Lipoprotein lipase in lungs, spleen, and liver: synthesis and distribution

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Abstract Lipoprotein lipase (LPL, E C 3.1.1.34) is the enzyme responsible for hydrolysis of triacylglycerols in plasma lipoproteins, making the fatty acids available for use by subjacent tissues. LPL is functional at the surface of endothelial cells, but it is not clear which cells synthesize the enzyme and what its distribution within tissues and vessels is. In previous studies we reported that in the major LPL-producing tissues (muscles, adipose tissue, and mammary gland) the enzyme is made by the major cell types. In the present work we have studied in adult guinea pigs some tissues that present LPL activity but in lower amounts (lung, spleen, and liver). On cryosections of these tissues we have searched for specific cell expression of the LPL gene (by in situ hybridization using a RNA probe) and for the corresponding protein distribution (by immunocytochemistry). Based on morphological criteria we can suggest that, contrary to the main LPL-producing tissues, in these tissues the enzyme is made by scattered cells, such as macrophages in the lung and spleen and Kupffer cells in the liver; endothelial cells present but do not synthesize the enzyme, indicating that the endothelial LPL originates in other cells. In the liver strong immunoreaction was detected in the sinusoid in contrast to the low level of mRNA expression, suggesting that liver takes up circulating LPL from blood.—**Camps, L., M. Reina, M. Llobera, G. Bengtsson-Olivecrona, T. Olivecrona, and S. Vilaró.** Lipoprotein lipase in lungs, spleen, and liver: synthesis and distribution. *J. Lipid Res.* 1991. **32:** 1877–1888.

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A major lipid transported with lipoproteins is triacylglycerides, which are carried in large chylomicra and VLDL particles (1). These lipoproteins bind transiently to endothelial sites where their triacylglycerides are hydrolyzed by lipoprotein lipase (LPL) (2). Fatty acids and monoglycerides are released and move readily within the tissue, down a concentration gradient created by their use, or reesterification within cells (3, 4). Recent studies (5, 6) have shown that LPL is synthesized by a variety of cell types: adipocytes, myocytes, mammary epithelial cells, skeletal and smooth muscle cells, and neurons. LPL activity has been demonstrated in a variety of other tissues, including lung, kidney, ovaries, and liver (7) but it is not clear which cells produce it in these tissues.

The cells synthesizing the LPL release it for transfer to endothelial sites, where it binds to glycan chains of heparan sulfate proteoglycans (8). This locates the enzyme in the outer part of the glycocalyx where it can interact with the large lipoprotein particles. Heparan sulfate proteoglycans are present at the surface of virtually all resident cells (9, 10). This raises the question of whether there is a specific path for LPL to certain heparan sulfate proteoglycans at the endothelium or whether the enzyme spreads over all cell surfaces.

In a previous study (5) we used a combination of in situ hybridization and immunohistochemistry to explore where LPL is synthesized and where the enzyme protein is located. In white and brown adipose tissue, heart and skeletal muscle, and in lactating mammary gland there was positive hybridization for LPL mRNA over all members of the major cell types, and there was strong immunoreaction for LPL protein at the vascular endothelium. No LPL mRNA was detected in endothelial cells in any of the tissues studied. Nonetheless, there was immunoreaction for LPL protein at the endothelial surface of all blood vessels, even in tissues with little or no LPL mRNA. For instance, in the glomeruli of the kidney there was strong immunofluorescence for LPL protein, despite no LPL mRNA in the surrounding cells. This LPL must have come from blood and the relatively high concentration of LPL here cannot be ascribed to local synthesis, but indicates the presence of high-affinity binding sites. A further variation in the disposition of LPL is its synthesis (6) and presence (11) in some discrete areas of the brain.

Abbreviations: LPL, lipoprotein lipase; VLDL, very low density lipoprotein.

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Several studies have shown that the liver extracts LPL from the circulating blood, suggesting that the liver may be a major site of degradation for LPL. Perfusion studies have shown that LPL bound in the liver can initially be released again by heparin (12). A likely candidate for these binding sites are heparan sulfate proteoglycans. It is known that the liver has a high-sulfated type of heparan sulfate (13, 14) which should bind the enzyme tightly. According to this view, initial binding of LPL to the heparin-sensitive sites in the liver would be similar to the binding of the enzyme in other tissues. In support of this it has been shown that exogenous LPL bound by the liver is functional in the sense that it can engage and hydrolyze lipoproteins (12). However, LPL bound in the liver soon loses its catalytic activity, can no longer be released by heparin, and is degraded. There are two possible sequences of events for this. One is that LPL is internalized bound to heparan sulfate proteoglycans. Inactivation would then be promoted by lowered pH in endosomes (15). The other possible process is that the enzyme first transfers to other types of binding sites which mediate the internalization. The existence of these is indicated by perfusion studies that show that the liver avidly takes up inactive monomeric LPL at heparin-insensitive sites. It has been reported that there are substantial amounts of inactive LPL protein in blood, and it is possible that most of the transport of LPL form peripheral tissues to the liver occurs after dissociation of the enzyme into catalytically inactive monomers, with decreased affinity for heparan sulfate (16). This process has been referred to as a built-in mechanism for self-destruction in the active, dimeric LPL molecule (17).

In this report we extend our studies with combined in situ hybridization and immunolocalization to three tissues: lungs, spleen, and liver. In all three, LPL is synthesized in a minority type of cell which is scattered in the tissue. Would this be reflected by intense immunoreaction at endothelial surfaces around the LPL-producing cells? or would the enzyme spread along the endothelium? In the liver, would LPL be confined to cells that synthesize the enzyme and to extracellular locations as in other tissues, or might there be cell-associated immunoreaction indicating cells which extract and degrade the enzyme?

MATERIALS AND METHODS

Animals

Guinea pigs weighing 350–500 g (2–3 months old) were used. The animals were provided free access to pellets, vegetables, and drinking water. The illumination was automatically regulated with 14-h light and 10-h dark cycles. The animals were killed according to the UFAM Handbook (UK). All protocols and procedures were approved by the Animal Ethics Committee at the Universities of Umeå (Sweden) and Barcelona (Spain).

Measurement of lipolytic activity

Tissues were homogenized (1 g tissue, 9 ml buffer) at 4°C in 25 mM ammonia buffer, adjusted with HCl to pH 8.2, containing 5 mM EDTA and (per ml) 8 mg Triton X-100, 0.4 mg SDS, 5 IU heparin, 10 μg leupeptin, 1 μg pepstatin (Boehringer Mannheim, Germany) and 25 IU Trasylol (Sigma, St. Louis, MO). Tissues were homogenized with a Polytron (Kinematica Co., Switzerland, 3 × 10 sec at medium setting). The homogenates were centrifuged at 10,000 *g* for 20 min at 4°C and the clear supernatant was recovered and used for the assay of LPL activity (18). To assess what fraction of the activity was due to LPL, aliquots of the supernatant were incubated in ice with 0.5 vol of antiserum to LPL before assay of lipase activity; control aliquots were similarly incubated with non-immune serum.

