The development of lipoprotein lipase has been examined during adipose conversion of preadipocyte Ob17 cells. These cells have been previously shown to differentiate in clusters of fat cells. The lipoprotein lipase activity, which increases 20-50-fold during adipose conversion, is fully inhibited by anti-lipoprotein lipase γ-globulins (directed against the rat heart enzyme) and is heparin-releasable (EC₅₀ 0.15 μg/ml of heparin). Monoacylglycerol lipase activity, already shown to increase in parallel to lipoprotein lipase activity, is not inhibited by anti-lipoprotein lipase antibodies and thus represents a different molecular entity. Identical curves of immunotitration are obtained for the lipoprotein lipase activity present in cell homogenates and for the heparin-releasable activity, indicating no significant difference in antigenicity between the different activities.

Studies by indirect immunofluorescence reveal the absence of lipoprotein lipase in early confluent cells. Immediately thereafter (5-6 days post-confluence) adipose conversion is accompanied by the appearance of the enzyme in cells present in developing fat clusters. A double labeling procedure using fluorescent and rhodamine immunoglobulin conjugates allows a topographical distinction between cell surface and intracellular lipoprotein lipase. Lipoprotein lipase activities are enhanced in confluent cells after chronic exposure to physiological concentrations of insulin and triiodothyronine. Immunotitration experiments, performed at a constant number of enzyme units under each condition, lead to the conclusion that changes in activity correspond to parallel changes in enzyme levels. The results show a direct modulation by both hormones of the enzyme cell content of adipose cells in culture. Immunofluorescence detection of lipoprotein lipase could be a useful tool for in vitro studies on the cellularity of rat and mouse adipose tissue during development.

The hormonal control of lipoprotein lipase (tri-acylglycerol-protein acylhydrolase, EC 3.1.1.34) in white adipose tissue has been well documented (1). Experiments in vivo have shown a strong positive correlation between lipoprotein lipase activity and plasma insulin concentrations (2-4). These data are consistent with those obtained by immunological studies showing a net increase of lipoprotein lipase content in adipose tissue from fed rats as compared to that from fasted rats (5). Furthermore, chronic exposure to insulin has been reported to enhance in vitro the development of lipoprotein lipase activity in differentiating 3T3-L1, 3T3-F442A, and Ob17 cells (6-8), and in differentiating rat adipocyte precursor cells (9). Substitution of insulin by antibodies directed against insulin receptors leads to a similar development of lipoprotein lipase in 3T3-L1 cells (10). The early emergence of lipoprotein lipase activity in Ob17 cells is independent of insulin, but the presence of the hormone is necessary to sustain high activity levels (6,11).

Preadipocyte Ob17 cells have been established from the adipocyte fraction of the epididymal fat pad of the C57BL/6J ob/ob mouse (11). In contrast to 3T3-L1 cells, Ob17 cells present (i) a down-regulation of insulin receptor levels when exposed chronically to insulin (12), and (ii) a response on a long term basis to physiological concentrations of triiodothyronine by increasing the activity levels of fatty acid activating and esterifying enzymes (13).

Immunotitration experiments on Ob17 cells reported below show for the first time that adipose conversion is accompanied by a net increase in lipoprotein lipase cell content. This increase is modulated by physiological concentrations of insulin and triiodothyronine, both hormones which are specifically recognized by surface and nuclear receptors, respectively (12,13). In addition, our studies demonstrate by indirect immunofluorescence experiments that lipoprotein lipase is (i) only present in differentiating cells, and (ii) localized both at the surface and inside the cells.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dulbecco’s modified Eagle’s medium was purchased from Gibco (catalogue number H21), fetal calf serum was a product of Seromed, Munich, Germany. [2-3H]Glycerol monooleate was prepared from [2-3H]glycerol trioleate (Amersham) after hydrolysis by pancreatic lipase and purification by thin layer chromatography. Glycerol tri[9,10-3H]oleate was purchased from Amersham. Conjugated rabbit anti-gest-γ-globulins were products of Cappel Laboratories, Cochranville, PA. Other compounds were obtained as follows: crystalline insulin, heparin, T₃, colcemid, bovine serum albumin (fatty acid poor), Sigma; monoolein and triolein, Bast of Copenhagen, Denmark; Triton X-100 and sodium dodecyl sulfate, Merck.

