Biosynthesis of Lipoprotein Lipase in Cultured Mouse Adipocytes

I. CHARACTERIZATION OF A SPECIFIC ANTIBODY AND RELATIONSHIPS BETWEEN THE INTRACELLULAR AND SECRETED POOLS OF THE ENZYME*

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Polyclonal antibodies have been raised in rabbits against homogeneous lipoprotein lipase (LPL) purified from the media of adipose 3T3-F442A cells. The antibody is able to inhibit the apolipoprotein C-II-dependent activity of LPL, to immunoprecipitate LPL under non-denaturing conditions from media and cellular extracts. A dot-blot immunosassay of secreted LPL is also described (range 0.1–0.7 milliunits). The secretion potential, μ, taken as the ratio of total releasable activity or antigen to initial cellular activity or antigen, was determined. This was shown in cells treated with heparin and cycloheximide to be equal to 1 for LPL antigen but significantly greater than 1 for LPL activity assayed under standard conditions. No LPL was actually degraded within the cells. A dramatic enhancement of the intracellular activity was induced by a mere dilution of detergent-treated cell lysates with no change in LPL antigen. The total intracellular activity reached a plateau at a value which now became identical to that obtained in the medium of cells exposed to heparin and cycloheximide. The existence of an inhibitor of LPL activity has been excluded as well as that of an increase in the catalytic activity of LPL during its secretion, before or after exposure to heparin. Our results indicate a systematic underestimation of LPL intracellular activity and suggest that LPL is present within intracellular cisternae in a cryptic state. This potential activity can be fully unmasked in vitro. In agreement with other data (Vannier, C., and Ailhaud, G., (1989) J. Biol. Chem. 264, 13206–13216), our results appear to exclude the existence of a reservoir of catalytically inactive LPL molecules within adipose cells.

Lipoprotein lipase (LPL), synthesized in parenchymal cells of tissues of mesodermal origin, is the key enzyme responsible for the hydrolysis of plasma triglycerides from apolipoprotein C-II-containing lipoproteins at the capillary endothelium (1). It has long been known that rapid regulatory tissue-specific changes in its activity occur during fasting and refeeding, illustrating its inverse and coordinate expression in muscle and adipose tissue. This regulation allows fatty acids to be directed according to the metabolic requirements of the parenchymal cells (1–9). It is well established that, in response to nutritional changes, LPL activity at the endothelium can vary dramatically (4–6), whereas it is not greatly changed in adipocytes (1, 7).

Numerous studies performed on intact fat pads as well as on isolated adipocytes and cells of preadipocyte clonal lines have documented the role of hormones and other factors in the control of LPL synthesis and expression in these cells (8). In that respect, insulin and corticosteroids are now recognized to stimulate the synthesis of the enzyme (9–11), whereas catecholamines seem to be involved, as are other lipolytic agents, in the acceleration of its degradative process (12, 13). These various hormones could thus directly or indirectly control the movement of LPL between adipocytes and endothelial cells. However, the mechanisms whereby these events take place have received less attention. With few exceptions (14, 15), adipose cells exhibit a slow constitutive secretion of active LPL. Heparin is known to stimulate the secretion of active LPL without affecting its cellular activity (12, 16, 17), and this stimulation can also occur in the absence of protein synthesis; so far, heparin remains the most powerful effector of this process. It has been hypothesized that, independently of protein synthesis, a hormone-stimulated intracellular translocation of LPL, leading in turn to its secretion, is accompanied by an increase in its catalytic activity (7, 8, 18, 19). In adipocytes, Robinson et al. (10) have suggested that LPL exists before secretion partly as an inactive precursor. This putative precursor pool or storage form of inactive LPL molecules has not yet been characterized.

