ADIPOSE TISSUE

Morphological Changes Associated with Lipid Mobilization

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ABSTRACT

Morphological changes associated with mobilization of lipid were studied in epididymal adipose tissue from fasted and from alloxan diabetic rats. In both groups of animals a decrease in lipid content was accompanied by the formation of complex frond-like cytoplasmic processes and of loops and folds of basement membrane which extended from cell surfaces. These changes, evident after 1 day of fasting, increased in magnitude with increasing weight loss. As the lipid content of the cell decreased further, lipid-cytoplasmic interfaces became irregular and convoluted. Cytoplasmic microvesicles were prominent and appeared to be greatly increased in number. Rosette-like structures composed of microvesicles were observed in both lipid-depleted fat cells and endothelium. The interpretation of these changes and their physiological significance are discussed in terms of the physical and chemical properties of lipids and lipid metabolism. It is postulated that microvesicles may represent the mechanism of transport of free fatty acids in fat cells and in endothelium. Hypotheses are proposed and illustrated schematically for the mode of formation of microvesicular rosettes, for the mobilization and uptake of lipids by fat cells, and for the transport of lipids through endothelium.

INTRODUCTION

Despite great advances in the knowledge of lipid biochemistry and metabolism, many questions relating to the precise manner of lipid absorption, transport through vessels, and uptake and release by fat cells remain unanswered. Electron microscope studies have greatly clarified cellular mechanisms involved in the transport of lipids through intestinal epithelium and the entrance of lipids into lymphatic vessels (1–5). The magnitude of lipid depletion in fasted and in alloxan diabetic animals suggested that fine structural alterations associated with mobilization of lipid might be demonstrable in fat cells. The present investigations were undertaken to determine what changes occur in fat cells during lipid mobilization and to obtain morphological evidence of the mechanism of release of lipid from fat cells.

METHODS

Diabetic Animals

After a 24-hour fast, thirty-five male Wistar rats weighing from 150 to 250 gm were anesthetized with Nembutal[1] (30 mg per kg intraperitoneally) and given alloxan monochloride (45 mg per kg) through the saphenous vein. They were then fed and allowed water *ad libitum*. The urine was checked daily for glucose with TES-TAPE.[2] Animals were weighed and killed, as described below, at daily

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[1] Abbott Laboratories, North Chicago, Illinois.
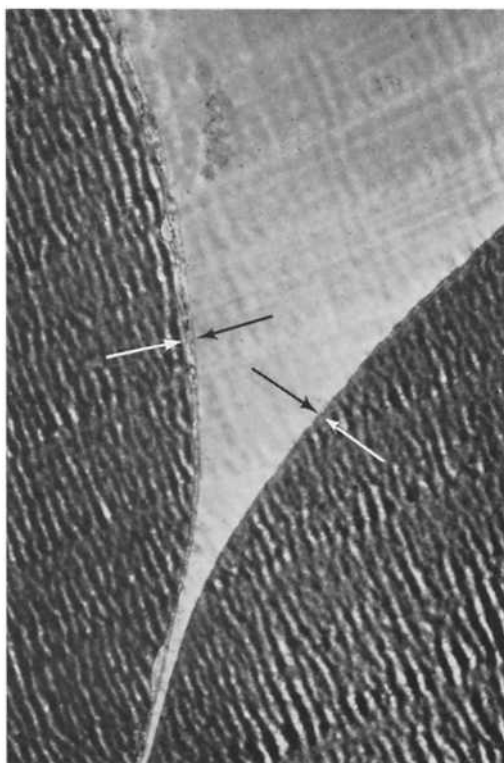
[2] Eli Lilly and Company, Indianapolis.

FIGURE 1 Portions of two fat cells from a normal rat fed *ad libitum*. A very thin rim of cytoplasm (between arrows) encompasses the large central fat droplets. × 4,000.
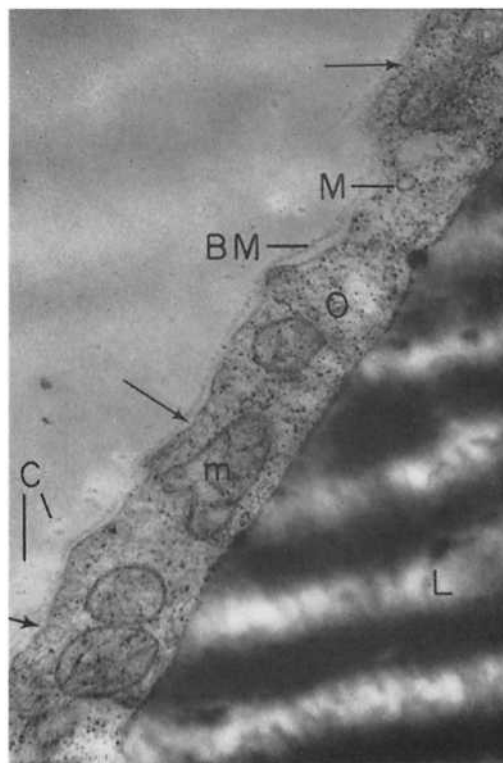
FIGURE 2 Higher magnification of cytoplasm of a normal fat cell showing mitochondria (*m*), microvesicles (*M*), and numerous flask-like invaginations of plasma membrane (arrows). Several collagen fibers (*C*) are shown in cross-section. The identity of the numerous very small black granules (encircled) was not ascertained. Lipid (*L*), basement membrane (*BM*). × 30,500.

intervals for 2 weeks. Blood samples for glucose determinations were taken from the inferior vena cava at the time the animals were killed. Glucose was estimated by the ferric-ferrocyanide method (6) using a Technicon autoanalyzer.

Fasted Animals

Thirty-six animals were weighed and placed in individual cages containing water but no food. Twenty-four were fasted for various lengths of time up to 10 days, reweighed, and killed as described below. Twelve were fasted 7 days and then killed at intervals of 1 to 48 hours after refeeding a diet rich in corn oil (75 per cent pulverized lab chow, 25 per cent corn oil).

Preparation of Tissue

Epididymal adipose tissue was used for microscope studies for the following reasons. It constitutes a relatively large accumulation of fat cells which can be readily located and identified even in lipid-depleted animals. Tissue samples can be easily removed

without trauma to the fat cells. The metabolism of rat epididymal adipose tissue has been studied extensively.