**Cell Culture**—After seeding 2 × 10⁵ cells (35-mm diameter dish) or 5 × 10⁵ cells (60-mm diameter dish), Ob17 cells were grown to confluence in Dulbecco’s Modified Eagle’s medium supplemented with 10% fetal calf serum, penicillin (200 units/ml), and streptomycin (50 μg/ml). This medium is defined as standard medium. Confluence was usually reached after 5 days in 35-mm diameter dishes (used for immunofluorescence staining and heparin release of

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* The abbreviation used is: T₃, triiodothyronine.
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lipoprotein lipase) as well as in 60-mm diameter dishes (used for immunocytolabeling experiments). Unless otherwise stated, 17 mM insulin and 2 mM T₃ were added to standard medium when cells reached confluence. Media were changed every two days and all experiments were carried out 18-24 h after media changes.

**Enzyme Preparation**—Acetone powder of epididymal fat pad from the C57BL/6J ob/ob (vide infra) was obtained according to Ash et al. (14). Cell-free extracts were obtained as follows. Cells were rinsed twice with ice-cold buffer, pH 7.4, containing 5 mM sodium barbital, 280 mM mannitol, 1.8 mM CaCl₂, 0.5 mM MgCl₂, and 1 mM glycerol. Cells were scraped with a rubber policeman, suspended in the same buffer, and recentrifuged at 50,000 g for 10 min. For the immunocytolabeling of lipoprotein lipase, homogenate was first incubated in the presence of 3 mM sodium dodecyl sulfate for 60 min at 4 °C. After centrifugation (30 min 12,000 × g), the supernatant, containing 90-100% of the lipoprotein lipase activity present in the homogenate, was used for assays.

Control experiments showed that sodium dodecyl sulfate, subsequently diluted in immunoaffinity experiments (<0.05 mM) and diluted further for lipoprotein lipase determinations (<0.025 mM), did not interfere in the formation of the immune complex and during the enzyme assay, respectively (vide infra). Studies of heparin-releasable lipoprotein lipase activity were carried out as follows. Culture medium was aspirated and cells were rinsed at 37 °C, first with phosphate-buffered saline, pH 7.4, and then with sodium barbital buffer, pH 7.4, as above. Unless otherwise stated, cells were incubated at 37 °C in the presence of sodium barbital buffer containing 10 mM heparin for 1 h. Control cells were incubated in parallel without heparin. Heparin-containing and control media were recovered and held at 0 °C for measurement of heparin-releasable lipoprotein lipase activity or for incubation with antibodies. Control experiments showed that, using lactate dehydrogenase activity as a marker, the contamination by suspended cells and debris was negligible (<5%). After medium removal, heparin-treated cells, which ranged fully viable (>95%), were then homogenized in sodium barbital buffer. 3 mg/mL of heparin were added and lipoprotein lipase activity was determined. 7-14 days of storage at −20 °C did not affect the enzyme activity.

**Enzyme Assays**—Lipoprotein lipase was measured as previously described, as the substrate-dependent hydrolysis of glycerol tri[1,2,3-3H]oleate (10 mCi/mmol) (8). Addition of 1 mM sodium chloride in the assay medium of cell homogenate causes a significant reduction not only in the serum-dependent but also in the serum-independent (referred to as basal) lipolytic activity (80-90% and 30-40%, respectively). Furthermore, both activities were fully inhibited in the presence of 0.4% Triton X-100 (8). In contrast, both basal and lipoprotein lipase activities of medium from heparin-treated cells (vide supra) were inhibited by 1 M sodium chloride. These and other results described below indicate that the basal lipolytic activity is closely related to lipoprotein lipase per se. Monocacylglycerol lipase was measured with [2-3H]glycerol monooctanoate (0.125 mCi/mmol) as substrate according to Ronquist et al. (15), except that Triton X-100 was used at 0.025% instead of 0.1%. These and other assays were performed at least triplicate aliquots of the same preparation obtained from three different preparations. Variability between assays was not more than 5%. Control experiments showed that variability between mean values from three separate dishes never exceeded 15%.

