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ELECTRON MICROSCOPIC STUDIES OF THE DIFFERENTIATION OF FAT CELLS IN HUMAN FETAL SKIN*

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ABSTRACT

1) Electron microscopic studies of primitive fat organs showing various degrees of fat storage from human fetuses 20 to 26 weeks of age were undertaken, with special reference to the origin of fat cells and the lipid formations in them. Based on this study, three types of cells, namely an undifferentiated-type cell, a young-type fat cell and a mature-type fat cell were discerned ultrastructurally, but they are considered to be identical concerning the morphology of mitochondria, smooth endoplasmic reticula and glycogen granules.

2) Concerning the origin of white fat cells, it was revealed that young-type and mature-type fat cells are not derived from reticuloendothelial cells or fibroblasts but from a certain definite type of mesenchymal cell which we have referred to as the undifferentiated-type cell.

3) The finding that cytoplasmic microvesicles are more prominent in young-type fat cells than in mature-type ones might be interpreted that the young-type cells may be actively releasing lipids or free fatty acids according to Williamson's concept of these organelles. But, contrary to Williamson's postulation, the young-type fat cells seem to be accumulating lipids or free fatty acids.

Despite previous embryological studies on the differentiation of fat cells in human skin made by light microscopy, important problems still remain unsolved (1–5). The present report concerns ultrastructural changes of fat cells in human fetal skins during their differentiation, with special reference to the origin of fat cells and the lipid formations in them.

MATERIALS AND METHODS

The materials were obtained from the skins of the limbs of human fetuses 20 to 26 weeks old (approximately 30 mm long in foot length and 191 mm long in crown-rump length). Previous histological studies have indicated that fat cells begin to develop in the human fetal skin at about 20 weeks (5).

The specimens were fixed with 4–6.5% glutaraldehyde for 2 hours followed by postosmification in 1% osmium tetroxide for one hour. After dehydration in graded ethanols, the materials were embedded in Epon 812. The sections for electron microscopy were stained with 2% uranyl acetate and 0.4% lead citrate.

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RESULTS

Light Microscopy

As seen in Figure 1, two types of fat cells were found. They are the cell containing multiple small lipid droplets (Y) and the signet, ring-type cell (M). Those cells were studied with the light microscope using primitive fat organs showing various degrees of fat storage in the skin of human fetuses 20 to 26 weeks old. Previously, as a result of light microscopic studies, these two types of cells were considered to represent the early to mid stage and end stage in maturation of *in utero* fat cells (3–5).

Electron Microscopy

Three types of fat cells were discerned by electron microscopic investigations of primitive fat organs from the same skin samples as those of the light microscopic observations mentioned above: (1), a cell which seems to correspond to the cell containing multiple small lipid droplets observed by light microscopy and which is referred to hereafter as the young-type fat cell; (2), a cell which seems to correspond to the light microscopic signet ring-type cell, which is referred to hereafter as the mature-type fat cell; (3), a cell which is considered to be the precursor cell developing to-

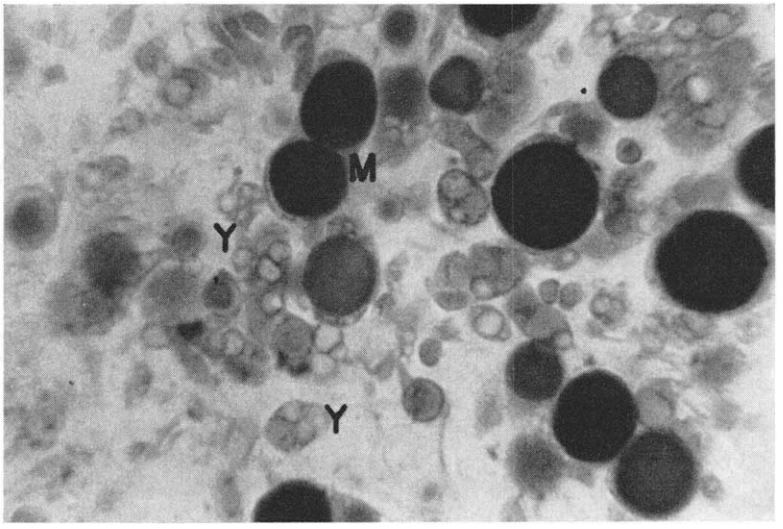


FIG. 1. Light micrograph of primitive fat organs with beginning fat storage found in the subepidermal mesenchyme of limb skin from a 21 week-old human fetus. Y: a cell containing two or more small lipid droplets. M: a signet-ring type cell with a large central lipid droplet. Stained with hematoxyline-eosin. (× 400)

ward the young-type fat cell though it has not yet developed any lipid droplets in its cytoplasm and which is referred to hereafter as the undifferentiated-type cell.

1. Young-Type Fat Cells

Cells of this type are variable in size, usually being thick and short in shape, with the cytoplasm having multiple lipid droplets accompanied by a large number of glycogen granules, numerous mitochondria, well developed smooth endoplasmic reticula, numerous cytoplasmic microvesicles compatible with those described by Williamson (6) and a relatively small number of rough endoplasmic reticula. A brief description of each of these elements follows:

Lipid droplets are generally round to ovoid in shape but some are irregular in shape. Some lipid-cytoplasmic interfaces of these droplets are bordered by thin dense lines over much of their area, others by an array of parallel fine filaments approximately 50 Å wide with nearly regular spacing which makes them appear as if they had been carefully brushed (Fig. 3). Wood (4) reported, in observations of adipose cells from the chick, fine filaments 40 to 50 Å wide showing a similar ordered array to that of the above-mentioned filaments. Luckenbill and Cohen (7) reported, in observations of avian sybsynovial adipose cells, a series of fine filaments 70 to 100 Å wide associated with lipid droplets. The pattern in which the latter filaments are arranged seems to differ from that of the filaments in the present investigations, in having a grid-like pattern.

The lipid droplets are variable in size and electron density. Some of them contain numerous electron dense substances, especially in their peripheral portion, while others contain electron less dense structures which are needle-shaped, cleft-shaped or tubular (Fig. 2). The majority of these structures is considered due to artifact, except for some of the tubular ones which seemingly come from endoplasmic reticula.

Glycogen granules are predominantly α-type and distributed throughout the cytoplasm (Figs. 3–6).

Mitochondria are usually round to ovoid in shape and show a topographically close relationship to groups of lipid droplets (Figs. 2, 3).

Smooth endoplasmic reticula are numerous, most of them being long and tubular in shape with random orientation among glycogen granules (Figs. 2, 4).