After Nembutal anesthesia (30 mg per kg intraperitoneally), the tip of an epididymal fat pad was excised, immersed in 1 per cent osmium tetroxide buffered with modified White's saline[3] (7), and cut into 1 mm cubes. The diced tissue was fixed for 1 hour at room temperature and then dehydrated by passage through a series of graded alcohols to absolute ethanol. Blocks of tissue were embedded in Epon and in methacrylate. Sections for examination

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[3] Equal parts of 2 per cent osmium tetroxide and double strength modified White's saline were combined just prior to use. The latter was modified by Dr. Walter Bauer of this department. Iron salts were eliminated and the calcium content was increased to three times that in the original formula.

by phase microscopy as well as thin sections for electron microscopy were cut on a Porter-Blum microtome with glass knives. Thin sections were stained with lead acetate (8) prior to examination in an RCA electron microscope Model 3F. In some instances, additional tissue was fixed in 10 per cent buffered formalin or Zenker-formol for light microscope studies.

of lipid (apparently separate from the large central droplet), and numerous very small dense granules 100 to 200 A in diameter (Fig. 2). Cytoplasmic microvesicles and flask-like invaginations of the plasma membrane are frequent. The entire fat cell is encompassed by a well defined basement membrane.

FIGURE 3 Fat cell from an alloxan diabetic rat (weight loss 14 per cent). The surface of the cell is irregular and deeply indented. Only a small droplet of lipid (L) remains in the cytoplasm in which mitochondria and vesicles (arrows) are prominent. Nucleus (N), vessel lumen (V), red blood cell (R). × 7,500.

OBSERVATIONS

Normal Adipose Tissue

Since the electron microscopic appearance of normal fat cells has been described (9–11), it will be recounted here only briefly. In cells from normal, fed animals the cytoplasm forms a very thin layer around the large fat droplet (Fig. 1) except in the region of the nucleus where it is somewhat thicker. Present in all parts of the cytoplasm are mitochondria, vesicles and membranes of agranular endoplasmic reticulum, scattered ribonucleoprotein granules, occasional small droplets

In addition to fat cells, which constitute the vast majority of cells present, rat epididymal adipose tissue contains a rich vascular network, scattered fibroblasts, macrophages, mast cells, collagen fibers, infrequent nerve fibers, and occasional leukocytes of all types. The peritoneal surface is lined by mesothelial cells. Vascular endothelium in adipose tissue is in no way remarkable except for its lack of fenestrations (12).

Lipid Depletion

All fasted rats lost weight, as much as 40 per cent, whereas weight loss in alloxanized animals

was related to the severity of the diabetes. Some diabetic animals lost as much as 20 per cent in 3 days, others maintained their weight, and a few even gained. Variation in the severity of the alloxan-induced diabetes was also reflected in blood glucose levels which ranged from 300 to 1000 mg per 100 cc. Although the microscopic

basement membrane frequently extended from the surface of lipid-depleted cells (Figs. 4 and 6), especially at the tips of cytoplasmic processes.

INTRACELLULAR CHANGES

MICROVESICLES: Cytoplasmic microvesicles were unusually prominent in fat cells depleted of
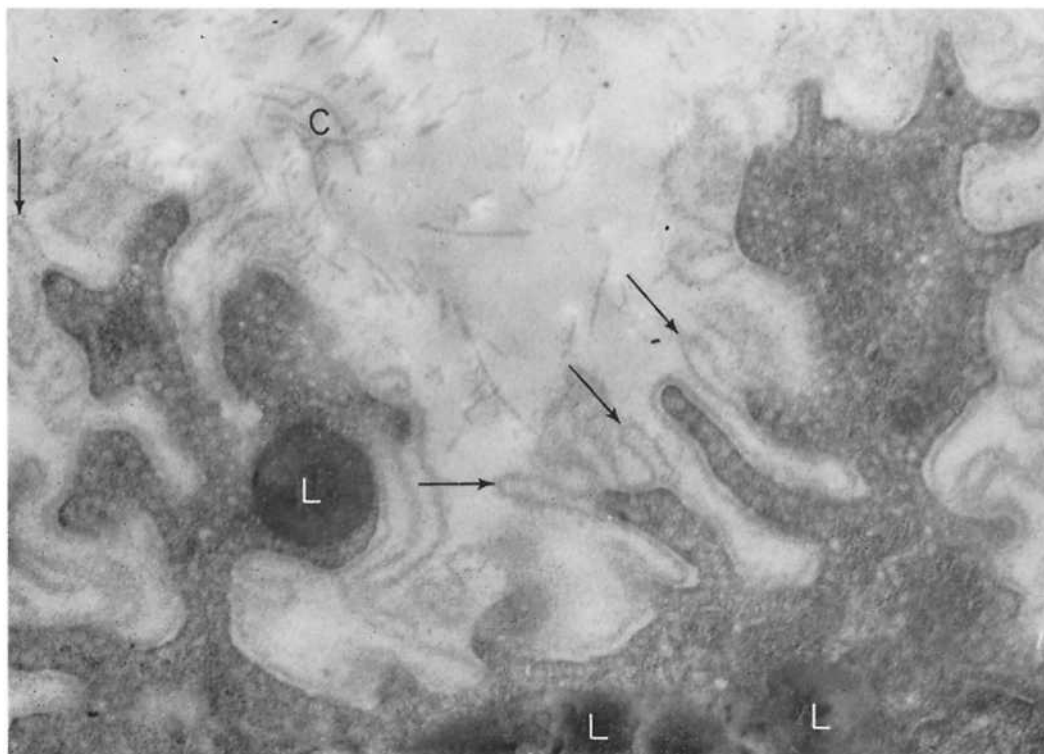

FIGURE 4   Portion of a lipid-depleted fat cell from a rat fasted for 6 days (weight loss 34 per cent) and refed 18 hours previously. Numerous loops and folds of basement membrane (arrows) extend from the surface of the cell. The intercellular space contains many collagen fibers (C). The frond-like cytoplasmic processes contain numerous microvesicles. Lipid (L). × 27,500.

changes to be described were identical in diabetic and in fasted animals, they developed only in those animals losing weight.