**Immunotitration**—Anti-lipoprotein lipase serum was obtained by immunizing a female goat with the purified rat heart LPL as described previously (16). Partially purified anti-lipoprotein lipase γ-globulins were isolated using ammonium sulfate and DEAE-cellulose ion exchange chromatography. The fraction was dialyzed extensively before use against phosphate-buffered saline containing 1 mg glycerol and adjusted to 2 mg/mL of protein. The formation of the immune complex was performed in a final volume of 0.18 mL in the presence of increasing concentrations of antibodies (20 h at 4 °C). Control experiments showed that under the conditions used, heparin either added to cell homogenate or present in media from heparin-treated cells, did not interfere with the formation of the immune complex. Moreover, no change in lipoprotein lipase was observed after addition of heparin to homogenates of cells previously treated or not pretreated with heparin (vide supra). Immunoreactive directed against rat liver monoacylglycerol lipase was a kind gift of Professor W. C. Hulsmann (Erasmus University, Rotterdam, The Netherlands).

**Fluorescence Microscopy**—Two different procedures were used to label lipoprotein lipase in differentiating and differentiated Ob17 cells. Unless otherwise stated, phosphate-buffered saline, pH 7.4, (in the absence or presence of reagents) was used at room temperature.

In the first procedure (single labeling), cells were first washed at 37 °C with phosphate-buffered saline and then fixed with 10% formaldehyde for 10 min. Cell permeabilization, if any, was performed in the presence of an acetone/water mixture (80/20, v/v) for 30 min at −20 °C. Cells, permeabilized or not, were subsequently incubated for 30 min in the presence of diazyl anti-lipoprotein lipase immunoglobulin (1:50 dilution in a final volume of 0.5 mL/25-mm culture dish). After washing, cells were stained with rabbit anti-goat γ-globulins labeled with fluorescein (1:50 dilution, 30 min at 20 °C). Cells were washed, mounted in glycerine saline buffer 0.5 M, pH 8.6, containing 90% glycerol under 25-mm coverslips, viewed, and photographed in Leitz Orthoplan microscope with epifluorescence optics.

In the second procedure (double labeling), cells were treated as described above but the permeabilization step with acetone was omitted. Under these conditions, all surface sites which bind lipoprotein lipase were saturated and the surface enzyme was labeled with the fluorescein conjugate. Cells were then made permeable in the presence of 0.1% Triton X-100 and anti-lipoprotein lipase immunoglobulins (30 min at 20 °C), washed with 0.1% Triton X-100, and finally with phosphate-buffered saline. Exposure to rabbit anti-goat γ-globulins conjugated to rhodamine isothiocyanate was next performed for 30 min and cells washed with phosphate-buffered saline. Prior permeabilization in the double staining procedure was carried out with Triton X-100, since a decrease in the fluorescein staining at the cell surface was observed when acetone was used. Before use, the different globulin fractions were routinely absorbed on nearly confluent Ob17 cells which had been fixed as described above, and which do not contain lipoprotein lipase, in order to reduce the nonspecific background fluorescence. For these absorptions, 2 mL of each dilution were absorbed for 15 min each on four 35-mm diameter culture dishes. In one experiment (see Fig. 2F), an immunoserum directed against homogeneous rat adipose tissue lipoprotein lipase was also used (kindly donated by Professor J. Etienne, Laboratoire de Biochimie, Hôpital St. Antoine, Paris, France). Nonspecific staining was carried out on differentiated cells by employing preimmune goat γ-globulins. Very little fluorescence was detected under these conditions.

**Miscellaneous**—Protein concentration was determined according to Lowry et al. (17). Cell viability was checked by exclusion of erythrosin B.

