

Lipoprotein lipase: cellular origin and functional distribution

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CAMPS, L., M. REINA, M. LLOBERA, S. VILARÓ, AND T. OLIVECRONA. *Lipoprotein lipase: cellular origin and functional distribution*. *Am. J. Physiol.* 258 (Cell Physiol. 27): C673-C681, 1990.—Lipoprotein lipase (LPL, E.C. 3.3.1.34) 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 is within tissues and vessels. 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 on cryosections of some LPL-producing tissues of guinea pigs. In white and brown adipose tissues, heart and skeletal muscle, and lactating mammary gland, there was positive hybridization for LPL mRNA over all members of the major cell types, indicating that mature and immature adipocytes, muscle cells, and mammary epithelial cells are main sources of LPL. In large vessels, LPL expression was detected in some smooth muscle cells in the media layer. There was no positive hybridization for LPL mRNA over endothelial cells in any of the tissues studied, but there was immunoreaction for LPL protein at endothelial surfaces of all blood vessels. In the kidney, there was strong immunofluorescence at the vascular endothelium, particularly in the glomeruli, but little or no LPL mRNA was detected in the surrounding cells. These observations suggest that in some tissues LPL is synthesized by parenchymal cells and spreads along the vascular mesh. Transfer to the vascular endothelium is, however, not the only route taken by LPL. In the mammary gland most of the enzyme protein appeared to be secreted, partly in association with milk fat droplets. Unsuckling led to near total loss of both positive hybridization and intracellular immunoreaction in the epithelial cells of the mammary gland, suggesting that downregulation of mammary LPL occurred at the level of gene regulation.

in situ hybridization; immunocytochemistry; endothelium; vessels

THERE ARE TWO GENERAL MECHANISMS for delivery of lipoprotein lipids to the cell. One is by receptor-mediated endocytosis of low-density lipoproteins (LDL) and other lipoproteins (4). This pathway is regulated within the cell that is to receive the lipid, and the receptor is expressed at the surface of these cells. This is possible for the rather slow turnover of these moderately sized lipoproteins which can pass from the circulating blood into extravascular spaces and engage the target cells directly. The major lipids transported with lipoproteins are, however, triglycerides, which are carried in large chylomicron and very low-density lipoproteins (VLDL)

particles (25). These lipoproteins bind transiently to endothelial sites where their triglycerides are hydrolyzed by lipoprotein lipase (LPL) (26). Fatty acids and monoglycerides are released and move readily within the tissue, down a concentration gradient created by their use or reesterification within cells (28). Adipocytes (10), myocytes (3), mammary epithelial cells (29), and macrophages (23) are known to synthesize the lipase. LPL activity has been demonstrated in a variety of other tissues, including lung and kidney (8), brain (11, 33), ovaries (5, 8), and aorta (9), but it is not clear what cells produce it in these tissues.

LPL is member of a gene family that also comprises the pancreatic lipase and the hepatic lipase (21) but is not related to the widespread hormone-sensitive lipase (15). The structures of guinea pig pancreatic lipase and hepatic lipase are not known, but available evidence makes it unlikely that these lipases are synthesized outside the liver (21) and pancreas (22), respectively. The cells that synthesize the LPL release it for transfer to endothelial sites, where it binds to glycan chains of heparan sulfate proteoglycans (24). This localizes the enzyme in the outer part of the glycocalyx, where it can interact with the large lipoprotein particles. This mode of attachment to the vascular endothelium is not unique for LPL. There are several other proteins in blood with affinity for heparan sulfate, e.g., antithrombin III (16), diamine oxidase (27), and the extracellular superoxide dismutase (19). Heparan sulfate proteoglycans are present at the surface of virtually all resident cells (14). This raises the question of whether there is a specific path for LPL to certain heparan sulfate proteoglycans at the endothelium or if the enzyme spreads over all cell surfaces.

To better understand where LPL is made and where it is localized, we have used "in situ" hybridization to determine what cells produce the lipase and immunofluorescence to determine how it is disposed within the major LPL-producing tissues. For this we have used guinea pigs, since from previous studies antibodies and cDNA for this species were available in our laboratory.

MATERIALS AND METHODS

In situ hybridization of LPL mRNA. We used a ³⁵S-labeled single-stranded RNA anti-sense probe, produced by T7 transcription from DNA templates derived by insertion of a 2.2-kb LPL-A cDNA described by Enerback et al. (12) into the EcoR1 site of PGEMR 3Zf (-)

(Promega). It is located within the coding sequence of the LPL mRNA and encompasses the COOH-terminal half of the mature protein. Plasmids were linearized with SmaI. Label reactions were made with Paired Promotor SP6 and T7 systems kits from Amersham. The average molecular weight of the probe used was 400 bp, with a specific activity of 2×10^8 counts·min⁻¹·μg⁻¹. The sense RNA probe was used as a control of nonspecific and background label.

For hybridization we followed the procedure described by Shivers et al. (32) 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, 10-μm sections of the tissues obtained in a cryostat (Reichert-Jung, FRG), mounted onto gelatin-coated slides, and stored at 30°C until use. 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(hydroxymethyl)aminomethane (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 ³⁵S-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 cover slips and incubated in a moist chamber at 48°C for 12 h. Each slide received 40 μl hybridization buffer containing 2×10^6 counts/min of anti-sense (LPL5) or sense (LPL3) RNA probe. Always two consecutive sections were used for each probe. After hybridization, sections were washed three times for 60 min each in 0.1× saline sodium citrate (SSC), 0.05% inorganic sodium pyrophosphate, and 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), diluted 1:1 with distilled water, air dried, and exposed in sealed boxes for 5–20 days at 4°C. Photoemulsion was developed with Kodak D-19 developer and stained with hematoxylin and eosin stain. The hybridization signal was observed and photographed 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 (30) and have been used to immunoprecipitate biosynthetically labeled LPL from several of

the tissues studied here (muscle, adipose tissue, heart, and mammary gland; Ref. 31) and to identify clones which directed synthesis of LPL-related proteins in a cDNA expression library (12).

To compare the results obtained with in situ hybridization and immunocytochemistry, indirect immunofluorescence (34) 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 fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (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 (Di-alux) fluorescence photomicroscopes.