CHANGES IN CELLULAR CONFIGURATION AND BASEMENT MEMBRANE

As cells lost lipid and the central fat droplet decreased in size, the cytoplasm was no longer stretched thin over the lipid droplet; instead, it formed numerous, long, frond-like processes (Figs. 3 to 5). These cytoplasmic processes, evident after 1 day of fasting, became progressively more prominent and complex with increasing weight loss and lipid depletion. Long loops and folds of

lipid and appeared to be increased in number (Figs. 5, 6, 9, 10). These microvesicles were identical in appearance and appeared to be as numerous as those normally present in endothelium. They were round or oval in configuration and 600 to 1000 A in diameter. Although some of them appeared to contain an amorphous material (Fig. 14), and occasional vesicles in endothelium, as well as in fat cells, contained irregular granular material of variable electron opacity (Figs. 7, 8), most vesicles contained no discernible material. Granular material similar to that in microvesicles was also observed extracellularly. Innumerable flask-like invaginations of plasma membrane (Figs.

5, 6, 10) were comparable in size and configuration to underlying microvesicles.

MICROVESICULAR ROSETTES: Rosette - like structures composed of microvesicles were often observed in lipid-depleted fat cells (Fig. 9). These rosettes consisted of from three to seven microvesicles grouped around and communicating with

involved the lipid-cytoplasmic interface which often became irregular and even somewhat convoluted (Figs. 13, 14). Occasionally, poorly defined membranes overlay portions of the lipid droplet surface (Fig. 14).

Numerous particles, presumably lipid or lipoprotein in nature (the serum from these animals
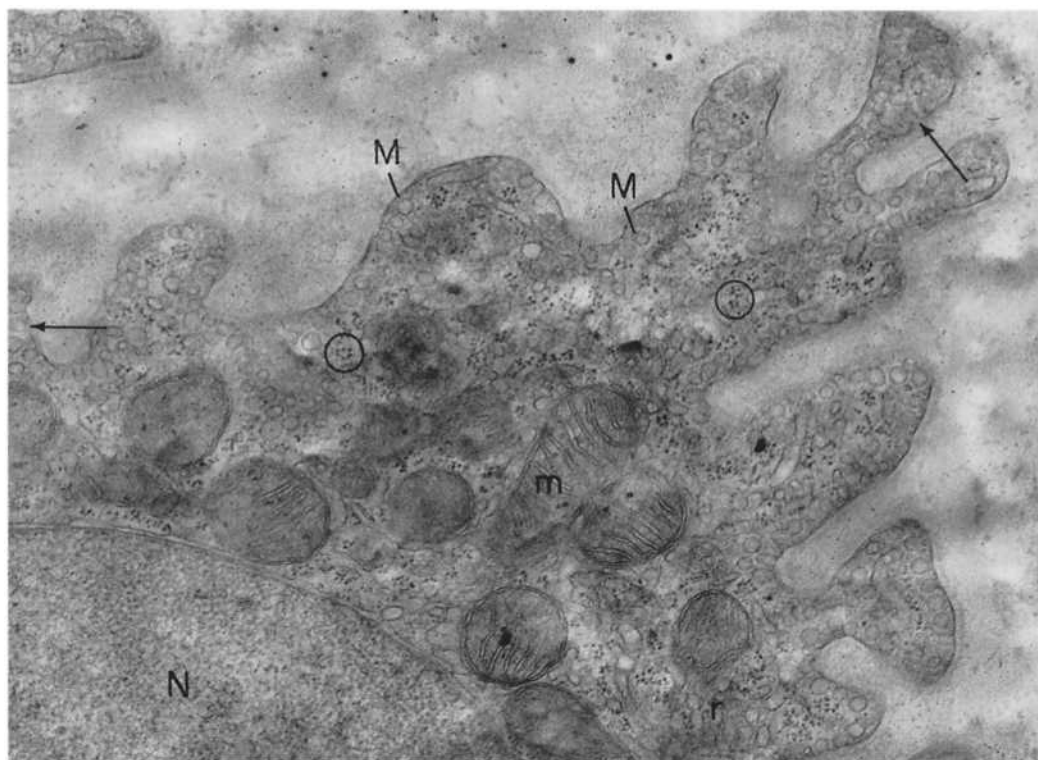

FIGURE 5 Portion of a lipid-depleted fat cell from a 6-day fasted rat (weight loss 29 per cent). The surface of the cell is irregular and the plasma membrane is indented by numerous small flask-like invaginations (arrows). Microvesicles (M) are numerous just beneath the plasma membrane, and tubules and elements of smooth endoplasmic reticulum (r) are prominent deeper in the cytoplasm. The cytoplasm also contains numerous mitochondria (m), and particulate ribonucleoprotein granules (encircled). Nucleus (N). X 34,000.

a slightly larger central vesicle. They were invariably located near the cell surface, and continuity was often demonstrable between the lumen of the rosette and the extracellular space (Fig 10). In these instances the limiting membrane of the rosette was continuous with the plasma membrane. Identical rosettes were also observed in vascular endothelium, in both adipose tissue and normal pancreas (Figs. 11, 12).

LIPIDS: Except for a decrease in lipid content, the only apparent alteration in intracellular lipid

was grossly lipemic) were frequently present in vessel lumina of diabetic animals (Fig. 12). These particles were 500 to 1500 A in diameter, round with slightly irregular surfaces, and were much less dense than the lipid inside fat cells. They were larger and less dense than the granular material in microvesicles and extracellular spaces (Figs. 7, 8), and than the granules in mitochondria (Figs. 6, 15).

Excluding the granular material occasionally observed in microvesicles and extracellular spaces,

FIGURE 6 Portion of a lipid-depleted fat cell from a 6-day fasted animal (weight loss 40 per cent). Microvesicles and flask-like invaginations of the plasma membrane literally rim the surface of the cell. Mitochondria (m) and elements of smooth endoplasmic reticulum (r) occupy the interior. Several loops of basement membrane protrude from the cell surface (arrows). The cleft-like spaces between plasma membrane invaginations and underlying cytoplasm are undoubtedly separation artefacts. Nucleus (N). × 36,500.

which may or may not be lipid in nature, particulate lipid (e.g., chylomicrons and lipoprotein aggregates) was not observed in fat cells, in endothelium, or in perivascular spaces. Neither were the very small, dense cytoplasmic granules (described in normal cells) observed in fat cells mobilizing lipid.

MISCELLANEOUS

Myelin figures (Figs. 9, 14) and intramitochondrial granules (Figs. 6, 15) were frequent in lipid-depleted fat cells.

Collagen fibers, no longer widely separated by lipid-filled fat cells, were prominent (Figs. 4, 16, 17).