Cytoplasmic microvesicles are located prin-

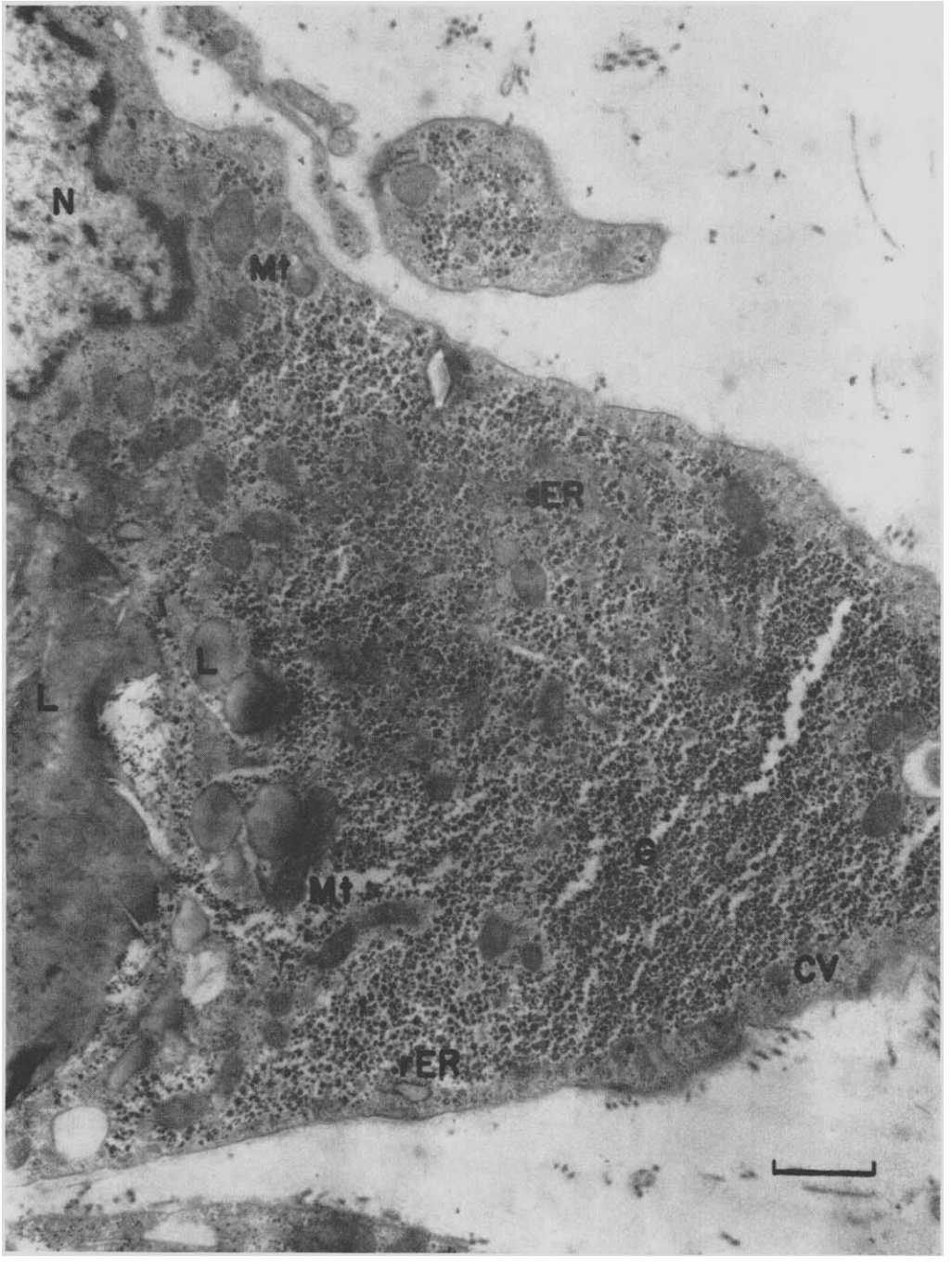


FIG. 2. Low power view of a portion of a young-type fat cell in the limb skin. Multiple lipid droplets (L) are clustered around the nucleus (N), and surrounded by numerous mitochondria (Mt), smooth endoplasmic reticula (sER) and abundant glycogen granules (G). CV: numerous cytoplasmic microvesicles in the peripheral zone of the cytoplasm. rER: rough endoplasmic reticula. ($\times$ 14,500, 1.45 cm = 1μ)

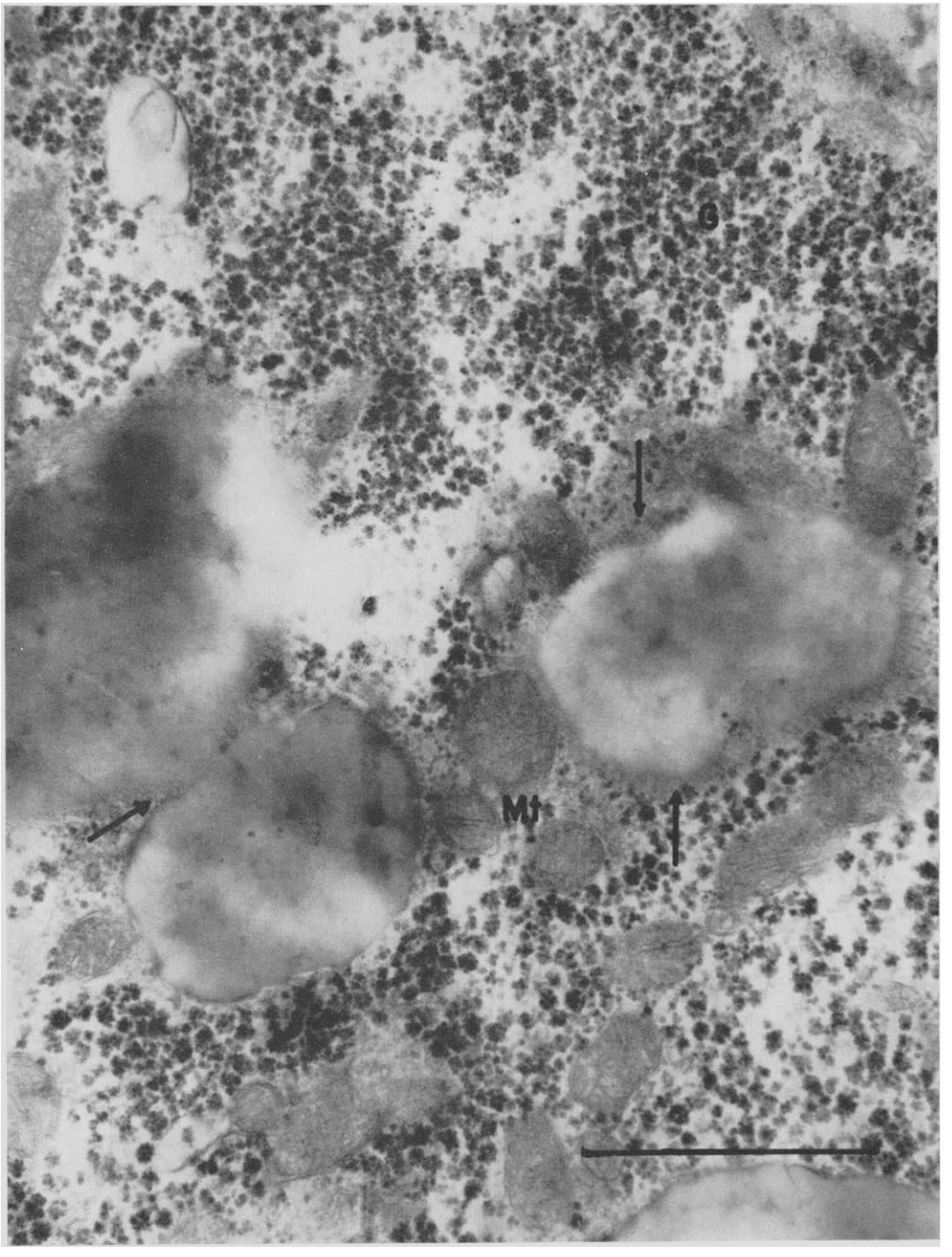


FIG. 3. An ordered array of fine filaments (arrow) bordering lipid-cytoplasmic interfaces in the cytoplasm of a young-type cell. Mt: mitochondria. G: α-type glycogen granules. (× 43,500, 4.35 cm = 1μ)

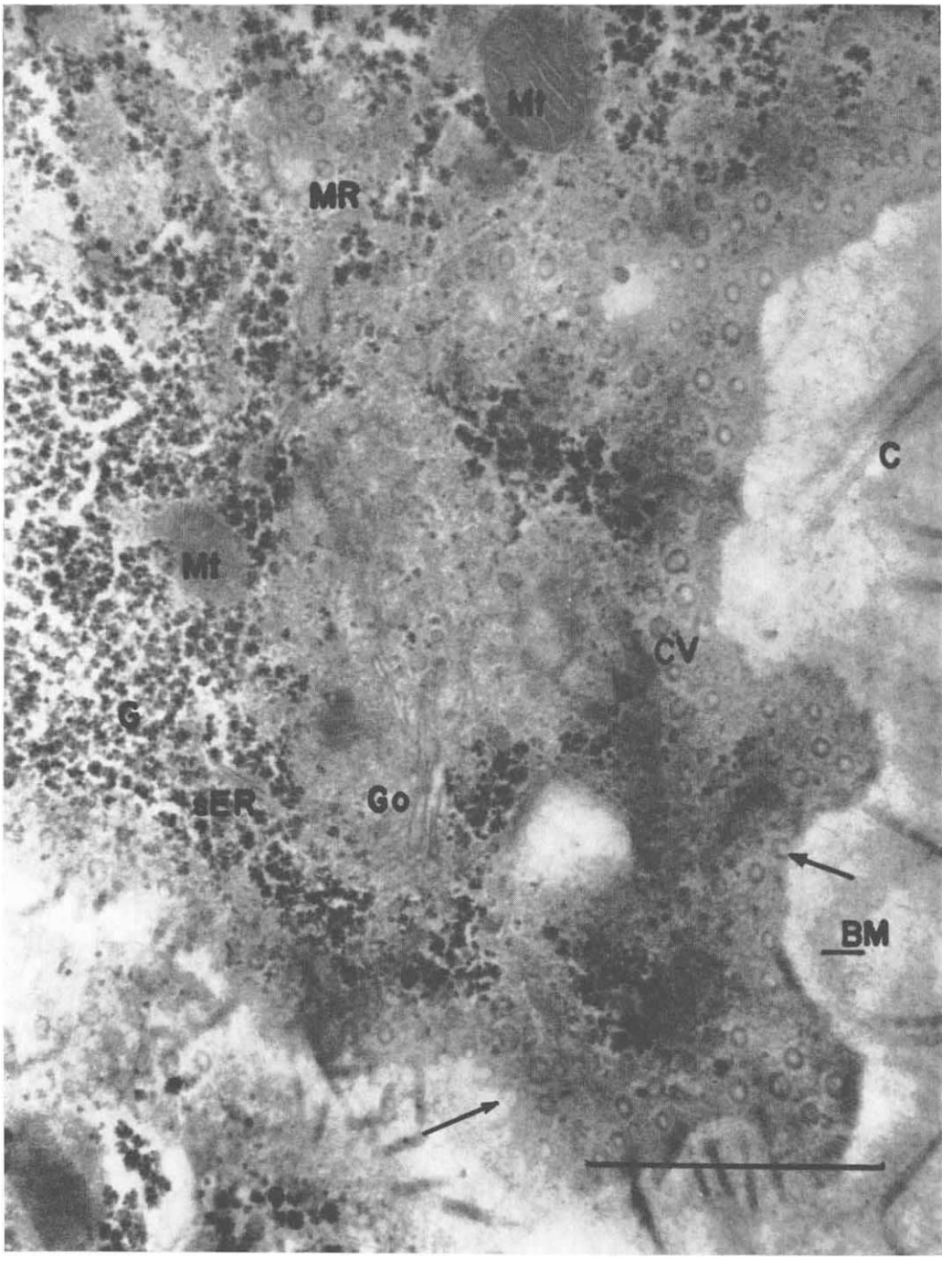


FIG. 4. Cytoplasmic microvesicles (CV) in the peripheral zone of the cytoplasm of a young-type fat cell. MR: microvesicular rosette which consists of several cytoplasmic microvesicles. Arrows indicate flask-like invaginations of the cytoplasmic membrane. Go: Golgi apparatus. sER: smooth endoplasmic reticula. Mt: mitochondria. G: α-type glycogen granules. BM: basement membrane. C: collagen fibers. ($\times$ 43,500, 4.35 cm = 1μ)

