STRUCTURE-FUNCTION RELATIONSHIPS

IN THE ADIPOSE CELL

I. Ultrastructure of the

Isolated Adipose Cell

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ABSTRACT

A method is described for preparing isolated rat adipose cells for electron microscopy. The ultrastructure of such cells and their production of $^{14}CO_2$ from U-glucose-^{14}C were studied simultaneously in the presence of insulin or epinephrine. Each adipose cell consists of a large lipid droplet surrounded by a thin rim of cytoplasm. In addition to typical subcellular organelles, a variety of small lipid droplets and an extensive system of membranes characterize the cell's cytoplasm. A fenestrated envelope surrounds the large, central lipid droplet. Similar envelopes surround cytoplasmic lipid droplets occurring individually or as aggregates of very small, amorphous droplets. Groups of individual droplets of smaller size also occur without envelopes. The system of membranes consists of invaginations of the cell membrane, vesicles possibly of pinocytic origin, simple and vesiculated vacuoles, vesicles deeper in the cytoplasm, flattened and vesicular smooth surfaced endoplasmic reticulum, and Golgi complexes. Neither insulin nor epinephrine produced detectable ultrastructural alterations even when cells were incubated under optimal conditions for the stimulation of $^{14}CO_2$ evolution. Structural responses of the isolated adipose cell to hormones, if such occur, must, therefore, be dynamic rather than qualitative in nature; the extensive system of smooth surfaced membranes is suggestive of compartmentalized transport and metabolism.

INTRODUCTION

The mature white adipose cell is roughly 95% triglyceride by weight. It consists of a large, central lipid droplet surrounded by an extremely thin rim of cytoplasm (1–4). This cell is nevertheless highly active metabolically and is subject to delicate nervous and hormonal control and nutritional influences (5–8). The adipose cell, during periods of caloric excess, readily converts glucose into fatty acids and stores these, together with those taken up from the extracellular space, as triglyceride. During periods of caloric deficit, stored triglyceride is released by the cell as fatty acids and glycerol for transport to other tissues.

The fine structure of the adipose cell has been examined at moderate resolution by a number of investigators (1–4). The extremely high lipid content of the adipose cell has, however, rendered such electron microscopic studies technically difficult and limited in scope. Few studies have undertaken a systematic examination of relationships between the cell's fine structure and its function. One report claims that insulin stimulates pinocytic activity in the adipose cell (9), and several others, that prolonged starvation of the experimental animal increases pinocytic activity in adipose tissue (1, 2, 4). Pinocytosis, however,

has never been conclusively demonstrated in the adipose cell under any experimental conditions. A single study at the phase-contrast microscopy level describes dense bodies, tentatively identified as lipid droplets, in the cytoplasm of isolated adipose cells, an increase in the number of these bodies upon exposure of the cells to epinephrine, and a rapid decline in their number upon subsequent exposure of the cells to insulin (10).

The publication of a method for the preparation of an isolated adipose cell suspension from rat epididymal fat pads (11) prompted the initiation of this series of studies specifically aimed at seeking relationships between the adipose cell's ultrastructure and its function under carefully controlled environmental conditions. This paper describes a method for the preparation of isolated adipose cells for electron microscopy and examines at high resolution the ultrastructure of such cells incubated under basal metabolic conditions and in the presence of insulin or epinephrine. The metabolism of radioactive glucose has been monitored in cells incubated in parallel with those to be studied morphologically.

METHODS

Epididymal fat pads were obtained from male Sprague-Dawley rats maintained on Purina Fox Chow (Ralston Purina Co. Inc., St. Louis, Mo.). Rats weighing from 200 to 250 g were sacrificed by decapitation. All incubations were carried out in freshly prepared Krebs-Ringer-bicarbonate (KRB) buffer, pH 7.4, 37°C, containing 0.1 or 1.0 mg glucose per ml and 5% bovine serum albumin, fraction V (Armour Pharmaceutical Co., Kankakee, Ill.), from which fatty acids had been extracted as described by Goodman (12)

Adipose cells were isolated by treating fat pads with a crude collagenase (Worthington Biochemical Corp., Freehold, N. J.) as described by Rodbell (11) with the following modifications: All procedures were carried out in polyethylene tubes and vials maintained at 37°C either by a 37°C water bath or by 37°C water jackets. All incubation vessels were flushed with 95% air, 5% CO_2. Fat pads were incubated in the presence of collagenase with rapid agitation for 15–20 min. The volume of final stock adipose cell suspension was adjusted with buffer to yield roughly 50 mg of cells per ml.

Polyethylene scintillation vials were used as incubation vessels. All test substances to be included in an incubation were placed in the vials shortly before addition of cells. Each vial was flushed for 10 sec with 95% air, 5% CO_2, capped, and warmed in a 37°C bath. Portions of cell suspension were trans-

ferred to the warmed vials and the samples so prepared were incubated with moderate agitation at 37°C for desired times.

Test substances were added to the incubation vials in small volumes of KRB-albumin buffer as follows: A stock solution of U-glucose-[14]C (New England Nuclear Corp., Boston, Mass.), 20 μCi per ml in water, was diluted in buffer and the diluted solution was added to the incubation vials to yield 0.05–0.10 μCi per mg glucose. A commercial solution of epinephrine (Parke, Davis adrenalin chloride,[1] 1000 μg per ml) was diluted in buffer and the diluted solution was added to the incubation vials to yield 0.001–1.0 μg per ml. A stock solution of recrystallized bovine pancreatic insulin (Mann Research Labs Inc., New York, 25 U per mg) was prepared fresh weekly by dissolving approximately 4 mg of insulin in 0.5 ml of 0.01 N HCl and adjusting the volume to 50 ml with 1% albumin in water. Daily the stock solution of insulin was diluted serially in buffer and the diluted solution was added to the incubation vials to yield 0.1–1000 μU per ml.

Test substances were added to the incubation vials in volumes such that at least 80% of the final incubation volume came from the stock adipose cell suspension. The volume of cell suspension added to each incubation vial was constant within a given experiment except where variations in cell concentration were being examined experimentally. In each experiment, a series of control vials incubated without cells, incubated for 0 time, or incubated without test substances was included. Because quantitative differences between experiments could not be eliminated, sufficient samples were included within each experiment to allow all of the desired comparisons. All incubation vials were prepared at least in duplicate.

The ultrastructure of isolated adipose cells was examined by electron microscopy. A portion of incubated cell suspension was transferred by polyethylene pipette to a 10 ml polyethylene centrifuge tube containing an equal volume of fresh, cold, 4% glutaraldehyde in KRB buffer, pH 7.4. Cells were fixed in the cold with occasional agitation for 30–60 min. Glutaraldehyde-fixed cells were washed with two 5-ml volumes of cold KRB-albumin buffer and resuspended in a small volume of the same buffer. An equal volume of fresh, cold, 4% OsO_4 in KRB buffer was added and the cells were postfixed in the cold with occasional agitation for 4 hr. During the OsO_4 postfixation, the cells settled to the bottom of the tube. OsO_4-fixed cells were usually stained with uranyl acetate before being embedded (13). Fixed cells were washed thoroughly, usually overnight, in cold Veronal-acetate buffer, pH 6.1, and exposed

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[1] Parke, Davis, Co., Detroit, Mich.

for 30 min at room temperature to 0.5% uranyl acetate in Veronal-acetate buffer. Fixed and stained adipose cells were dehydrated in a graded series of ethanol-water solutions and in propylene oxide, and infiltrated overnight in the cold with a 1:1, volume: volume, propylene oxide:Epon, solution (14). Best results were obtained with an Epon mixture of 7:3, volume:volume, nadic methyl-anhydride (NMA) plus Epon 812:dodecenyl-succinic anhydride (DDSA) plus Epon 812. The propylene oxide of the infiltration solution was evaporated and small portions of infiltrated cells were layered onto the surface of fresh Epon contained within embedding capsules. During several hours at room temperature, the cells settled to the bottom of the capsule; polymerization was then carried out at 60°C for at least 48 hr. Thin sections were cut from each block, stained with uranyl acetate (15) and lead citrate (16), and examined and photographed with an Hitachi HS-7S electron microscope.

