THE DIFFERENTIATION OF WHITE ADIPOSE CELLS

An Electron Microscope Study

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ABSTRACT

Differentiating white adipose tissue from presumptive and developing fat pads of newborn and young rats was fixed in buffered osmium tetroxide, embedded in Vestopal W, and examined in an electron microscope. Pre-adipose cells were found to be fibroblasts characterized by their spindle shape, long tenuous cytoplasmic extensions, and profuse endoplasmic reticulum. The developmental stages traced from fibroblast to mature adipose cell show a gradual change in cell shape, an accumulation of cytoplasm and non-membrane-bounded lipid, a decrease in the endoplasmic reticulum, and a change in shape of mitochondria. Transitory glycogen appears at mid-differentiation. Numerous smooth-membraned vesicles occur in the cytoplasm throughout differentiation. Pinocytosis is constantly evident. Cells of the multilocular stage are shown to differ from brown fat cells, particularly with respect to cytoplasmic membrane systems and mitochondria. No transport of particulate lipid from the lumen of the capillary to, or within, the adipose cell was detected, nor could any cell organelle be demonstrated to be visibly related to lipid synthesis and/or deposition.

INTRODUCTION

The physiology and biochemistry of lipid metabolism in general, and of adipose tissue in particular, are currently under intensive investigation in many laboratories. The recent development of refined techniques for the measurement of lipids has shown adipose tissue to be highly sensitive (both *in vivo* and *in vitro*) to a wide variety of stimuli. However, problems of fixation and ultramicrotomy associated with tissues rich in lipids are such that the cytologist's modern tool of electron microscopy has not yet been extensively applied to the study of the fine structure of adipose cells. Various aspects of the ultrastructure of mature adipose cells have been reported (2–4, 8, 9, 14, 16, 19, 20, 24, 30), but it is apparent in many of these studies that the technical problems mentioned above have made interpretations difficult.

Although a number of light microscope studies have been made on the maturation of adipose cells, and indeed this process of lipid storage is a routine laboratory exercise in most histology courses, confusion still exists as to whether fat cells always arise from specific, predetermined *anlagen*, or, in fact, may also arise from fibroblasts. Differences of opinion have existed for nearly a century, and, unfortunately, recent studies have not resolved the problem. In 1909, Bell (5) reviewed the early investigations; these, and more contemporary studies, have been extensively discussed by Tedeschi (28) and Barrnett (3). To our knowledge, no elec-

tron microscope observations of differentiating white adipose cells have yet been reported. Such observations would be useful in establishing the progressive fine structural changes in the development of a cell from the pre-adipose stage to the mature fat cell. Further, an ultrastructural study of actively growing, differentiating white fat cells might shed light on the subject of lipid transport-to and/or synthesis-within the adipose cell. Finally, such a study might contribute to a better understanding of the relationship of white fat to brown adipose tissue (thought by some (1, 23, 25) to be an immature form of white fat).

The present study was undertaken in an attempt to answer the following questions: (1) What is the fine structure of the pre-adipose cell, and can it be identified with any connective tissue element; (2) What changes occur in this fine structure with the accumulation of lipid; (3) Is brown adipose tissue an immature form of white fat; and (4) Can one observe the passage of particulate lipid from the vascular system into the adipose cell?

MATERIALS AND METHODS

The observations are based on the study of inguinal and epididymal fat pads of rats ranging from newborn to 9 days old. For electron microscopy these tissues were fixed *in situ* by immersion in osmium tetroxide adjusted to pH 7.2–7.6 with veronal acetate or phosphate buffer. After 5 to 10 minutes the fat pads were excised, and small blocks of tissue (1 × 1 × 2 mm) were cut and transferred to fresh fixative for approximately 2 hours. The tissues were dehydrated in increasing concentrations of ethanol (60, 75, 95, and 100 per cent). As a final step, the tissues were placed in dry 100 per cent acetone (3 changes of ½ hour each) and infiltrated with Vestopal W for 1 to 3 hours. The material was then transferred to Vestopal W with 1 per cent initiator and 1 per cent activator and left overnight. Polymerization of the plastic was carried out in a 65°C oven for 72 hours. Sections showing light gold interference colors were cut on either a Porter-Blum or Huxley microtome and examined directly without removal of the plastic. Some grids were stained with lead hydroxide (17). Micrographs were made on either a Philips 100-B or 200 electron microscope at original magnifications of 2000 to 9100, and enlarged photographically to the desired final magnification.

For light microscopy, thick sections of the material described above were examined in a phase contrast microscope. In addition, material was fixed in formol-Zenker, embedded in paraffin, and stained in hematoxylin-eosin.

Electron microscope observations of brown adipose tissue were made on material reported earlier (19).

OBSERVATIONS

Depending on their location and the age of the animal, presumptive and developing fat pads contain cells of varying morphology. In newborn rats, the inguinal fat pad is composed of adipose cells in many stages of differentiation; this is designated in the present study as a developing fat pad. At this time it consists of a loose aggregate of cells which vary in size, shape, and fine structure from spindle-shaped fibroblasts, containing little lipid, to mature signet-ring forms. The accumulation of lipid is an obvious criterion for distinguishing the progressive stages of fat cell maturation. Comparable stages are not found in the epididymal fat pad, however, until the 7th day *postpartum*. Until this time the cells, primarily fibroblasts, are generally devoid of lipid inclusions and, for this reason, the early epididymal fat pad is designated in this study as a presumptive fat pad. By the 9th day, fat cell differentiation in the epididymal fat pad is readily apparent, and there are present in the cell population a large number of signet-ring cells, which differ from adult fat cells only in the size of the lipid inclusion. In developing fat pads, differentiating adipose cells are observed both immediately adjacent to, and at some distance from, capillaries. A diagram which summarizes the observations made on the differentiation of adipose cells is presented in Fig. 1.

The cell type present in the presumptive epididymal fat pad of the 6-day-old rat is illustrated in Fig. 2. The spindle-shaped stem cells generally possess 4 to 5 protoplasmic extensions directed in the long axis of the cell. The processes are long, tenuous, and generally devoid of inclusions. The perinuclear mass of cytoplasm, on the other hand, is rich in cell organelles. There is an abundant and highly organized endoplasmic reticulum. The mitochondria are small, spherical, and possess a simple internal structure. The nuclei are round to ovoid, have 1 or 2 small nucleoli, and a homogeneous distribution of intranuclear material of intermediate electron opacity. No remarkable changes in the internal morphology of the nuclei are observed in the course of the differentiation of adipose cells. However, the accumulation of lipid within the cytoplasm displaces the nucleus to a peripheral position, and it frequently acquires a crescent shape.