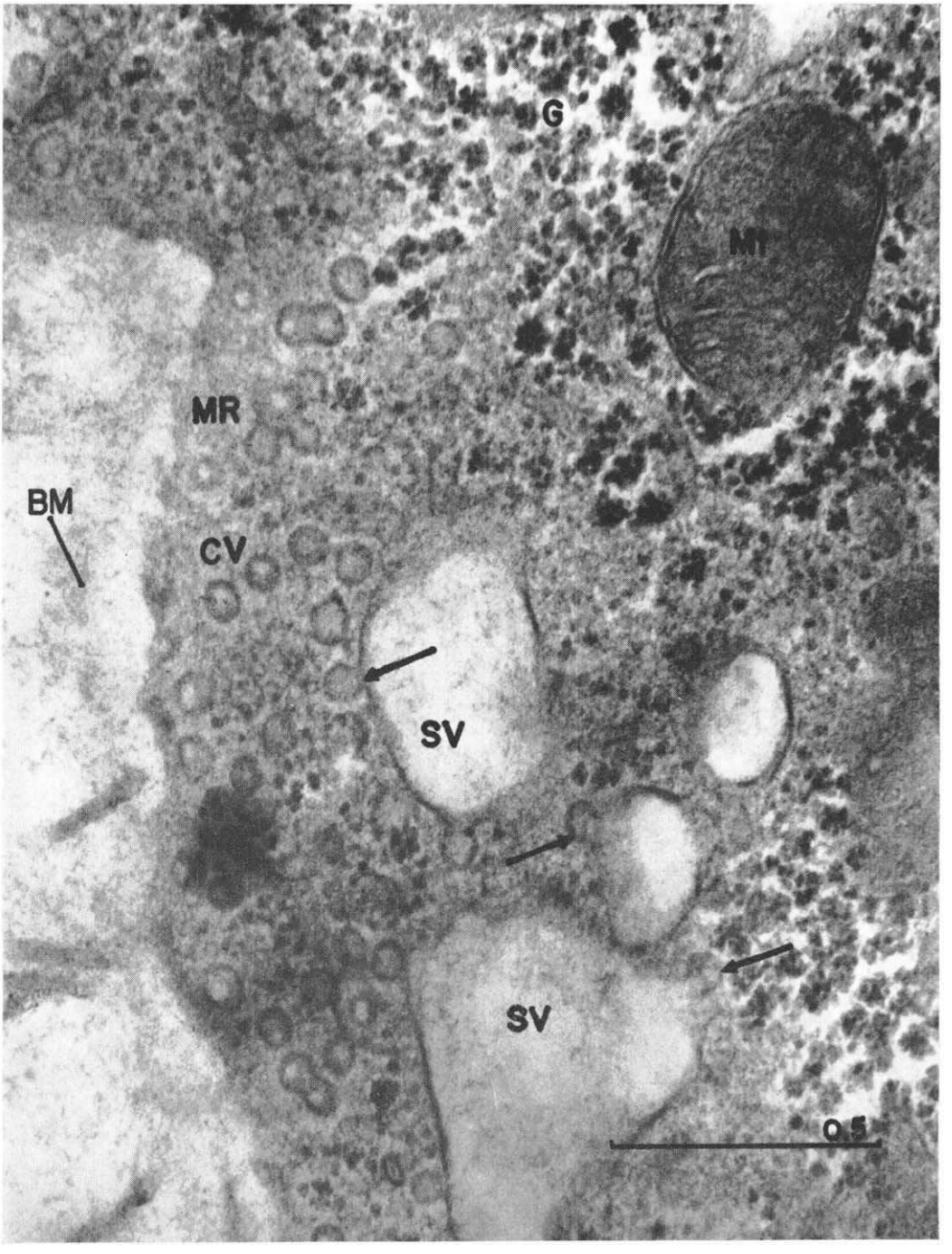


FIG. 5. Microvesicular rosette (MR) and flask-like invaginations. CV: numerous cytoplasmic microvesicles in the peripheral zone of the cytoplasm. Arrows indicate flask-like invaginations of smooth surfaced vacuoles (SV). Mt: mitochondria. G: α-type glycogen granules. BM: basement membrane. (× 79,500)

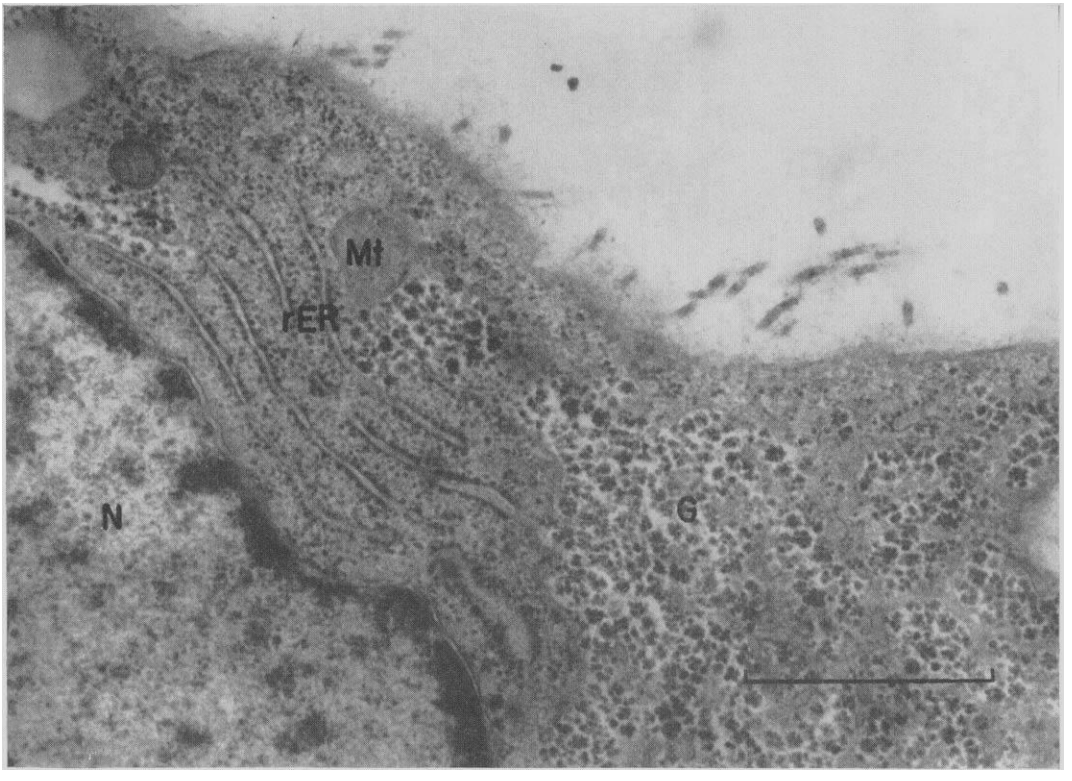


FIG. 6. Rough endoplasmic reticula (rER) arranged in lamellar form of a young-type fat cell. Mt: mitochondria. G: α-type glycogen. N: nucleus. (× 32,400, 3.6 cm = 1μ)

cipally in the peripheral zone of the cytoplasm. Among them are rosette-like structures which consist of several microvesicles (Figs. 4, 5, MR). In addition to these rosette-like structures, there are some microvesicles, whose limiting membrane fuse with those of smooth surfaced intracytoplasmic vacuoles, with infolded cavities of cytoplasmic membranes, or directly with cytoplasmic membranes, forming flask-like invaginations in each of these cases, similar to the observations of Williamson (Fig. 5, arrows).

Rough endoplasmic reticula are generally few in number and short and tubular in shape. Occasional cells show them well developed in which case they have a lamellar arrangement (Fig. 6), or are dilated and cystiform.