Glucose metabolism by isolated adipose cells was monitored by measuring the incorporation of counts from U-glucose-^{14}C into CO_2 as described by Glie-mann (17). Scintillation fluid consisted by volume of 750 parts toluene, 750 parts ethyl cellosolve, and 63 parts liquid scintillator containing 100 g 2,5-diphenyloxazole (PPO) and 1.25 g p-bis[2-(5-phenyl-oxazolyl)]benzene (POPOP) per liter in toluene (Liquifluor, Pilot Chemicals, Inc., Watertown, Mass.); ^{14}C-counting was carried out in a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Because of the reproducibility among replicate samples and the need only for a relative metabolic index, the results were generally expressed as CO_2 ^{14}C-counts per minute per ml of incubated cell suspension and estimates of lipid or cell weight or of cell number per incubation vial were not made.

Isolated adipose cells were examined by electron microscopy and their metabolism was monitored under four conditions: immediately after isolation, after incubation for 30–240 min in the absence of hormones, after incubation for 60 min in the presence of 1 μg epinephrine per ml, and after incubation for 60 min in the presence of 100 μU insulin per ml. Incubation conditions were chosen to yield optimal basal metabolism and hormonal responses while remaining within approximately physiological limits. Only cell preparations exhibiting normal metabolism were examined electron microscopically.

RESULTS

The incorporation of ^{14}C-counts into CO_2 from U-glucose-^{14}C in the incubation medium is a linear function of the volume of stock isolated adipose cell suspension present in a constant incubation volume (Fig. 1). $^{14}CO_2$ evolution in the
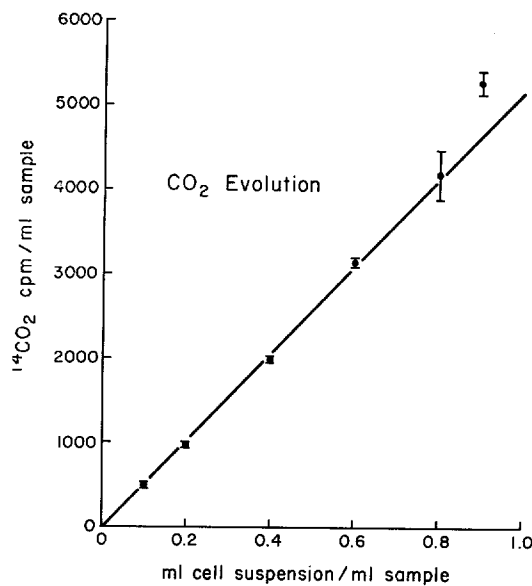

FIGURE 1 $^{14}CO_2$ evolution *versus* volume of stock cell suspension present in 1 ml of final incubated sample. Isolated adipose cells incubated 60 min in KRB-albumin buffer in the presence of 0.1 mg glucose and 100 μU insulin per ml. (Values ± standard deviation.)

absence of hormones and in the presence of insulin or epinephrine is linear with time for at least 120 min (Fig. 2). Both insulin and epinephrine stimulate $^{14}CO_2$ evolution, insulin to a greater degree than epinephrine. While the responses are dependent upon hormone (Fig. 3) and glucose concentration in the incubation medium, the shapes of the dose response curves are essentially the same at the two glucose concentrations tested. Parameters other than $^{14}CO_2$ evolution have been examined periodically in order to verify the specificity of each hormonal response (data not shown), and metabolic data similar to those described here have been obtained in each electron microscopic experiment.

The gross features (Figs. 4 and 5) of the isolated adipose cell are a large, central lipid droplet, a very thin rim of cytoplasm, a thickened perinuclear cytoplasm containing small lipid droplets, and a nucleus, in which a nucleolus is occasionally visible. Commonly, even the perinuclear cytoplasm is relatively thin and the nucleus flattened. Considerable variation in the electron opacity of the cell's lipid droplets, central or cytoplasmic, suggests that variable amounts of lipid have
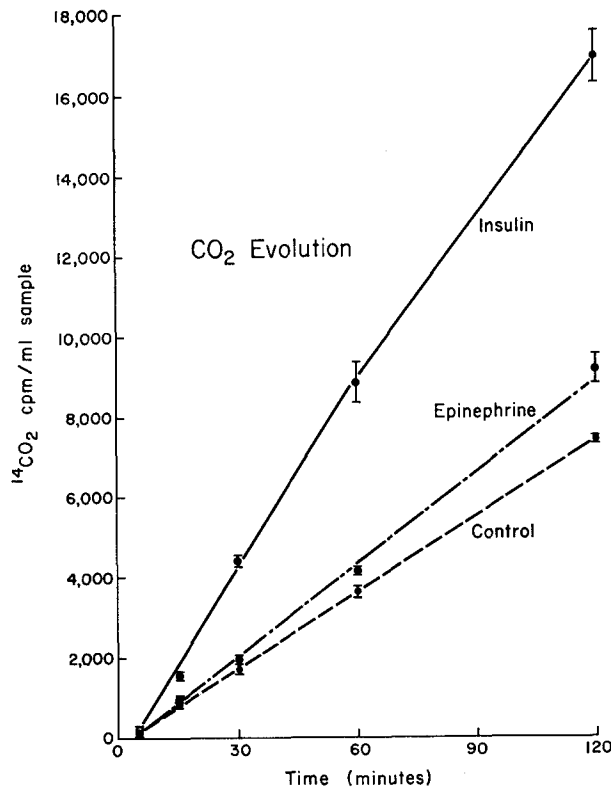
CO$_2$ Evolution

Insulin

Epinephrine

Control

^{14}CO$_2$ cpm/ml sample

18,000
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0

0   30   60   90   120

Time (minutes)

FIGURE 2 ^{14}CO$_2$ evolution *versus* time in the presence of 100 μU insulin or 1.0 μg epinephrine per ml or in the absence of hormones. Isolated adipose cells incubated in KRB-albumin buffer in the presence of 0.1 mg glucose per ml.

been extracted during the processing of the cells for electron microscopy. However, sufficient fixed lipid remains to maintain the spherical shape of the droplets and of the cell. Typical subcellular elements of the adipose cell include mitochondria, endoplasmic reticulum membranes, vesicles, vacuoles, and the cell membrane and its invaginations (Fig. 5). Breaks in the cell membrane occasionally occur due to the fragility of the fixed cells (Fig. 5).

While most cells have only a single large, central lipid droplet, some cells contain more than one separated from each other by a thin band of non-membranous electron-opaque material. The junction between the large, central lipid inclusion and the cytoplasm (Fig. 6) consists of an electron-opaque line immediately separating the lipid from the cytoplasmic matrix, electron-opaque debris on the lipid side of the junction, and a fenestrated envelope on the cytoplasmic side. This fenestrated envelope has, in general, the appearance of flattened endoplasmic reticulum (ER). However,

only on occasion are there direct continuities between these two subcellular elements. Those few continuities which do exist connect the envelope with either smooth-surfaced (SS) or rough-surfaced (RS) ER. Ribosomes are sometimes attached directly to the envelope, but only on its cytoplasmic surface. The fenestrations dividing the flattened envelope into closed segments vary greatly in dimension. Cytoplasm fills each fenestration and appears to separate slightly the envelope from the lipid.