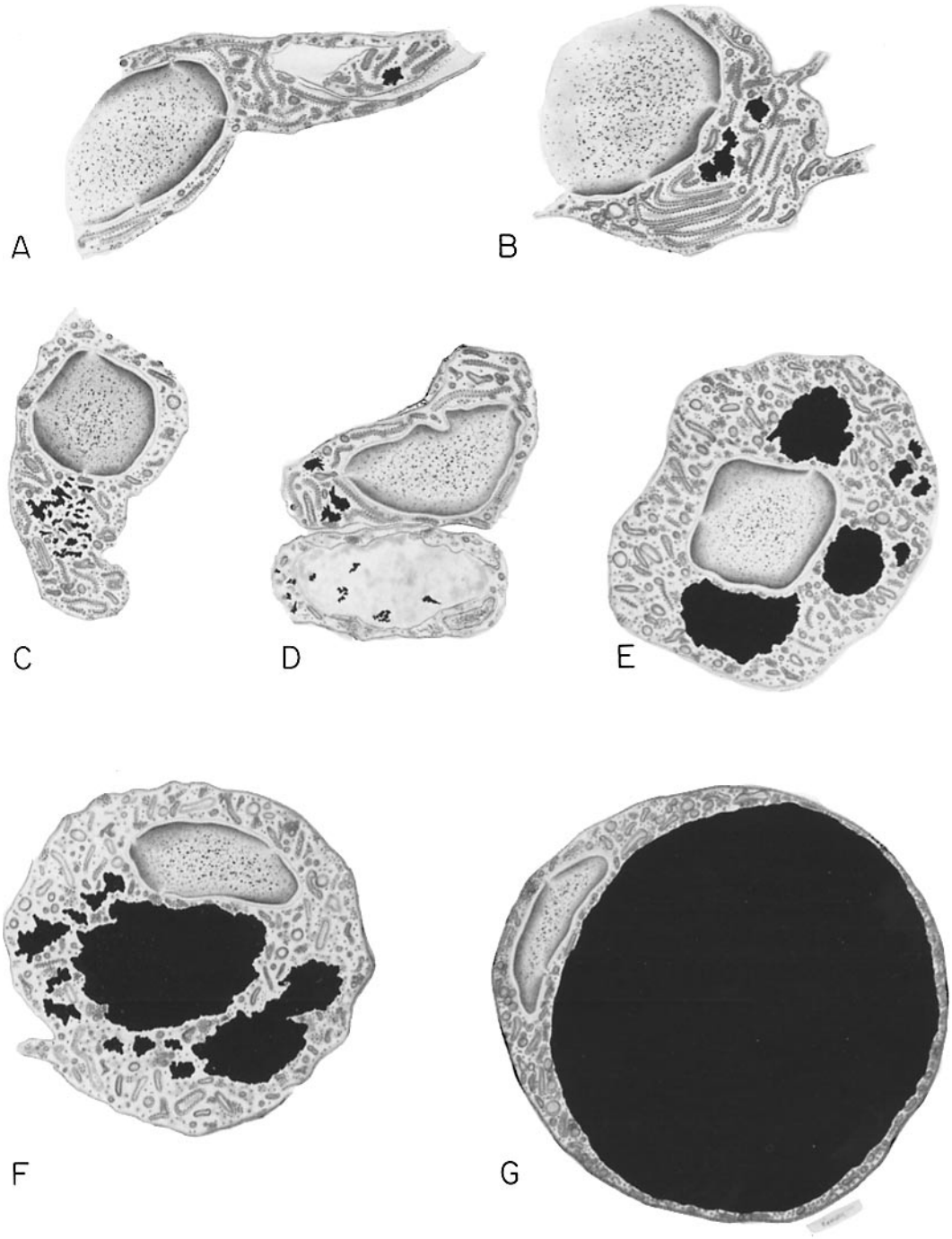


FIGURE 1 contains line drawings of several stages in the differentiation of white fat cells.

FIGURES 2 through 12 are electron micrographs of presumptive or developing fat pads. Tissue was fixed in buffered OsO_4 and embedded in Vestopal W.

FIGURE 1 Diagram of stages in adipose-cell differentiation. (*A*) is a pre-adipose fibroblast; (*B*, *C* and *D*) are early stages in differentiation; (*E* and *F*) are late stages; (*G*) is a signet-ring cell. (*D*) shows the close relation between a presumptive adipose cell and a capillary. Not drawn to scale.

The stem cell just described appears throughout the presumptive epididymal fat pad, and cells with similar ultrastructural characteristics are observed, especially in the pericapillary regions, in rapidly enlarging adipose organs such as the inguinal fat pad of newborn animals and the epididymal fat fat pad of the 9-day-old animal (Fig. 3). The interstitial areas in both presumptive and developing fat pads possess a large number of collagen fibers (Fig. 2). There is a marked vascularity of the presumptive and developing fat pads, and, after feeding, the capillaries contain numerous chylomicrons (Fig. 3).

The earliest stage in differentiation to which the name adipose cell may be given is indicated by the appearance of numerous lipid inclusions toward one pole of the still spindle-shaped cell (Figs. 4 and 5). The tenuous protoplasmic extensions characteristics of the stem cell are less frequently observed. The cell is bounded by a plasma membrane and a thin layer of material of lighter density which is commonly interpreted by electron microscopists as the basement membrane. Collagen fibers appear in close association with the cell. Due to the occurrence of numerous pinocytotic vesicles the plasma membrane has a ruffled appearance in many regions. The mitochondria are usually spherical, though filamentous forms are sometimes observed. Profiles of endoplasmic reticulum are distributed throughout the cytoplasm, but in a less organized form than in the stem cell; their cisternae are sometimes distended and filled with a granular material of moderate electron opacity but this is not a constant feature. A small Golgi zone is usually located adjacent to the nucleus, though such areas are sometimes observed elsewhere in the cytoplasm. A remarkable feature of the cytoplasm is the abundance of smooth surfaced vesicles of varying size, which often contain a material of low electron opacity. It is not clear whether these are agranular components of the reticulum, vesicular components of the Golgi apparatus, or small vesicles derived from the pinocytotic activity of the plasma membrane. These vesicles continue to be present throughout the advancing stages of differentiation.

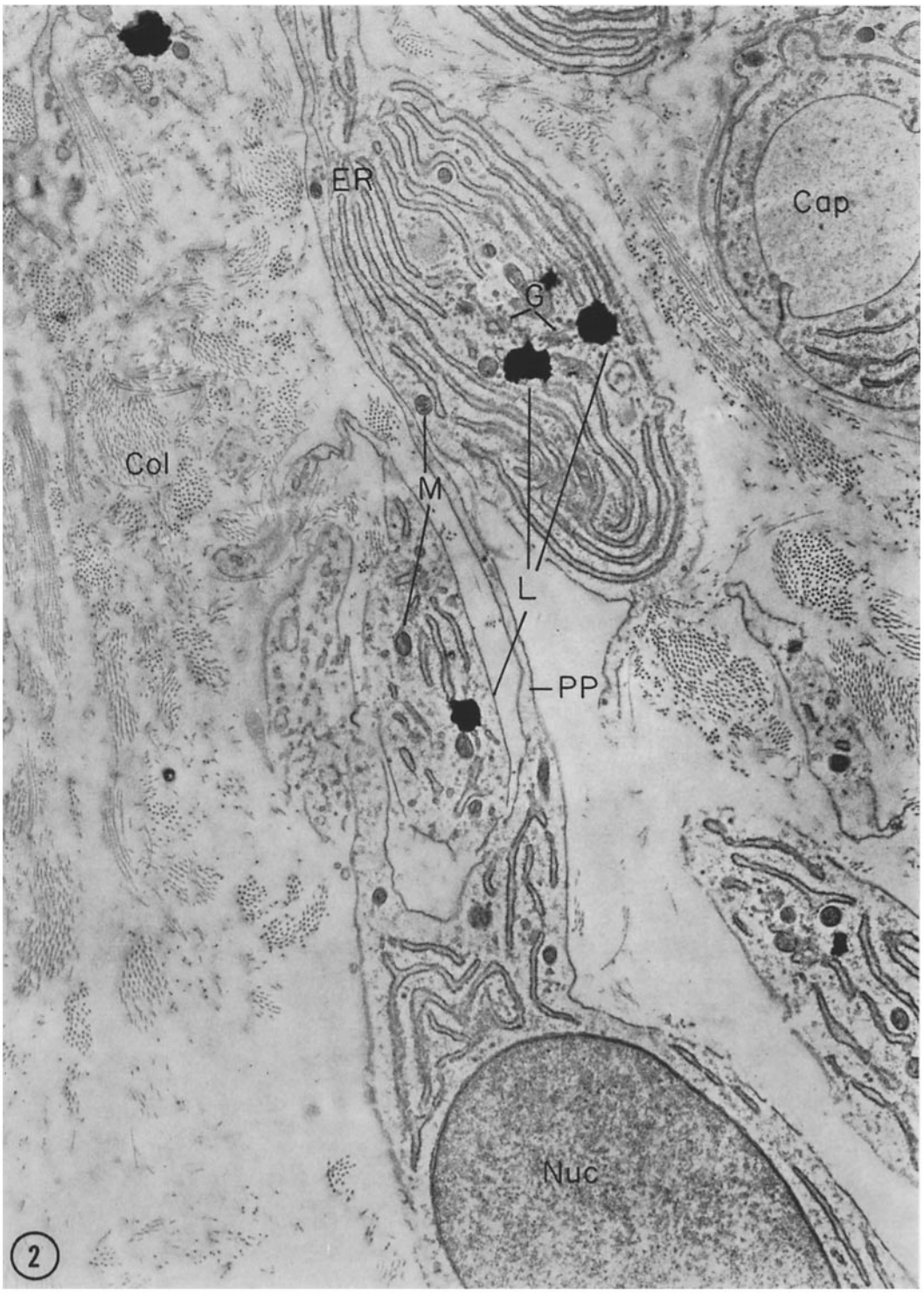
With increasing accumulation of lipid, the differentiating adipose cells go through a sequence of stages (Figs. 6 through 10) in which there are modifications of certain cell organelles and inclusions. All these stages of mid-differentiation are found in developing fat pads, often in close association with the endothelial cells lining the capillaries.