In situ hybridization of LPL mRNA

We used a ^{35}S-labeled single-stranded RNA antisense probe, produced by T7-transcription from DNA templates derived by insertion of a 2.2 kb LPL-A cDNA described by Enerbäck et al. (19) into the Eco R1 site of PGEM® 3Zf(−) (Promega). It is located within the coding sequence of the LPL mRNA and encompasses the C-terminal half of the mature protein. Plasmids were linealizated with SmaI. Label reactions were made with "Paired-promotor SP6 and T7 systems" kit from Amersham. The average molecular weight of the probe used was 400 bp, with a specific activity of 2.10^8 cpm/μg. The sense RNA probe was used as a control of nonspecific and background label.

For hybridization we followed the procedure described by Shivers, Schachter, and Pfaff (20) with some modifications. Briefly, tissues from adult guinea pigs were fixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) at 4°C for 12 h, rinsed two times for 1 h, and immersed in 30% sucrose in 0.1 M PBS at 4°C for 2 h; they were frozen, and 10-μm sections of the tissues were obtained in a cryostat (Reichert-Jung, Germany), mounted onto gelatin-coated slides, and stored at −30°C until use. On the day of the experiment sections were warmed at 37°C for 10 min, hydrated in 0.1 M glycine in PBS for 20 min, deproteinized with 0.2 M HCl for 10 min, postfixed with 4% paraformaldehyde in PBS for 20 min, washed with 0.1 M glycine in PBS, and acetylated in acetic anhydride–triethanolamine, pH 8.0, for 10 min. A prehybridization step was done by incubating sections with 1.2 M NaCl, 20 mM Tris-HCl, 2× Denhart's solution, 2 mM EDTA, 0.1% yeast total RNA, 0.01% *Escherichia coli* tRNA, 0.1% inorganic sodium pyrophosphate, 20 mM diethylpyrocarbonate, 20 mM L-methionine, 0.04% salmon sperm DNA, and 50% formamide in a humidified box at 48°C for 2 h. For the hybridization step, the ^{35}S-labeled single-stranded RNA probe was added to identical prehybridization buffer complemented with

20% dextran sulfate. The probe/buffer mixture was applied to the sections, which were then covered with coverslips, and incubated in a moist chamber at 48°C for 12 h. Each slide received 40 μl hybridization buffer containing 2×10^6 cpm of antisense (LPL5) or sense (LPL3) RNA probe. Two consecutive sections were always used for each probe. After hybridization, sections were washed three times for 60 min in 0.1×SSC, 0.05% inorganic sodium pyrophosphate, 30% formamide at 42°C, followed by ribonuclease A (20 μg/ml) treatment for 30 min at room temperature and three further washes under the same conditions as above. Then, the sections were dehydrated with 0.03 M ammonium acetate in ethanol and air-dried. Slides were dipped in a nuclear track emulsion (Ilford (Warrington, PA) K5), diluted 1:1 with distilled water, air-dried, and exposed in sealed boxes for 5 to 20 days at 4°C. Photoemulsion was developed with Kodak D-19 developer and stained with hematoxylin-eosin stain. The hybridization signal was observed with the bright and dark field optics of a Leitz photomicroscope.

Immunolocalization of LPL

For immunocytochemistry, polyclonal antibodies raised in rabbits against LPL purified from guinea pig milk were used. These antibodies identify a single band in adipose tissue homogenates by Western blots (21), and were previously used to study LPL synthesis by immunoprecipitation (21) and to identify clones that directed synthesis of LPL-related proteins in a cDNA expression library (19).

In order to compare the results obtained with in situ hybridization and immunocytochemistry, indirect immunofluorescence (5) was performed over sections consecutive to those used for in situ hybridization. Sections obtained as described above were washed with 0.1 M glycine in PBS, permeabilized with 1% Triton X-100 in 0.1 M glycine in PBS, washed with 0.1% Triton X-100 (buffer A), and incubated with LPL antiserum or preimmune serum diluted 1/10 with 1% bovine serum albumin (Sigma) in buffer A for 2 h at room temperature. Then sections were washed with buffer A and incubated with FITC-conjugated goat anti-rabbit IgG (Nordic) diluted 1/25 in 1% bovine serum albumin in buffer A for 1 h at room temperature, washed, and mounted with 70% glycerol, 5% n-propyl galleate-buffered medium. The immunostaining was observed and photographed with Olympus IMT2-RFL or Leitz (Dialux) fluorescence photomicroscopes.

RESULTS

Lipoprotein lipase activity in guinea pig tissues

Table 1 shows lipoprotein lipase activity displayed by several guinea pig tissues and the effect of LPL antiserum on the activity. The highest activity was detected in adipose tissue, heart, and lungs. Liver and spleen presented 8.6 and 6.9%, respectively, of the epididymal adipose tissue activity. Lower activities were present in muscle (not shown) and in kidney (1.6% of epididymal adipose tissue activity). All the activities detected were inhibited by anti-LPL serum to variable degrees. The highest inhibition was detected in adipose tissue, heart, and lungs, while lower inhibitions were detected for the other tissues, suggesting the presence of other lipolytic activities.

TABLE 1. Lipoprotein lipase activity in some tissues of untreated male guinea pigs

Tissue	Total	Anti-LPL
	mU/g (wet weight)	
Heart	3360 ± 311	85 ± 24
Epididymal adipose tissue	4068 ± 263	142 ± 16
Perirenal adipose tissue	2052 ± 138	115 ± 10
Liver	353 ± 29	109 ± 3
Spleen	284 ± 26	191 ± 10
Lung	1029 ± 71	47 ± 3
Kidney	67 ± 3	22 ± 2

For details, see Materials and Methods. Values are mean ± SEM for five animals. Activities were measured in duplicate.

Lung

In the lung there was immunoreactivity in several different places (**Fig. 1**). Label was present encircling alveolar spaces (Fig. 1 A-B), mainly associated with the capillary network (Fig. 1 C-D) but also in the cytoplasm of some alveolar cells (Fig. 1 G and H), which cannot be identified by this technique. In addition, immunoreactive material was present in the connective tissue that envelops bronchioles (Fig. 1 J), as well as at the endothelial walls of arterioles (Fig. 1 F) and veins (Fig. 1 E) and inside the lumen of some veins (Fig. 1 A and I). There was immunofluorescence over the perichondrial layer of the bronchial cartilage, and over the cytoplasm of chondrocytes but there was little or no labelling over the cartilage matrix (Fig. 1 K).