Basement membranes are occasionally found overlying portions of the cytoplasmic membrane of some fat cells of this type. They usually have tufts of fine filaments along the membrane (Figs. 4, 5).

2. Mature-Type Fat Cells

Most cells of this type are larger than the young-type cells, nearly ovoid in shape and have a large central lipid droplet which seems to be made up by the enlargement and coalescence of the multiple lipid droplets in young-type cells. This central lipid droplet displaces the nucleus to the periphery of the cell, being occasionally accompanied by a few small droplets. So, this type of cell frequently shows a very thin rim of cytoplasm which encompasses a large central lipid droplet (Fig. 8). Cytoplasmic microvesicles and microvesicular rosettes markedly decrease in number, occasionally being absent. Except for these differences, this type of cell shows features almost identical concerning mitochondria, smooth endoplasmic reticula and glycogen granules to those of young-type cells (Fig. 7).

3. Undifferentiated-Type Cells

Cells of this type are mostly thick and short in shape, have no lipid droplets, but show fea-

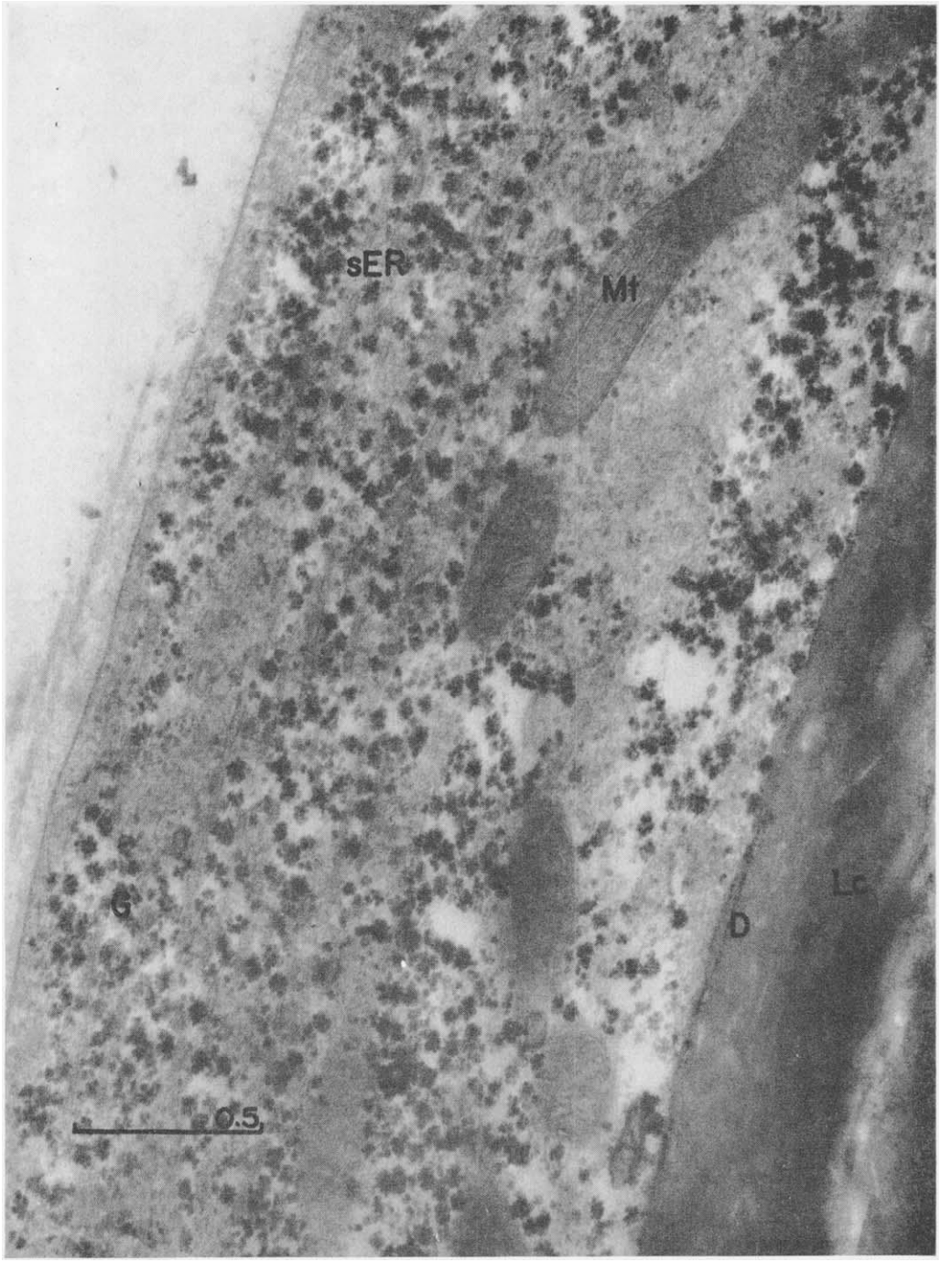


FIG. 7. Cytoplasm of a mature-type fat cell in the limb skin. Lc: Portion of a large central lipid droplet (Lc) contains numerous electron dense substances (D). The cytoplasm shows nearly same morphology of mitochondria (Mt), smooth endoplasmic reticula (sER) and glycogen granules (G) as in the young-type cell (Fig. 2), except for the evident decrease of microvesicles in contrast to the young-type. (× 54,000)

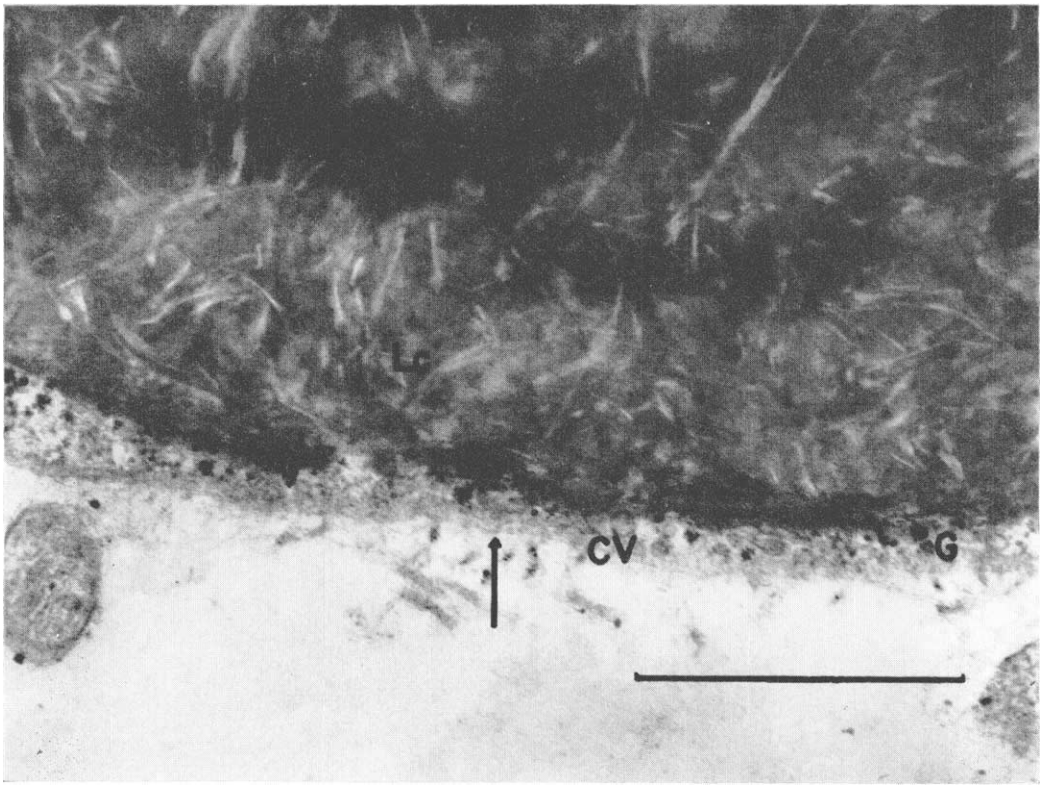


FIG. 8. Thin rim of cytoplasm (arrow) encompassing a large central lipid droplet (Lc)
G: α-type glycogen granules. CV: cytoplasmic microvesicles. (× 43,500, 4.35 cm = 1μ)

tures almost identical to those of young-type cells, especially with respect to the morphology of mitochondria, smooth endoplasmic reticula and glycogen granules (Figs. 9, 10). The number of cytoplasmic microvesicles is relatively less, compared to those in young-type cells. The cell shown in Figure 11 seems to have made the first step in the transformation from the undifferentiated-type into the young-type, with only one small lipid droplet forming in the cytoplasm. This droplet is ovoid in shape, located near a Golgi apparatus and shows a slight degree of homogeneous electron density, except for some small areas in its peripheral zone which are less electron dense (Fig. 12). The other structures of the cell in Figure 11 are similar to those of undifferentiated-type cells.