Lipid Repletion

Although studies of lipid uptake by lipid-depleted fat cells are incomplete, they do permit the following general comments. In contrast to fasted animals, in refed rats numerous lipid particles (presumably chylomicrons) were present in vessel lumina and in the peripheral cytoplasm of fat cells (Figs. 16, 17). The intravascular particles in refed animals were larger, much more dense, and more nearly spherical with smoother surfaces than the particles in vessels of diabetic rats. They appeared to be adherent to the vessel wall. Lipid droplets in fat cells were frequently present in the tips of cytoplasmic processes where they were bounded by a very thin layer of cytoplasm containing numerous microvesicles (Figs. 4, 17). Unidentified small, dark granules similar to those in the cytoplasm of normal fat cells were also observed after refeeding. As was true during lipid mobilization, nothing was identifiable as lipid in endothelium or in perivascular spaces.
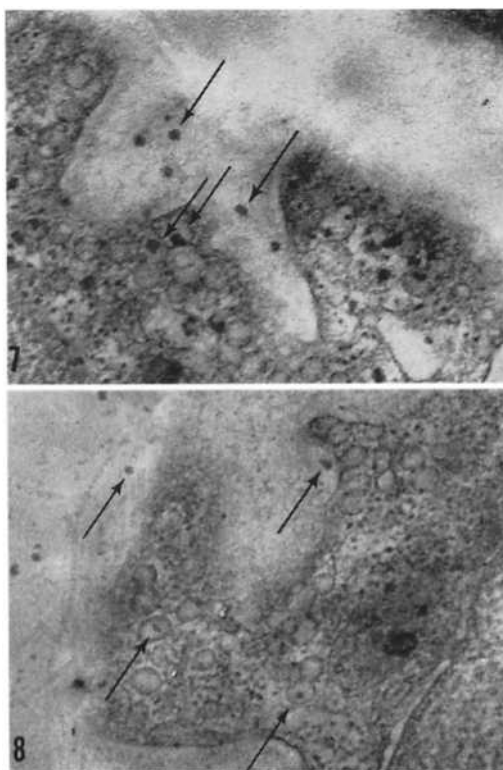
Figures 7 and 8 Portions of fat cells from an alloxan diabetic rat (duration 10 days, weight loss 14 per cent) demonstrating irregular granular material (arrows) in microvesicles and extracellular spaces. × 49,000 and 46,000, respectively.

DISCUSSION

Lipid-depleted fat cells developed striking changes in size and shape as well as in internal structure. Since the lipid content of fat cells was reduced regularly in fasted animals, all of which lost weight, but was reduced only in those diabetic animals losing weight, the changes described are considered in both instances to be related to lipid depletion.

Cellular Configuration and Basement Membrane Changes in Lipid-Depleted Fat Cells

One of the most remarkable changes in lipid-depleted cells was the development of long, frond-like cytoplasmic processes. Similar cytoplasmic processes were described by Wasserman and McDonald (10), and by Sheldon, Hollenberg, and Winegrad (13). These changes can probably best be explained by studies of plasma membrane

tension and the behavior of interfacial membranes (14–16). The relatively acute loss of large amounts of intracellular lipid would decrease intracellular pressure, permitting the plasma membrane to collapse spontaneously into folds and convolutions as predicted by Goldacre (16).

The folds and loops of basement membrane suggest that this structure is not removed and does not shrink as rapidly as the surface area of the plasma membrane decreases. Their frequent occurrence at tips of cytoplasmic processes suggests that the form of the latter may be inconstant and that the basement membrane may lag behind the retracting cytoplasmic processes. The possibility that some of the cytoplasmic processes represent pseudopodia cannot be excluded.

Intracellular Changes Associated with Lipid Mobilization in Fat Cells

The interpretation of intracellular changes associated with the mobilization or uptake of lipid requires first a consideration of factors influencing the forms and the distribution of lipids in tissues. General discussions pertinent to the present considerations are those of Dervichian (17), Engström and Finean (18), and West and Todd (19).

Lipids are recognized in electron micrographs in two forms: aggregates or droplets, and lipoprotein membranes. The polarity and state of ionization would appear to be important determinants of the lipids constituting these two forms. Neutral triglycerides, cholesterol, and cholesterol esters, comprising the bulk of lipid aggregates, are essentially unionized, non-polar lipids (17) held together as spherical aggregates by cohesive van der Waals forces. Long-chain fatty acids (at physiological pH) and most phospholipids are ionized, highly polar lipids (17–20). These lipids accumulate at lipid-aqueous interfaces where they are oriented with their non-polar ends in the lipid phase and their polar groups in the aqueous phase (20, 21). This orientation is apparently achieved in the double-layered "unit" membrane structure of cellular lipoprotein membranes (22) and at surfaces of triglyceride droplets (20).

At normal concentrations, over 90 per cent of the free fatty acids of plasma are bound to albumin by processes involving both polar and non-polar groups (23). They accumulate in triglyceride

droplets and lipoprotein particles only when the binding capacity of albumin is saturated (23, 24).

MICROVESICLES

The most striking change within cells mobilizing lipid was the increased prominence of microvesicles. These microvesicles are identical to those in endothelium, where they appear to be equally

in fat cells are also involved in transport, and that microvesicles may represent the mechanism of transport of free fatty acids in fat cells, endothelium, and muscle.