In situ hybridization showed that, in contrast to adipose, muscle, and mammary gland tissue (5), LPL mRNA was expressed in a specific and not in a major cell type (**Fig. 2**). Positive signal to the RNA probe was detected at the alveolar level associated with cells that looked like macrophages (Fig 2 A-B and F-G). In agreement with the immunofluorescence pattern, high expression was seen in scattered cells in the connective tissue that surrounds bronchioles (Fig. 2 D-E). According to their position in the alveoli as well as their typical morphology, these cells were probably macrophages (**Fig. 3**). No positive signal was detected in endothelial cells of capillaries or larger vessels or in alveolar epithelial cells (Fig. 3 A).

Spleen

Immunoreactive material was located in specific areas of both white and red pulp (**Fig. 4**). In the white pulp immunofluorescence was at the periphery of lymphatic nod-

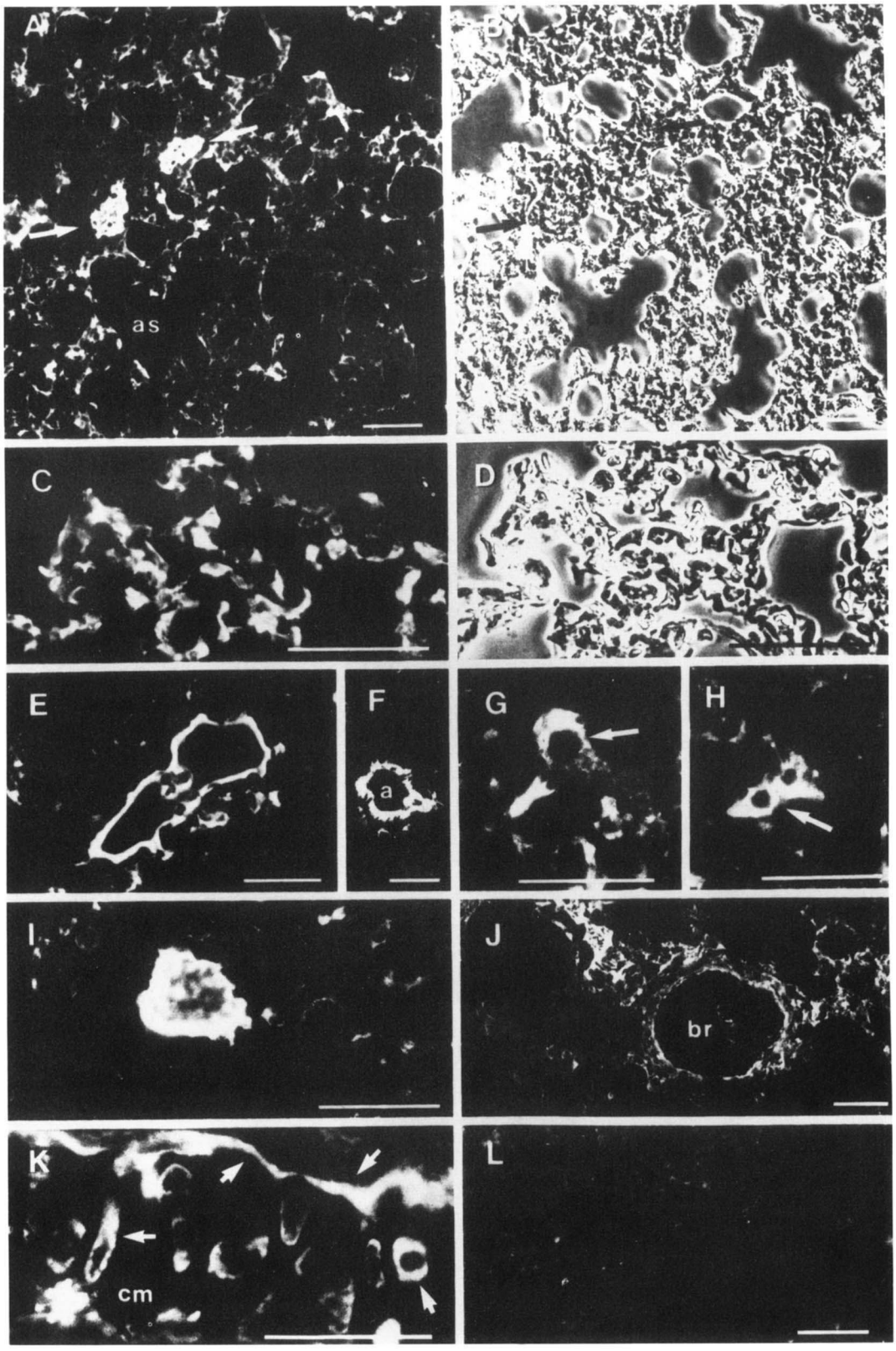

Fig. 1. Distribution of LPL immunoreactivity in lung. (A): Low magnification of a lung section showing the pattern of immunoreactivity. Note that immunofluorescence is present encircling alveolar spaces (as) and inside vessels (arrows) (D). (B): Phase contrast of the same section shown in A. (C): capillary immunoreactivity encircling alveolar spaces. (D): Phase contrast of the same section shown in C. (E): Immunofluorescence in the endothelial walls of a venule. (F): Immunofluorescence in the endothelial walls of an arteriole (a). (G and H): Cytoplasmic immunoreactivity (arrows) in cells of epithelial alveoli. (I): Immunofluorescence in the lumen of venules. Note that the label is not cell-associated. (J): Low magnification of a bronchiole (br). Note that the label is present in the tissue that surrounds this bronchiole. (K): Immunoreactivity in cartilage. Note that immunofluorescence is in the perichondrial layer (short arrows) and inside chondrocytes (large arrows), but not in cartilage matrix (cm). (L): Section incubated with preimmune serum where no positive staining is seen. Bars 50 μm except for panel F where the bar is 20 μm.