RESULTS

Adipose tissue. Figure 1 shows the fluorescence patterns obtained when cryosections of epididymal adipose tissue were immunostained with antibodies to LPL. The immunoreactivity was intensive compared with other tissues, and almost all adipocytes showed reaction to the antibodies (Fig. 1A). In mature adipocytes, immunoreactivity was confined to the cytoplasmic areas surrounding the lipid droplets (Fig. 1E). LPL protein was also detected in the cytoplasm of other cells (Fig. 1B), characterized by the presence of small lipid droplets in their cytoplasm. Some cells with no lipid droplets, located in the interlobular connective tissue, also showed some cytoplasmic immunoreactivity (Fig. 1C). In addition, there was immunofluorescence over some pericytes at the periphery of the capillary network (Fig. 1D), and there was a diffuse immunofluorescence over the connective tissue (Fig. 1, A and C).

Immunohistochemically detectable LPL was prominent in capillaries (Fig. 1, D and E) and larger vessels, arterioles (Fig. 1, F and G) and venules (Fig. 1H), in agreement with previous studies in human adipose tissue (18). In both arteries and veins the greatest density of immunolabeling was seen at the endothelial walls (Figs. 1, G, H, and J, and 2). In addition, there was also immunoreactive material in the lumen of vessels, mainly in veins (Fig. 1H). An interesting observation came from serial sections of the tissue (Fig. 2). As the diameter of the vessel increased, the endothelial label increased in arterioles, whereas in venules both endothelial and luminal labeling increased (Fig. 2, compare D with A). A weak immunoreaction was detected in the mesenchymal tissue surrounding vessels (Fig. 1, F and G). In some larger arteries immunoreactivity could be seen in the smooth muscle layer (Fig. 1J).

In situ hybridization with a ³⁵S-RNA probe complementary to LPL mRNA revealed that the LPL gene is expressed in mature adipocytes (Fig. 3, A and B), as well

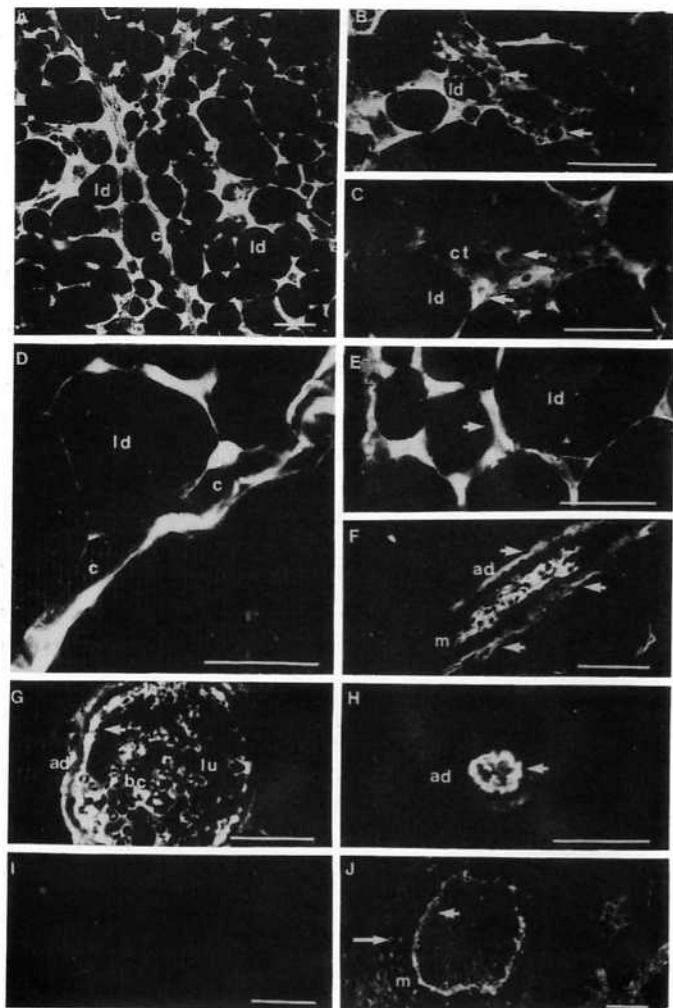


FIG. 1. Distribution of lipoprotein lipase (LPL) immunoreactivity of white adipose tissue. *A:* immunofluorescence staining is confined to the cytoplasmic region surrounding lipid droplets (ld) of mature adipocytes. Some immunofluorescence is also present in bundles of connective tissue (ct). *B:* immunoreactivity in immature adipocytes which present small lipid droplets (arrows) in their cytoplasm. *C:* positive immunoreactivity in cells (arrows) of connective tissue (ct) with fibroblast-like morphology. *D:* presence of LPL immunoreactivity in capillaries (c) of white adipose tissue. *E:* high magnification of mature adipocytes, showing positive immunoreactivity (arrow) in the cytoplasmic areas surrounding lipid droplets. *F:* tangential section of an artery which shows positive immunoreactivity to LPL in the lumen and adventitia layer (ad) (arrows), whereas media layer (m) is negative. *G:* section of a vein with positive staining in the lumen (lu), endothelial wall (arrow), and adventitia layer. *H:* cross section of an artery with positive label in the endothelial wall (arrow). *I:* control section at low magnification incubated with preimmune serum and photographed at the same exposure time as sections incubated with LPL antiserum. No staining was detected. *J:* large artery from white adipose tissue, showing immunoreactivity in the endothelial wall (small arrow) and in some cells of the media layer (large arrow). Bars, 50 μm.

as in preadipocytes and unknown cells located in the connective tissue (not shown). The signal was more concentrated around the nucleus but also was present in the nonlipidic cytoplasmic region (Fig. 3, *C* and *D*). In addition, some autoradiographic signal was seen in mesenchymal cells surrounding vessels (Fig. 3, *F* and *G*). No significant signal was detected in the cytoplasm of endothelial cells or pericytes (Fig. 3, *C* and *D*).