Droplets containing lipid vary not only in size but also in distribution and structure. In addition to the large, central lipid droplet, three classes of lipid inclusions are observed within the adipose cell's cytoplasm. The first, and largest, class consists of lipid droplets of moderate and relatively uniform size (Figs. 7 and 10). These droplets are randomly located in the cytoplasm, are separated from the cytoplasmic matrix by an electron-opaque line, and are surrounded by a fenestrated envelope similar to that surrounding the large,
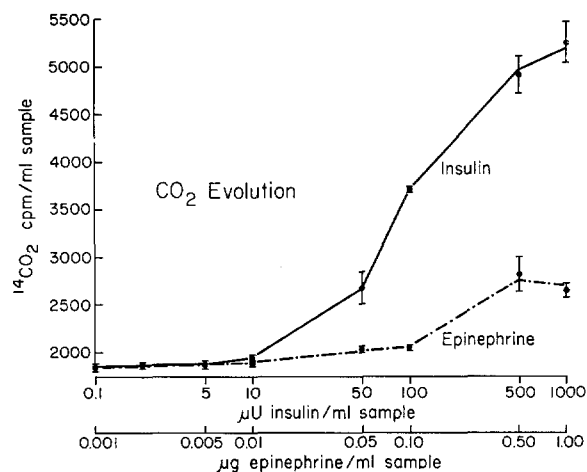
FIGURE 3 $^{14}CO_2$ evolution *versus* insulin and epinephrine concentration. Isolated adipose cells incubated 60 min in KRB-albumin buffer in the presence of 1.0 mg glucose per ml.
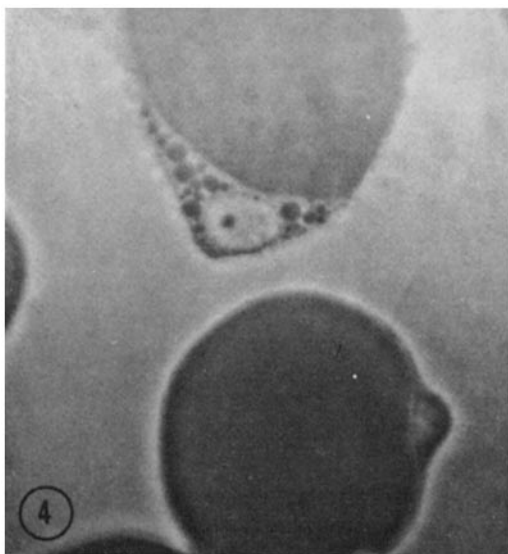

FIGURE 4 Isolated adipose cells incubated 60 min in KRB-albumin buffer in the absence of hormones. 0.5 μ section, phase optics. Approximately X 1300.

central droplet. Occasionally a single envelope encloses more than one droplet. Lipid droplets of this class have been readily identified in the roughly 1 in 20 cells sectioned through a region of thickened cytoplasm.

Lipid droplets of the second class, characterized by their lack of association with a fenestrated envelope, were observed within the cytoplasm of about 1 in 200 cells sectioned (Fig. 8). The elec-

tron-opaque line at the lipid-cytoplasmic junction is prominent. Although such droplets may be of moderate size, they are generally small and occur in groups. Groups of small droplets are most frequently located adjacent to larger droplets or to the large, central droplet. Only amorphous ground substance separates the members of these groups. About 75% of lipid droplets of this class were seen in cells exposed to epinephrine. This organelle was identified in a relatively small number of sections, however, and it was not possible to make a statistical correlation between changes in its appearance or numbers and alterations of glucose metabolism caused by epinephrine.