Although it is known that lipid is released from fat cells in the form of free fatty acids (31, 32), the site at which stored triglycerides are hydrolyzed to fatty acids is not known. If hydrolysis occurred
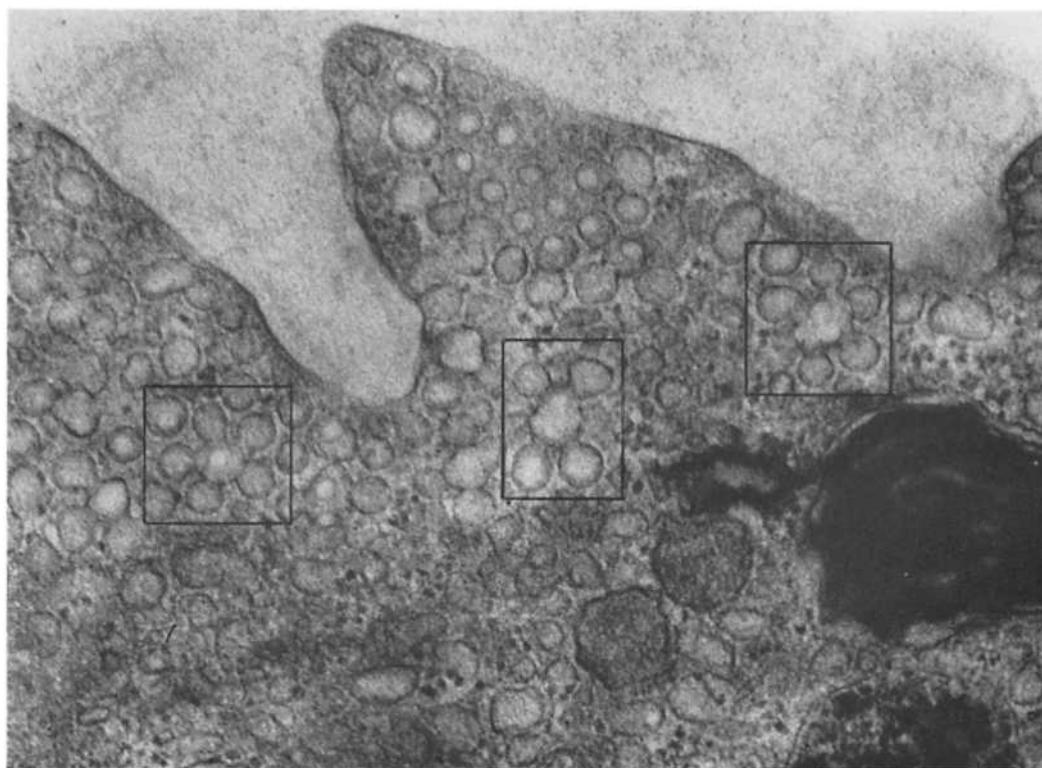

FIGURE 9   Three microvesicular rosettes (rectangles) in cytoplasm of a lipid-depleted fat cell from a rat fasted 6 days (weight loss 40 per cent). A myelin figure is present at the right margin just below midline. X 77,000.

numerous, and to those in muscle, where they are somewhat less numerous (25, 26). Although the capacity of these vesicles to transport particulate matter through endothelium has been demonstrated (27–29), most convincingly by Jennings, Marchesi, and Florey (29), and although Bennett (30) has postulated a mechanism for vesicle formation, the physiological stimulus for formation of vesicles and the materials normally transported by them are not known. A number of circumstances and morphological features to be discussed suggest very strongly that microvesicles

at the cell surface, one might expect to see small lipid droplets formed from the large central droplet and transported through the cytoplasm to the plasma membrane. The absence of such lipid droplets in cells known to be mobilizing lipid argues against this possibility. On the other hand, if hydrolysis took place at the surface of the lipid droplet, the resulting polar fatty acids would more likely be transported to the cell surface in association with lipoprotein membranes or as fatty acid–protein complexes similar to albumin–fatty acid complexes in plasma. The configuration of
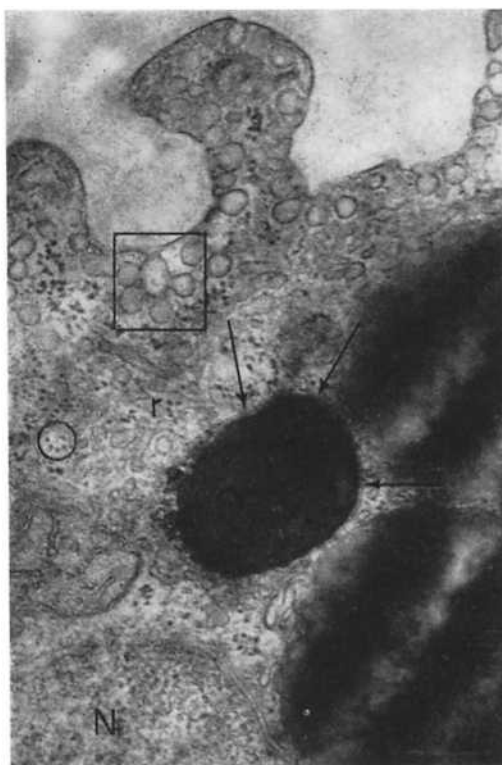
FIGURE 10 Portion of a partially lipid-depleted fat cell from a rat fasted 6 days (weight loss 40 per cent). A microvesicular rosette is shown in cross-section, demonstrating continuity of the limiting membrane of the rosette with the plasma membrane, and communication of the lumen of the rosette with the extracellular space. Flask-like invaginations of plasma membrane are numerous. Elements of smooth endoplasmic reticulum (r) and particulate ribonucleoprotein granules (encircled) are prominent deeper in the cytoplasm. The central lipid droplet appears to be partially bounded by membrane (arrows). Nucleus (N). × 39,500.

FIGURE 11 Microvesicular rosette in vascular endothelium in a normal islet of Langerhans of the pancreas. Vessel lumen (V). × 54,000.

FIGURE 12 Cross-section of a microvesicular rosette in vascular endothelium in adipose tissue of an alloxan diabetic animal. Several "lipid" particles (arrows) are present in the lumen of the vessel (V). Nucleus (N). × 46,500.

microvesicles might be particularly well suited for the transport of free fatty acids in either of these forms. Polar groups could be "bound" to cationic groups of proteins lining the inner wall of the vesicle membrane, and hydrophobic nonpolar ends could be concentrated in the interior. Alternatively, fatty acid–protein complexes could be carried in the lumen of the vesicle.