4. Mesenchymal Cells Other Than Fat Cells

Besides the three types of cells described above, other mesenchymal cells which are ultrastructurally distinguished from any of them were found in primitive fat organs as follows:

Reticuloendothelial cells. Cells which belong to the reticuloendothelial system of the skin, and showing no close relationship to fat cells were found. They are thick and short in shape, having many thin, pseudopodia-like protrusions or micro-villi along their periphery. Their cytoplasm is rich in lysosomal, dense bodies, phagocytic vacuoles and smooth endoplasmic reticula, which are more conspicuously developed than rough surfaced ones (Fig. 13).

Fibroblasts. A relatively small number of fibroblasts were found in primitive fat organs. They are fusiform in shape and usually located a short distance from fat cells (Fig. 14). The cytoplasm has numerous rough endoplasmic reticula, relatively well developed Golgi apparatus, many mitochondria, partially developed filamentous structures and

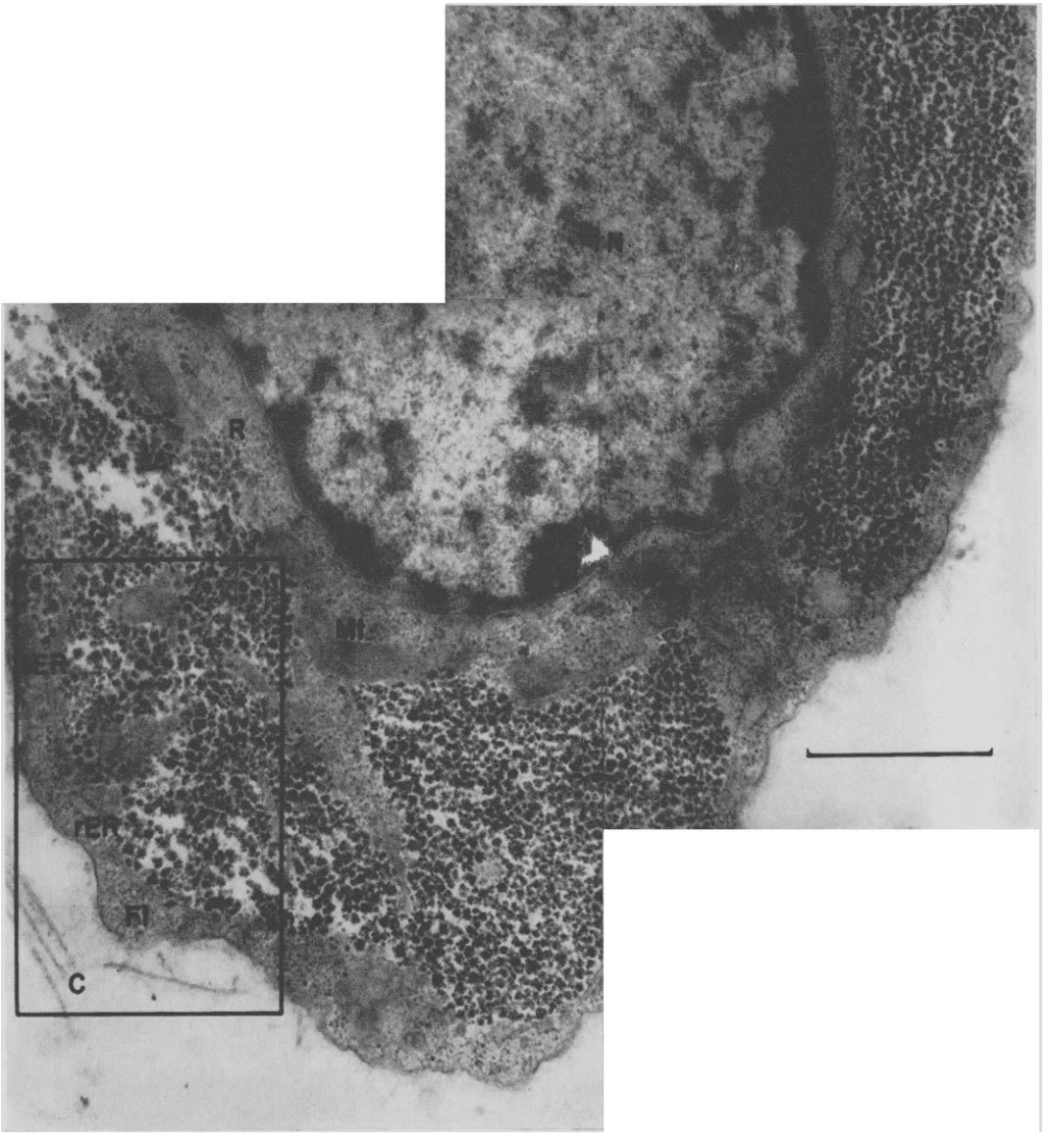


FIG. 9. Low power view of an undifferentiated-type cell in the limb skin. Though the cytoplasm contains no lipid droplet, it reveals nearly the same morphology of mitochondria (Mt), smooth endoplasmic reticula (sER) and glycogen granules (G) as in young-type fat cells. FI: flask-like invaginations. (× 24,495, 3.45 cm = 1μ)

occasional small dense bodies which have not been definitely identified.

Mast cells. They are commonly thick and short in shape, having numerous microvilli or pseudopodia-like projections around the cytoplasmic membrane. The cytoplasm has numerous mast cell granules, most of which are in smooth surfaced vacuoles, mitochondria and a well developed Golgi apparatus. The details of these mast cells will be reported in a separate paper (8).

Ultrastructural features of these mesenchymal cells, i.e., reticuloendothelial cells, fibroblasts and mast cells, differ from those of the fat cells, especially in that the morphology of mitochondria and smooth endoplasmic reticula

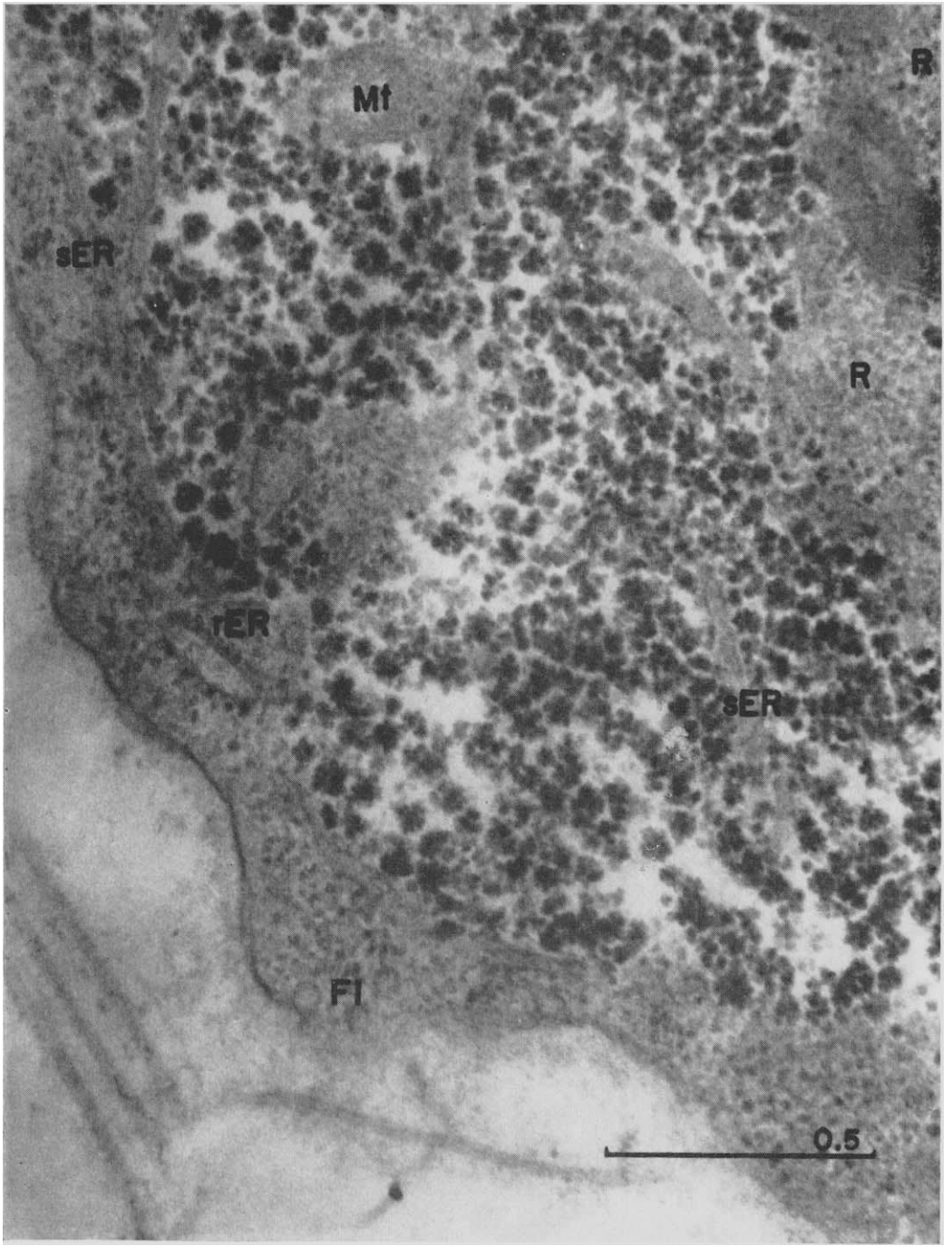


FIG. 10. Enlarged view of the area marked by the rectangle in Fig. 9. SER: numerous smooth endoplasmic reticula long and tubular in shape running among glycogen granules (G). R: free ribosomes. Fl: flask-like invaginations of the cytoplasmic membrane. Mt: mitochondria. rER: rough endoplasmic reticula. (× 79,500)