Packed aggregates of very small droplets of variable appearance, associated with amorphous material of variable electron opacity, constitute the third class of lipid inclusions observed in the adipose cell cytoplasm (Figs. 9 and 10). Larger lipid droplets may be dispersed among the smaller droplets. These aggregates usually occur closely associated with the large, central lipid droplet (Fig. 9), but can be observed free in the cytoplasm (Fig. 10). In addition, each aggregate is surrounded by a fenestrated envelope which, for aggregates juxtaposed to the central lipid droplet, appears to be continuous with that surrounding the large droplet. Occasionally the flattened envelope is vesicular in appearance. The ground substance of the cell is continuous with the matrix of the aggregates through the fenestrations in the envelope. Such aggregates were seen about twice
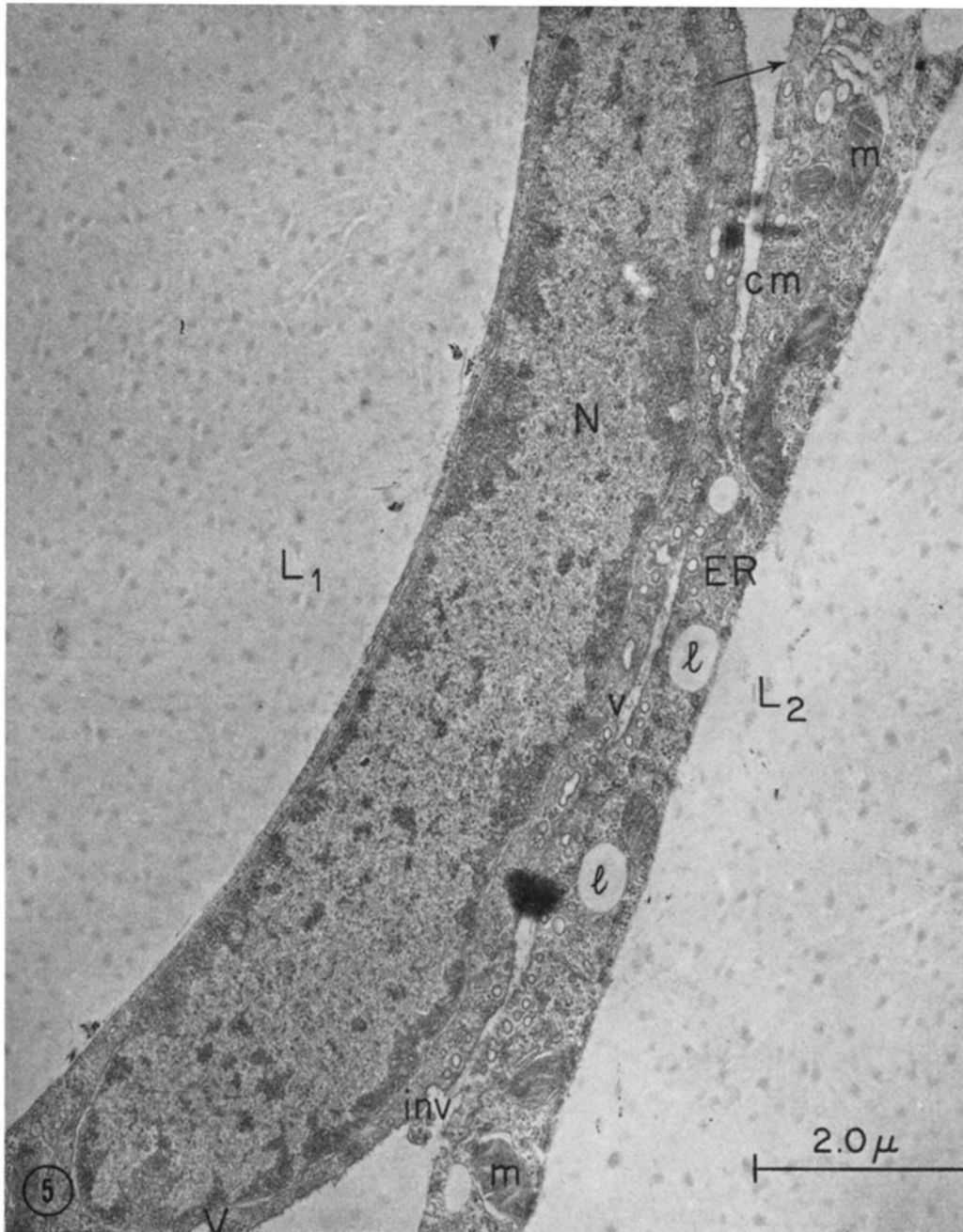
FIGURE 5 Isolated adipose cells incubated 60 min in KRB-albumin buffer in the presence of 1.0 μg epinephrine per ml. L, large, central lipid droplet; l, cytoplasmic lipid droplet; cm, cell membrane; m, mitochondrion; N, nucleus; V, vacuole; v, vesicle; inv, invagination; ER, endoplasmic reticulum; $\downarrow$, break in cell membrane. × 15,000.