Similar patterns of immunofluorescence and in situ

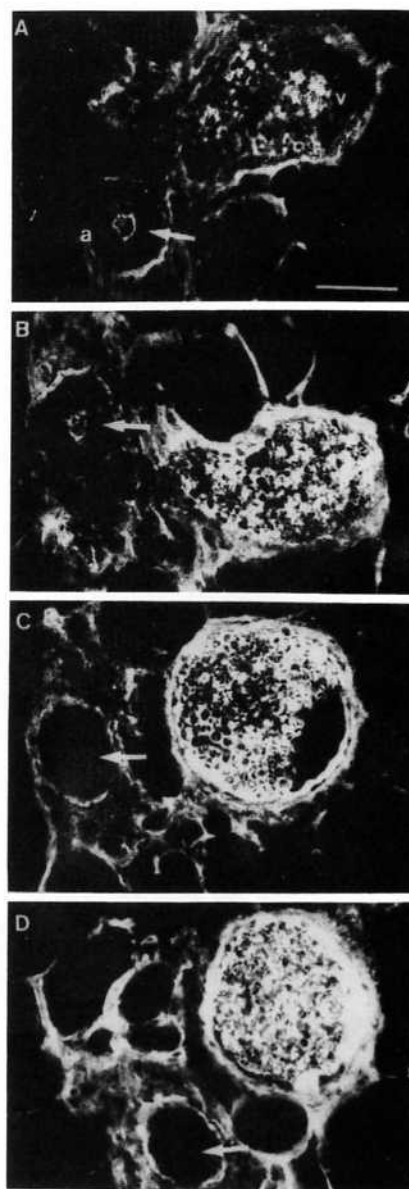


FIG. 2. LPL distribution in serial sections of vessels from white adipose tissue. All sections and pictures were processed in the same way. Note that as diameter of vessel increased, endothelial label (arrows) increased in arterioles (a) (*A* compared with *D*), and in venules (v) both endothelial and luminal labeling increased (*D* compared with *A*). Bar, 50 μm.

hybridization were seen in adipose tissue from other locations (mammary and adrenal glands, heart, etc.) as well as in brown adipose tissue from newborn guinea pigs (not shown).

Heart. LPL immunoreaction was found mainly in the myocardium but could also be detected at the endocardium associated with endothelial cells (Fig. 4*H*). There was immunoreactive material both in the walls of capillaries (Fig. 4, *A* and *B*) and in the periphery of muscle cells (Fig. 4*C*). Like in adipose tissue, immunofluorescence was also detected in larger vessels of the heart and at the endothelial walls of arterioles (Fig. 4, *D* and *E*) and venules (Fig. 4*F*). In venules there was also prominent label in the lumen (Fig. 4*G*) which was not associated with blood cells or the endothelial wall. Consecutive

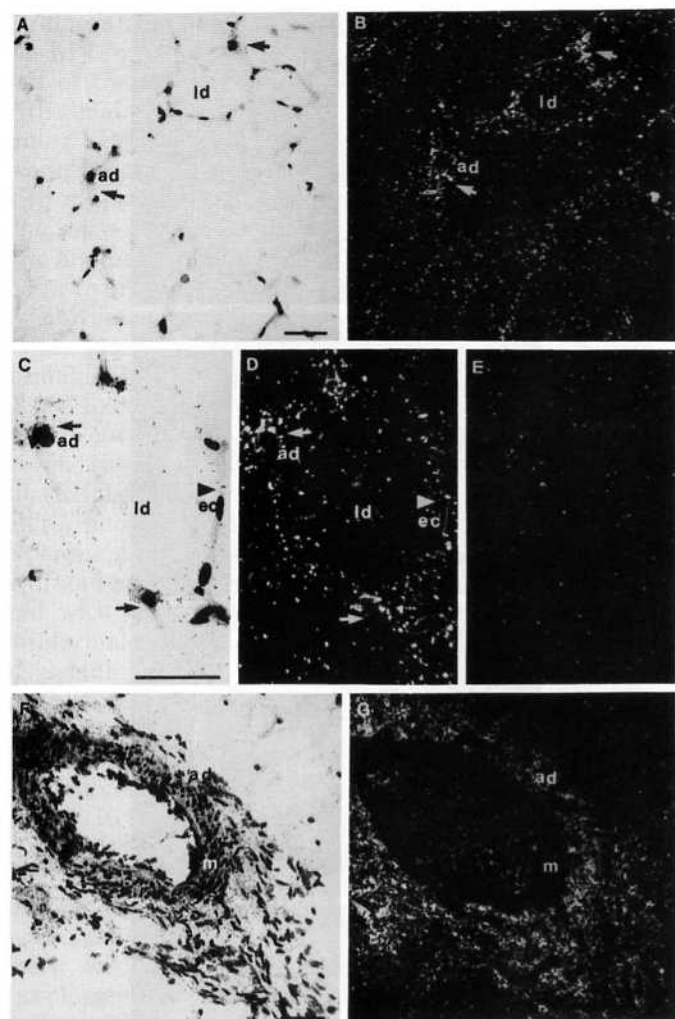


FIG. 3. Distribution of LPL mRNA in white adipose tissue. A, C, and F: bright-field photomicrographs of same fields shown in B, D, and G, respectively, which were photographed under dark ground illumination. A–D, F, and G: autoradiographies of sections hybridized with anti-sense RNA probe. E: autoradiograph of a corresponding section hybridized with sense RNA probe. A and B: positive hybridization signal (arrows) in all mature adipocytes (ad). C and D: highest density of silver grains is near adipocyte nucleus (arrow), but some signal is also over cytoplasmic region that surrounds lipid droplets (ld). Label in the endothelial cells (ec) is not over background (E). F and G: high density of label in connective tissue and adventitial layer that surrounds arterioles of white adipose tissue. Note that no significant label is in the media layer (m). Bars, 50 μ m.

serial sections confirmed that this immunoreactive material was present in the lumen of the vessels.

Figure 5 shows the pattern of expression of LPL mRNA as revealed by in situ hybridization. In contrast to immunolocalization results, LPL gene expression was located at the myocardium. Endocardial cells did not present any significant autoradiographic signal (Fig. 5, A and B). In the myocardium, LPL expression was detected in myocytes and associated with cytoplasmic areas surrounding the nucleus (Fig. 5, A–D). No significant signal could be detected in endothelial cells either in capillaries (Fig. 5, C and D) or large vessels (Fig. 5, E and F).

Thoracic aorta. Intensive immunoreactivity was present at the endothelial wall of the thoracic aorta (Fig. 6,

A and B). There was also reaction over cells of the smooth muscle layer (Fig. 6C). This immunofluorescence had a clustered appearance, suggesting that the protein could be inside transport vesicles. In situ hybridization experiments with serial sections showed significant presence of LPL mRNA in smooth muscle cells, but in a clearly lower abundance than in adjacent cardiac muscle cells (Fig. 6, D and E). There was no significant hybridization in endothelial cells (Fig. 6, F and G).

