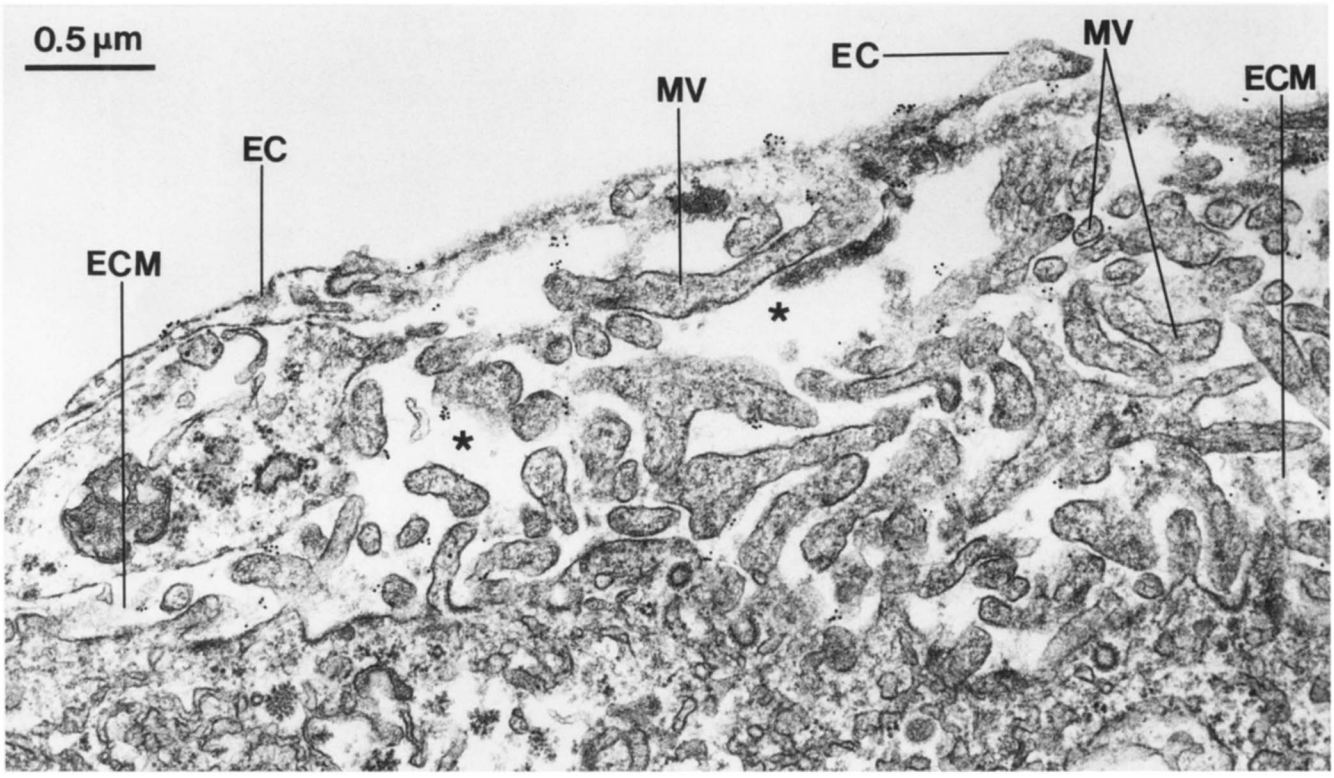

Fig. 6. Hepatic lipase is found in the space of Disse in transgenic rabbit livers. Transgenic rabbit livers were prefixed with paraformaldehyde and examined for HL location by immunogold labeling and electron microscopy as in Fig. 5. Gold-labeled particles are visible as black dots on endothelial cell (EC) surfaces and throughout the space of Disse (*) on hepatocyte microvilli (MV), and the extracellular matrix (ECM). The magnification is indicated by the 0.5-µm scale bar.

dant in the extracellular matrix (37). Furthermore, this fixative maintains glycoprotein antigenicity and preserves cell ultrastructure nearly as effectively as glutaraldehyde. In comparison, plain paraformaldehyde may be somewhat more capable of preserving antigenicity, but at the cost of some loss in the structural integrity of the extracellular matrix. Both fixatives were successful, but periodate-lysine-paraformaldehyde appeared to be more effective in revealing the distribution in vivo of HL on the surface of both hepatocytes and endothelial cells.

Applying the HL antibody to tissue sections after gentle fixation maximized its ability to penetrate into intercellular spaces before the potential degradation of extracellular proteoglycans. Tissue sections were reacted with primary antibody within 48 h of collection, and they were labeled with immunogold before embedding for electron microscopy to minimize the possible loss of immunoreactivity or the extraction of HL from exposure to the denaturing preparative reagents. Mouse monoclonal antibodies to human HL were used to minimize potential cross-reaction with nonspecific antigens. In this regard, there was no significant antibody reaction to proteins in the nontransgenic control rabbit livers, supporting the likelihood that human HL was detected specifically in the transgenic rabbits. The use of transgenic rabbits permitted the rapid fixation and collection of liver tissue, maximizing the preservation of extracellular structures that otherwise might undergo partial degradation, resulting in a loss of bound protein. The rapid fixation of transgenic rabbit livers would favor the preservation of sensitive associations between proteoglycans and surface-bound proteins, thereby enhancing the detection of HL.