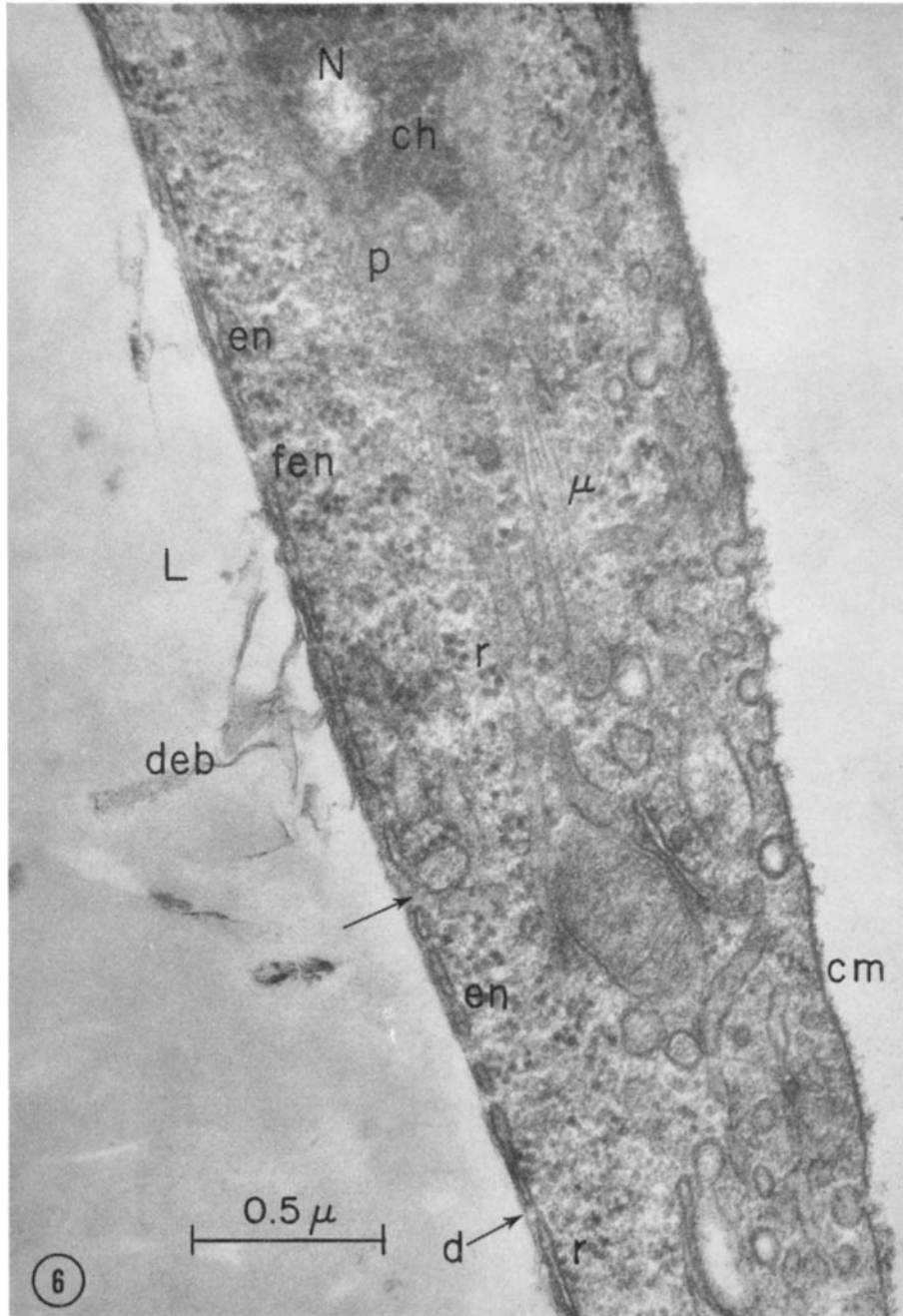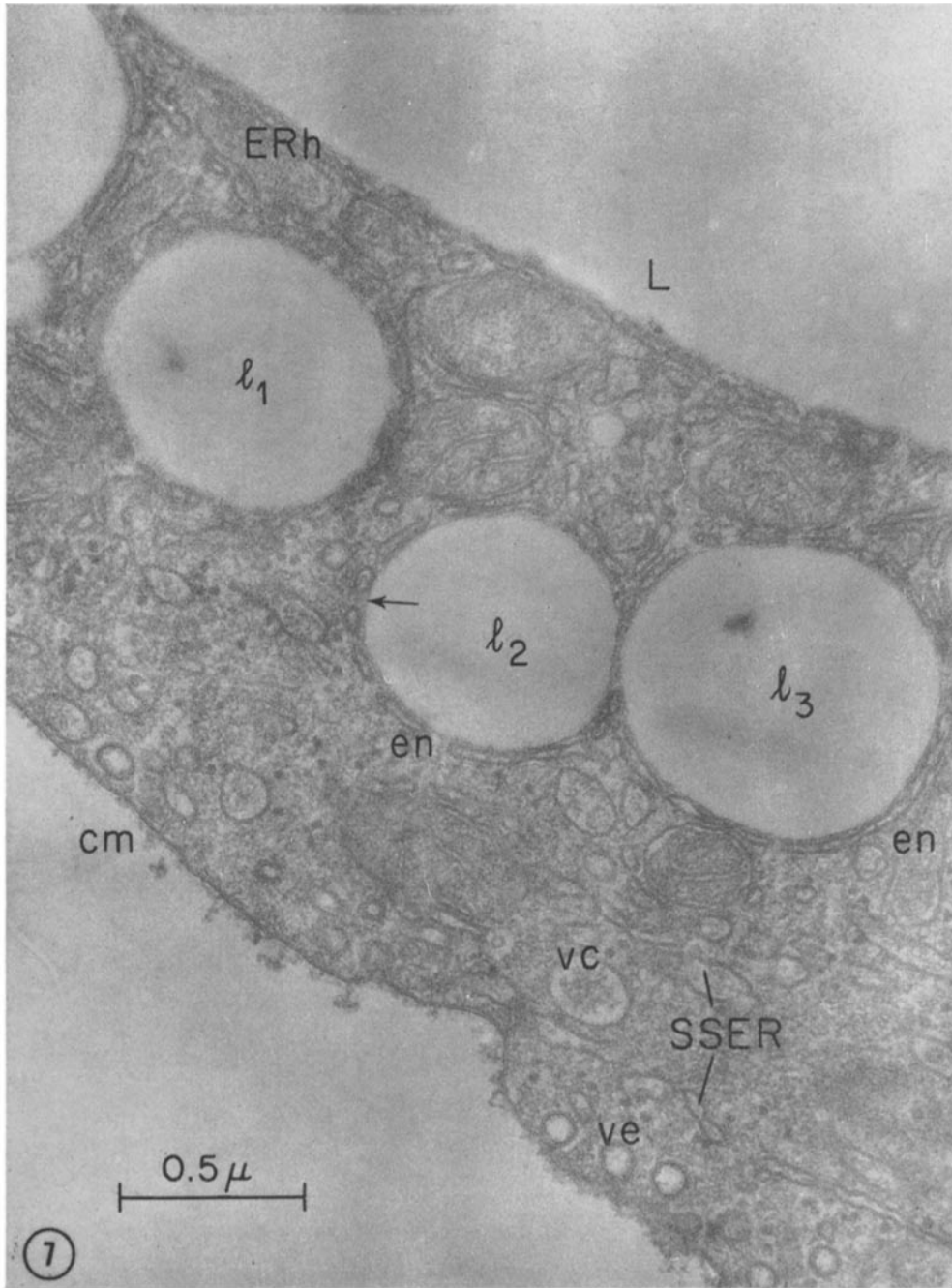
FIGURE 6   Isolated adipose cell incubated 60 min in KRB-albumin buffer in the presence of 100 μU insulin per ml. *L*, large, central lipid droplet; *cm*, cell membrane; *N*, nucleus; *ch*, condensed chromatin; *p*, nuclear pore; μ, microtubule; *en*, fenestrated envelope; *fen*, fenestration; *r*, ribosome; *deb*, electron-opaque debris; *d* ↓, electron-opaque line; ↓, cytoplasm in fenestration. × 51,000.
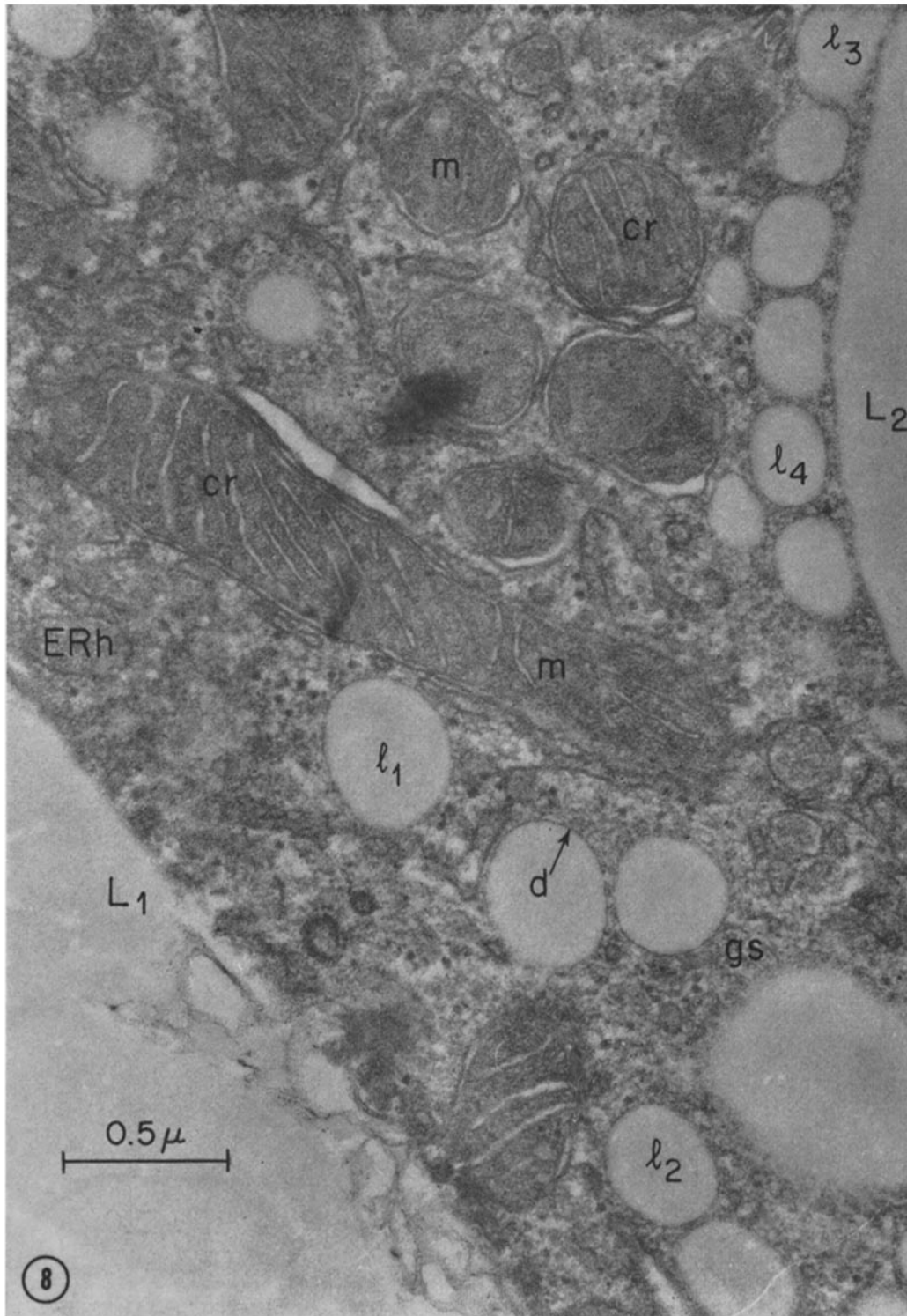
FIGURE 7 Isolated adipose cell incubated 60 min in KRB-albumin buffer in the presence of 1.0 µg epi-
nephrine per ml. *L*, large, central lipid droplet; *cm*, cell membrane; *l*, cytoplasmic lipid droplet; *en*, fenes-
trated envelope; ↓, electron-opaque line; *SSER*, smooth surfaced endoplasmic reticulum; *ERh*, dilated
endoplasmic reticulum; *ve*, empty vesicle; *vc*, vesicle with centrally condensed electron-opaque material.
× 51,000.