Our previous studies have shown that the effect of heparin on LPL secretion from differentiated Ob17 adipose cells, among the few which do not exhibit a constitutive enzyme secretion, was to induce a rapid mobilization of an intracellular store of active molecules (9, 20). In full agreement with other data from subcellular fractionation and immunotitration studies (21), we have provided compelling morphological and biochemical evidences for LPL being mainly localized in the Golgi apparatus (20, 22), thus establishing its status as a secretory protein in the adipocyte. These investigations in Ob17 cells have also shown that LPL acquired its enzymatically active conformation in the proximal cisternae of the Golgi apparatus (20) and that no further increase in its catalytic activity occurred during secretion.

The aim of the present and accompanying papers (23) was to delineate more precisely the relationships between catalytic
activity and protein content during the export of LPL, using a combination of immunological and biochemical approaches. These studies have been carried out with Ob17SA16 and 3T3-F442A adipose cells which display both a constitutive and a heparin-stimulated secretion. In this first paper, we report the characterization of a rabbit antibody raised against mouse LPL and its use in immunoassays of the cellular and secreted enzyme. It is shown that no inactive precursor is present within the cells but instead it is proposed that active LPL molecules are stored as a pool whose activity is masked upon condensation. In the second paper, the main features of the biosynthesis and intracellular processing of LPL, as well as its oligomerization, are reported. The existence of a regulated pathway for LPL secretion in adipocytes is documented.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

**Lipoprotein Lipase Activity and Lipoprotein Lipase Antigen in Adipose Cells and in Medium**—Our previous investigations on LPL secretion had been focused on the determination of the true LPL activity releasable under heparin stimulation by Ob17 cells (15, 20). This activity was accurately measured only by using the continuous flow technique by means of the combined knowledge of the secretion rate and the rate constant of enzyme inactivation (15). A typical time course of LPL secretion from Ob17SA16 cells is shown in Fig. 3a. The first phase of secretion, which terminated 40–45 min after heparin addition, was independent of protein synthesis as it was not altered by cycloheximide. This phase thus corresponds to the secretion of a preexisting pool of intracellular molecules and occurred at a higher rate than that of newly synthesized molecules which are released during the second phase of secretion. The curves indicate that the constitutive secretion of LPL occurred at a constant rate which was similar to that found for the release of newly synthesized enzyme molecules in cells exposed to heparin. The conditions of Fig. 3a allow the comparison of the actual sizes of the initial cellular and releasable pools of LPL activity. It was found that the secretion potential μ (see Miniprint, Table I), measured in the absence of protein synthesis and under heparin stimulation was higher than 1. Therefore, two hypotheses could be proposed to explain this increase of LPL activity upon secretion. First, some cellular or operational factor(s) may have led to an underestimation of LPL intracellular activity. Second, a large majority of the intracellular LPL molecules might be enzymatically inactive but were activated upon secretion.

The first hypothesis was excluded because cell lysates, depleted of LPL by affinity chromatography, were unable to inhibit the activity of the purified enzyme. Additional control experiments excluded also the possibility of lipids and other intracellular components as well as Triton X-114 molecules interfering with LPL assays (not shown). Moreover, the linearity of LPL assays is maintained for activities up to 20–25 milliunits/ml, corresponding to the release of 12–15% of the total fatty acid chains. Because the amounts of LPL protein in the cell lysates and in the secretion media were adjusted to be similar and because the activities of LPL determined in the secretion media were below 20–25 milliunits/ml, under-

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Fig. 3. Kinetics of constitutive and heparin-stimulated secretions in Ob17SA16 and 3T3-F442A cells. Secretion of LPL was studied in differentiation medium containing 7.5% serum using the continuous flow technique (see “Experimental Procedures”). a, kinetics of LPL secretion by Ob17SA16 cells in the absence (Δ, Δ) or the presence (○, ○) of 3 μg/ml heparin, without (Δ, ○) or with (Δ, ○) 15 μM cycloheximide. Plotted are the integral curves (f(t) dt), calculated from the secretion rates obtained by assaying the enzymatic activity secreted every 5 min (dU/dt), estimated of LPL activity due to a lack of substrate could be also excluded.