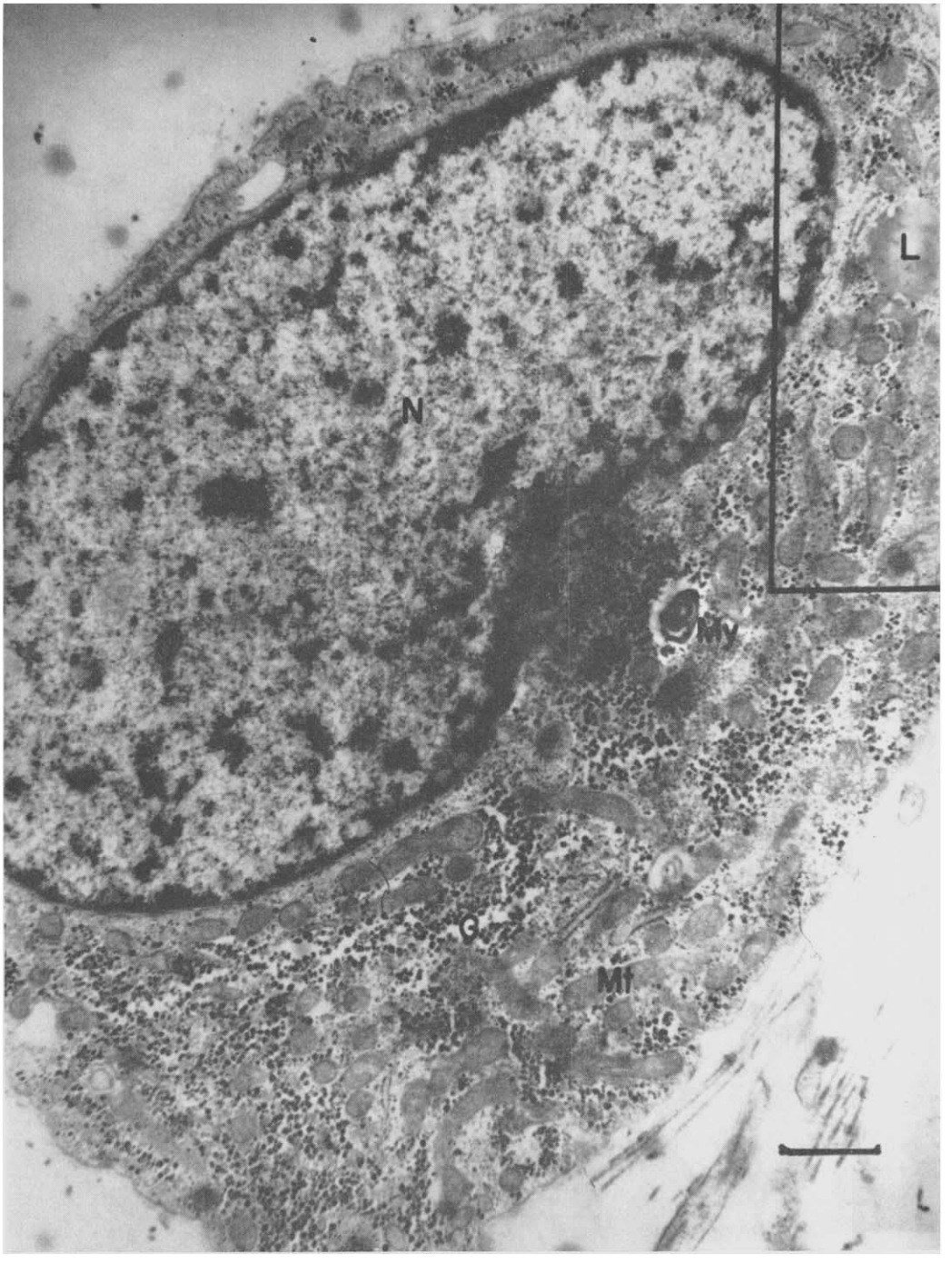


FIG. 11. Low power view of a cell which seems to have made the first step in the transformation from the undifferentiated-type cell toward the young-type fat cell. N: nucleus. L: only one small lipid droplet forming in the cytoplasm. My: electron dense myelin-like structure. Mt: numerous mitochondria. G: glycogen granules. (× 12,500, 1.25 cm = 1μ)

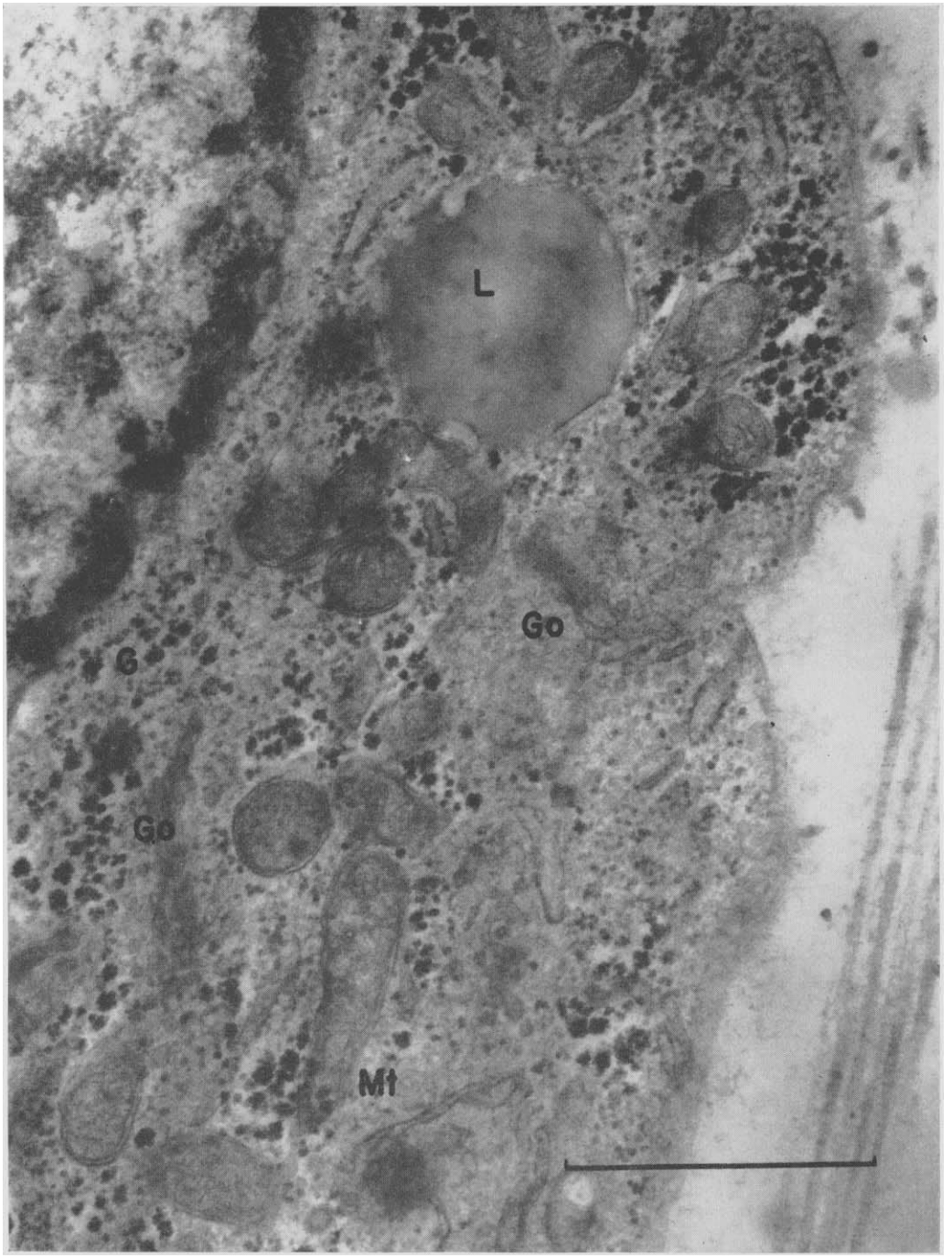


FIG. 12. Enlarged view of the area marked by rectangle in Fig. 11. L: the lipid droplet. Go: Golgi apparatus. Mt: mitochondria. G: α-type glycogen granules. (× 46,500, 4.65 cm = 1μ)