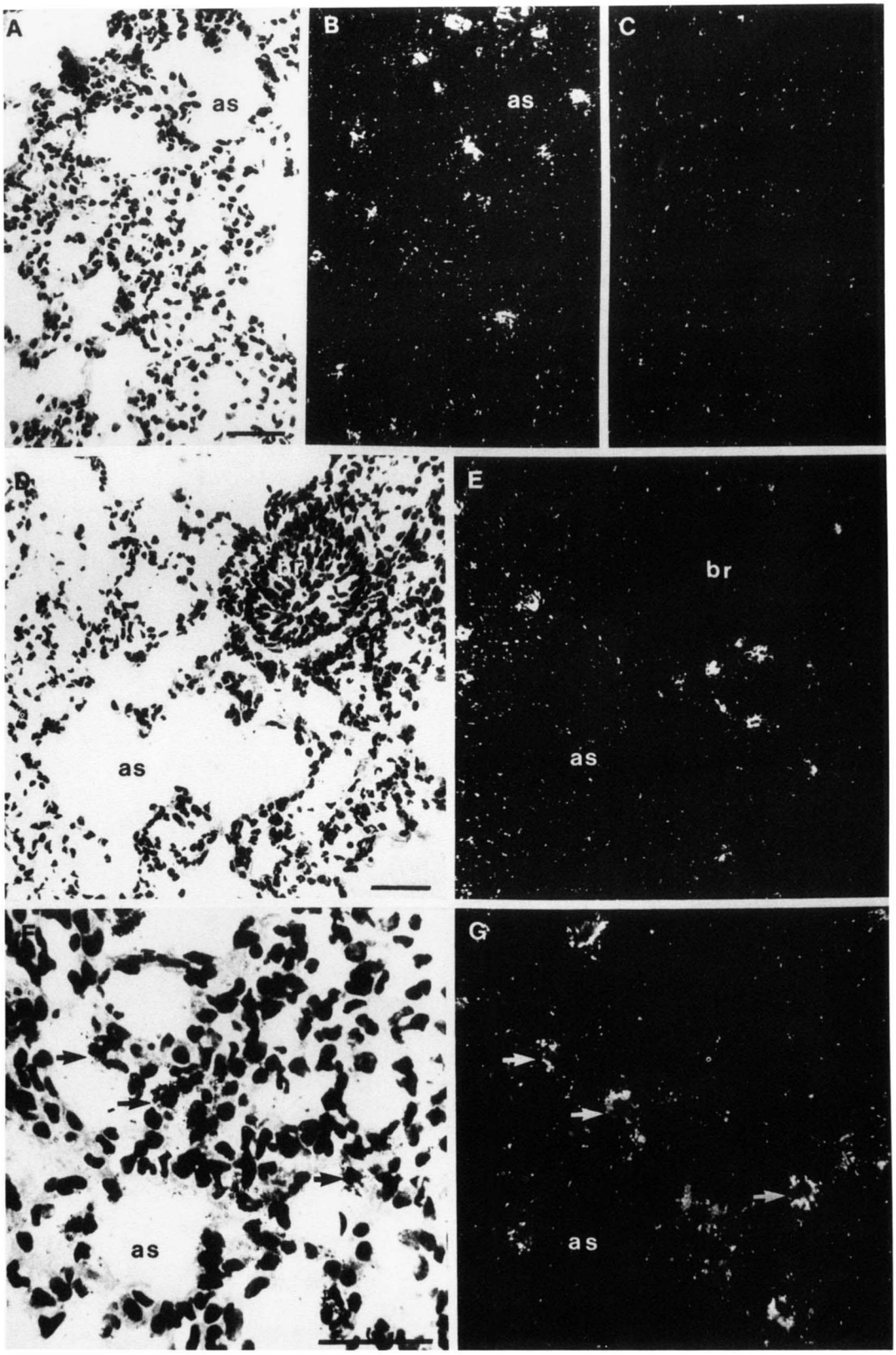

Fig. 2. Distribution of LPL mRNA in lung. Panels A, D, and F are bright field photomicrographs of the same fields shown in B, E, and G, respectively, photographed under dark ground illumination. Panels A-B, D-E, and F-G are autoradiographs of sections hybridized with antisense RNA probe and panel C is an autoradiograph of a corresponding section hybridized with a sense RNA probe as a control of background. (A-C): Low magnification of lung sections showing that positive signal to antisense LPL probe is present in scattered cell types, encircling alveolar spaces (as) (panels A-B). This label is clearly over the background shown in panel C. (D-E): Positive signal to LPL probe is also near bronchioles (br). (F-G): High magnification of positive cells show hybridization (arrows) located in the epithelia of alveoli. Bar 50 μm.

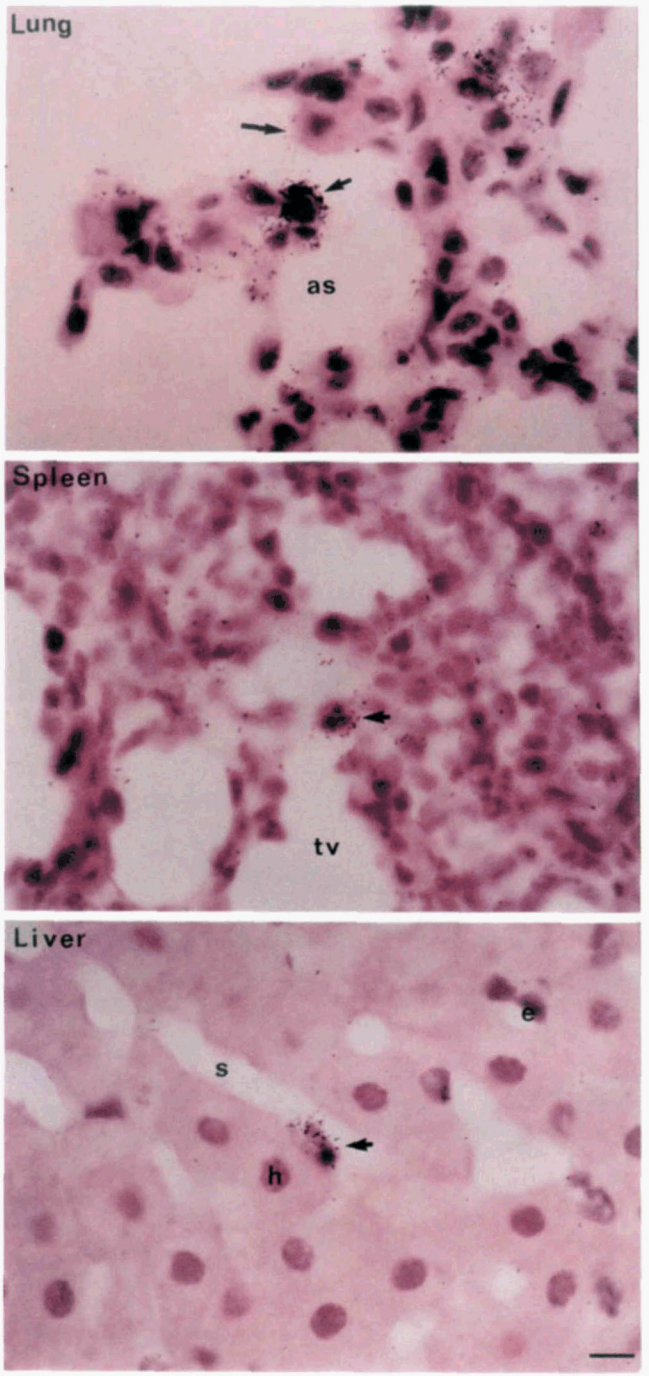

Fig. 3. Summary of the cells positive to in situ hybridization for LPL probe in lungs, spleen, and liver viewed at high magnification and by hematoxilin-eosin stain. Note that in all three cases the cells present a lobular shape of their nucleus. In lungs, positive cells (short arrows) are in contact with alveolar space (as) and can be distinguished from alveolar cells (large arrows). In spleen and liver, the positive cells are close to trabecular veins (tv) and sinusoidal space (s), respectively, and in the case of liver can be distinguished from endothelial cells (e) and hepatocytes (h). Bar 25 μm.