The youngest cells in this sequence (Fig. 6) are elongate and have a central nucleus. The most characteristic feature of cells at this stage is the extensive concentration of small lipid droplets, generally on either side of the nucleus towards both poles of the cell. It can be seen from the earliest stages that the lipid droplets appear not to be bounded by a membrane. Throughout all stages of development they appear crenated and are adielectronic to varying degrees. The basement membrane of developing cells is clearly evident, and, in favorable planes of section, collagen fibers appear to be in intimate contact with it (Fig. 6). When the plane of section is tangential to the plasma membrane, the marked vesicular formations of pinocytosis are apparent. The cytoplasm adjacent to the capillaries frequently contains a delicately filamentous material of low electron opacity (Fig. 6).

Key to Abbreviations Used in Figures

BM, basement membrane
Cap, capillary
Chy, chylomicron
Col, collagen
End, nucleus of endothelial cell
ER, endoplasmic reticulum
F, filamentous material
G, Golgi zone
Gly, glycogen
L, lipid inclusion
M, mitochondrion
Nuc, nucleus of adipose cell
PP, protoplasmic process
PV, vesicles of pinocytosis
RBC, red blood cell
Ves, cytoplasmic vesicles

FIGURE 2 Portions of three stem cells, surrounded by masses of collagen. The cells are spindle-shaped, and long protoplasmic processes (*PP*) are evident in the central cell. Endoplasmic reticulum (*ER*) is abundant and well organized. Also shown are spherical mitochondria (*M*), a small Golgi zone (*G*), and a few small lipid inclusions (*L*). A cross-section through a capillary (*Cap*) appears at the upper right. × 15,000.


ER
Cap
G
Col
M
L
PP
Nuc
2

The cells gradually grow in size and assume an ovoid form (Fig. 7). There seems to be a moderate increase in the amount of cytoplasm as well as in the amount of lipid. Much of the lipid has coalesced into one droplet, though there still may be several small lipid inclusions scattered throughout the cytoplasm. These stages, with their multiplicity of lipid droplets, may be correlated with the multilocular stage of white fat as viewed in the light microscope. The endoplasmic reticulum and Golgi membranes are less evident, but smooth membraned vesicles of varying size are still abundant. At this stage of mid-differentiation significant amounts of glycogen are observed (Fig. 8), most frequently as puddles around the lipid droplets.

In the late stages of differentiation the cells become nearly spherical and the nucleus occupies a peripheral position. The lipid is concentrated in relatively large masses towards the central region of the cell. The final stage of this series (Fig. 10) is considered to occur just prior to the true signet-ring stage. It shows the nucleus flattened out at one side of the cell, and large accumulations of lipid appearing to coalesce in the center. During these stages there is a progressive decrease in the number of profiles of endoplasmic reticulum. The mitochondria present a variety of shapes, from spheres to long filaments. Their cristae are not numerous and rarely extend across the organelle. The cytoplasm continues to contain large numbers of vesicular profiles, and pinocytosis is evident.

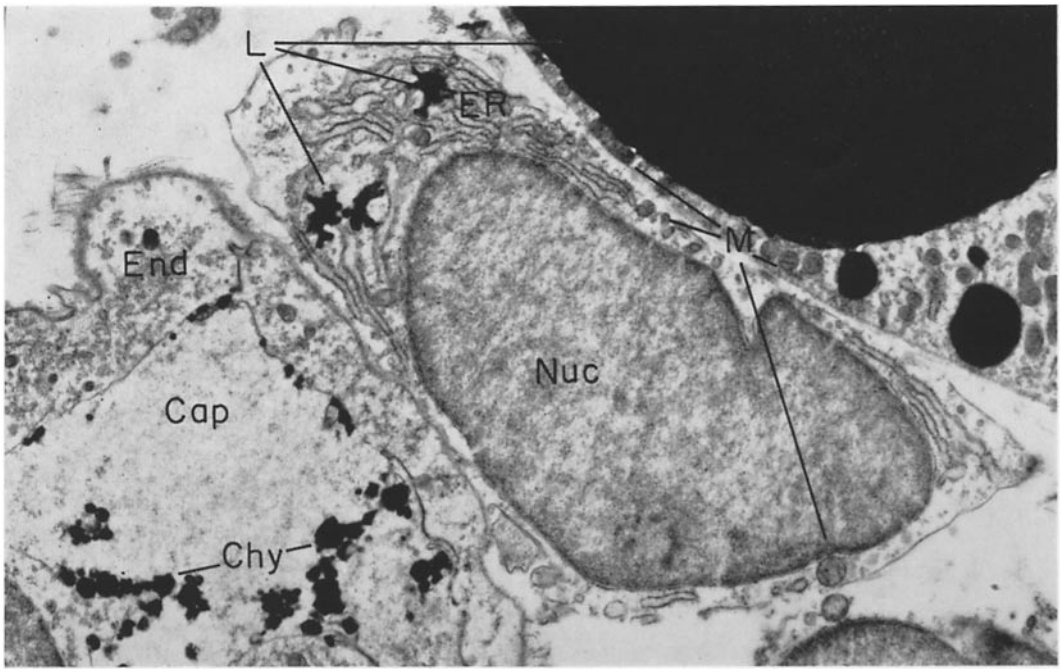


FIGURE 3 Section from a developing fat pad, showing a capillary (at left) with chylomicrons (*Chy*) in the lumen, a spindle-shaped stem cell with abundant endoplasmic reticulum and spherical mitochondria, and a portion of a signet-ring cell (upper right) showing part of the enormous lipid inclusion and the peripheral rim of cytoplasm with its contained mitochondria. Both the spherical and filamentous forms of mitochondria appear in the mature cell. Note the close relation of capillary and adipose cell. × 17,000.

The cytological changes in a cell as it proceeds through the foregoing stages of differentiation can be summarized in the following manner. There is a change in the shape of the cell, from the spindle-shaped fibroblast to the nearly spherical form of a mature adipose cell. There is an ever-increasing accumulation of lipid, first as small inclusions concentrated towards one pole of the cell, then as larger inclusions distributed towards both poles, and finally as large lipid masses in the central region of the cell. The large masses appear to be derived in part from the coalescence of medium-sized inclusions formed at an earlier time in the life of

the cell, and in part from small droplets of lipid which continue to be formed near the interface between the large droplets and the cytoplasm (Figs. 7 and 10). Initially, all mitochondria are of spherical shape, but, as differentiation progresses, more and more filamentous forms are observed. The internal structure of the mitochondria, however, remains simple and unchanged throughout differentiation. The cristae are not numerous, nor do they appear to extend across the organelle as complete septa. There is no consistent orientation of the cristae with respect to the axis of the mitochondrion. The endoplasmic reticulum is abundant and highly organized in the earliest stages, and sometimes the cisternae are distended and filled with homogeneous material. By the time differentiation is complete, the reticulum has almost disappeared and now occurs only as short, discrete sections of granulated membrane lying scattered in the cytoplasm with no apparent spatial relation to other cell organelles. The Golgi apparatus is never very prominent in the developing adipose cell. However, vesicular components of the cytoplasm, whatever their derivation, are abundant throughout the whole of differentiation, beginning to appear just after the stem-cell stage when lipid droplets also first appear. Glycogen is first observed at a mid-stage in differentiation, appearing as an aggregation of particles approximately 200 to 300 A in diameter in the immediate vicinity of lipid droplets. Its occurrence is transitory, for, at the time the adipose cell has matured, glycogen is rarely observed.