In either case, microvesicles engaged in this process would be formed in the interior of the cell rather than from the plasma membrane, and hence would not be pinocytotic in origin. It should
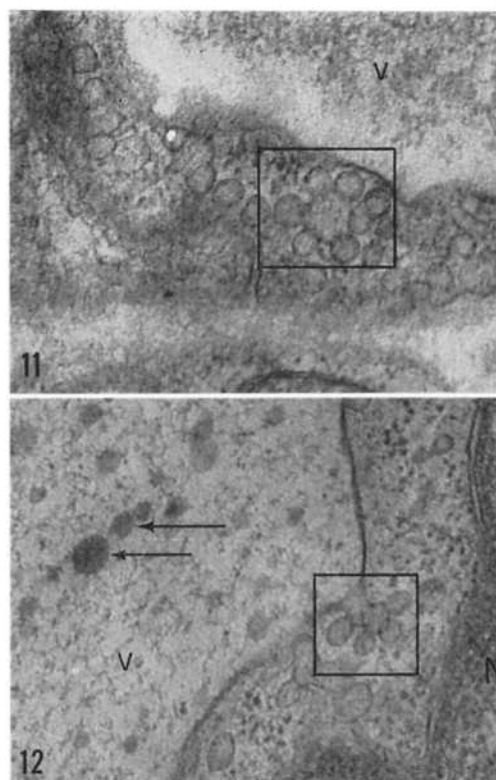
be emphasized that in none of the published electron microscope studies of adipose tissue (9–11, 13) has the precise origin of microvesicles been demonstrated, in either normal or fasted animals. The origin, content, and direction of transport of microvesicles may well vary according to the metabolic state of the cell. That microvesicles in fat cells mobilizing lipid may not be primarily pinocytotic in origin is suggested by the absence of particulate mercuric sulfide (injected 1½ hours prior to death) in microvesicles of fat cells despite considerable accumulations in adjacent macrophages (unpublished observations). Furthermore, the occasional convoluted lipid-cytoplasmic interfaces and interfacial membranes observed in partially lipid-depleted cells are consistent with
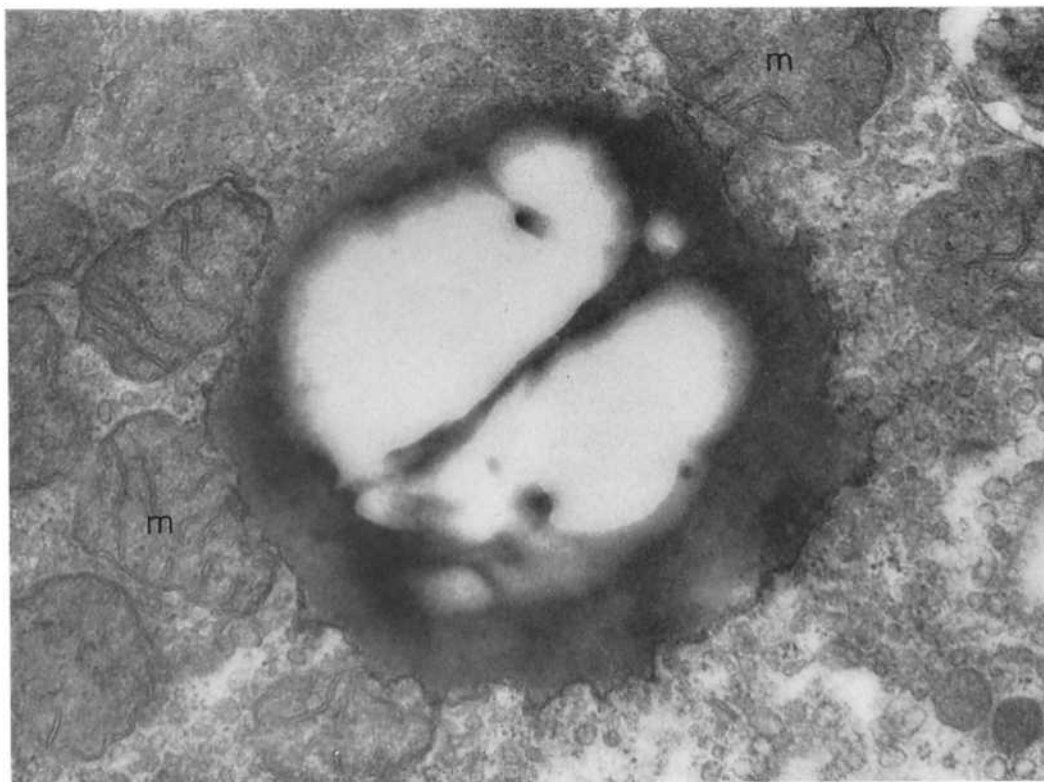
FIGURE 13 Small lipid droplet in a fat cell from a 6-day fasted animal (weight loss 40 per cent), demonstrating irregularity of the lipid-cytoplasmic interface. The surface of the lipid droplet is intimately associated with cytoplasmic microvesicles over much of its area and has a vesicular appearance itself. Mitochondria (m) are numerous and elements of smooth endoplasmic reticulum are prominent in the left upper corner. Clear spaces in the fat droplet are artefacts produced by loss of lipid. × 42,000.

the hypothesis of vesicle formation in the region of the lipid-cytoplasmic interface. Association of cytoplasmic proteins and/or proteins of lipase enzymes with fatty acids at interfaces might be expected to result in "membrane" formation just as proteins and fatty acid monolayers form films *in vitro* (33, 34). [That protein films also exist *in vivo* in association with lipid droplets has been demonstrated (35, 36).]

Transport in microvesicles implies isolation of the vesicle contents from the remaining cytoplasm. The compartmentalization and transport within microvesicles of fatty acids derived from lipolysis would be analogous to the compartmentalization of secretory products in general (37–40), and would be consistent with biochemical evidence suggesting that intracellular fatty acids derived from lipolysis are anatomically separate from those coming in (41).

Fig. 18 is a schematic representation of the process of lipid mobilization in which the mechanisms postulated in the preceding discussion are depicted.