**RESULTS**

**Inhibition of Lipoprotein Lipase Activity by Anti-lipoprotein Lipase γ-Globulins**—The inhibition of lipoprotein lipase activity by increasing concentrations of antibodies directed against rat heart lipase is depicted in Fig. 1. Extracts of acetone powder from epididymal fat pads of C57BL/6J ob/ob mice were first used. Curves of Fig. 1A show an excellent cross-reactivity with the mouse enzyme. Lipoprotein lipase activity is completely inhibited by anti-lipoprotein lipase immunoglobulin. Similar results are obtained with cell homogenates of differentiated Ob17 cells (Fig. 1A). The corresponding nonimmune fraction has no effect in both cases. It could be argued that, since immune γ-globulins were obtained after immunization with lipoprotein lipase purified from rat heart, the antibodies could be directed against different molecular forms of the enzyme which should be present in rat heart (18, 19).

However, even if this were so, our experiments indicate that the antigenicity is very similar for all possible molecular species of mouse lipoprotein lipase possibly present in acetone extracts of adipose tissue and in Ob17 cell homogenates.

Anti-lipoprotein lipase γ-globulin does not inhibit monocacylglycerol lipase activity of homogenates from differentiated Ob17 cells, and immunoserum directed against monocacylglycerol lipase does not inhibit lipoprotein lipase activity. These results demonstrate directly that monoacylglycerol lipase and lipoprotein lipase activities belong to separate molecular entities, as already strongly suggested through the use of differential assays for both enzymes (8).

Heparin is known to enhance lipoprotein lipase secretion in mature adipocytes, adipocyte precursors, and differentiated 3T3-L1, F442A, and Ob17 cells (6-9, 11, 20, 22). A full inhibit-
tion of the heparin-releasable activity is obtained with the immune fraction. This inhibition (Fig. 1B) is superimposable with that obtained with an identical number of enzyme units from cell homogenate. Therefore, it is most likely that the antigenicity of the releasable and the nonreleasable form of lipoprotein lipase is quite similar.

Altogether, these data allow us to conclude that in differentiated Ob17 cells the different molecular forms of lipoprotein lipase, if any, do show similar antigenicities.

Therefore, anti-lipoprotein lipase immunoglobulin was used in order to study the emergence of the enzyme in differentiating cells and its localization in differentiated cells, as well as to demonstrate changes in the cellular lipoprotein lipase content during adipose conversion.

Localization of Lipoprotein Lipase in Differentiating and in Differentiated Cells—No lipoprotein lipase can be detected in early confluent cells (Fig. 2A), in agreement with the low enzyme activities determined at that stage of adipose conversion (8).

Differentiation of the preadipocyte cell lines so far described occurs after confluence with a formation of adipose clusters. These clusters correspond to colonies of triglyceride-filled cells separated by cells devoid of lipid droplets. The latter cells (insusceptible cells) maintain a fibroblastic morphology even after many weeks in culture and do not differentiate. However, they can acquire the susceptible state if they are allowed to divide under certain conditions. In the above experiments, one cannot exclude the possibility that insusceptible cells might express some early phenotype such as the presence of lipoprotein lipase. These cells would not accumulate late triglycerides only because late phenotypic enzymes involved in the esterification process would not be expressed. Fig. 2, B and C clearly show that this is not the case: lipoprotein lipase is not detectable in cells present outside the clusters of fat cells. Identical observations have been made on late confluent cells (not shown). Therefore, this lack of lipoprotein lipase in insusceptible cells does not seem to be related to a delay in the expression of the differentiation program. It is most likely that these cells did not enter the differentiation process. Examination of cells present in fat clusters, with or without cell permeabilization, indicates that lipoprotein lipase is localized both at the surface and inside the cells (Fig. 2B compared to Fig. 2C). This becomes more apparent in Fig. 2E. As expected, observations on permeabilized cells show that lipoprotein lipase is excluded from areas corresponding to intracellular lipid droplets.

The double localization of lipoprotein lipase was ascertained by double labeling experiments on differentiated cells. Cell surface sites for the enzyme were first saturated with anti-lipoprotein lipase γ-globulins and detected with the fluoresceine conjugate. Next, the cells were made permeable to the same antibodies, the immune complex formed inside the cells was detected on the same cells with the rhodamine conjugate.

As shown in Fig. 2F, fluoresceine staining is localized at the cell surface but is never detectable in the cells (not shown), insuring the validity of the procedure for surface labeling. In contrast, rhodamine staining is detected inside the permeabilized cells.