ules (Fig. 4 A-B and C-D) and it appears that most of the label was associated with tissue cells, but at this level of resolution it cannot be excluded that some immunoreactive material was associated to the capillary network. Central splenic arteries showed immunoreactivity at their endothelial walls (Fig. 4 E and F-G). In the red pulp the immunofluorescence was clearly over the cytoplasm of cells located preferentially close to the vessels (Fig. 4 H-I and J-K). Some immunoreactive cells were also seen dispersed in the red pulp matrix (Fig. 4 J-K). In the area there was little or no labelling of blood vessels (Fig. 4 H-I). In situ hybridization gave positive reaction over some tissue cells (**Fig. 5**) located in the areas intensively labeled by immunofluorescence. However, the number of positive cells was lower than the immunoreactive cells. Fig. 5 A-B shows at low magnification, and Fig. 5 D-E at high magnification, a few cells positive to the LPL-mRNA probe located at the periphery of white pulp (compare with immunofluorescence this area, Fig. 4A). Also, in the red pulp there were some positive cells near the trabecular veins (Fig. 5 F-G). By their morphology, it appears that most, perhaps all, labeled cells were macrophages (Fig. 5 D-E and F-G, and Fig. 3).

Liver

Liver showed a heterogeneous immunoreactivity pattern for LPL (**Fig. 6**). The greatest reactivity was in the periportal areas (Fig. 6 A), whereas perivenous areas showed much weaker reactivity (Fig. 6 A). Hence, there was a clear gradient of total label within the lobule. There was immunofluorescence at the endothelial walls of both portal tracts (Fig. 6 D) and arteries (Fig. 6 B). In addition, there was immunoreactive material over the cytoplasm of a few periportal hepatocytes (Fig. 6 A and D). In the sinusoid the immunoreactivity was quite intensive (Fig. 6 E-F), although at this level of resolution it may not be resolved if the label is associated with endothelial or with parenchymal cells at the Disse space. In contrast to other tissues reported, such as adipose tissue, heart, diaphragm muscle (5), and lung (present study), luminal reactivity within vessels was limited to arteries and portal tracts (Fig. 6 C); the lumen of centrolobular veins never presented any mark (Fig. 6 A), suggesting that the liver removes LPL from blood. In situ hybridization experiments (**Fig. 7**) showed significant signal with ^{35}S-RNA probe only in a few nonparenchymal cells, that possibly corresponded to Kupffer cells because their position in the tissue was in the sinusoidal lumen and by their morphology they were clearly not endothelial cells (Fig. 3 C). There was no significant labelling over hepatocytes (Fig. 7 D-E). In this figure is also shown a circulating blood cell with monocyte morphology which presented positive signal for the LPL probe (Fig. 7 F-G).