Diaphragm and skeletal muscle. Immunofluorescence in diaphragm muscle (Fig. 7) showed a pattern similar to that in heart ventricles. Intensive label was detected in the periphery of muscle cells (Fig. 7, B–D). This agrees with the results obtained by in situ hybridization (not shown), which gave positive hybridization over muscle cells, indicating that this cell type is the principal source

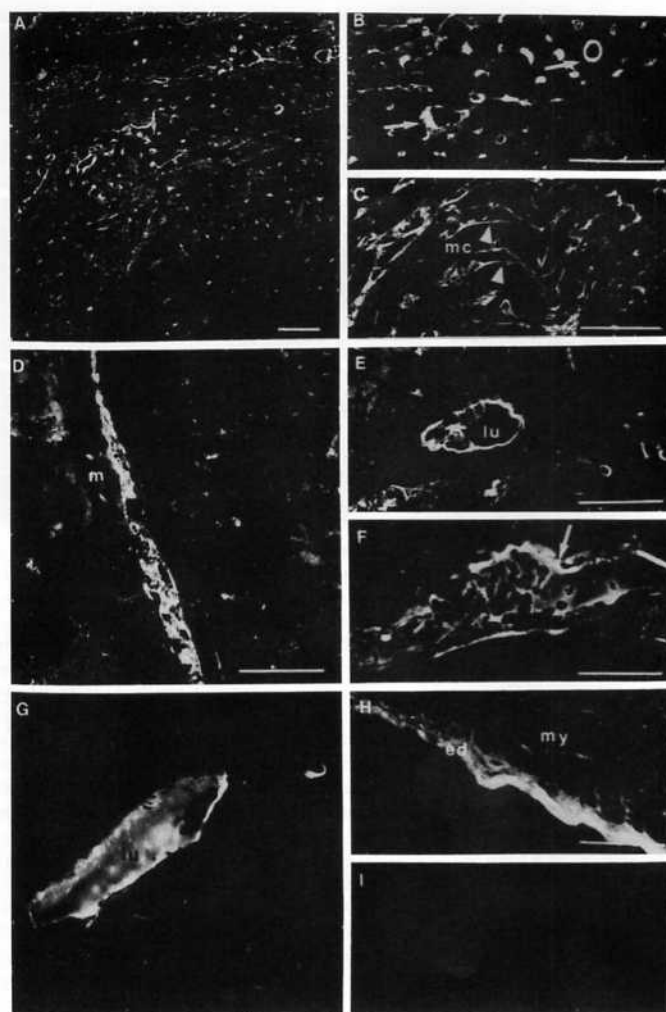


FIG. 4. Distribution of LPL immunoreactivity in heart. A: general pattern of immunoreactivity in myocardium. B: positive fluorescence in endothelial cells of capillaries (arrows). C: inside muscle cells (mc) immunoreactivity is higher in the cytoplasmic periphery (arrowhead). D and E: tangential (D) and cross section of an arteriole from myocardium with fluorescence signal in the lumen associated with the endothelial wall (arrow) and absence in the media layer (m). F and G: section of venules with immunoreaction in the endothelial wall (arrow) and lumen (lu). H: high positive immunoreaction is detected in the endocardium (ed) (my, myocardium). I: heart section incubated with preimmune serum and photographed at same exposure time as sections incubated with LPL antiserum. No reaction is seen. Bars, 50 μ m.

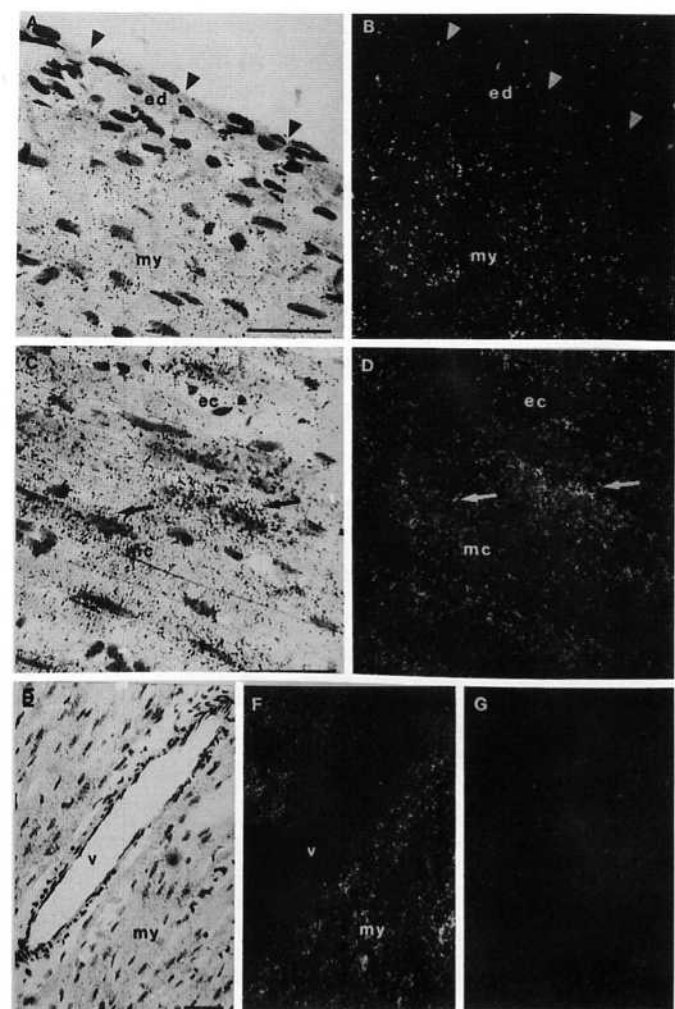


FIG. 5. Distribution of LPL mRNA in heart. A, C, and D: bright-field photomicrographs of same fields shown in B, D, and F, respectively, which were photographed under dark ground illumination. A–F: autoradiographs of sections hybridized with anti-sense RNA probe. G: autoradiography of a corresponding section hybridized with sense RNA probe. A and B: distribution of LPL mRNA in endocardium (ed) and adjacent myocardium (my). Note the high concentration of autoradiographic signal in the myocardium while label was absent from the endocardium (arrowheads). C and D: positive signal (arrow) in myocardium is over muscle cells (mc) but not in endothelial cells (ec). E and F: section of a venule (v) that shows no reaction to anti-sense RNA probe (my, myocardium). Bars, 50 μ m.

for diaphragm LPL. There was intense immunoreactive material in blood vessels, both in capillaries (Fig. 7, A and E) and at the endothelial walls of arteries (Fig. 7, F–H and L) and veins (Fig. 7, A and I). In addition, there was immunoreaction in the lumen of vessels which was not cell associated. This was seen in the lumen of arteries (Fig. 7H) and more prominently in the lumen of veins (Fig. 7, J and K). There was also immunoreaction in the connective tissue surrounding the muscle (Fig. 7M).

Similar patterns of immunofluorescence and in situ hybridization were seen in skeletal muscle (not shown).