FIGURE 8   Isolated adipose cell incubated 60 min in KRB-albumin buffer in the presence of 1.0 μg epinephrine per ml. *L*, large, central lipid droplet; *l*, cytoplasmic lipid droplet; *gs*, ground substance; *d* ↓ , electron-opaque line; *ERh*, dilated endoplasmic reticulum; *m*, mitochondrion; *cr*, crista. × 51,000.

334

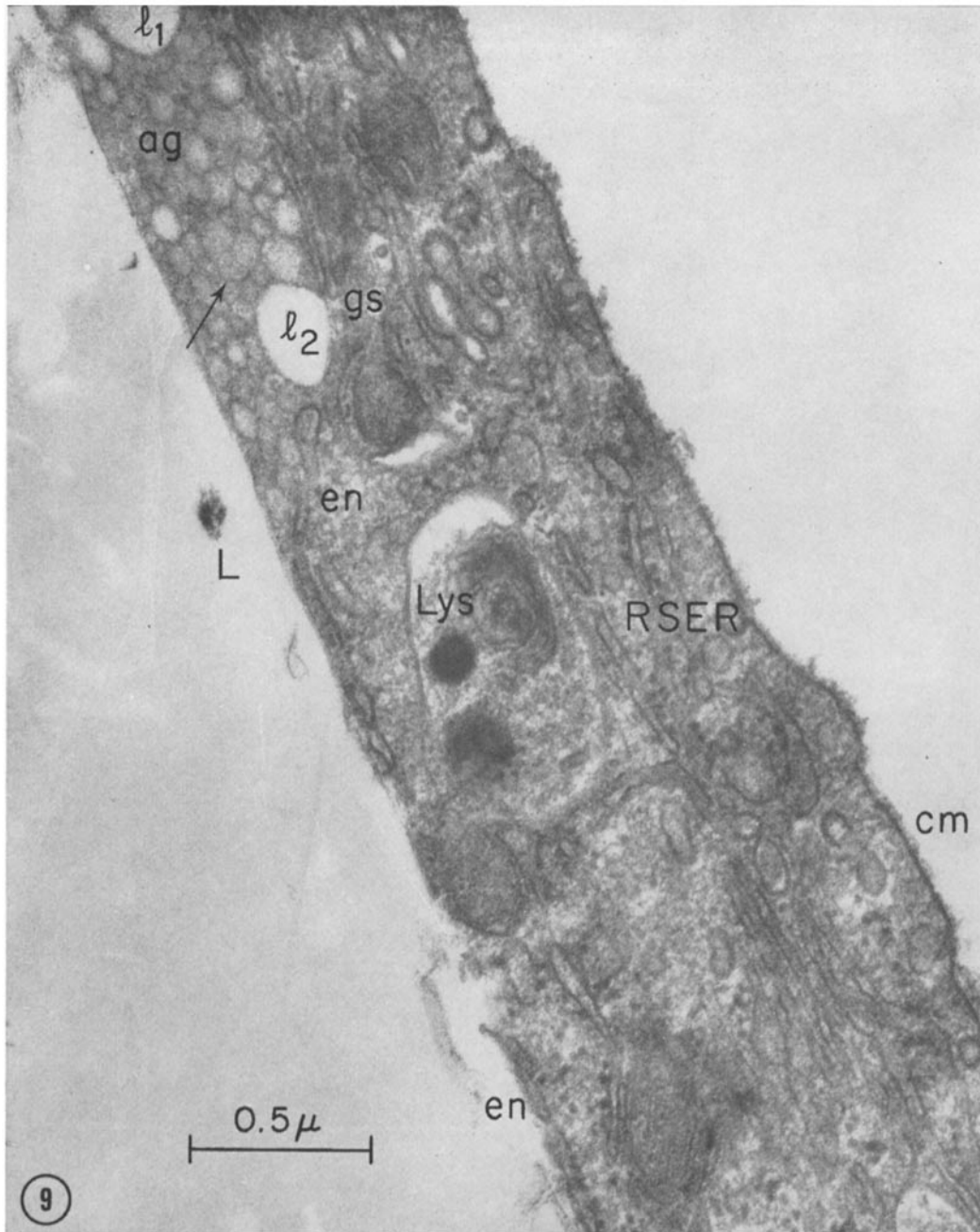FIGURE 9 Isolated adipose cell incubated 60 min in KRB-albumin buffer in the presence of 100 µU insulin per ml. *L*, large, central lipid droplet; *cm*, cell membrane; *l*, cytoplasmic lipid droplet; *ag*, aggregate of amorphous lipid droplets; ↓, electron-opaque material; *gs*, ground substance; *en*, fenestrated envelope; *RSER*, rough surfaced endoplasmic reticulum; *Lys*, lysosomal vacuole. × 51,000.
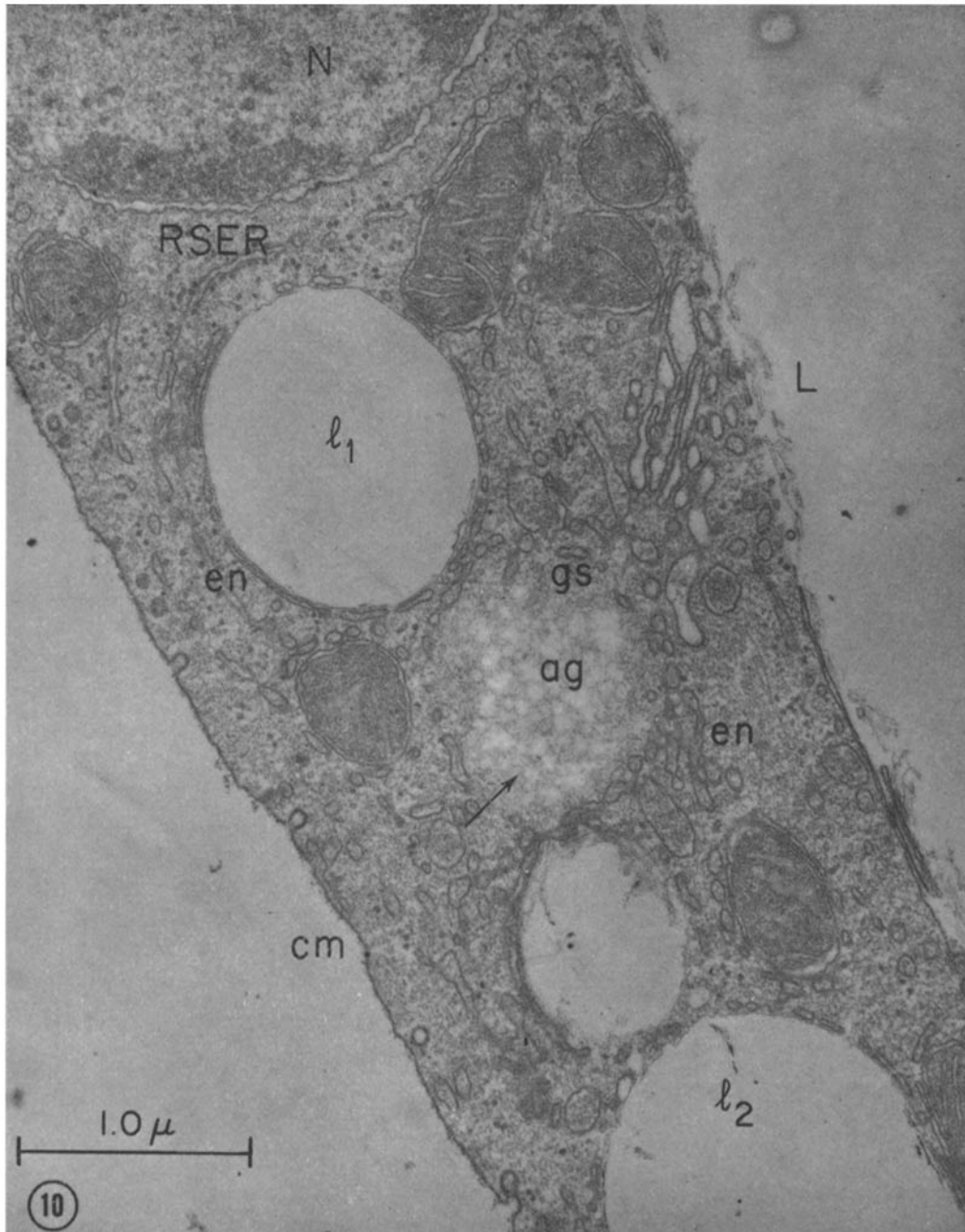
FIGURE 10 Isolated adipose cell incubated 120 min in KRB-albumin buffer in the absence of hormones. *L*, large, central lipid droplet; *l*, cytoplasmic lipid droplet; *cm*, cell membrane; *N*, nucleus; *RSER*, rough-surfaced endoplasmic reticulum; *en*, fenestrated envelope; *ag*, aggregate of amorphous lipid droplets; *gs*, ground substance; ↓, electron-opaque material. × 33,000.
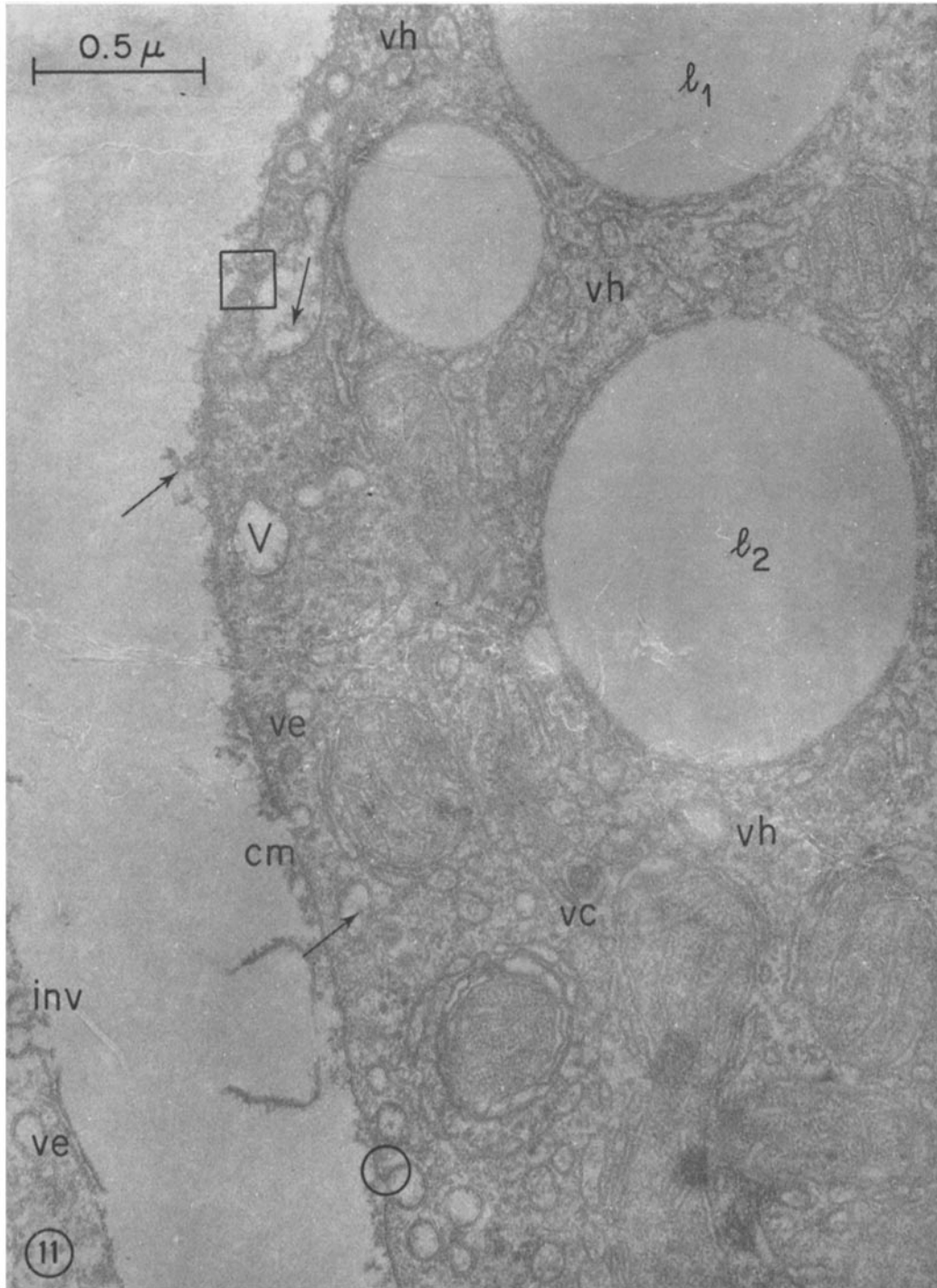
FIGURE 11   Isolated adipose cells incubated 60 min in KRB-albumin buffer in the presence of 1.0 μg epinephrine per ml. *cm*, cell membrane; *l*, cytoplasmic lipid droplet; ↓ , electron-opaque debris; *inv*, invagination; *ve*, empty vesicle; *vc*, vesicle with centrally condensed electron-opaque material; *vh*, vesicle with homogeneously distributed electron-opaque material; *V*, vacuole; ◯, differentially stained leaflets; ☐, vesicular opening continuous with cell membrane. ✕ 51,000.