However, when cell lysates were diluted above the standard 2-fold dilution routinely used, the total activity of LPL increased in parallel and as a function of the dilution factor. At final dilutions ranging from 32 to 128, a plateau was reached. This plateau gave the true intracellular LPL activity as its value (determined before heparin addition) was equal to that found for the total heparin-releasable LPL activity (Fig. 3b and inset). Thus, as determined for LPL antigen, the secretion potential μ determined for activity had now become equal to 1. The fact that this value was simply reached upon dilution of cell lysates prepared in detergent-containing buffer, rather than supporting the hypothesis of a precursor of LPL being activated upon secretion, argued for a systematic underestimation of the cellular activity.

In order to support this tentative conclusion, further experiments were carried out using the solubilized membrane fraction, of the post-nuclear supernatant obtained from cell homogenates of 3T3-F442A cells (Fig. 4) because (i) this fraction contains more than 90% of the cellular activity in Ob17 cells (22), and (ii) immunoprecipitation assays indicated that it contained the total antigen pool of 3T3-F442A cells (not shown). As indicated by the results of Fig. 4a, very low LPL activity was detected in the concentrated membrane lysate. This activity was dramatically increased upon dilution. A plateau was obtained, corresponding to a 50-fold increase in the total activity when compared to the initial value obtained at the standard dilution. This increase could vary from experiment to experiment according to the activities of LPL determined before dilution and which were within the limits of sensitivity of the assay. When heparin was added to the dilution buffer at a concentration of 3 μg/ml (maximal heparin concentration used in secretion media and in experiment of Fig. 3b), a further 2-fold increase in the plateau value was observed. This increase remained unchanged with higher concentrations of heparin (Fig. 4a). The effect of heparin was not specific since it could be mimicked by adding heat-inactivated LPL (Fig. 4a). Additional experiments showed that (i) the higher the LPL concentration in the lysate, the higher was
the increase due to the addition of heparin or heat-inactivated LPL and (ii) the higher the LPL concentration in the cell lysate, the higher was the half-maximally effective concentration of heparin. The same phenomenon of “masked” intracellular activity is further illustrated in the experiment of Fig. 4b which shows clearly a 300-fold increase in the LPL activity after a 64-fold dilution of the cell lysate whereas extrapolation of the curve indicates that no LPL activity should be detectable in the absence of dilution, i.e. before homogenization, that is to say intact cells. The results of Fig. 4b also clearly indicate that no effect of dilution was observed on the total activity of the secreted enzyme. For LPL concentrations in the range of 0.1–6.5 milliunits/ml, not surprisingly because determinations were performed in the linear part of the assay curve (see above), the measured activity is a hyperbolic function of the dilution factor. By contrast, at low dilutions a bell-shaped curve, instead of a plateau, is obtained for the cellular activity (Fig. 4b, inset).

Secretion of Lipoprotein Lipase in the Presence or Absence of Heparin—A dot-blot immunoassay was used to study the kinetics of LPL secretion carried out in the absence and then in the presence of heparin (Fig. 5). This was performed using the continuous flow technique, by taking advantage of the irreversible adsorption of LPL to nitrocellulose in the absence or in the presence of very low concentrations of contaminant proteins, which are conditions occurring in ITT medium (see “Experimental Procedures”). The integral secretion curves show that, before and after heparin addition, there was a parallel increase in both enzyme activity and titratable antigen illustrated by the dramatic increase in the secretion rates of both parameters (Fig. 5, inset). For the fractions collected from 0 to 90 min a linear correlation (r = 0.97) was obtained for the two parameters. Whether or not heparin was present, the specific activity of the secreted LPL was found to be 0.71 ± 0.04 absorbance unit/millunit, in agreement with the values reported in Fig. 2. It must be stressed that the experiment of Fig. 5 failed to detect any change in the molecular activity of LPL. This result demonstrates unambiguously that LPL did not become more active during secretion into the medium in the presence of heparin.