Mammary gland. Cryosections of lactating mammary glands gave intensive immunostaining with the anti-LPL serum at several sites in the tissue (Fig. 8). Most of the fluorescence was in the cytoplasm of epithelial cells of milk-producing alveoli (Fig. 8, A, B, and E), mainly in

the apical part of the cell, but also in the basal cytoplasm (Fig. 8B). There was also labeling in the lumen of alveoli, mainly associated with lipid droplets (Fig. 8D). Immunoreactive material was detected in endothelial cells of capillaries (Fig. 8, B and C), arteries, and veins (not shown), but the intensity was lower than over alveoli. In alveoli distended by milk, immunoreactivity over epithelial cells was depressed, and unsuckling for 12 h dramatically reduced the label over alveolar epithelial and vascular endothelial cells (Fig. 8, compare F with A). Nevertheless, immunoreactive material was still present in the lumen of alveoli (Fig. 8F) and ducts associated with milk lipid droplets (Fig. 8G).

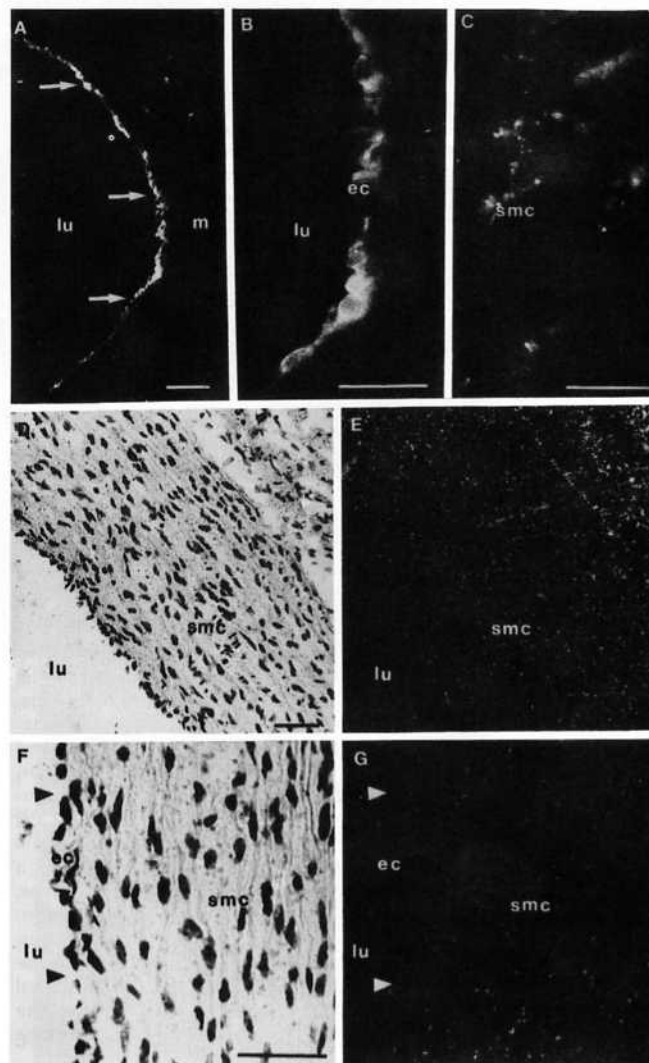


FIG. 6. Distribution of LPL immunoreactivity and mRNA in thoracic aorta. A–C: immunofluorescence results from sections incubated to anti-LPL serum. D–G: autoradiographs of sections hybridized with anti-sense RNA probe. D and F: bright-field photomicrographs of same fields showed in E and G, respectively, which were photographed under dark ground illumination. A: low magnification of cross section of aorta. Positive immunoreactivity is located in the endothelium (arrows) (lu, lumen; m, media layer). B: high magnification of endothelial wall which shows that immunoreactivity is over endothelial cells. C: high magnification of media layer that shows immunofluorescence in smooth muscle cells. D and E: positive signal for LPL mRNA is located over smooth muscle cells (smc) (lu, lumen). F and G: high magnification of endothelial wall. Note that label (arrowheads) over endothelial cells (ec) is not significantly above background. Bars, 50 μ m.