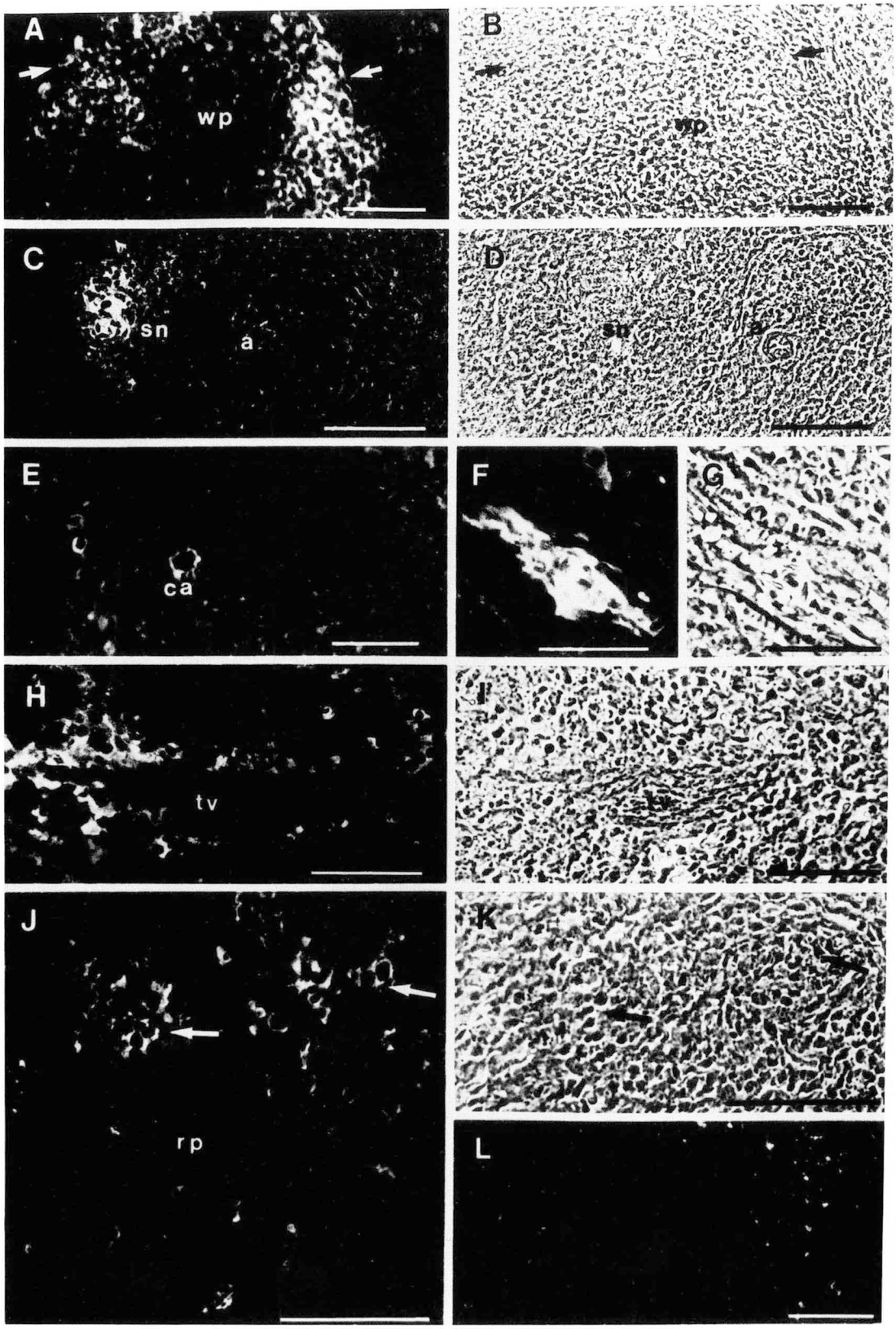

Fig. 4. Distribution of LPL immunoreactivity in spleen. (A): In the white pulp (wp) immunostaining is associated to marginal zone (arrows). (B): Phase contrast of the same section shown in A. (C): Some splenic nodules (sn) also show immunoreactivity to LPL antiserum. (D): Phase contrast of the same section shown in C. E (cross section) and F (longitudinal section) of central arteries (ca) in white pulp present endothelial label (E) and luminal immunoreactive material (F). (G): Phase contrast of the same section shown in F. (H): low magnification of red pulp. Immunostaining is located in cells near the trabecular veins (tv). (I): Phase contrast of the same section shown in H. (J): Red pulp immunoreaction is over the cytoplasm of some peritrabecular cells (arrows). (K): Phase contrast of the higher part of the area shown in J. (L): Portion of a red pulp from a section incubated with preimmune serum, where some nonspecific signal is present, but clearly different from the specific signal in the other panels. Bar 50 μm.

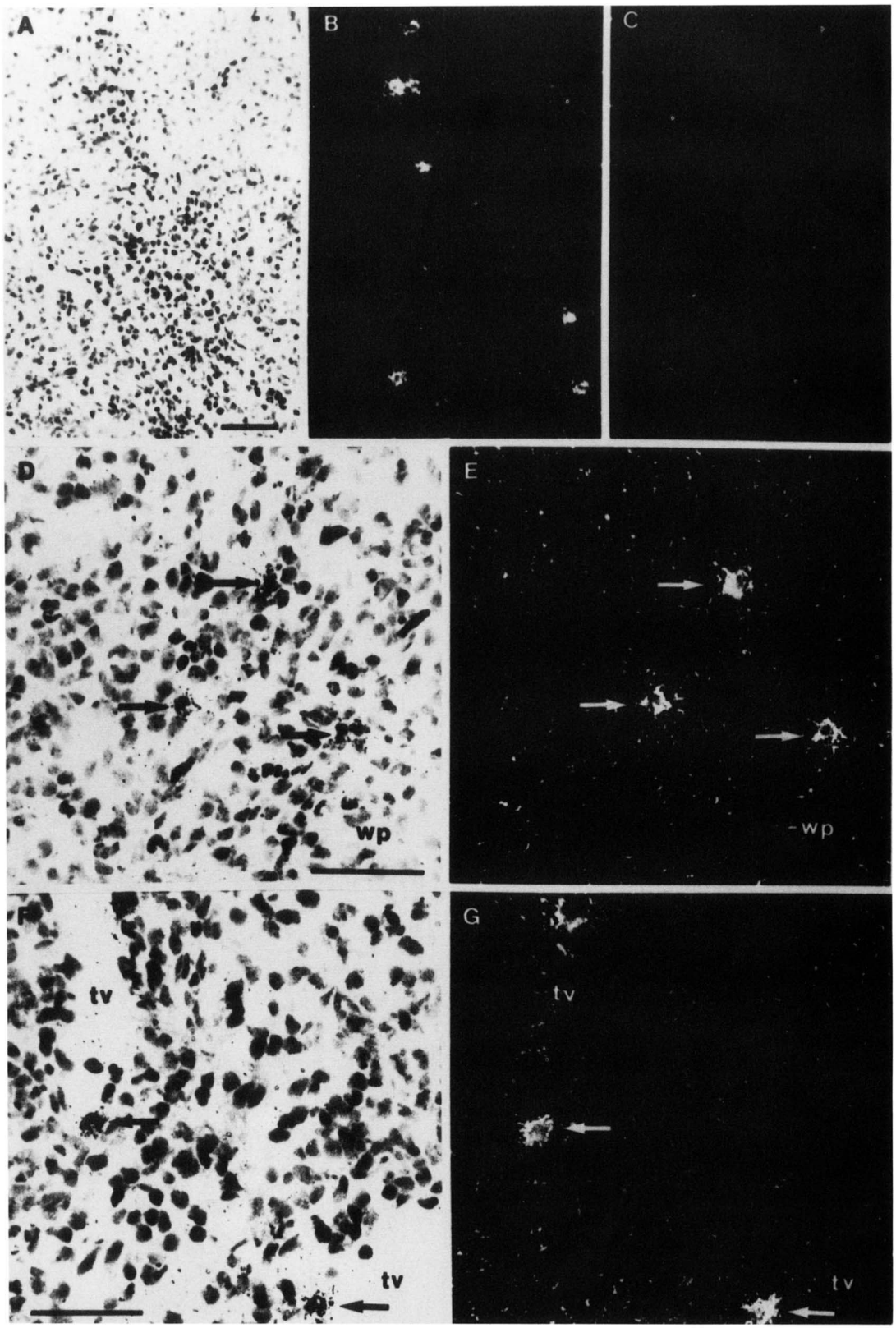

Fig. 5. Distribution of LPL mRNA in spleen. Panels A, D, and F are bright field photomicrographs of the same fields shown in B, E, and G, respectively, photographed under dark ground illumination. Panels A-B, D-E, and F-G are autoradiographs of sections hybridized with antisense RNA probe and panel C is an autoradiograph of a corresponding section hybridized with sense RNA probe, as a background control. (A–C): Low magnification of spleen section, where a specific signal for RNA probe is located over scattered cells dispersed in the tissue (A-B). The signal is clearly over the background shown in panel C. Labeled cells (arrows) in the marginal sinuses of white pulp (wp). (F-G): Cells giving positive hybridization (arrows) in red pulp are located close to the vascular lumen of trabecular veins (tv). Bar 50 μm.