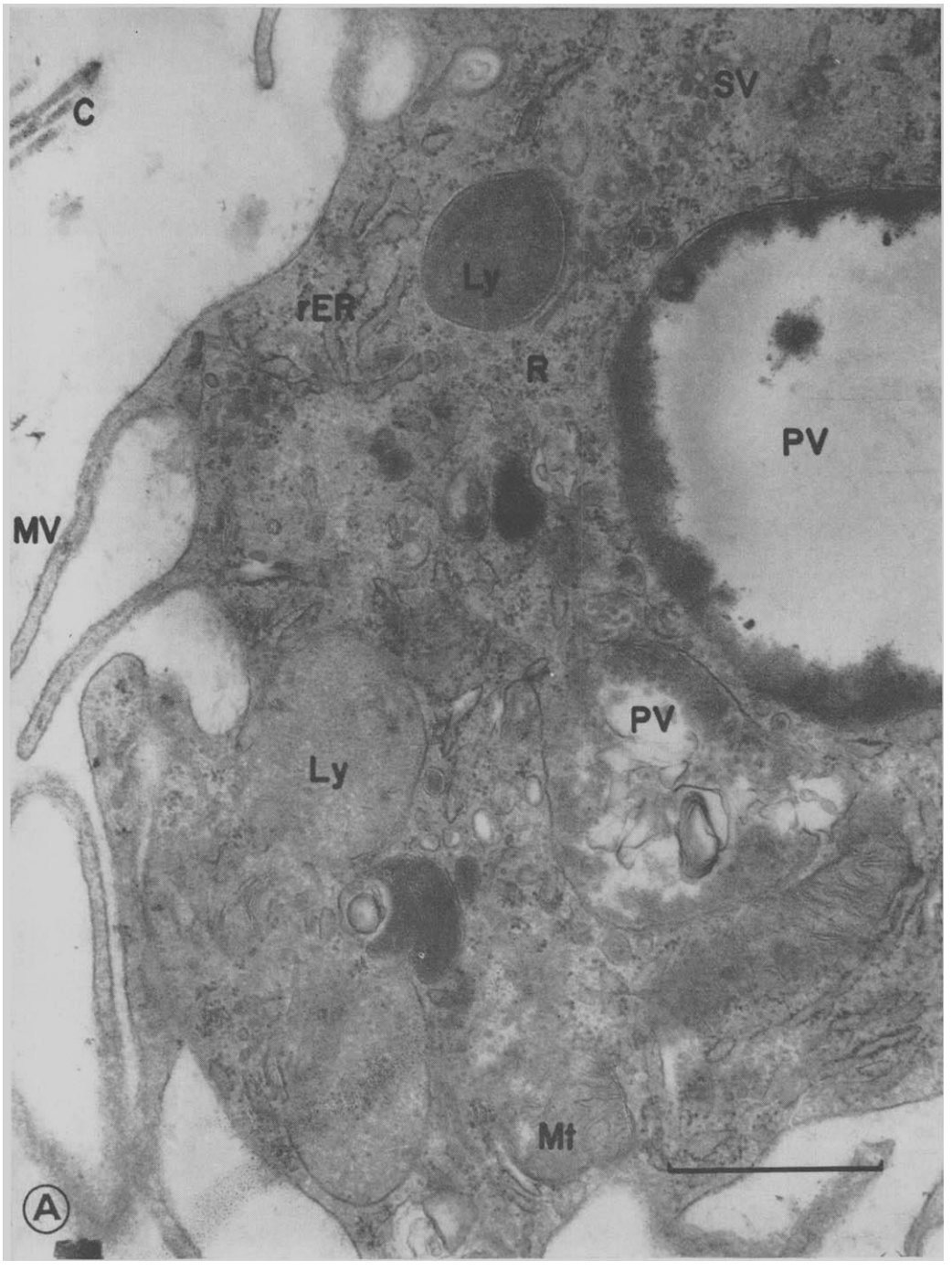


FIG. 13-a. A reticulo-endothelial cell in the primitive fat organs of the limb skin.

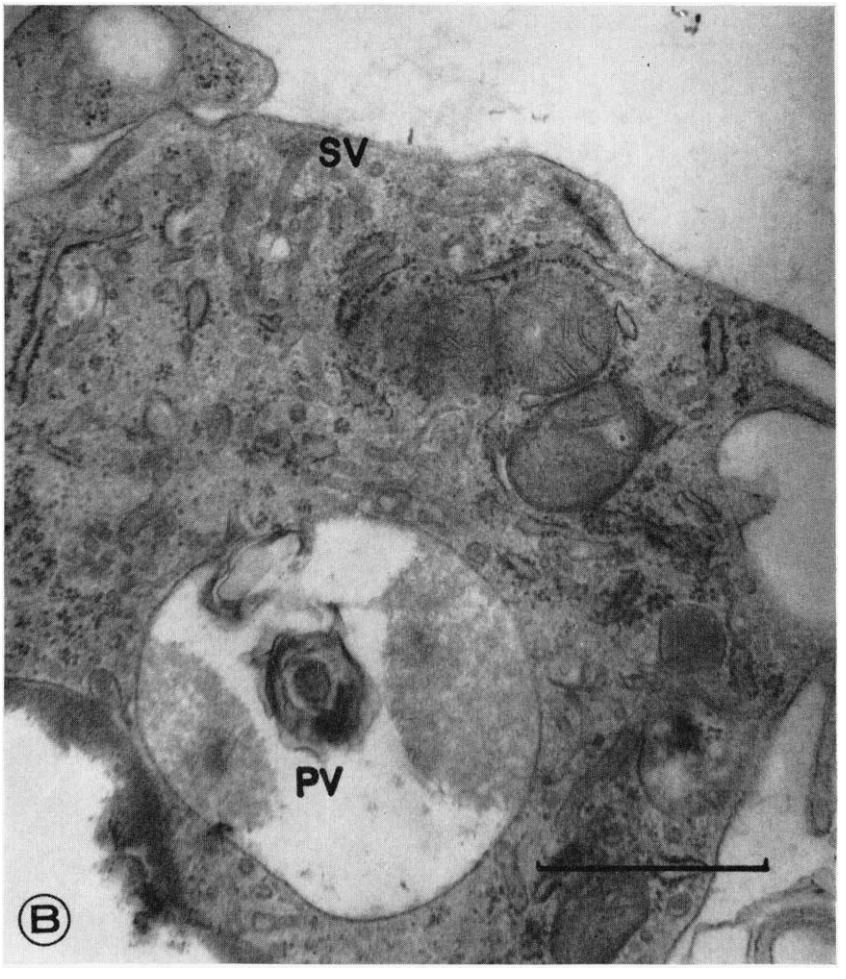


FIG. 13-b. Another portion of the cytoplasm of the same cell in Fig. 13-a. The cytoplasm contains lysosomal dense bodies (Ly), phagocytic vacuoles (PV), numerous smooth vesicles (sV), but no lipid droplets nor glycogen granules. MV: numerous, thin pseudopodia-like protrusions. R: polysomes. rER: rough endoplasmic reticula. Mt: mitochondria. (× 31,000, 3.1 cm = 1μ)

is different, and they have no lipid droplets or glycogen granules in contrast to the fat cells.

DISCUSSION

1. The Origin of White Fat Cells

In the last century, Fleming stated that fat cells were not derived from specific cells, but from fibroblasts in the connective tissue, while Toldt contended that they were derived from specific primitive cells (lipoblasts) in certain areas distinct from surrounding connective tissue (1–5).

This argument concerning the origin of fat cells in white adipose tissue has not yet been conclusively settled, but recently most investigators have agreed with the compromise opinion proposed by Hammar and supported by Wassermann (1–5), that fat cells in white adipose tissue are formed by the differentiation of primitive mesenchymal cells without passing through the stage of fibroblast or reticuloendothelial cell in their process of differentiation.

Our electron microscopic findings of fat cells in human fetal skin favor the last opinion for our observations reveal that they are not derived from reticuloendothelial cells, fibroblasts

or mast cells, but from a certain definite type of mesenchymal cell which we have refered to as the undifferentiated-type cell.

These undifferentiated-type cells are found in the vicinity of young-type and mature-type fat cells, and except that they have no lipid droplets show ultrastructural features identical to the young-type and mature-type fat cells. The ultrastructures of these three types of mesenchymal cells are incompatible with those of the other mesenchymal cells, including reticuloendothelial cells, fibroblasts and mast cells found in primitive fat organs of the same fetus, especially in that the morphology of mitochondria, smooth endoplasmic reticula and glycogen granules is different and they do not show phagocytic phenomena.

Napolitano, in reporting on the differentiation of white adipose cells, based his report on studies of the inguinal and epididymal fat pads of rats ranging from newborn to nine days of age (9). He reported that the pre-adipose cells were found to be fibroblasts characterized by their spindle shape, long tenuous cytoplasmic extensions and profuse endoplasmic reticula and traced their development from fibroblasts to mature adipose cells.