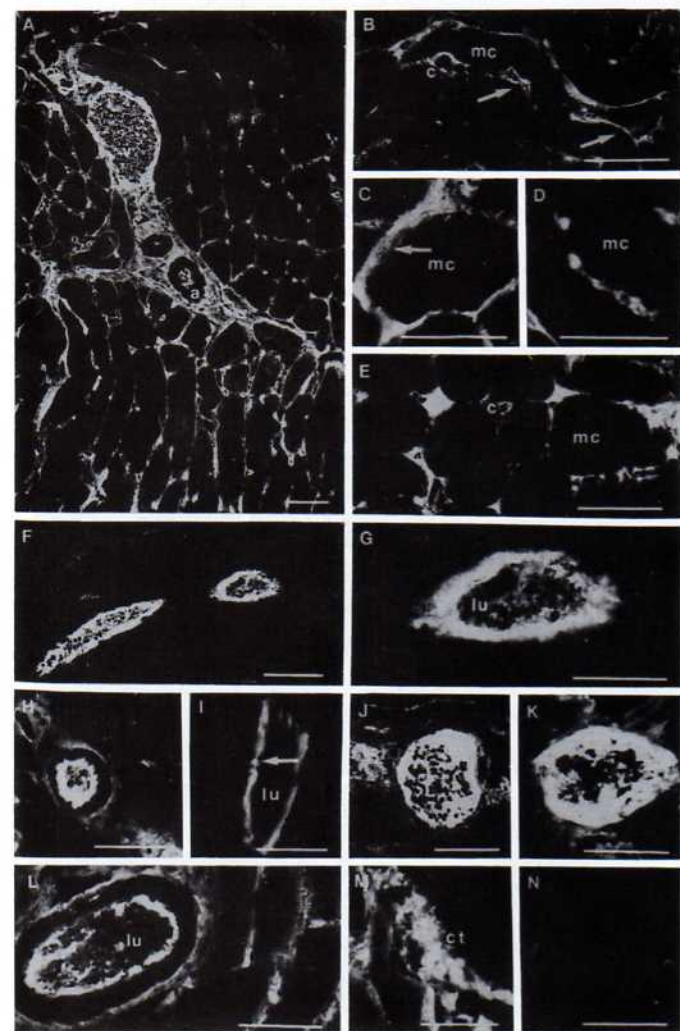


FIG. 7. Distribution of LPL immunoreactivity in diaphragm muscle. A: general pattern of LPL immunoreactivity in diaphragm muscle. Some diffuse immunofluorescence is over connective tissue that surrounds vessels (a, arteriole; v, venule). B-E: cytoplasmic signal (arrows) is located at periphery of muscle cells (mc). This could be seen in longitudinal (B and D) and cross sections (C and E). High immunoreactivity is also present in capillaries (c) (B and E). F and G: LPL immunoreactivity is present in the lumen (lu) of arterioles (F). This label is quite diffuse and is not cell associated (G). H and L: high immunostaining is also associated with endothelial walls of arterioles. I-K: venules also have positive immunostaining (arrow) in their endothelial walls (I). Luminal label is somewhat higher than present in arterioles (J and K). M: positive immunoreaction is also present in the connective tissue (ct) that envelops the muscle. N: in sections incubated with preimmune serum and photographed at the same exposure time as sections incubated with LPL antiserum, no specific label could be seen. Bars, 50 μ m.

The pattern obtained by in situ hybridization (Fig. 9) revealed that expression of the LPL gene in mammary gland takes place in alveolar epithelial cells (Fig. 9, A, B, F, and G). No significant signal was detected over other cell types of mammary alveoli (Fig. 9, D and E).

Kidney. In the kidney there was immunofluorescence over blood vessels but little or no signal over parenchymal cells. In the cortical areas the immunoreactivity was related mainly to the glomeruli (Fig. 10, A and B) and to the walls of interlobular arteries and veins (Fig. 10, D and E). In addition, peritubular capillaries also showed

some immunoreactive material in their walls (Fig. 10C). No immunofluorescence was detected in epithelial cells of tubules. In medullar areas, the immunofluorescence was seen only in capillary vessels of the vasa recta (Fig. 10F). In situ hybridization experiments gave no positive signal for the LPL probe (not shown).

These findings suggested that LPL in kidney was present mainly at the endothelial surface of blood vessels and was not synthesized to any major extent within the kidney. To test this hypothesis one kidney was perfused with heparin. There was a dramatic decrease in immunofluorescence in all types of blood vessels, as illustrated in Fig. 11 for glomeruli (compare B with A) and arteries (compare D with C).

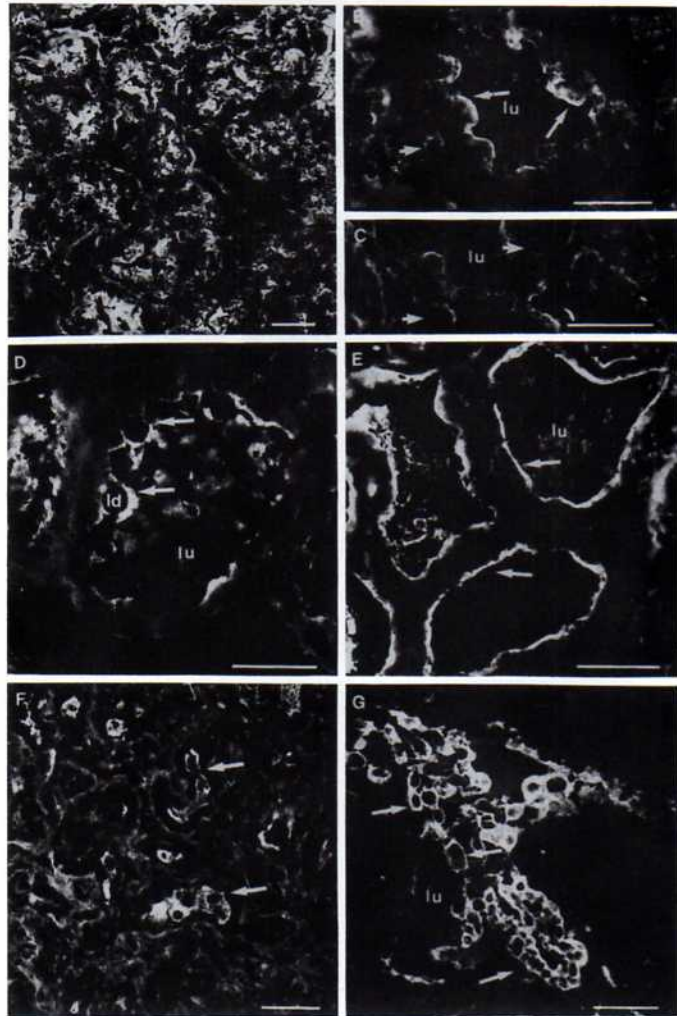


FIG. 8. Distribution of LPL immunoreactivity in suckling (A-E) and unsuckling (F and G) mammary glands. A: intense immunostaining in active alveoli of suckling gland. B, C, and E: high magnification of alveoli shows that immunoreactivity is intense in apical part of epithelial cells (B) (large arrows). Some weak signal (short arrows) is also present in capillaries (B and C) (lu, lumen). D: in secreting epithelial cells immunoreactivity (large arrows) is associated with lipid droplets (ld). F: unsuckling for 12 h reduces size of mammary alveoli as well as LPL immunoreactivity in epithelial cells. Some diffuse immunofluorescence could be seen in the lumen of alveoli (arrows). G: in the lu of mammary ducts the remaining milk presents positive immunostaining associated with fat globules (arrows). Bars, 50 μ m.