**DISCUSSION**

The elucidation of the mechanisms involved in the synthesis and secretion of functional LPL at the molecular level was facilitated by the preparation of an antibody specific to the mouse enzyme permitting the use of a homologous antigen-antibody interaction. Changes in the catalytic properties of LPL during its processing can only be interpreted accurately by means of an antibody allowing access to the antigenic structure specific to inactive and/or precursor forms. This goal has been reached in the present work, which represents the first successful attempt to immunize a rabbit against mouse LPL. Owing to its monospecificity and high titre, this antibody, which interacts specifically with inactive and/or precursor forms, has proven to be suitable for immunoprecipitation, immunotitration and immunoblotting experiments (see Ref. 23).

So far, a few polyclonal antibodies have been successfully raised against LPL from rodents. The anti-rat LPL antisera obtained by Jansen et al. (35) and Etienne et al. (36) have allowed us in previous studies to delineate the induction, the intracellular activation and the localization of LPL in mouse adipose Ob17 cells (9, 20, 22), since these anti-rat LPL antisera raised in goats were able, via a cross-reaction with the mouse enzyme, to inhibit the enzymatic activity and to recognize LPL antigenic sites in formaldehyde-fixed cells. Unfortunately, these antibodies proved not to be suitable for the immunological studies reported herein and performed with mouse Ob17SA16 and 3T3-F442A cells. On the other hand, Al-Jafari and Cryer (14, 21) have recently used antisera directed against rat heart LPL, raised in chicken or guinea
pig, to develop an immunoassay for LPL in rat adipocytes. Recently, Schotz and colleagues (37) also reported an enzyme-linked immunosorbent assay for rat LPL using an antibody raised in chicken against the bovine enzyme. Other antisera have been prepared by Olivecrona and colleagues by immunizing rabbits and hens with bovine milk LPL (38), and goats with guinea pig milk LPL (39). The cross-reactivity displayed by these antibodies with mouse LPL, checked by their ability to inhibit the enzyme activity, did not seem to have an evolutionary basis. In one of these cases only, advantage was taken of the inhibitory effect of the antiserum to detect LPL in mouse adipose cells by immunoprecipitation (34,40).

We have reinvestigated in the present study the quantitative relationships between the intracellular and the releasable pools of LPL antigen and LPL activity by determining their respective size in mouse adipose cells. Two questions were posed: (i) are some inactive LPL molecules present within the cells? (ii) do changes in the catalytic activity of the enzyme occur upon secretion?

The immunoprecipitation assay has allowed us to determine the actual amounts of LPL involved in the constitutive and the heparin-stimulated secretions exhibited by 3T3-F442A and Ob17SA16 cells. At the steady state, e.g. in the absence of heparin, only part of the protein pool is exported through constitutive secretion. Remarkably, when heparin-stimulated secretion occurred, the entire pool of preexisting molecules was released into the extracellular medium. The fact that this release remained possible even in the absence of protein synthesis (µ = 1, see Miniprint, Table I) is not surprising but strengthens the idea that, under appropriate stimulation by a secretagogue, adipocytes become able to secrete their entire LPL content in vivo. Moreover, no change in the specific activity of LPL was observed whether or not the enzyme secretion was constitutive or heparin-stimulated (Fig. 5). In that respect, immunoprecipitation data (see Miniprint, Table I) have been confirmed by the dot-blot immunoassay of LPL (Fig. 5).