As already mentioned, a distinctive property of lipoprotein lipase is its release from cells by heparin. Since the enzyme could be visualized at the cell surface (Fig. 2F), dose-response curves of lipoprotein lipase release by heparin were performed on differentiated Ob17 cells (Fig. 3).

Lipoprotein lipase recovered in the medium is maximal at 0.6 µg ml⁻¹ of heparin. The activity released by heparin is usually 2-fold higher than that recovered in treated cells. Half-maximal release is obtained at an Eₕ value of 0.15 µg ml⁻¹ of heparin while a small but significant release occurs spontaneously in the absence of the sulfated polysaccharide. This Eₕ value is close to those found for the release by heparin of lipoprotein lipase from mature adipocytes (20), of bovine milk lipoprotein lipase bound to bovine artery endothelial
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**Fig. 2. Immunofluorescence of lipoprotein lipase in Ob17 cells.** Cells were processed according to the first procedure (A to E) or to the second procedure (F) as described under "Experimental Procedures." A, early confluent cells exposed to anti-lipoprotein lipase without treatment for permeabilization. Fluorescence is very weak. Permeabilized cells give identical pictures (not shown); B, 9-day-old postconfluent cells without treatment for permeabilization and exposed to anti-lipoprotein lipase; C, 9-day-old postconfluent cells made permeable and exposed to anti-lipoprotein lipase; D, same cells as in B but exposed to the nonimmune γ-globulin fraction. Very little fluorescence is observed. Identical pictures are obtained after permeabilization (not shown); E, 9-day-old postconfluent cells treated as in B (left) or as in C (right); F, cells present in a fat cluster (9 days after confluence) are double labeled as described under "Experimental Procedures" and observed under fluorescein (left) or rhodamine (right) fluorescence. Magnifications are: A-D, ×250; E, ×1000; F, ×190. In A and D, due to the very low fluorescence detected, photographs are overexposed in order to visualize the cells. Typical examples of undifferentiated cells are indicated by arrowheads in B and C.

Lipoprotein lipase activities per culture dish are 0.1 milliunit (A) and 3.4 milliunits (B-F).

Cells (23), and of rooster lipoprotein lipase bound to calf aorta endothelial cells (24).

Changes in Cellular Lipoprotein Lipase Content during Adipose Conversion—Lipoprotein lipase activity increases 20–50-fold during adipose conversion of Ob17 preadipocytes to adipose cells (8, 11). The enzyme activity rises sharply after confluence, reaches a maximum between day 10 and day 15 postconfluence and decreases afterwards (Fig. 4A). This pattern is reproducibly found in preadipocyte cell lines (6–8).

Immunotitrations of lipoprotein lipase were performed on homogenates of variable enzyme specific activities by using in each case an identical number of enzyme units and increasing concentrations of anti-lipoprotein lipase γ-globulins. Results of Fig. 4B demonstrate that inhibition curves of lipoprotein lipase activity are identical. Therefore, during adipose conversion, the changes in lipoprotein lipase activity are a direct
reflection of parallel changes in the lipoprotein lipase content per cell. A remote possibility could be that these changes in activity are due, at least in part, to changes during adipose conversion in the maximal stimulation of the enzyme by apo-C\(_2\). This hypothesis can be excluded since the activity ratio, that is the activity in the presence of serum (containing apo-C\(_2\) as activator) versus the activity in the absence of serum, remains constant at any given time of adipose conversion (Fig. 4A).

Long Term Effects of Insulin and Triiodothyronine on the Development of Lipoprotein Lipase—Chronic exposure of confluent Ob17 cells to insulin leads to increased activity levels of characteristic phenotypic enzymes of adipose conversion (11, 25). In addition to insulin, triiodothyronine is able at physiological concentrations to further amplify the expression of these phenotypes (13). It was therefore of interest to see whether or not both hormones, either alone or in combination, could affect the lipoprotein lipase content of Ob17 cells.

Dose-response curves reported in Fig. 5A show that insulin is maximally effective at 17 nM and that the presence of T\(_3\) cells are activity levels at any given concentration of insulin. The EC\(_{50}\) value for insulin is approximately 2 nM. This concentration is within physiological range and close to the Kd values (0.45–1 nM) determined for the high-affinity binding sites characterized on these cells (12).