Because of the differences in species, location of the adipose tissues and age of the organisms, it isn't possible to determine the relationship between the fibroblasts observed by Napolitano, which he identified as pre-adipose cells, and the undifferentiated-type cell of our studies. The time element, i.e. age of the organism, is of particular importance. Our studies have been concerned with the embryonic differentiation of white fat cells *in utero* and we have postulated their embryonic development from undifferentiated-type cells to mature-type fat cells (see arrow 1 in chart) and have negated the development of young-type fat cells from fibroblasts, reticulo-endothelial cells or mast cells. Napolitano's study was concerned with the *post partum* differentiation of white adipose cells and he has postulated a sequence of transitional changes in *post partum* cells (see arrow

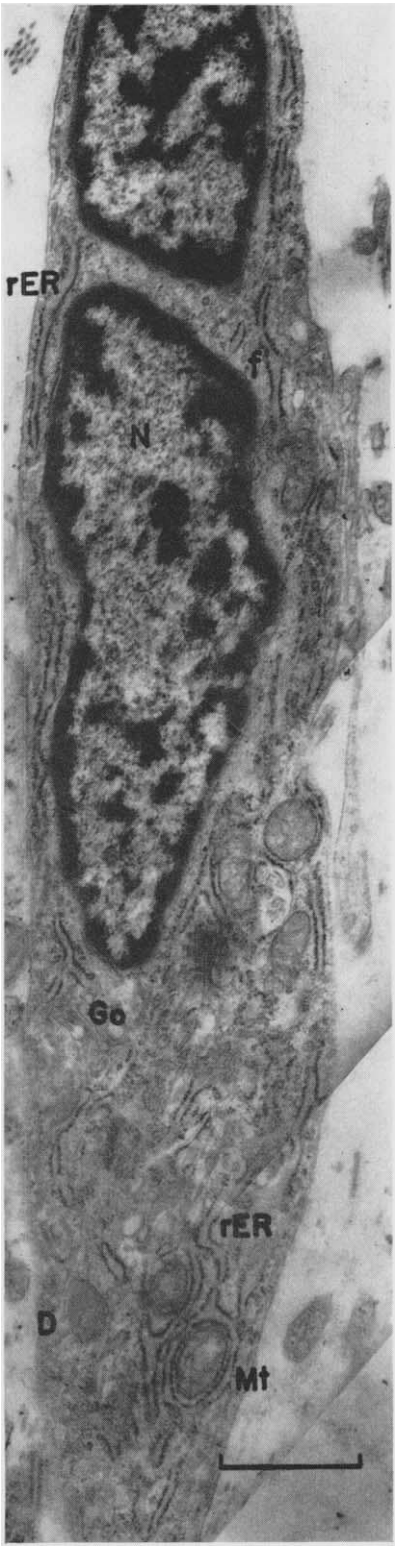
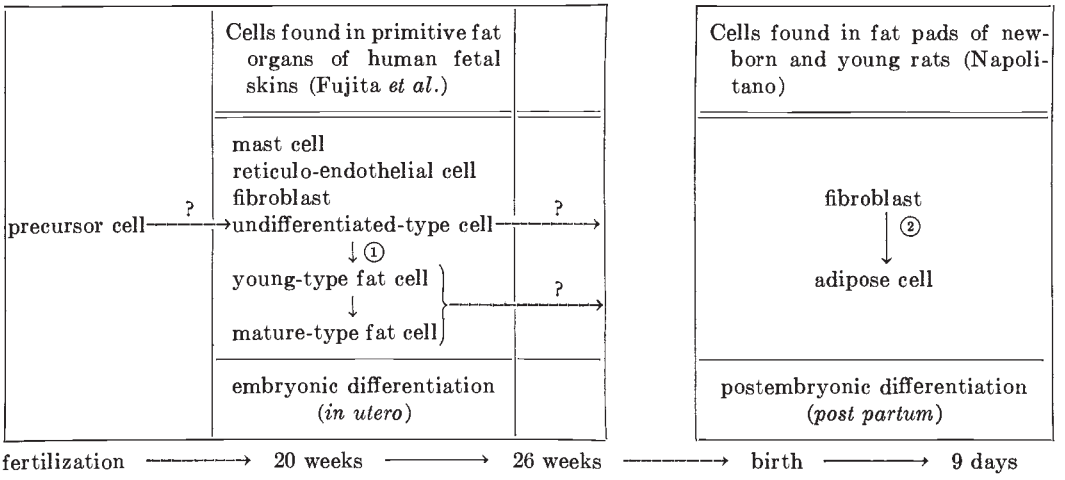


FIG. 14. A fibroblast found in a primitive fat organ, located a short distance from fat cells. rER: rough endoplasmic reticula are well developed and distributed throughout the cytoplasm. Mt: mitochondria. Go: a Golgi apparatus. D: unidentified small dense body. f: filamentous structures. (× 18,860, 2.3 cm = 1μ)

CHART. Possible sequence of steps in the differentiation of white fat cells.



2 in chart). Because of these differences between our studies and Napolitano's studies, we cannot further discuss whether the *in utero* undifferentiated-type cell or the *post partum* pre-adipose fibroblast is the archtype. Napolitano also stated it was not the purpose of his study to pursue the question of the ultimate embryonal origin of adipose cells.

Simon (2) reported that the following five stages of cell development were discernible in adipogenesis: 1) the primitive cell (the perivascular reticular cell); 2) the adipogenic reticular cell; 3) the adipoblast; 4) the preadipocyte; and 5) the adipocyte. Simon based his report on light microscopic examinations of adipose tissues from human fetuses 26 to 30 weeks of age, from children, and from adults, and on electron microscopic examinations of human biopsies and fats of albino rats. From the description by Simon it may be that what he calls the primitive cell corresponds to the undifferentiated-type cell of this study. The adipogenic reticular cell, the adipoblast and the preadipocyte may correspond to what we have called the young-type fat cell and the adipocyte may correspond to our mature-type fat cell. But, as his electron microscopic examinations are not concerned with embryonic fat cells it is not possible to make an exact comparison between our results and the observations by Simon.

It was not within the scope of this study to ascertain the following: 1) Whether or not the formation of subcutaneous fatty tissues may be the result of cell division of undifferentiated-type fat cells which might be retained until parturition. It may be that the young-type and the mature-type fat cells of the human skin lose the capacity to divide as Simon has described. 2) Whether or not the subcutaneous fatty tissue of the human skin may be formed by the cell division of the young-type or mature-type fat cells in the *post partum* period in the event that the undifferentiated-type cells do not persist into the *post partum* period.

Finally, for our purposes, it is desirable to clarify the ultimate origin of human fat cells. In order to do that it seems useful to trace back the embryological differentiation of fat cells *in utero* to identify the precursor cell of what we have called the undifferentiated-type cell. Following that it will be useful to trace the development from 26 weeks *in utero* to birth, of the undifferentiated-type cell, young-type and mature-type fat cells and then their changes and/or development in the immediate *post partum* period.

2. Intracellular Changes Associated with Lipid Mobilization in Fat Cells

Williamson (6), as a result of his electron microscopic observations of experimentally induced lipid-depleted fat cells, postulated that in *post partum* organisms, cytoplasmic microvesicles and microvesicular rosettes in fat cells represent the mechanism of extracellular release of lipids or free fatty acids. If the results of the present investigations were to be inter-

preted from the viewpoint of Williamson's postulation, then it could be presumed that the activity of young-type fat cells in releasing lipids or free fatty acids is presumably higher than that of mature-type fat cells, because cytoplasmic microvesicles and microvesicular rosettes are more prominent in young-type cells than in mature-type ones. But according to previous histological studies (2, 3, 5) on the lipid formation of embryonic fat cells, the activity of young-type cells (cells containing multiple small lipid droplets) in storing lipids should be higher than mature-type cells (signet ring-type cells), a postulate which conflicts with the presumption made above.

Barnett *et al.* (10), in electron microscopic observations of the epidermal fat pad from young albino rats, found that in adipose cells lipid synthesis was stimulated by the addition *in vitro* of insulin which also produced prominent infoldings of the plasma membranes, numerous small vesicles just under the cell surface (some of which resemble the flask-like invaginations of both types of fat cells of this investigation) and numerous small membrane-bounded vesicles in the cytoplasm (some of which resemble our cytoplasmic microvesicles). Barrnett and his colleagues postulated that such morphological changes of adipose cells and lipid synthesis seem to be interdependent events.

With such controversial findings in mind, the results of this study may be postulated as follows: the young-type fat cells are accumulating lipids or free fatty acids through cytoplasmic organelles which are able to perform this function *in utero*.

This postulation may be contrary to the concept of Williamson who postulated that in *post partum* organisms the fat cells release lipids or free fatty acids through such organelles. However, there may be some degree of agreement between our findings and those of Barrnett *et al.* because of the resemblances in cell structure which have been noted and our finding that the young-type fat cells are accumulating lipids or free fatty acids. But, again, we note that the observations of both Williamson and Barrnett *et al.* were on *post partum* rats whereas ours have been on human fetuses (*in utero*). The differences in species and in stage of development may be of major importance.

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