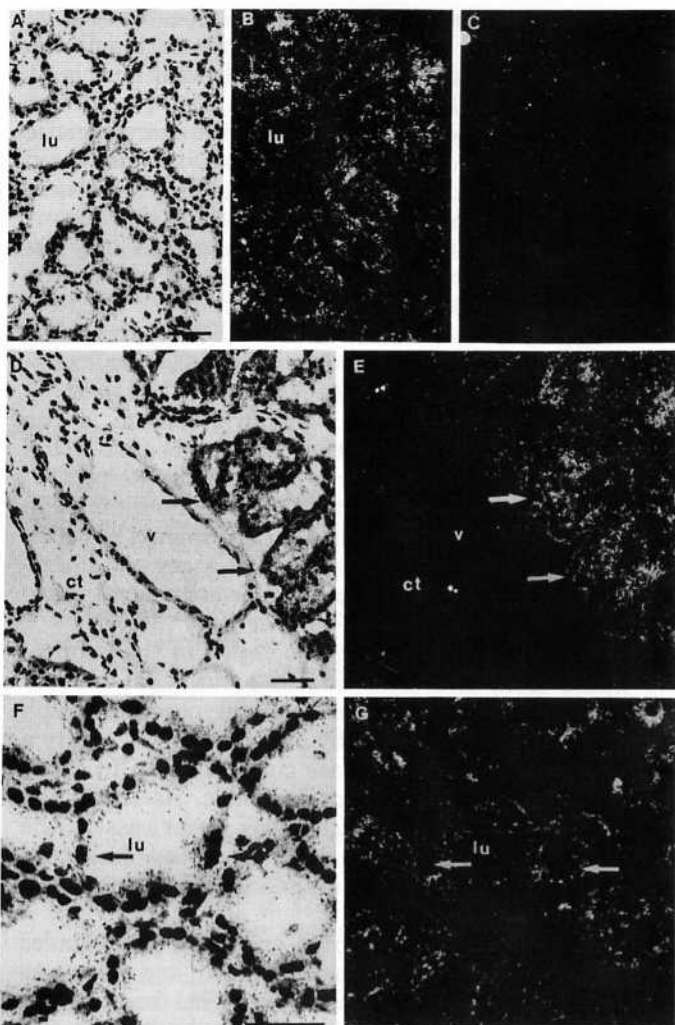


FIG. 9. Distribution of LPL mRNA in mammary gland. *A*, *D*, and *F*: bright-field photomicrographs of the same fields shown in *B*, *E*, and *G*, respectively, which were photographed under dark ground illumination. *A* and *B* and *D*–*G*: autoradiographs of sections hybridized with anti-sense RNA probe. *C*: autoradiograph of a corresponding section hybridized with sense RNA probe, as a control of background. *A*–*C*: high intensity of hybridization signal obtained in mammary tissue (*A* and *B*). Signal is clearly over background (*C*). *D* and *E*: presence of LPL mRNA is exclusively located over mammary alveoli (arrows), whereas vessels (*v*) and adjacent connective tissue (*ct*) are negative to LPL probe. *F* and *G*: high magnification of active alveoli that shows presence of positive signal over epithelial cells (arrows). Bars, 50 μ m.

DISCUSSION

Cellular sites of LPL synthesis. The in situ hybridization experiments indicate that in the major producing tissues LPL is made by the predominating cells. This is further supported by the finding that there was immunofluorescence over these cells.

There are three examples of this in the present study: adipocytes in white and brown adipose tissue, muscular cells in heart and skeletal muscle, and epithelial cells in the lactating mammary gland. Previous studies have established that in these tissues the production and turnover of LPL is regulated in relation to substrate needs (3, 11, 29). This makes it logical that LPL is made in the predominant parenchymal cell type, since this is where regulation of other aspects of energy metabolism

is exerted, e.g., free fatty acid mobilization and utilization, glucose uptake, and metabolism.

There is ample biochemical evidence that adipocytes express LPL (11). In fact, LPL appears to be an early marker of adipocyte differentiation (36). As far as we could discern there was positive hybridization over all mature adipocytes. In addition, there was reaction both by immunofluorescence and by hybridization over cells which had few or no fat droplets. These cells could represent early stages of adipocyte differentiation, in accord with previous demonstrations that LPL is expressed not only in fat-filled cells but also in preadipocytes (36).

Blanchette-Mackie et al. (2) have recently visualized LPL within endoplasmic reticulum, Golgi, and transport vesicles of heart myocytes, providing strong evidence that the enzyme is made there. There is also biochemical

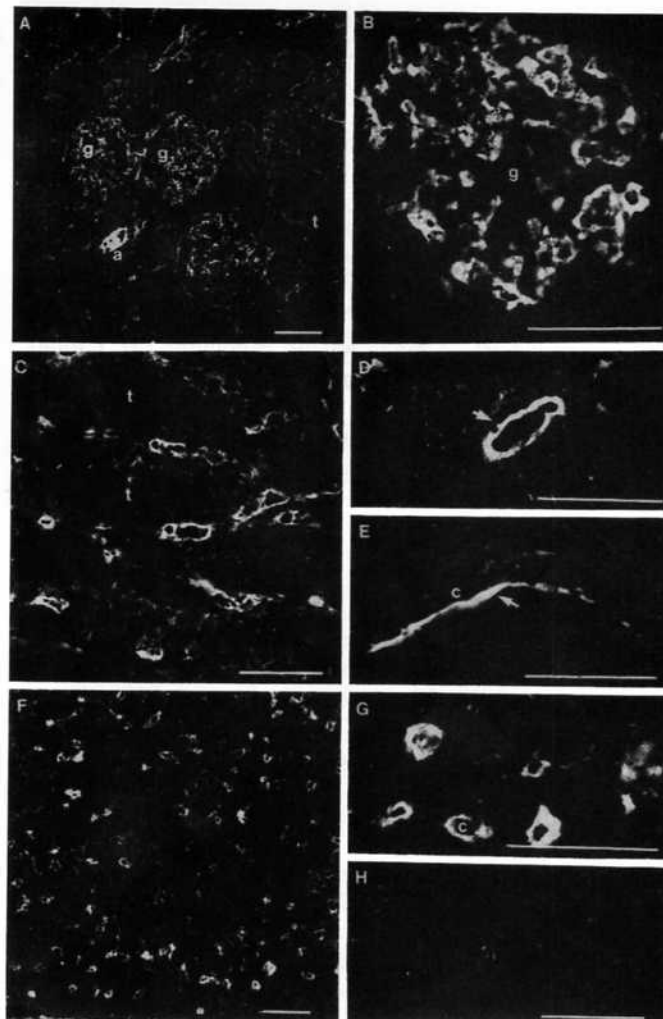


FIG. 10. Distribution of LPL immunoreactivity in kidney. *A*: low magnification of cortex area. Immunoreactivity is present in glomeruli (*g*) and arteries (*a*) but not in tubules (*t*). *B*: high magnification of renal corpuscle that shows positive signal located to capillary network. *C*: positive immunostaining in endothelium of peritubular capillaries. *D* and *E*: high magnification of positive (arrows) immunoreaction in endothelial walls of an arteriole (*D*) and peritubular capillary (*c*) (*E*). *F* and *G*: low (*F*) and high (*G*) magnification of positive immunostaining in medullary areas located by walls and lumen of capillaries. *H*: control section incubated with preimmune serum and photographed at same exposure time as sections incubated with LPL antiserum. Bars, 50 μ m.