The most striking observation, with regard to intracellular LPL activity, remains the discrepancy between the values of the secretion potential µ measured by the enzymatic assay and the immunoprecipitation assay. When intracellular activity was assayed using cell lysates containing LPL at a concentration near or equal to that found in the secretion media where the enzyme amount is accurately determined, the secretion potential appeared to be largely over-estimated (µ > 1). Until now such an observation, e.g. a secretion-coupled increase in enzymatic activity in the absence of protein synthesis, has represented the main argument in favor of the existence of an inactive precursor of LPL within the cells (10). Among hypotheses, the inactive form of LPL could be represented by that transiently present in the endoplasmatic reticulum of Ob17 cells (20). Alternatively, the inactive forms of LPL described in 3T3-L1 cells by Olivecrona et al. (34) could also be candidates. However, because of the protocol used by these latter authors to extract and concentrate LPL from acetone-ether powders, it is not possible to decide whether these inactive molecules actually represented an inactive precursor form of LPL. Our results presented above and elsewhere (23) show that, in the presence of heparin and in the absence of protein synthesis, no enzyme degradation takes place; they also show that the enzyme activity being released is identical to that determined within the cells providing that the cell lysates are extensively diluted (Figs. 3 and 4).

Our findings indicate also that the maximal increase in LPL activity requires the synergistic effects of dilution and heparin addition. The structure in which potentially active LPL molecules are present remains unclear. However, two types of interactions could be responsible for the observed cryptic character of the intracellular LPL activity. We envision that in situ, a first interaction would take place between LPL and some intracellular molecules, giving rise to a specific complex (first level of interaction), and that a second interaction would take place between these complexes, giving rise to a network (second level of interaction). If this were so, one could postulate that (i) by mere dilution which lowers the concentration of the network components, the second interaction would be disrupted, leading in turn to a partial access of LPL molecules to the substrate; (ii) the first interaction would then be disrupted by displacing LPL from its binding site with either heparin or heat-inactivated LPL.

Some observations are in favor of the hypothesis of LPL being bound to intracellular proteoglycan structures. First, although proteoglycans in mammalian tissues are known to be components of the extracellular matrix, it has been demonstrated that heparan sulfate proteoglycans and sulfated glycosaminoglycans are part of the internal content of secretory organelles (41), including the chromaffin granule (42), cholinergic synaptic vesicles (43), the zymogen granule (44), the prolactin granule (45), and the adrenocorticotropic hormone granule (46). The fact that proteoglycans represent potential binding sites for LPL is well illustrated by the recent work of Klinger et al. (47) who used affinity chromatography on immobilized LPL to purify the heparan sulfate proteoglycans from rat brain. Second, some secretory proteins such as adrenocorticotropic hormone (48), and in a more extreme form represented by the crystalline state of insulin in β granules (49) are stored intracellularly in a condensed state. It remains to be shown that granules containing LPL and proteoglycans are indeed present in adipose cells. If it were so, the condensed state of LPL within the cells would be compatible with the existence of a concentrative sorting process in the regulated secretory pathway (50, 51).

Another consequence of our findings is that the significance of intracellular LPL activity now becomes questionable as this is probably minimal or absent in intact cells. For instance, our previous studies had shown that the catalytically active conformation of LPL was acquired at the time of its emergence in the cis- and/or medial-Golgi cisternae (20); this activity can now be regarded as a mere indicator of the functional maturation of LPL. In other words, apart from the cryptic of LPL within the subcellular compartments which can be unmasked by detergent solubilization (22), an additional level of crypticity appears to be due to the presence of LPL within a network. Thus the intracellular LPL activity so far described is an unavoidable reflection of the true potential value. In vivo, this phenomenon of masking the activity of LPL is advantageous for the cell owing to the phospholipase A1 activity of the enzyme on glycerophospholipids.

In conclusion, the proposal regarding the existence of a putative reservoir of inactive LPL molecules appears to be related to a systematic underestimation of its intracellular activity. In that respect, because active LPL is a homodimer (33), the monomer cannot be regarded as an inactive precursor form as, in vitro, activation by dimerization would not be favored by lowering the protein concentration.