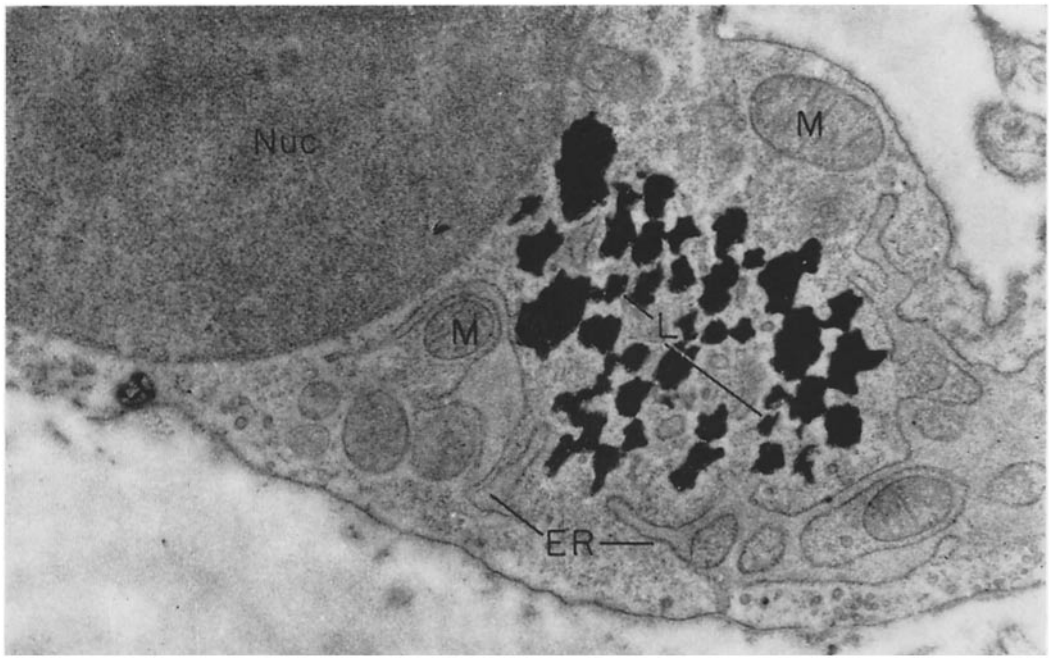


FIGURE 4 A portion of an adipose cell at an early stage in differentiation. The cell is still spindle-shaped, and mitochondria are mostly spherical. There are many small lipid inclusions concentrated at one pole of the cell. The endoplasmic reticulum is abundant but less well organized than in earlier stages. × 20,000.

Details from typical signet-ring cells are shown in Figs. 3, 11, and 12. The enormous size of the mature white adipose cell is due to the central lipid droplet whose dimensions dwarf the cell organelles. The nucleus of such a cell (Fig. 11) is displaced to a peripheral position and is surrounded by a small amount of cytoplasm. The numerous mitochondria, varying in shape from round to filamentous forms, still show an unremarkable internal structure. The cytoplasm contains many agranular vesicular components and an occasional profile of endoplasmic reticulum (Fig. 12). Cell organelles occur more abundantly in the perinuclear region, but can be found throughout the peripheral rim of cytoplasm (Fig.

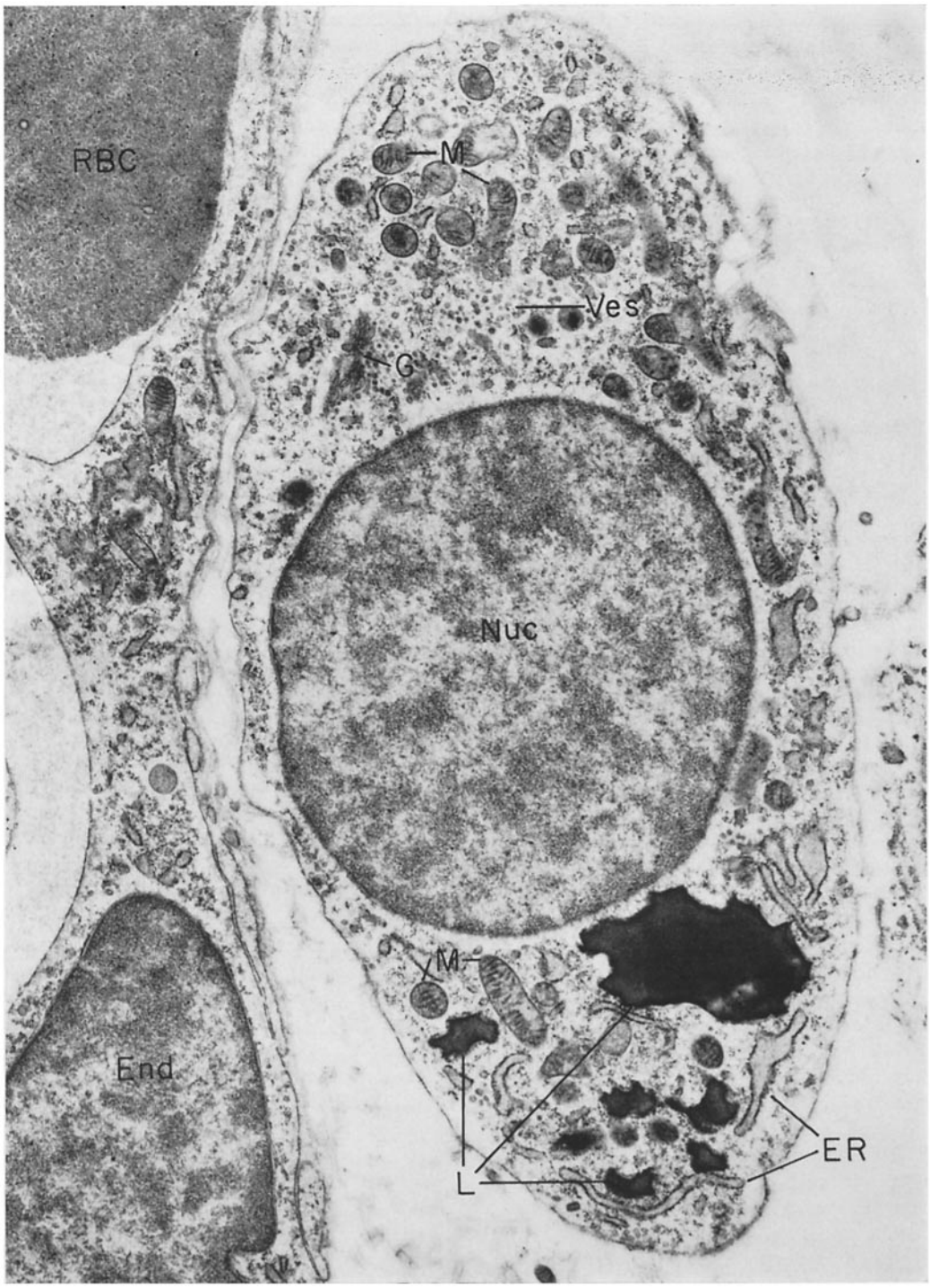


FIGURE 5 A slightly later stage than that shown in Fig. 4. The cell is becoming ovoid, and more filamentous mitochondria are seen. Lipid is still concentrated at one pole of the cell. A small Golgi zone appears near the nucleus. Patches of endoplasmic reticulum and many smooth-surfaced vesicles are scattered throughout the cytoplasm. × 13,000.