337

as often in cells treated with insulin as in untreated cells and very rarely in epinephrine-treated cells. Here, as was the case with lipid droplets of the second class discussed above, the observations were too few to allow statistical correlations.

The additional ultrastructural feature which particularly characterizes the adipose cell is the extent of its systems of membranes (Figs. 7, 9–12). A cell membrane bounds the cytoplasm of the adipose cell; a system of cytoplasmic vesicles and vacuoles appears to originate from it (Fig. 11). In place of the amorphous, but regular, basement membrane which surrounds each adipose cell within intact adipose tissue (1, 2), an electron-opaque debris is found which adheres to the cell membrane of the isolated cell. The cell membrane itself directly separates the cytoplasm from the extracellular space. At irregular intervals, invaginations of varying dimensions distort the cell membrane. Each invagination contains electron-opaque debris identical in appearance to that adhering to the cell membrane. In addition, the staining pattern which characterizes the cell membrane, that is, an outer leaflet of higher electron opacity than the inner leaflet, likewise characterizes the invaginations, but in the reverse order. Invaginations range from slight indentations of the cell membrane to fully developed vesicles which remain connected to the cell membrane only by a narrow neck.

Complex invaginations of the cell membrane appear as rosettes, with many fused vesicles, or as small vacuoles, with only one or two fused vesicles which remain identifiable (Fig. 11). While many of these complex invaginations are continuous with the cell membrane, others appear to lie free in the cytoplasm adjacent to it. Single vesicles also appear to lie free in the cytoplasm near the cell membrane. The morphological similarities among simple invaginations, rosettes, small vacuoles, and vesicles suggest their common origin at the cell membrane. These structurally similar subcellular elements may, however, represent portions of a highly contorted cell membrane, and their subdivision into distinct classes may reflect sectioning artifacts.

Although vesicles and small vacuoles are observed throughout the cytoplasm (Fig. 11), those characterized as being of cell membrane origin are localized solely to the narrow region of cytoplasm adjacent to the cell membrane. They are devoid of electron-opaque material save the debris ad-

hering to their membranes. Those vesicles and vacuoles localized predominantly to the interior of the cytoplasm characteristically have uniformly stained membranes and contain either centrally condensed or homogeneously distributed electron-opaque material. Such vesicles are found only occasionally near the cell membrane.

The extensive SSER of the adipose cell is usually flat in appearence but can be observed in configurations which are sufficiently vesicular as to render difficult a distinction between them and those vesicles localized to the interior of the cytoplasm (Figs. 7, 8, and 11). Their lumina, in either case, generally contain abundant electron-opaque material. RSER is generally scarce in adipose cells; in addition, for those few profiles visualized, ribosomes are attached in irregular numbers at irregular intervals (Figs. 6, 9, and 10). The adipose cell does, however, contain an occasional RSER cistern on which many ribosomes are closely and regularly packed.

Golgi complexes in the adipose cell (Figs. 10 and 12) vary widely in the relative proportion of cisternal and vesicular elements constituting them and in the structure of their cisternal elements. The number of vesicles associated with each Golgi complex varies from few to a quantity constituting almost the entire complex. The cisternal membranes appear to vary in structure from closely packed, well organized layers (Fig. 10) to dispersed arrays of contorted, swollen sacks (Fig. 12). Golgi vesicles typically contain amorphous electron-opaque material while the cisternal membranes are electron-lucent. Golgi complexes in the adipose cell, independent of their organization and membrane structure, are frequently associated with regions of the cytoplasm which are devoid of all formed subcellular elements save a few free ribosomes and vesicular profiles. Microtubules are characteristically observed in these regions (Fig. 12).