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Intracellular and Secreted Pools of Lipoprotein Lipase

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SUPPLEMENTARY MATERIAL TO
Biogenesis of Lipoprotein Lipase in Cultured Mouse Adipocytes. I. Characterization of a Specific Antibody, Relationships Between the Intracellular and Secreted Pools of the Enzyme.

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EXPERIMENTAL PROCEDURES

Mannitis — Glycerrhizic acid (10, 20 g) was extracted from the Radiochemical Centre (Amersham, Buckinghamshire, U.K.). Pseudo-A. S. Ehlmann was from Pharmacia France S.A. Hepatocult® H4 was from MERCK (Darmest, Germany). Puriﬁcation by organic solvent was performed by chromatography on E. coli (Carss, France) and 3, 4-diaminobenzylidene from Sera Fine Biotechnics (Heidelberg, FRG). Nicotinamide was a product of Bio-Rad Laboratories (Richmond, USA).
Intracellular and Secreted Pools of Lipoprotein Lipase

Secretion of Lipoprotein lipase — Secretion was measured in the absence or in the presence of heparin, either in differentiating medium containing 0.1 or 0.5 mM fetal bovine serum or in ITT medium as indicated. Two protocols were used as already described (13). The first protocol (buffer A containing NaCl and fetal bovine serum) was used for the preparation of LPL samples for analytical chromatography on Hepatitis- Ulpergic or quantitative immunoprecipitation. Cells were washed with DMEM or ITT medium and further incubated in the medium used for secretion for the indicated periods. This medium was then collected and rapidly chilled at 4°C. When it was intended for LPL assays, the secretion medium was diluted 1:5 with physiological buffer (pH 7.4, 1.5 mM glycerol, 1% BSA, 0.3 mM MgCl2) to stabilize the enzymatic activity and maintained at 4°C or stored at -70°C until use. Under these conditions, the full volume of the differentiated medium was 100% recovered per ml of culture. For preparative purposes, the secretion media were stabilized by addition of glycerol to a final concentration of 30-40% (w/v).

The second protocol (continuous flow technique) was used for the accurate determination of the amounts of active LPL, secreted by the cells maintained in a perfusion chamber (15). With this protocol, LPL activity was measured by the clearance of the effluent, which is collected with a flow rate of 0.8 ml/min into the popliteal lymph nodes was filtered through glass fibers to remove any cellular debris and incubated at 4°C for 4 h with 0.05 M Tris-HCl buffer pH 8.0, containing 0.1 M NaCl, 0.02% Tween 20, and 0.001% sodium azide. After heat treatment at 30°C to pellet Triton X-114 (29), the detergent-depleted LPL was further purified by a 2-fold dilution in a medium containing 0.1% Triton X-114 and 0.5% sodium azide. Membrane-bound LPL secreted into the culture medium was then treated with 200 U/ml of heparin-Ultrogel for 2 h. After several washes at 4°C, the proteins were solubilized with 0.5 M NaCl and 0.05% Triton X-114, and washed for 0.5% Triton X-114, and stored at -20°C before analysis.

Membrane-bound LPL was then treated with 200 U/ml of heparin-Ultrogel for 2 h. After several washes at 4°C, the proteins were solubilized with 0.5 M NaCl and 0.05% Triton X-114, and stored at -20°C before analysis.

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Intracellular and Secreted Pools of Lipoprotein Lipase

TABLE I

| Chase | LPL activity | Immunoprecipitable LPL activity | Time | Addition | Cell | Medium | Cell | Medium |
|-------|-------------|-------------------------------|------|----------|------|--------|------|--------|
| none  | 17.5 (100)  | 71.5 (100)                    | 0    | (0)      | 0    | (0)    | 0    | (0)    |
| 90 min| 17.1 (94.8) | 12.0 (170)                    | 90   | (0)      | 0    | (0)    | 0    | (0)    |
| 90 min| 14.8 (80.7) | 103 (803)                     | 90   | (0)      | 0    | (0)    | 0    | (0)    |
| 90 min| 2.3 (151.6)| 19.7 (1152)                   | 90   | (0)      | 0    | (0)    | 0    | (0)    |
| 90 min| 0 (327)     | 87 (410)                      | 90   | (0)      | 0    | (0)    | 0    | (0)    |