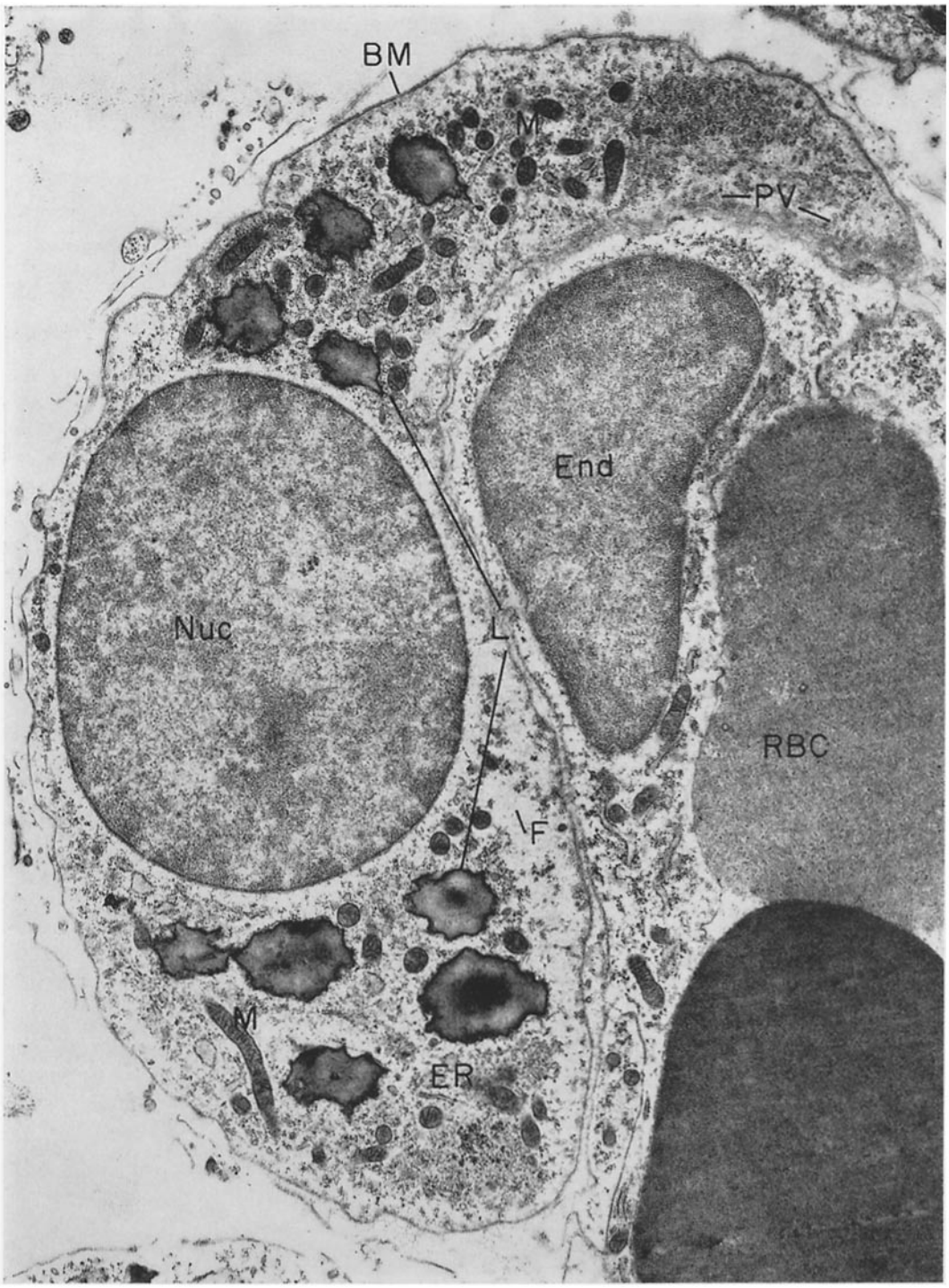


FIGURE 6 An adipose cell at a midstage in differentiation. The lipid inclusions occur at both poles of the cell. The crenated shape and irregular density of the lipid inclusions are considered to be artefacts. Many vesicles of pinocytosis are evident at the plasma membrane, and this cell shows a filamentous material in the cytoplasm adjacent to the endothelial cell of the capillary. The basement membrane is clear, especially in the upper part of the micrograph. × 18,000.

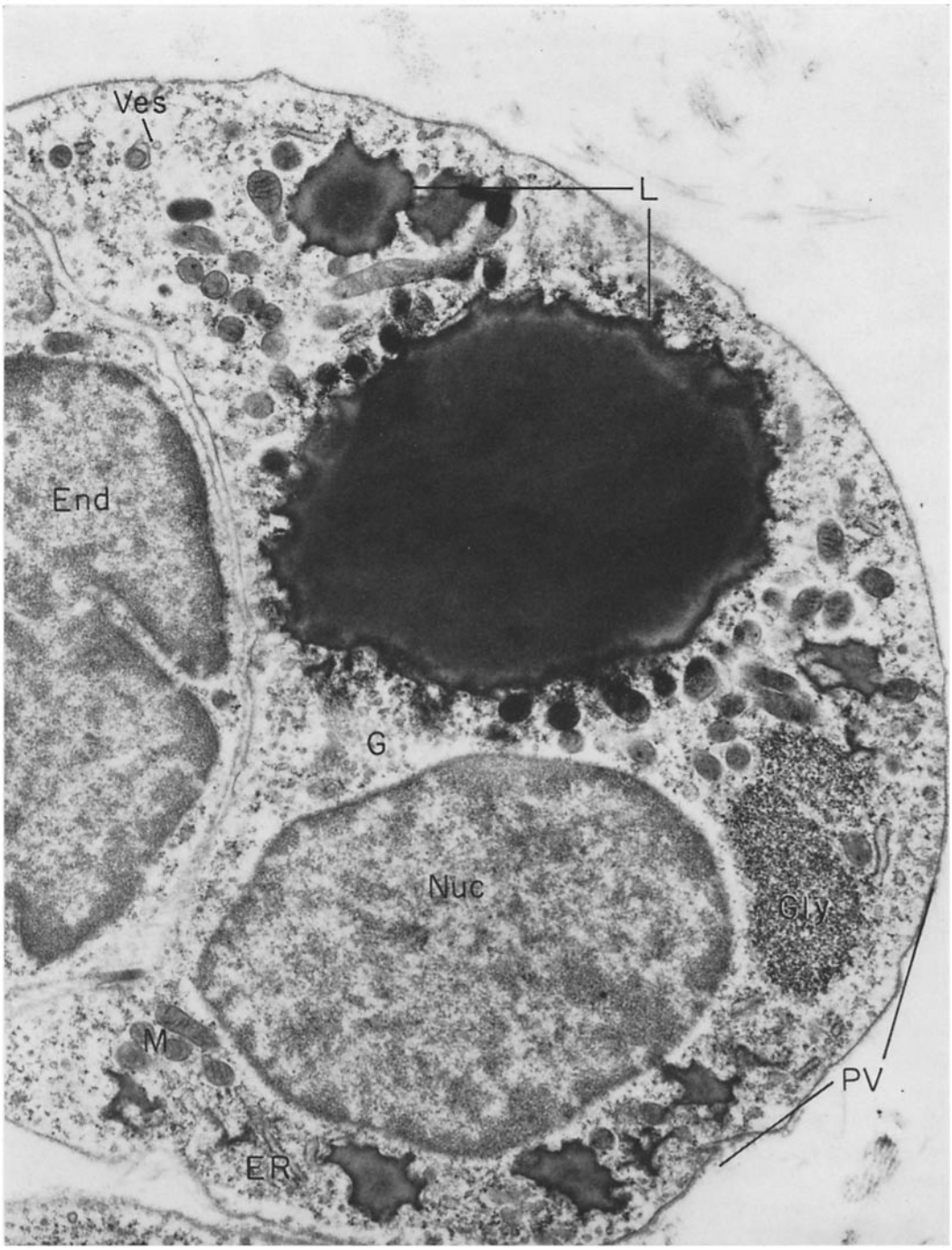


FIGURE 7 A section of an adipose cell at the stage when lipid is being concentrated in large amounts in one area of the cell. The cell has increased in total size as compared to earlier stages. A deposit of glycogen is seen to the right of the nucleus. Sections of endoplasmic reticulum are scattered throughout the cytoplasm, and a Golgi zone appears in the perinuclear region. Pinocytosis is evident and the basement membrane clearly shows. × 15,000.

3). The cell itself is embraced by a network of collagen fibers. The limiting membranes of the endothelial and adipose cells are often observed closely opposed to one another. The basement membranes of the two cells and unit collagen fibers are apparent in the intercellular space between them.