The activity ratio, as defined above, remains constant with varying insulin concentrations, in the presence or absence of T\(_3\). Therefore, changes in lipoprotein lipase activity possibly due to changes in the maximal stimulation brought by apo-C\(_2\) can be excluded (Fig. 5A).

The reproducible decrease in lipoprotein lipase activity observed above 17 nM insulin is not specific for this enzyme and it has been observed for other key enzymes of adipose conversion (not shown). This observation is related, at least in part, to the continuous mitogenic action of insulin on susceptible cells which no longer significantly affects existing fat cell clusters.

Immunotitrations of lipoprotein lipase reported in Fig. 5B were performed under conditions similar to those of Fig. 4B. When a constant number of enzyme units for each condition were incubated with increasing amounts of antibodies, identical curves were obtained for insulin-treated cells in the presence or in the absence of T\(_3\). However, in cells not exposed to insulin, the binding of the lipolytic activities to antibodies, which initially was the same, showed a significant difference at higher concentrations of antibodies. These data, with the exception of cells not exposed to insulin, lead to the conclusion that changes in the specific activities of lipoprotein lipase (Fig. 5B) are only due to parallel changes in enzyme content per cell.
has been put forward for adipocytes by different authors. Changes in lipoprotein lipase activity are a direct reflection of the lipase activity of adipose tissue obtained during adipose conversion (Fig. 12392).

Our experiments on Ob17 cells show for the first time that changes in lipoprotein lipase activity are a direct reflection of corresponding changes in the cell enzyme content. The lipoprotein lipase activity has been defined as the serum (apo-CII)-dependent hydrolysis of triacylglycerol. However, the basal activity, i.e. the serum-independent activity, should be related to lipoprotein lipase per se since (i) a constant activity ratio is obtained during adipose conversion (Fig. 4A) or when confluent cells are chronically exposed to increasing concentrations of insulin, in the presence or in the absence of T3 (Fig. 5A), (ii) as for lipoprotein lipase, the basal activity is not detectable in 5-bromo-2-deoxyuridine-treated cells in which adipose conversion is blocked, (iii) inhibition of the basal activity is parallel to that of lipoprotein lipase, in the presence of 0.4% Triton X-100 in the assay medium (vide supra).

**DISCUSSION**

It has been well substantiated that lipoprotein lipase is synthesized in vivo by subendothelial cells and transferred by an unclear mechanism to the luminal surface of endothelial cells (1). Insulin has been shown to modulate lipoprotein lipase activity of adipose tissue in vivo and that of differentiating 3T3 and Ob17 cells in vitro. Moreover, the phenotypic expression of the differentiation program in preadipocyte cell lines and cell strains is characterized by the early emergence of lipoprotein lipase activity (6-8).

Our experiments on Ob17 cells show for the first time that changes in lipoprotein lipase activity are a direct reflection of corresponding changes in the cell enzyme content. The lipoprotein lipase activity has been defined as the serum (apo-CII)-dependent hydrolysis of triacylglycerol. However, the basal activity, i.e. the serum-independent activity, should be related to lipoprotein lipase per se since (i) a constant activity ratio is obtained during adipose conversion (Fig. 4A) or when confluent cells are chronically exposed to increasing concentrations of insulin, in the presence or in the absence of T3 (Fig. 5A), (ii) as for lipoprotein lipase, the basal activity is not detectable in 5-bromo-2-deoxyuridine-treated cells in which adipose conversion is blocked, (iii) inhibition of the basal activity is parallel to that of lipoprotein lipase, in the presence of 0.4% Triton X-100 in the assay medium (8), and (iv) using cell homogenate as an enzyme source, the curve of inhibition in the presence of anti-lipoprotein lipase y-globulins of the basal activity is superimposable to that of the lipoprotein lipase activity when using identical amounts of cell homogenate (Fig. 5C). Moreover, the immunotitration curves of the serum-independent activity are identical when using medium from heparin-treated cells as an enzyme source instead of homogenate (not shown).