Our results indicate that a major portion of HL in the liver is located on the sublumenal extracellular matrix component of endothelial cells and on the microvillar surfaces of hepatocytes in the space of Disse, and in nearby interhepatocyte spaces. In addition, we have confirmed that HL is located on the lumenal surfaces of sinusoidal endothelium. A broad distribution of HL in the liver and a direct association of this enzyme with hepatocyte surfaces are consonant with the known metabolic roles of HL.

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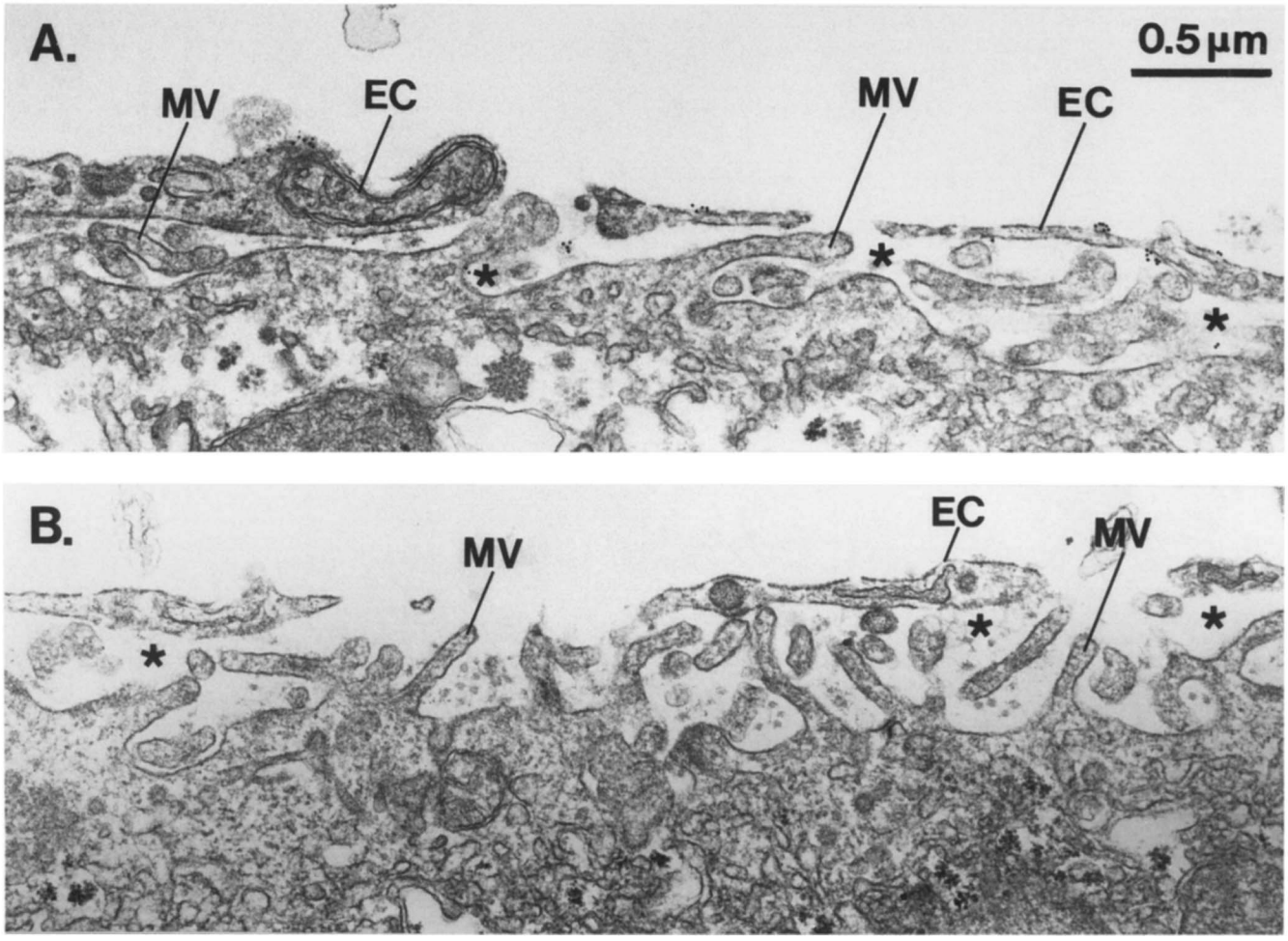

Fig. 7. Detection of HL on sinusoidal endothelial cells. Livers from transgenic (A) and nontransgenic control (B) rabbits were prefixed with paraformaldehyde and examined as in Fig. 6. Details of the endothelium (EC) are shown revealing clusters of HL-bound gold particles on lumenal and, to a lesser extent, sublumenal cell surfaces. Note also the occasional HL label on hepatocyte microvilli (MV) in the space of Disse (*). The magnification for each panel is indicated by the 0.5-µm scale bar.

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