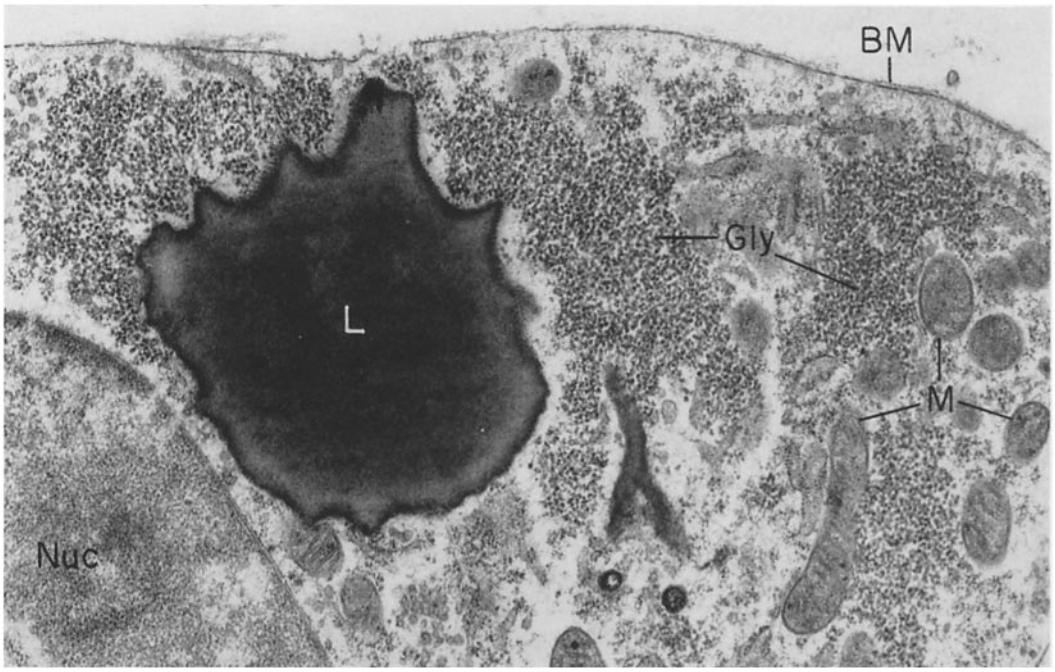


FIGURE 8 A portion of a cell at the same stage as that in Fig. 7, showing large concentrations of glycogen in the vicinity of the lipid inclusion. × 21,000.

DISCUSSION

Origin of Adipose Cells

The question of the ultimate origin of adipose cells, both brown and white, is one which has plagued cytologists for many years. On the one hand, it has been averred that adipose cells arise from fibroblast cells of the connective tissue system, while on the other hand it has been contended that these cells arise from mesenchymal cells closely related to the reticulo-endothelial system.

Clark and Clark (10), for example, in their unique observations of tissue growth in a transparent chamber in a rabbit's ear, described the *in vivo* differentiation of a fibroblast into a mature fat cell. Sidman (25) has also reported the development of adipose cells from "fibroblast-like" cells migrating outward from an explant of brown adipose *anlage*. The observations of Clark and Clark record the continuing modulation of cell form with the accumulation of lipid, and the ultimate coalescence of small lipid inclusions to create a single droplet. The profiles of presumptive and developing fat cells in the electron micrographs presented here are remarkably similar to their illustrations.

The contention that adipose cells are derived from elements of the reticulo-endothelial system and bear a closer relationship to macrophages than to fibroblasts is supported by studies on the uptake of particulate dye by adipose cells (6, 7, 15), and on the apparent interchangeability of function between blood-cell formation and adipose-cell formation of reticulo-endothelial cells of bone marrow (28). It is also supported by Wassermann (29) who has described adipose tissue as arising from mesenchymal elements existing in close conjunction with capillaries in primitive adipose organs. They were fundamentally the same in structure as the reticulo-endothelial organs which form blood and lymphoid tissue. A rather special case which relates the adipose cell to macrophages has

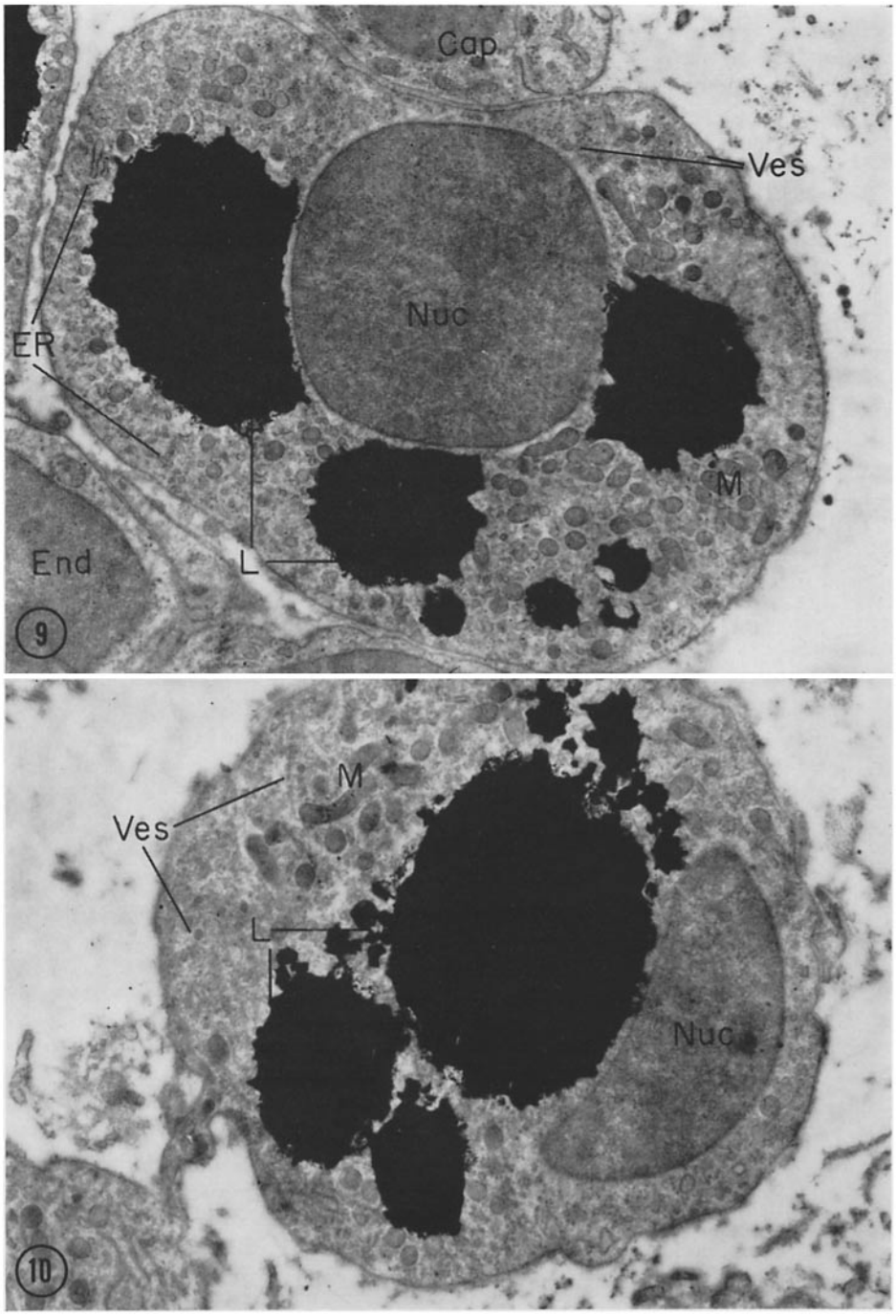


FIGURE 9 A late stage in differentiation when the cell is assuming a spherical shape. The nucleus is still central. Mitochondria are both spherical and filamentous. Many cytoplasmic vesicles occur throughout the cell. × 7,000.

FIGURE 10 A very late stage when the lipid masses are assuming a central position. The nucleus is displaced to the periphery. × 12,000.

been reported by Fisher and Paar (11) in a study of the effects of carrageenin injections. This material stimulates the formation of new collagen at the site of injection, but the collagen later breaks down and is replaced by adipose tissue, the latter being derived, according to Fisher and Paar, from alterations within the macrophages. From the foregoing studies it appears possible, then, that the development of adipose cells may proceed in some cases from mesenchymal elements other than fibroblasts.