Adipose cell organelles are most easily observed in the thickened cytoplasm containing the nucleus (Fig. 10) or in regions of the cytoplasm between large lipid droplets (Fig. 8). The nucleus, although usually flattened, is characterized by its pores, peripherally condensed chromatin, and associated microtubules (Fig. 6). Mitochondrial profiles are usually circular or filamentous (Fig. 8). Cristae, originating on either side of the mitochondrion, extend in parallel well into the lumen, but are not closely packed. Mitochondria are occasionally associated with flattened segments of SSER. The
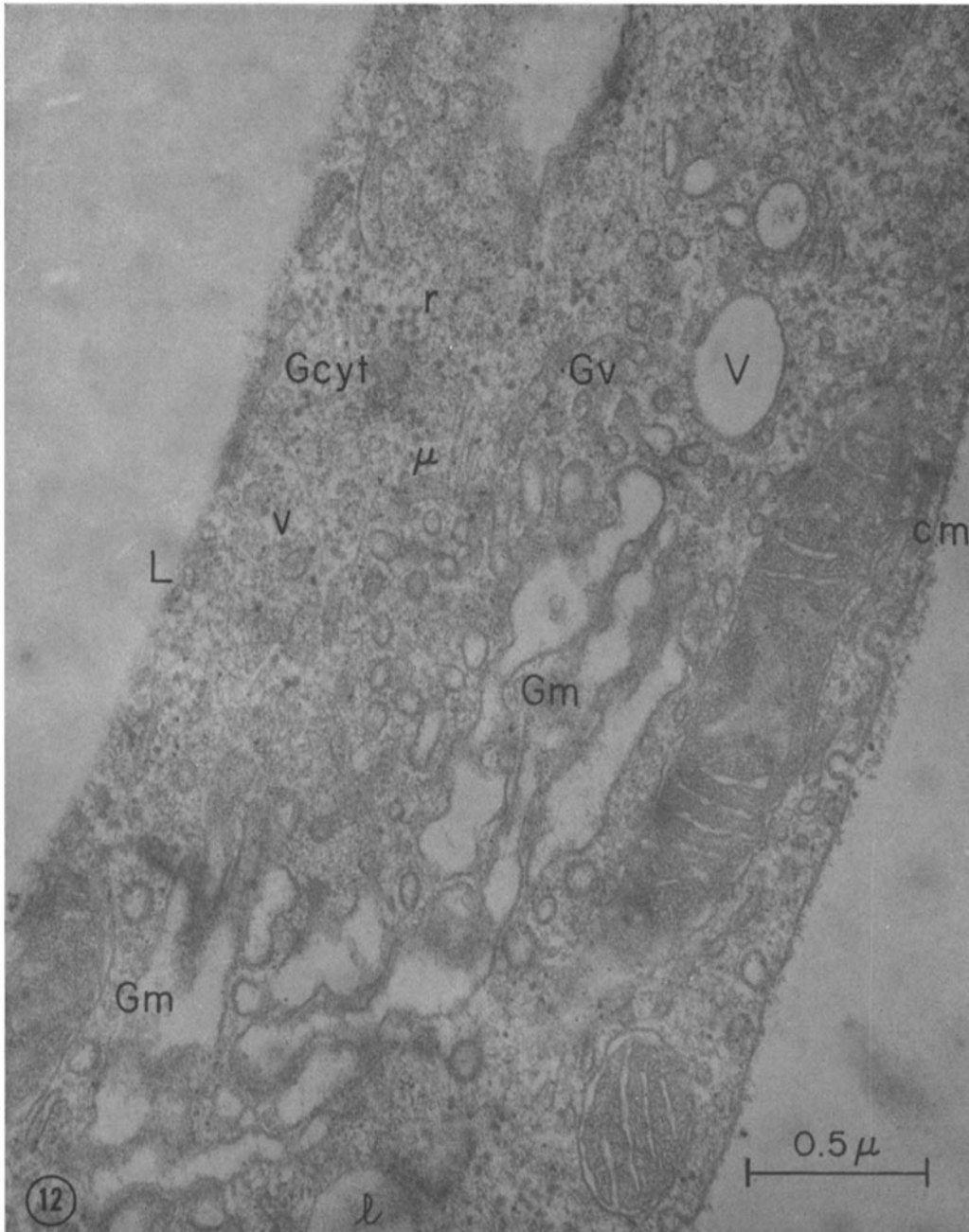
FIGURE 12   Isolated adipose cell incubated 60 min in KRB-albumin buffer in the presence of 1.0 μg epinephrine per ml. *L*, large, central lipid droplet; *cm*, cell membrane; *l*, cytoplasmic lipid droplet; *V*, vacuole; *Gm*, cisternal membrane of Golgi complex; *Gv*, vesicle of Golgi complex; *Gcyt*, special region of cytoplasm associated with Golgi complex; *v*, vesicle; *r*, ribosome; *μ*, microtubule. × 51,000.

adipose cell contains numerous free ribosomes (Figs. 6, 8, 11, and 12). Lysosomes, containing condensed electron-opaque material and membrane profiles, are also observed (Fig. 9). Mitochondria, free ribosomes, some SSER, and vesicles of cell membrane origin do occur even in the thinnest regions of the adipose cell cytoplasm.

DISCUSSION

A variety of lipid droplets and a complex and extensive network of vesicular and smooth membraned subcellular elements are the most prominent among the ultrastructural features of the isolated adipose cell. The collagenase treatment used in the preparation of isolated adipose cells from rat epididymal fat pads leaves the cells essentially intact save a destruction of their basement membranes (1-4, 18).

Insulin and epinephrine, well known stimulators of triglyceride synthesis and mobilization, respectively, do not produce qualitative alterations in the ultrastructure of the adipose cell under the relatively physiological conditions examined. The suspected increase in the number of lipid droplets noted above was not based on a sufficient number of observations to allow quantitation.

Of potential interest as structural elements which might function specifically in the adipose cell's metabolic responses to insulin and epinephrine are the three classes of cytoplasmic lipid droplets. Individual lipid droplets, surrounded by fenestrated envelopes, have been readily identified in all cells in which a region of thickened cytoplasm has been sectioned (3, 10). The smaller droplets, characterized by their lack of association with fenestrated envelopes, have been identified in roughly one-tenth this number of cells cut, although most of these smaller droplets occurred in cells exposed to epinephrine. Aggregates of small, amorphous droplets have been identified even less frequently; they occurred approximately twice as often in cells exposed to insulin than in control cells and practically not at all in cells exposed to epinephrine. No statistical validity can be attached to these apparent alterations, however, due simply to the small total number of positive identifications obtained and the absence of a clearly definable parameter on which to base a statistical comparison.

The presence in the cytoplasm of the adipose cell of a large number and variety of vesicles and smooth-surfaced membranous elements suggests the operation of a complex compartmentalized transport and metabolic system. Such elaborate membranous systems have been shown to function in the intracellular transport of substances taken up from or to be released into the extracellular space by other cell types, most notably by the mouse peritoneal macrophage (19, 20) or the acinar cell of the pancreas (21, 22), respectively. The presence, in the adipose cell, of vesicular structures at or near the cell membrane (1-4, 9) and of membranous envelopes around most of the cell's lipid droplets and the well established bidirectional transport and metabolism of fatty acids by the adipose cell (5-8) offer the possibility that a compartmentalized transport and metabolic system, if such exists in the adipose cell, might function in fatty acid uptake and release and in triglyceride synthesis and mobilization (23). Invaginations of, and vesicles near, the cell membrane have been identified in sections from every cell cut. A statistical analysis of alterations in the number of such structures observed, however, could not be obtained since the variation observed in sections from the same cell or from cells treated together proved to be as large as in cells exposed to different experimental conditions.

The ability to observe the metabolism and the ultrastructure of the isolated adipose cell simultaneously permits correlations between alterations in structure and alterations in function. This study describes the methodology and provides the high resolution ultrastructural characterization necessary for such studies. The lack of hormonally produced structural alterations shows that the adipose cell is prepared to respond rapidly to either lipogenic or lipolytic hormones. Dynamic structural responses to hormones may occur in those of the cell's subcellular organelles which contain lipid. A vesicular transport system responding in this fashion (1, 2, 4, 9) could be analyzed by nonmorphological techniques. In the following paper some of the factors influencing pinocytosis are measured quantitatively using radioactive colloidal gold (24).

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