MICROVESICULAR ROSETTES

Microvesicular rosettes, although occasionally noted in endothelium (29), have not been described as such in detail. If considered in three dimensions, they would resemble hollow spheres with numerous outpouchings. The hypothetical steps leading to the formation of rosettes in endothelium and in fat cells (assuming that microvesicles transport products of lipolysis to the surface of the cell) are depicted in Fig. 19. When the limiting membrane of a microvesicle fuses with the plasma membrane (Fig. 19, *1*) and bursts (Fig. 19, *2*), the lining of the vesicle remains, for a time at least, as a flask-like invagination of the
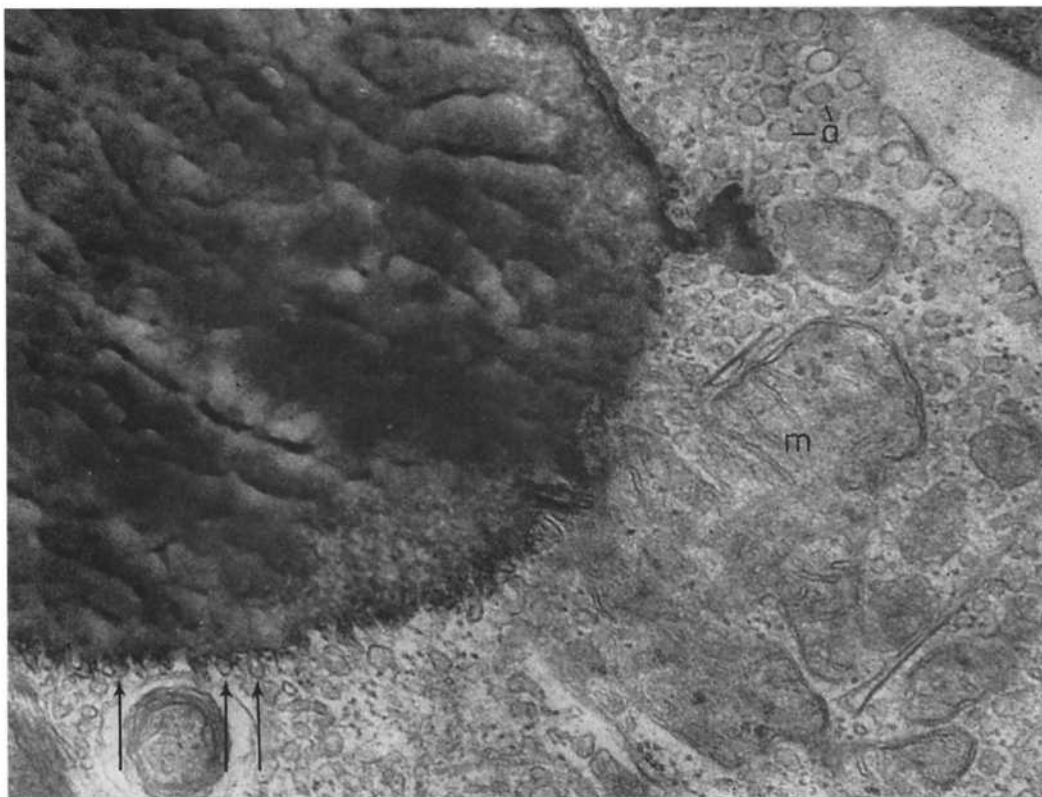
FIGURE 14 Small lipid droplet in a fat cell from a 6-day fasted animal (weight loss 40 per cent), showing irregularity of the lipid-cytoplasmic interface. Poorly defined short lengths of convoluted membrane (arrows) outline the lipid-cytoplasmic interface along the lower portion of the droplet. The lamellar structure in the left lower corner is probably a myelin figure. Occasional vesicles appear to contain amorphous material (a). Mitochondria (m). × 54,000.

plasma membrane. This step corresponds to changes in membranous sacs associated with the release of granules in secretory cells (39, 40). If this flask-like invagination possesses the properties of the plasma membrane, it is reasonable to expect that microvesicles in the immediate vicinity could then fuse with (Fig. 19, 3) and open into it (Fig. 19, 4) thereby forming the rosette-like structures observed (Fig. 19, 4, 5; compare with Figs. 9 and 10). It seems less likely that pinocytotic vesicles originating from plasma membrane would form such orderly structures; however, this possibility cannot be excluded.

Transport of Lipid through Endothelium and into Fat Cells

Differences in the appearance of intravascular "lipid" particles in diabetic animals and chylo-

microns in refed fasted animals are consistent with evidence that lipid particles of hepatic and of alimentary origin differ in size and composition (42) and with the unsaturated lipids fed to fasted animals.

The presence of numerous lipid droplets in the peripheral cytoplasm of fat cells after refeeding indicates that these cells are accumulating lipid, and the numerous chylomicrons in vessel lumina suggest themselves as the source of the newly acquired lipid. The absence of comparable lipid droplets or particles in endothelium and in perivascular spaces, however, indicates that chylomicrons are hydrolyzed or altered in some way prior to or during transport through endothelium, if they are, indeed, the source of the lipid in fat cells. Considerable circumstantial evidence is consistent with and supports the hypothesis that

hydrolysis of chylomicron triglycerides occurs prior to, or during transport through blood vessels in general, and that microvesicles represent the vehicle of transport of free fatty acids through endothelium.

Evidence that lipoprotein lipase is located in, or is intimately related to endothelium (43, 44)

endothelium lining hepatic sinusoids (45, 46) and lymphatic vessels in the small intestine (1, 2, 5, 45) is characterized by frequent discontinuities or open junctions through which the lipid particles pass. Basement membranes around these same vessels, and around lymphatic vessels in general (47), are poorly formed and often discontinuous
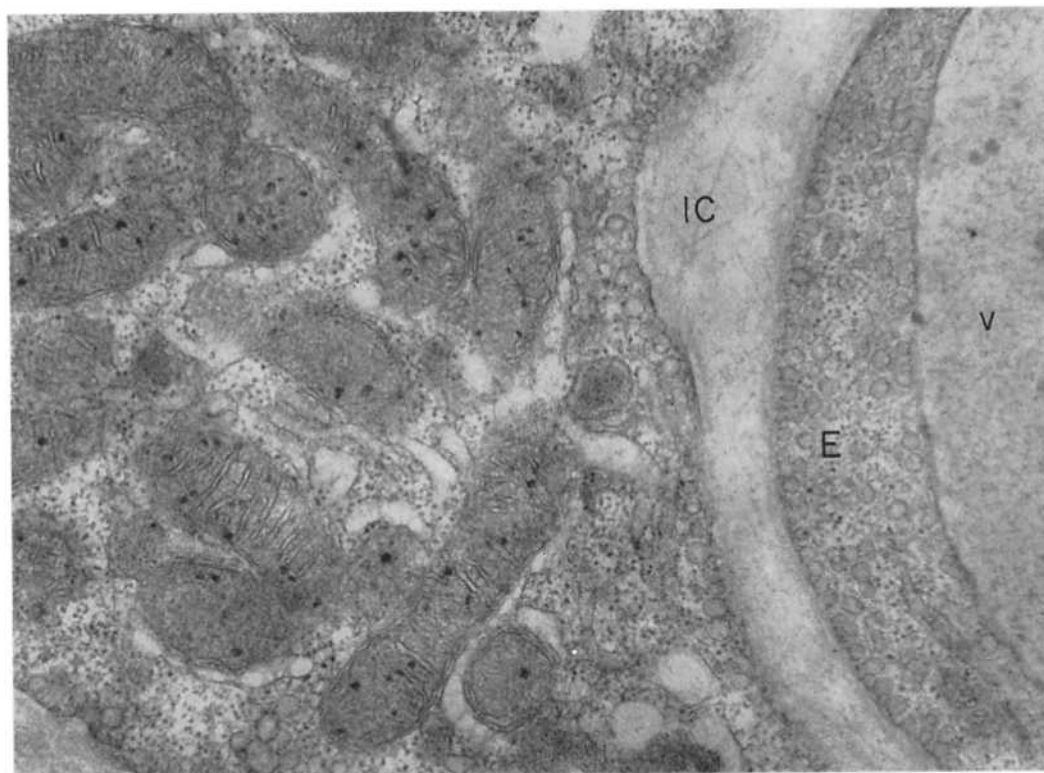