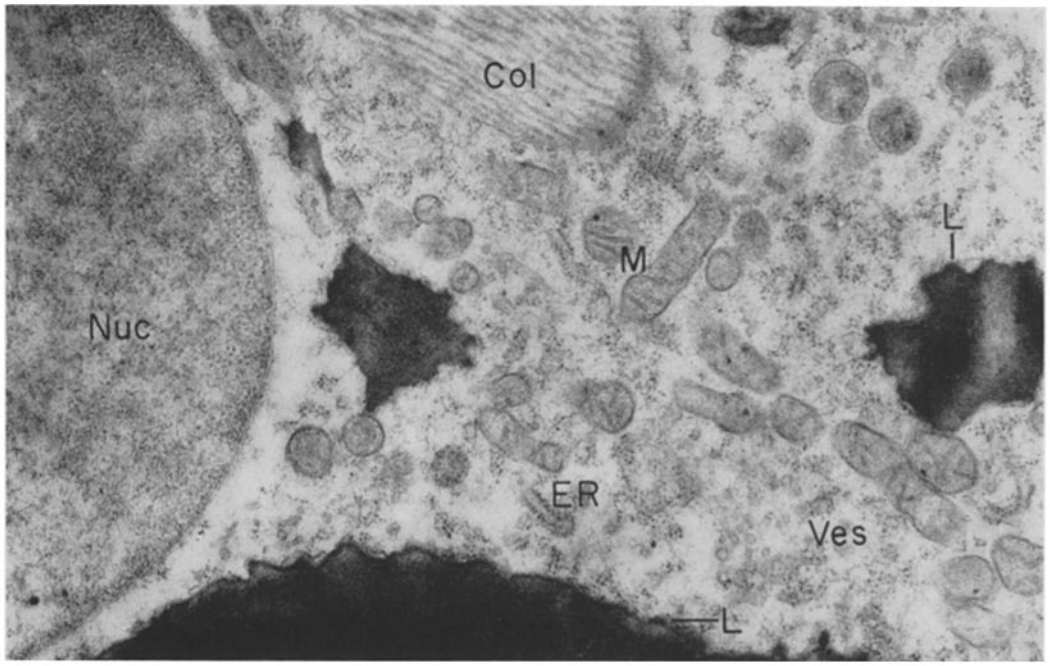


FIGURE 11 A portion of a signet-ring cell showing part of the nucleus and a small part of the large, central lipid inclusion. The cytoplasm contains an unusually large number of particles (150 A), which are interpreted as free ribonucleoprotein particles. × 21,000.

It is not the purpose of this study to pursue the question of the ultimate embryonal origin of adipose cells. Whether they be derived from connective tissue or reticulo-endothelial elements (or perhaps from both, when all sites of adipose cell development are considered), these cells are mesodermal in nature, and the exact path (or possibly paths) by which a cell arrives at the state of being a pre-adipose cell is not within the scope of the present study. Experimental conditions in other studies seem to have elicited the differentiation of adipose cells from both fibroblast and mesenchymal archetypes, and it would seem that there may be in the cells of both systems a multipotency which permits the differentiation of cells into adipose cells if the environmental situation stimulates it. It does seem important to emphasize, however, that the present study shows that, under normal conditions in at least two sites in the rat, there is a gradual ultrastructural differentiation of white adipose cells from precursor cells which are morphologically similar to typical fibroblasts found in many areas in the rat (Napolitano, unpublished observations) and, indeed, in other animals (22).

One of the first ultrastructural changes in the differentiation of an adipose cell from a fibroblast is the development of the basement membrane which becomes thereafter an integral characteristic of the fat cell and is not lost even when the differentiated cell is depleted of its fat (3, 18, 24, 30). However, to designate this postfibroblast, prelipid-inclusion stage as the immediate ancestor of the true adipose cell, and to give it an individual name, would probably only add to the confusion in the literature. It seems more prudent simply to designate this as a stage in which the cell is con-

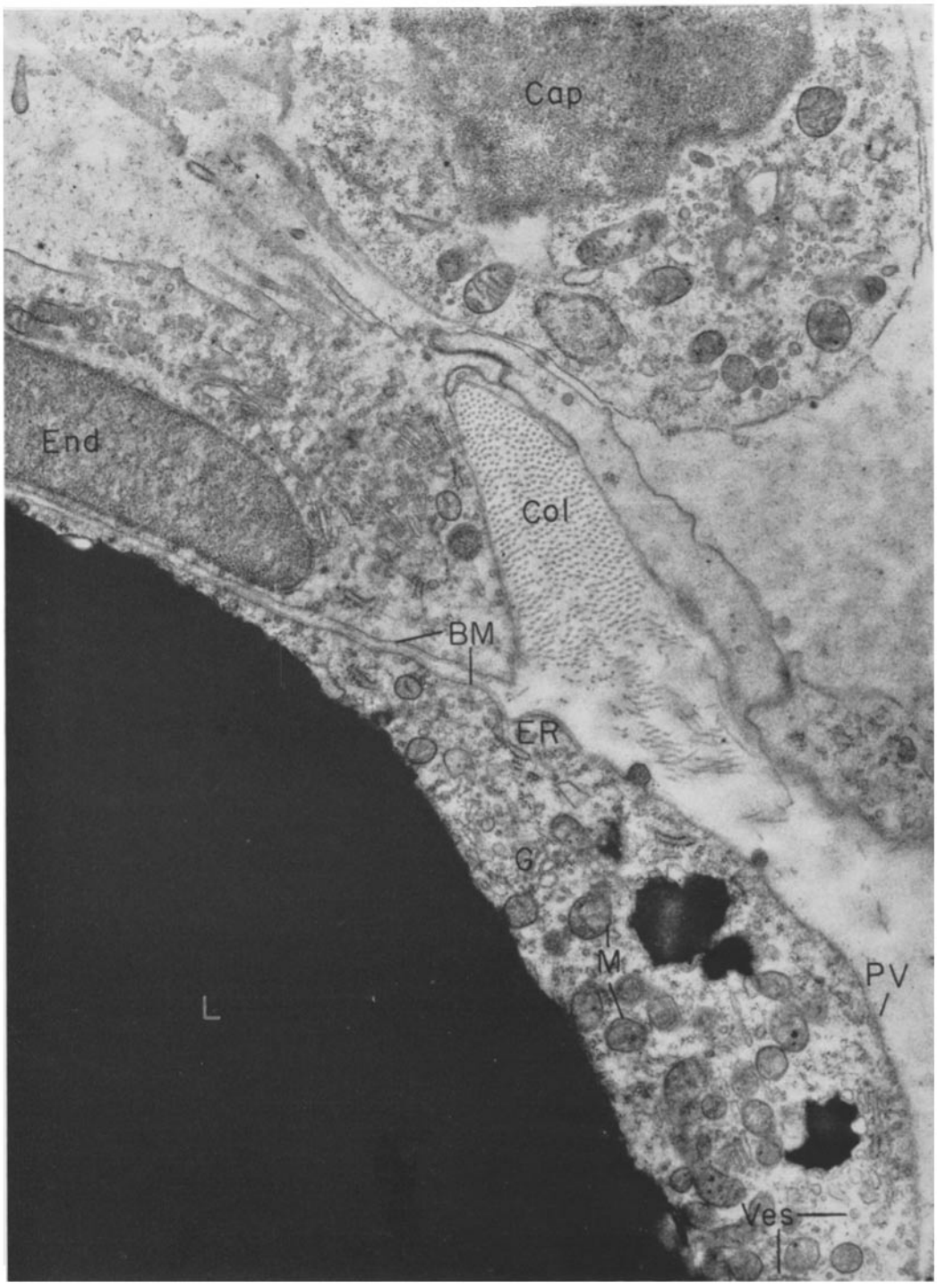


FIGURE 12 A small portion of a signet-ring cell and an adjacent capillary. A small Golgi zone, scattered profiles of endoplasmic reticulum, mitochondria, and smooth surfaced vesicles fill the cytoplasm. Pinocytosis is readily apparent. Note the relation of adipose cell to endothelial cell, their basement membranes running closely parallel for some distance. × 20,000.

comitantly losing certain of its fibroblast characteristics and beginning to acquire the characteristics of an adipose cell—in other words, an early stage in adipose cell differentiation.

The factors which may influence the differentiation of precursor cells into adipose cells are not well understood, though it is readily apparent that many are involved. A few examples will serve to illustrate the curious specificity of fat cell development. It is well known, for instance, that massive accumulations of adipose tissue occur in predetermined anatomical locations in certain races of man and in certain species of animals. The amount and distribution of adipose tissue is influenced by the sex of an individual. The caloric intake, also, has an important influence upon the mechanisms governing fat cell development. Finally, regardless of the above statements, there remain certain regions of the body (ear lobe, eyelid, penis, scrotum, scalp) which are generally lacking in adipose tissue even though fibroblasts and mesenchymal elements are present. It would appear that, for the differentiation of adipose cells, there must be a proper localized environment incorporating genetic, nutritional, and hormonal influences.