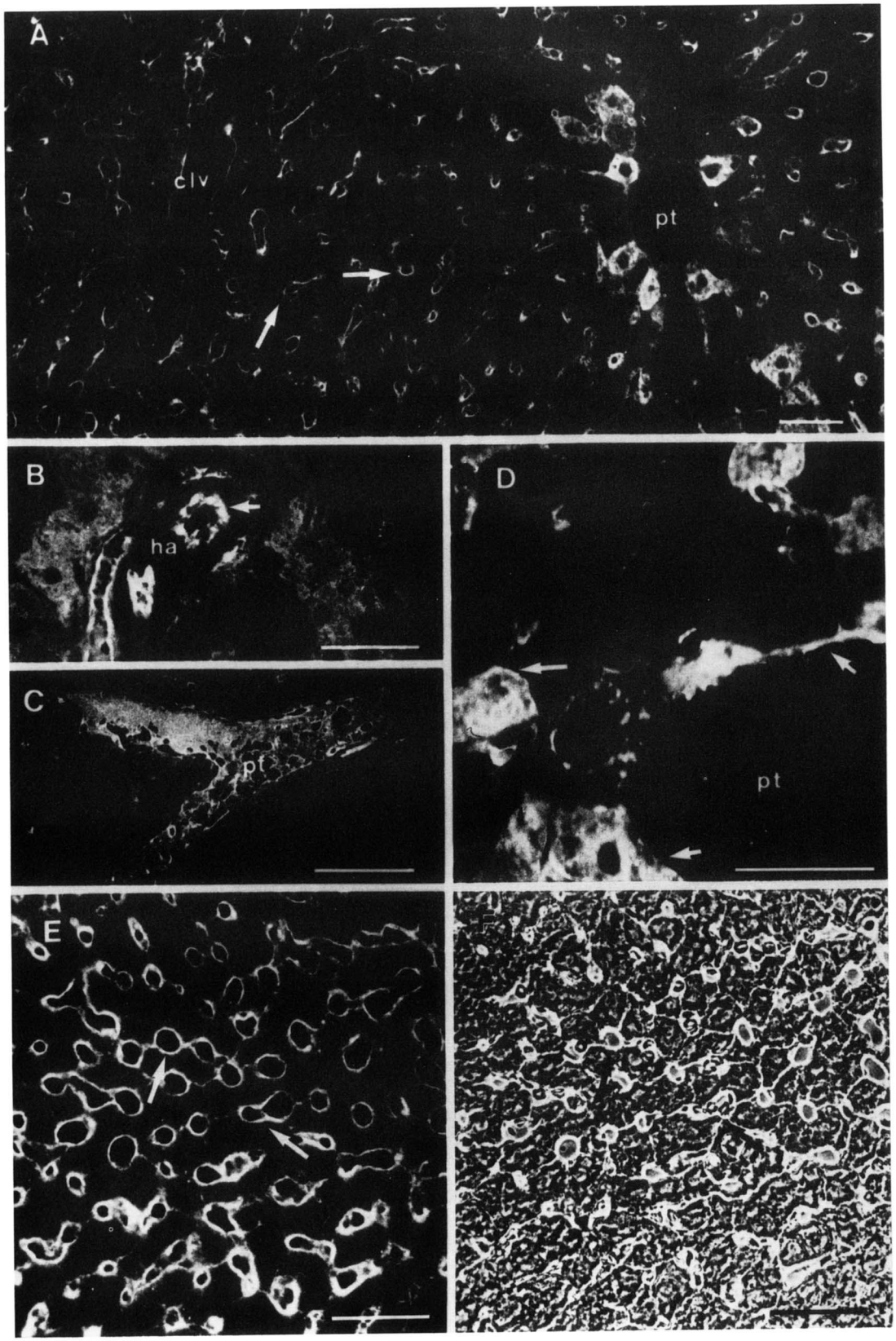

Fig. 6. Distribution of LPL immunoreactivity in liver. (A): Low magnification of an hepatic lobule. General pattern of immunoreactivity shows that label is present in liver sinusoid (arrows) and inside cells of periportal areas; pt, portal tract; clv, central lobular vein. Perivenous areas present low intensity of immunostaining. Note the absence of signal in the lumen and walls of a central vein. (B): Hepatic arteries (ha) with immunostaining in endothelial walls (arrow). (C): Label in lumen of portal tract. (D): High magnification of a periportal area. Immunoreaction inside periportal hepatocytes (large arrows) and in endothelial walls of portal tract (short arrows). (E): Immunoreaction in hepatic sinusoid. (F): Phase contrast of the same section shown in E. Bar 50 μm.