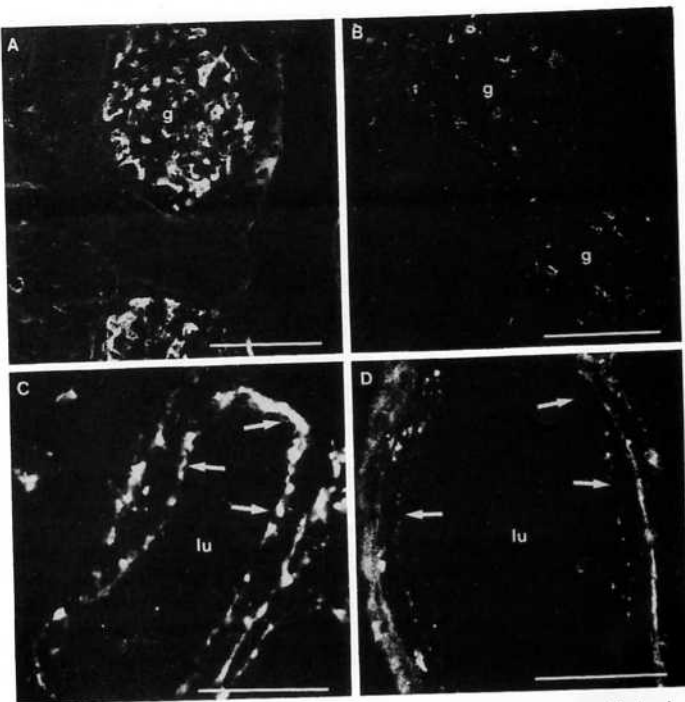


FIG. 11. Effects of heparin perfusion on LPL immunoreactivity in kidney. A and C: sections from control kidney. B and D: heparin-perfused kidney. Heparin perfusion decreases LPL immunoreaction in glomeruli (g) (B compared with A) and in arteries (arrows) (D compared with C) (lu, lumen). Bars, 50 μ m.

evidence that LPL is made within myocytes (3). Chajek et al. (6) have, however, concluded on the basis of cell culture experiments that the main source of LPL within heart is a nonbeating cell population (6). It is not clear what cell type this corresponds to in the living heart. In our study hybridization for LPL mRNA was over muscle cells both in the heart and in diaphragm and skeletal muscle, indicating that these cells are the main source of LPL.

In mammary tissue hybridization for LPL was over the milk-producing cells. Distension of the alveolus leads to diminished rates of milk production as seen when the dam is removed. In the present study unsuckling for 12 h led to a near total loss of positive hybridization and intracellular immunofluorescence, in accord with previous studies on LPL activity (29). Our results indicate that the rapid downregulation of LPL occurs at the level of gene expression.

Distribution of LPL within the tissues. There was no hybridization for LPL mRNA over endothelial cells in any of the tissues studied. This is in accord with previous reports that cultured endothelial cells do not produce LPL (20). The implication is that endothelial LPL originates in parenchymal cells. Recently Blanchette-Mackie et al. (2) demonstrated by immunoelectron microscopy that in mouse hearts LPL moves along cell surfaces, from synthesis in myocytes to the vascular endothelium. Our data are in general accord with this. There was immunofluorescence over vascular surfaces, as well as over cells which, according to the hybridization data, produce the lipase, e.g., adipocytes and myocytes. The distribution of the enzyme was, however, not confined to these sites. There was diffuse immunofluorescence also

over connective tissue, as has previously been noted by Jonasson et al. (18). This was most prominent in adipose tissue but was also seen in heart and skeletal muscle. It was not seen in kidney. This suggests that the diffuse labeling over connective tissue may be a characteristic of tissues where much LPL is synthesized and delivered to the vascular endothelium (1). LPL can bind to a variety of polyanions (24). Therefore, some of the enzyme molecules released from lipase-producing cells may associate with negatively charged structures within the connective tissue.

Presence of LPL in blood vessels. There was LPL immunoreactivity in all the vessels studied, even in tissues where *in situ* hybridization indicated little or no synthesis of the enzyme, e.g., within glomeruli in the kidney. This is in accord with the facts that 1) endothelial cells throughout the body carry heparan sulfate proteoglycans (7), 2) there is a low but definite amount of LPL in the circulating blood (24), and 3) experiments in which labeled LPL was injected have shown a general distribution of the label, with no particular concentration to tissues with high endogenous LPL activity (35).

There must be continuous dissociation of the lipoprotein lipase from and rebinding to endothelial binding sites (1). Therefore, the enzyme will chromatograph along the vessel wall carried by blood. This will create a gradient with the highest lipase concentration in capillaries adjacent to lipase-producing cells. As the lipase emerges into venules and successively larger vessels, the distribution will increasingly favor the blood. In accord with this we saw strong immunoreaction in venous vessels in several tissues.

The immunofluorescence over arterioles and venules was intense in most of the tissues studied, and there was LPL also in large vessels, as exemplified here by the aorta. Significant *in situ* hybridization, and immunofluorescence, was obtained for scattered cells in the media layer of the aorta, which probably corresponds to smooth muscle cells, but not over cells in smaller vessels. Our results coincide with immunofluorescence results of Jonasson et al. (17) who suggested that smooth muscle cells in arteries produce LPL. The reaction was scattered and at considerable distance from the endothelium. It is not clear how enzyme made in these cells could transfer to the endothelium. It seems more likely that most of the endothelial labeling was due to enzyme taken up from blood.

Some tissues take up LPL from blood. In the kidney LPL synthesis was low; *in situ* hybridization showed little or no reaction, and there was very little immunofluorescence over parenchymal cells. Nevertheless, there was rather intense staining over some vessels in the tissue, notably over the glomeruli. It seems likely that this is largely LPL picked up from blood. This agrees with the known high concentration of heparan sulfate at the glomerular endothelium (13). In further support, perfusion with heparin removed almost all the immunoreactive material, showing that it was indeed located at the luminal surface of the vascular endothelium.

LPL is secreted into milk. In the lactating mammary gland LPL immunofluorescence was most intense over the apical cytoplasm of milk-producing cells and over

milk in alveoli and ducts. The labeling over capillaries was less intense. This is in contrast to most other tissues and is also in apparent conflict with the view that the main purpose of mammary synthesis of LPL is to deliver the enzyme to the vascular endothelium to effect uptake of blood lipids for synthesis of milk lipids (29). Previous studies have, however, shown that in guinea pigs LPL activity in milk is as high or higher than in the gland (29). Semb and Olivecrona (31) recently found that the rate of LPL synthesis in mammary tissue corresponds roughly to the proportion of LPL among milk proteins. They concluded that LPL is released into milk as efficiently as other milk proteins are. The function of LPL in milk is, however, an unresolved question.

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