Dot-blot immunosassay of secreted LPL — The data of Figure 2, which summarize the characteristics of the assay, illustrate the ability of anti-LPL antiserum to quantitatively assay the antigen. Figure 2a shows a dot-blot obtained with serial dilutions of purified mouse and bovine milk LPL. No or little porcine liver staining was observed when non-immune rabbit serum was used (Fig.2a, lane C). By cutting out and counting the dots containing radioactive antigens, it was first ascertained that the amount of adsorbed material was a linear function of that used for dotting onto the membrane (Fig.2b, left). In the case of mouse LPL, the recovery of the [3H]leucine radioactivity associated with the membrane was 92 ± 4% when compared to that of the initially soluble antigen, over the 0.1 to 3.6 ml range studied. The corresponding figure for bovine LPL, which was diluted such that the response was within the range of the standard curve, was 94 ± 5%. The intensities of the corresponding porcine controls given by the two antigens are shown in Figure 2b (right). The absorption remained a linear function (up to 0.7 optical density units) of the antigen concentration providing that the latter was below 0.9 and 0.2 ml of 200 ml of initial sample for mouse and bovine LPL respectively. The standard titer curve of purified mouse LPL remained unchanged when the antigen was dotted after dilution in IIT medium instead of buffer A containing 150 mM NaCl.

The sensitivity and the specificity of the assay are established by the data of Figure 2c. When the low background was subtracted from the total absorbance measured in the absence of antiserum, the slope of the linear phase of the curve was 0.66 0.048 optical density units/ml of purified LPL in the assay. With LPL secreted by 3T3-F442A cells for 5-10 min in IIT medium, a value of 0.74 ± 0.055 was obtained for the curve slope (not shown). The reasons for this difference remain unclear but could well be related, in view of the instability of the secreted enzyme, to differences in the percentage of inactive forms of LPL in the two samples.

Immunostaining of LPL secreted constitutively and after heparin stimulation — The relationships between the cellular and releasable LPL pools have been investigated in 3T3-F442A cells. The amounts of LPL activity and immunoreactive protein were compared under various conditions (Table I). For that purpose the cells were labeled with 3H-leucine or with 35S-methionine then chased for 90 min with or without heparins, in the presence or absence of cycloheximide. Both the cellular compartment and the secretion medium were assayed for LPL activity and antigen at the end of the labeling and chase periods. The immunoprecipitation assay revealed that LPL protein could be quantitatively secreted by the cells under heparin stimulation whether or not cycloheximide was present (97,6 and 104%, respectively). By contrast, only 20-30% of the LPL protein was released constitutively during the same time, whereas approximately 10% was recovered within the cell (versus 2.5% in the presence of heparins).

At this point, it is necessary to define the secretion potential, α, taken as the ratio of total releasable activity or antigen to initial cellular activity or antigen determined in cells exposed simultaneously to heparin and cycloheximide. Results of Table I indicate that α was equal to 1 for LPL antigen; this means that the cells were able to secrete α unit of their LPL content and that no LPL activity was actually degraded in cells exposed to heparin. A quite different situation emerged for LPL activity since α was equal to 4.1. This result could not be explained by an increase in LPL specific activity since no change was observed for the secreted enzyme activity which remained around 1.12±0.3 ml/55Kd (as calculated from the values obtained in the presence of cycloheximide). In other words this finding excluded the possibility that changes in molecular activity of LPL occurred in the presence of heparins.

This discrepancy between the α values determined for LPL activity and LPL antigen was not unique to 3T3-F442A cells and was also observed in others cell lines and rat uterine vascular cells (52).