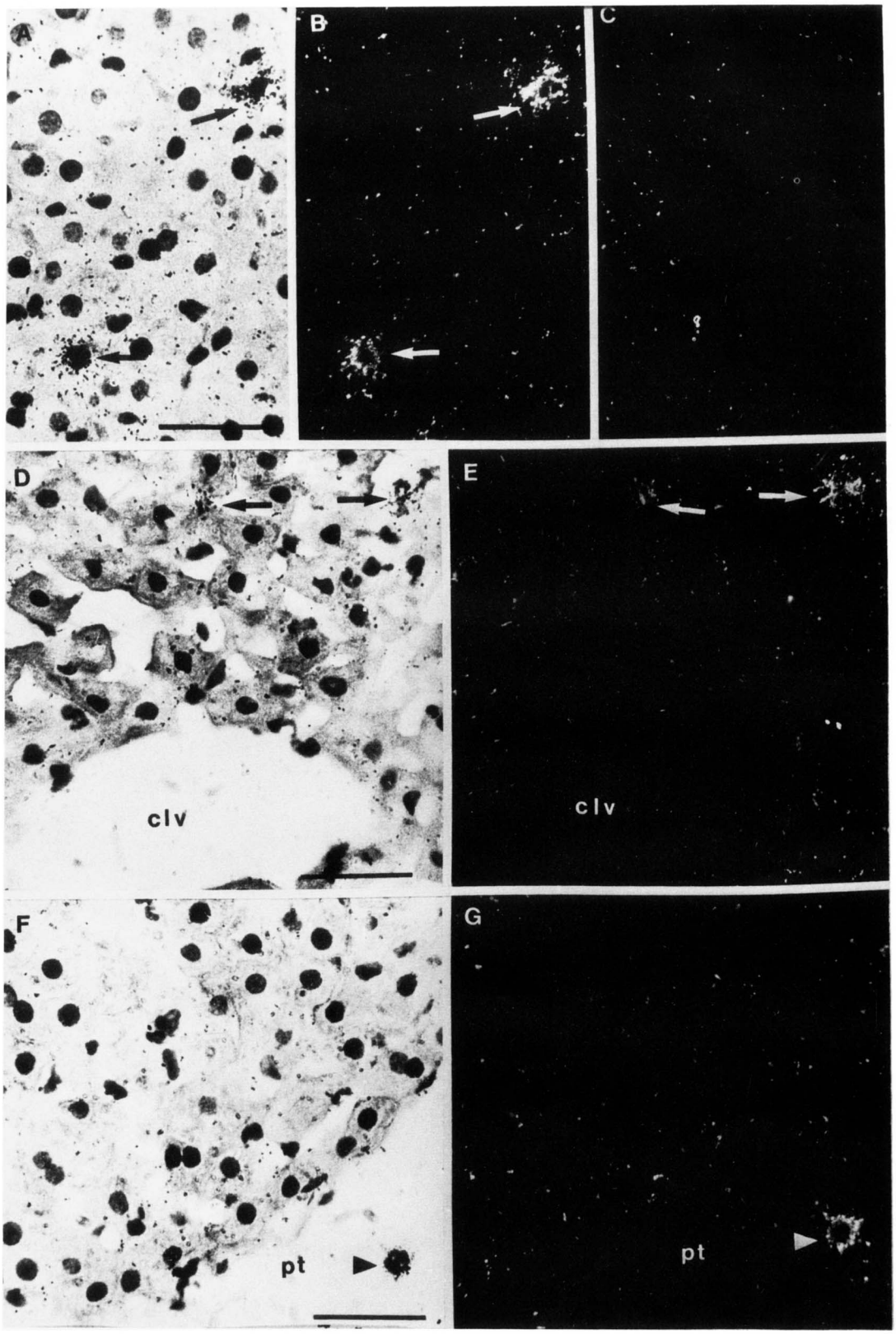

Fig. 7. Distribution of LPL mRNA in liver. Panels A, D, and F are bright field photomicrographs of the same fields shown in B, E, and G, respectively, photographed under dark ground illumination. Panels A-B, D-E, and F-G are autoradiographs of sections hybridized with antisense RNA probe and panel C is an autoradiograph of a corresponding section hybridized with sense RNA probe, as a background control. (A-B): Dispersed sinusoidal cells in the hepatic lobule with LPL mRNA expression (arrows). (D-E): Sinusoidal cells (arrows) near central lobular vein (clv) with positive signal to RNA probe. (F-G): Portion of a portal tract (pt) that shows a circulating monocyte with positive signal (arrow head) to RNA probe. Bar 50 μm.

DISCUSSION

The present study shows that in lung, spleen, and liver, LPL is synthesized by a minority type of cells, scattered in the tissue. This is in contrast to major LPL-producing tissues (e.g., adipose tissue, muscles) where the main cell type produces the enzyme. In the present three tissues, according to their morphology and tissue position (Fig. 3), all the hybridization-positive cells appeared to be macrophages or related cells. In addition, we detected LPL expression in circulating cells, which showed monocyte-like morphology (Fig. 6 F-G). It is known that monocytes-macrophages produce LPL (22, 23), and that their expression of the enzyme is triggered by activation of the cells.

In agreement with previous studies (23) we detected that lungs presented relatively large quantities of LPL activity. Previous studies indicate that macrophages are the main LPL-producing cells in the lung (23). Our observations support this, although we cannot rule out that some of the hybridization positive cells were alveolar type II cells. By immunocytochemistry there was strong LPL immunoreaction along the vascular endothelium encircling alveolar spaces. Coonrod, Karathanasis, and Lin (24) have recently shown that there is LPL in bronchoalveolar fluid. This LPL could be secreted by the local LPL-producing macrophages and then transported in the tissue in the same way as LPL is in adipose tissue and muscles. The enzyme could, however, to a large extent, originate in other tissues and be taken up from blood. Another possibility is that some of the enzyme comes from the bronchoalveolar fluid by way of transcytosis through alveolar type II cells. However, it is clear that there was no particular concentration of the enzyme at endothelial sites adjacent to LPL-producing cells, but there was a rather even distribution along the vascular endothelium of both capillaries and larger vessels.

Mindham and Mayes (25) have recently reported that there is substantial hydrolysis of chylomicron triglycerides on perfusion through rat spleen, indicating a role for this organ in lipoprotein metabolism. In accordance with this, the spleen contained LPL activity that was inhibited by anti-LPL antibodies, but we cannot exclude that other lipolytic activities participate in degradation of plasma triglycerides in the spleen. In situ hybridization revealed that the spleen contained a substantial number of LPL-positive macrophages. In contrast to lung, however, there was little immunofluorescence along vessels in the spleen, suggesting that the macrophages here make the lipase for use in their own immediate surrounding to acquire fatty acids for metabolic purposes or as part of their scavenging role. Another implication is that the vessels in the spleen have a low ability to bind LPL, otherwise they should pick up the enzyme both from local macrophages and from the circulating blood.

Low but definite LPL synthesis has been reported in adult rat and guinea pig liver (26, 27). Our data show that the synthesis is confined to non-parenchymal cells. These cells were probably Kupffer cells, which would be in accordance with the fact that these cells are closely related to macrophages. However, from this study we cannot know if all Kupffer cells contain LPL mRNA. On the other hand, there has been evidence that, in the liver of newborn rats, the main LPL-producing cell type is the hepatocyte (27, 28). LPL activity is much higher in the liver of fetal and newborn rats than in adult rats (26). Hence, LPL synthesis in the hepatocytes is probably suppressed as part of the differentiation these cells undergo in the developing liver.

Previous studies indicate that the liver extracts LPL from the circulating blood and may be a main site of LPL degradation (12). In accordance with this there was strong immunoreaction over the lumen of the hepatic artery and the portal vein, whereas the reaction was weak over the hepatic vein. This would represent LPL in blood and at vascular surfaces. We observed intracellular immunofluorescence over some hepatocytes in periportal areas, indicating that these are the cells that internalize LPL. They did not show any reaction of LPL mRNA, demonstrating that they do not synthesize LPL. Some of the periportal hepatocytes showed rather intense immunoreaction, whereas other nearby cells displayed little or no reaction. The basis for this interesting observation is presently not understood.

These observations suggest the following model for LPL transport. In the main LPL-synthesizing tissues the enzyme moves from synthesis in parenchymal cells to binding sites in adjacent capillaries. It then moves along the vascular endothelium from one binding site to the next, carried by blood. The concentration of LPL in the general circulation is kept low by uptake and degradation in the liver. Hence, the system is not in equilibrium, but there is a concentration gradient from areas with LPL synthesis to other parts of the vascular mesh, and there is net flow of LPL to the liver. The distribution of endothelial LPL is, however, not governed only by where the enzyme is synthesized, but also depends on the distribution of binding sites, as illustrated here by the difference between lung and spleen, and in previous studies by the presence of LPL in glomeruli of the kidney (5) and by the uptake of LPL by heparin-sensitive sites in the liver (12).

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