Altogether, these results do indicate that the different forms of lipolytic activity, stimulated or not by apo-CII, present in the cells, or released by heparin, display a quite similar antigenicity when being titrated by the antibody fraction used in this study. The results reported in Fig. 1B do not support the existence in Ob17 cells of an activation process of lipoprotein lipase during enzyme release triggered by heparin, which has been put forward for adipocytes by different authors (26, 27). If such process is operative in Ob17 cells, one would have to assume for the heparin-releasable form that the percentage of increase in the catalytic efficiency of the released enzyme would be identical to the percentage of increase in its antigenicity. This possibility cannot be excluded but is not very likely. Thus, the possibility remains that differentiated Ob17 cells are different from isolated adipocytes in that respect.

The antigenicity of lipoprotein lipase in confluent Ob17 cells never exposed to insulin seems quite different from that of lipoprotein lipase in cells chronically exposed to the hormone, whether or not triiodothyronine is included in the culture medium (Fig. 5A). This observation may be related to the acute effect of insulin described in differentiated 3T3-L1 cells by Spooner et al. (28). Insulin was shown to rapidly liberate a small fraction, or pool, of the total enzyme activity of the cells. It is possible that insulin-ununtreated Ob17 cells accumulate a similar pool of enzyme which presents a higher antigenicity. Whether or not this pool, if any, corresponds to a "proenzyme" form remains an open question. When confluent Ob17 cells are chronically exposed to insulin, it cannot be excluded that this proenzyme form is present in low proportions and therefore escapes detection by immunotitration experiments. For this reason, the existence of a precursor form was approached in a different way since the existence in rat heart of a storage form of low affinity for triglycerides has recently received experimental support (19). Our results are not in favor of this hypothesis since no significant difference in the apparent Kd values for glycerol trioleate (=0.5 mM) was found between the lipoprotein lipase activity from cell homogenate and from medium of heparin-treated cells (not shown).

Direct immunofluorescence studies have revealed directly the presence of lipoprotein lipase at the cell surface of Ob17 cells. Double labeling experiments with fluorescein and rhodamine immunoglobulin conjugates allow the identification of an intracellular pool of lipoprotein lipase distinct from the surface pool. This topographical distinction is supported by the release of lipoprotein lipase in the presence of heparin which presumably acts at the cell surface of adipose cells as it has been shown directly for the release of lipoprotein lipase from endothelial cells (23, 24). In addition, preliminary investigations indicate that colcemid-pretreated cells, incubated with heparin and stained for lipoprotein lipase, present a significant decrease in the cell surface fluorescence.

Direct proof that lipoprotein lipase is present only in cells entering the differentiation program is also supported by immunofluorescence studies. The differentiating cells can be easily distinguished in the culture dish from insusceptible cells even in the absence of visible lipid accumulation since their morphology is different and since most of them are joined in clusters. The dissociation during adipose conversion between the appearance of lipoprotein lipase and that of lipid droplets is not surprising since (i) the accumulation of triglycerides lags behind the increase in enzyme activity observed early after confluence and (ii) Ob17 cells maintained in delipidized serum (11) or 3T3-F442A cells maintained in biotin-deprived medium (29) do not accumulate lipids while the emergence of the enzyme is unaffected.

It should be pointed out that detection by immunofluorescence techniques of lipoprotein lipase-positive cells may be of interest for estimating in a more satisfactory way the number of adipose cells during adipose tissue development in rat and mouse, that is under physiological situations in which the lipid-filling process of adipocytes is underway (30).

The pattern of appearance of lipoprotein lipase during adipose conversion of Ob17 cells shows clearly that Ob17 cells are responsive to insulin but also, in contrast to 3T3-L1 cells, to triiodothyronine within a physiological range of concentrations (28). The usefulness of Ob17 cells as a model to study long term hormonal effects is supported by the reversible modulation by insulin of the activities of key enzymes involved in the synthesis, activation, and esterification of fatty acids. In conclusion, changes in lipoprotein lipase are directly correlated with changes in enzyme content, and these changes are directly modulated by physiological concentrations of insulin. It is thus tempting to postulate that in vitro the regulation of lipoprotein lipase activity levels in adipose tissue, in response to variations of plasma insulin concentration, occurs through the same mechanism.

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