It must also be considered that an adipose cell is a highly differentiated form. Present observations (18) indicate that the cells in fat depots of animals maintained on a chronic low caloric intake do not de-differentiate into fibroblasts, but retain a number of the characteristics of the mature adipose cell, except that they are devoid of lipid and have altered cell profiles. Similar observations have been made on the adipose cells of acutely fasted animals by Wassermann (30), Barrnett (3), and Sheldon *et al.* (24). The inherent tendency of adipose tissue to maintain this differentiated form is further substantiated by the classic observations of Strandberg (27). He noted, in the transplantation of skin from the anterior abdominal wall to the dorsum of the hand, that the subcutaneous fat layers which developed in the new site retained the characteristics of the panniculus adiposus that had been inadvertently carried along in the graft.

While both descriptive and experimental studies at the light microscope level, then, have in some cases demonstrated a differentiation of adipose cells from mesenchymal precursors, the observations of this study support the view that fibroblasts may give rise to adipose cells (5, 10, 12, 13), by demonstrating in normal development in two sites of the body the ultrastructural continuity between typical fibroblasts and mature adipose cells.

Modulations of the Ultrastructure of the Cell with the Accumulation of Lipid

Although certain cellular components remain essentially unchanged during the differentiation of an adipose cell, the morphology of the mature fat cell differs in many aspects from that of its stem cell. There is a gradual but obvious change in the size and shape of the cell. The increase in size appears to be due primarily to the acquisition of lipid, since the apparent increase in amount of cytoplasm may be ascribed mainly to a withdrawing of cytoplasm from the long tenuous processes of the stem stage with a concomitant massing of cytoplasm in the main body of the cell. The early spindle form with its long protoplasmic processes becomes progressively modified to form a sphere. The accumulation of lipid is undoubtedly responsible for this change in shape. Clark and Clark (10) have observed in the living cell a coalescence of small lipid inclusions to form a single droplet of such dominating size that it impresses its shape upon the cell.

Lipid inclusions are always spherical when examined in living cells by light microscopy. The appearance of the lipid inclusions in the present study, especially those of small size, is often crenated. This ink-blot outline is characteristic of lipid droplets in many cell types when observed in the electron microscope. No explanation for the crenated appearance of lipid droplets is made here other than to suggest that it probably represents a distortion set up by the forces of fixation, polymerization, and microtomy. Also, it may be true that the variations in the adielectronic properties of lipid inclusions, which are apparent in the present study and those published by others, reflect only an artifact of preparations. (Preparations even more obviously distorted than those presented here were frequently encountered during the course of this study).

There are minimal changes in the shape and internal structure of mitochondria from the early undifferentiated cell to the mature form. The cristae are generally few in number and rarely extend across the organelle to form complete septa. The more frequent occurrence of filamentous mitochondria in the maturing cells does not appear to be directly related to the process of lipid development. These mitochondria are not found in close association with fat droplets nor in greater numbers in those areas of the cytoplasm where lipid is being deposited. No observations have been made

of any structures, such as reported by Lever in brown fat (16), which would be regarded as transitional between mitochondria and lipid droplets.

As has been pointed out earlier, there is a large amount of highly organized endoplasmic reticulum in the stem cell. As differentiation proceeds, there is a decrease in the amount and degree of organization of this material. However, even in the mature cell, profiles of endoplasmic reticulum are occasionally observed. In the present study, no direct morphological evidence could be found to support the contention of Oda *et al.* (20), in their study of brown fat, that lipid accumulations occur in intimate relation to the endoplasmic reticulum. The accumulation of material in the cisternae of the endoplasmic reticulum in cells in early stages of differentiation appears only occasionally, which makes it unwarranted at this time to draw any conclusions as to its metabolic significance. The current concept that the endoplasmic reticulum is related to protein synthesis suggests that this subcellular system may be utilized in this instance for the synthesis of new cytoplasm in the growth of the cell and/or even the production of the enzyme systems of triglyceride synthesis.

The Golgi apparatus as an organized structure is not particularly well developed in maturing fat cells. The cytoplasm has, however, an abundance of smooth surfaced vesicles enclosed by a single membrane. Whether these vesicles are morphologically related to the Golgi apparatus, the agranular reticulum, the vesicles of pinocytosis, or are independent entities in themselves, is unclear. It seems premature to speculate that their role in the physiology of the cell may be the transporting of materials which are utilized in lipid biosynthesis. It is clear that these vesicles do not contain material with the same adielectronic properties as the material contained in lipid inclusions. This observation and the apparent lack of a limiting membrane around the lipid suggest that the lipid inclusions do not arise by a coalescence of such vesicles. In fact, it is most noteworthy that the appearance of lipid inclusions and their coalescence into large deposits, in developing white fat cells at least, appears unrelated, morphologically, to any cell organelle or cytoplasmic membrane system, an observation which is in keeping with biochemical demonstrations of the possibility of fatty acid synthesis by the soluble supernatant fraction of centrifuged material (26).

The occurrence of pinocytosis at the plasma membrane is characteristic of white fat cells in all stages of differentiation. However, in any one plane of section not all regions of the membrane appear to be involved. Whether this is only true of normal cells in young animals has not yet been determined, but the fact that regional variations do occur in this instance should make one cautious in utilizing pinocytosis as an index of activity in adipose cells subjected to experimental manipulation.

Particulate glycogen is not observed in presumptive fat cells. It first appears in developing fat cells but only after lipid has accumulated to some degree. Its occurrence in the developmental stages and its proximity to the lipid droplets suggest that it may be involved in the accumulation of lipid.

Relation to Brown Adipose Tissue

The presence of multiple lipid droplets within the cytoplasm of brown adipose cells and developing white adipose cells has led to the concept that the tissues are identical. This similarity is especially striking in lipid-stained preparations examined in the light microscope. Auerbach (1) was one of the earliest investigators to conclude from light microscope study that "brown fat" is really an arrested embryonal form of white fat, and this view has been supported by other investigators (23, 25) even to the present time. In 1956, Sidman (25) published a report of the transformation of brown adipose cells, in organ culture, into unilocular white fat cells. He stated that no fundamental distinction exists between brown and white fat cells, but that they differ only in the quantity of lipid stored. This common opinion, that the two kinds of cells represent the same tissue in different developmental states, is based, however, on light microscope examination of limited resolution. The increased resolution of the electron microscope affords an opportunity for visualizing in detail all of the cellular components. The fine structure of brown fat cells (19) is characterized by the large size, form, and complex internal structure of its mitochondria. The granular appearance of the cytoplasm and the near absence of cytoplasmic membranes are other distinguishing features of brown fat cells. The membranous components of the cytoplasm in developing white fat cells, on the other hand, are very prominent, and the mitochondria appear uncomplicated in form and structure. The earliest stages of differentiation of brown adipose cells from "fibroblastic" stem cells have not been documented by the electron microscope (by the 17th day *in utero* the rat embryo already

has brown fat cells which are identical in fine-structural detail to the brown adipose cells of the newborn (Napolitano, unpublished observation)) and it may, of course, be possible that in earlier stages of differentiation the size and complexity of mitochondria and/or the amount of cytoplasmic membrane systems more closely resemble those of these organelles in developing white fat cells. However, it is striking that, at the stage in the development of white fat cells which might be correlated with the multilocular brown fat cell, the cytoplasmic organelles of the white fat cell, when viewed in the electron microscope, are in several ways distinct from those of brown adipose tissue cells; *i.e.*, in the size and internal structure of mitochondria, and the presence of membrane systems. Therefore, while certain experimental conditions may modify white and brown fat so that they come to resemble one another more closely, it seems apparent that brown adipose tissue is not an embryonal form of white fat, but exists as a distinct morphological entity whose biological role remains undefined.

Passage of Lipid into the Cell

Palay and Karlin (21) have observed with the electron microscope the absorption of particulate lipid in the digestive tract. The subsequent passage of the lipid through and between the epithelial cells into the vascular and lymphatic systems suggested that a similar mechanism may be operative in the accumulation of lipid in developing adipose cells. Chylomicrons were readily apparent in the capillary lumen, and an occasional lipid inclusion, not limited by a membrane, was observed in the endothelial cells. Extensive observations, however, failed to reveal the presence of lipid bodies in the intercellular region between the capillary bed and the adipose cell or in regions of the cytoplasm subjacent to the surface of the fat cell. It would appear, then, that the cellular mechanisms responsible for absorption of particulate lipid in the digestive tract are not operative in the storage of lipid within the adipose cell. The accumulation of lipid in adipose cells does not involve the absorption of discrete lipoidal substances (at least at the resolutions afforded in this study), but may be due exclusively to *in situ* synthesis.

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