FIGURE 15 Lipid-depleted fat cell from a diabetic animal. Mitochondria are prominent and contain numerous irregular granules of varying density. Microvesicles and particulate ribonucleoprotein granules are numerous in endothelium (E) as well as in the fat cell. Vessel lumen (V); intercellular space (IC). X 38,000.

indicates the availability of an enzyme capable of hydrolyzing chylomicron triglycerides.

The presence of numerous microvesicles and rosettes in endothelium and fat cells and their relative sparsity in other cell types suggest that these structures may be related to a function or functions peculiar to both cell types, namely lipid transport.

The vascular structure of the only two organs, liver and small intestine, in which particulate lipids have been demonstrated to pass through vessel walls (1, 2, 5, 45) is of special interest. The

or not present. [An additional basement membrane has been described between lamina propria and intestinal epithelum (1, 2); however, it has not been fully characterized and just how lipid droplets pass through this region is not known.] In striking contrast, endothelial open junctions are absent and perivascular basement membranes are well defined in most tissues, including adipose tissue, in which transport of particulate lipids through endothelium has not been reported. [The author is aware of only a single reference to the
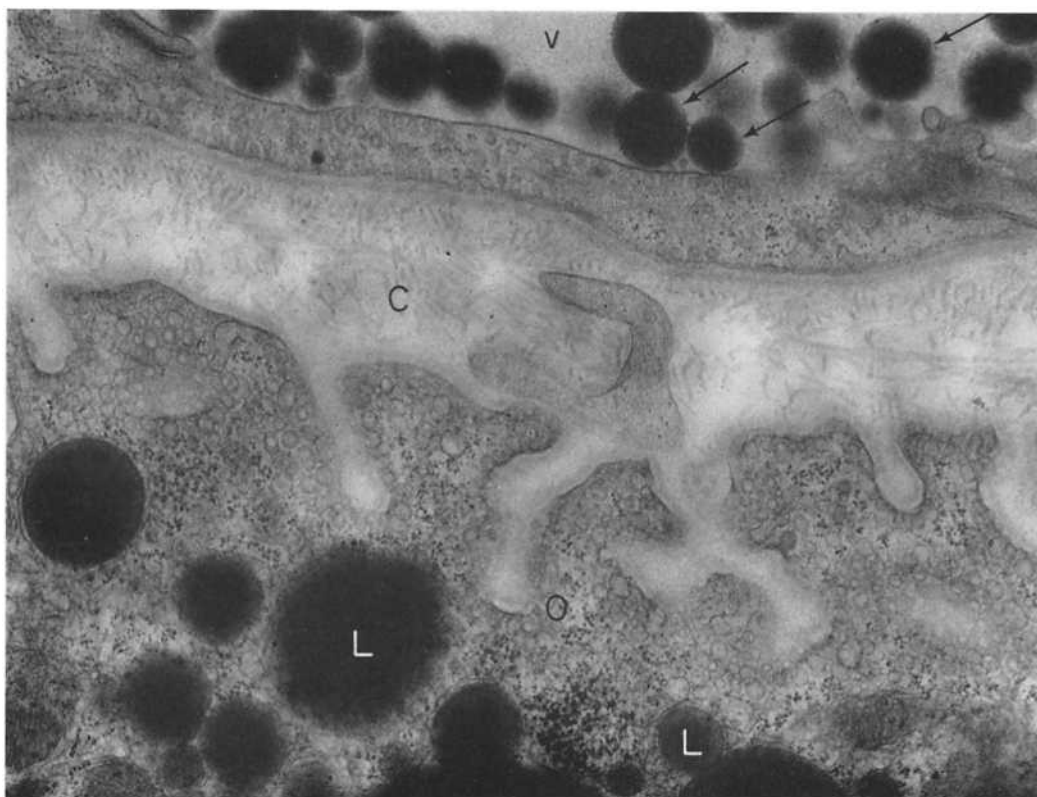
FIGURE 16 Portions of a fat cell and a venule from a 6-day fasted animal refed a diet rich in unsaturated fats (corn oil) 18 hours prior to death. Lipid is present in the form of chylomicrons (arrows) in the vessel lumen (V), and as small droplets (L) in the fat cell. Numerous collagen fibers (C), but no discernible lipid, are present in the perivascular space. The identity of numerous very small cytoplasmic granules (encircled) was not ascertained. × 25,500.

presence of "lipid particles" in endothelial microvesicles of blood vessels (48).]

More direct evidence also indicates that well developed basement membranes around endothelium and fat cells would almost certainly hinder the movement of particulate lipid. In many organs the perivascular basement membrane functions effectively as a sieve to filter and retain particulate materials (colloidal gold, mercuric sulfide, carbon, etc.) passing through the vessel wall (49, 50), and to hold back emigrating leukocytes (51, 52). Indeed, Majno and Palade (49) have demonstrated retention of lipid particles as well as of colloidal mercuric sulfide by the basement membrane of inflamed vessels in rat cremasteric muscle. Filtration and retention of colloidal mercuric sulfide particles by perivascular basement membranes in rat epididymal adipose tissue has also

been observed by the author (unpublished observations).

That chylomicrons may pass intact through vascular endothelium has been suggested in reports describing lipid particles in lymph collected from cervical and lower extremity lymphatics (53–55). However, the number of lipid particles in lymph compared to plasma in these experiments was small, and the manner in which they entered the lymph was not demonstrated.

Bragdon and Gordon (56) also concluded from their studies that chylomicrons were transported across endothelium and taken up intact by fat cells. Although they demonstrated that fatty acids of chylomicron triglycerides were taken up by fat cells and that widespread intravascular hydrolysis of chylomicron triglycerides did not occur, their data did not exclude the possibility
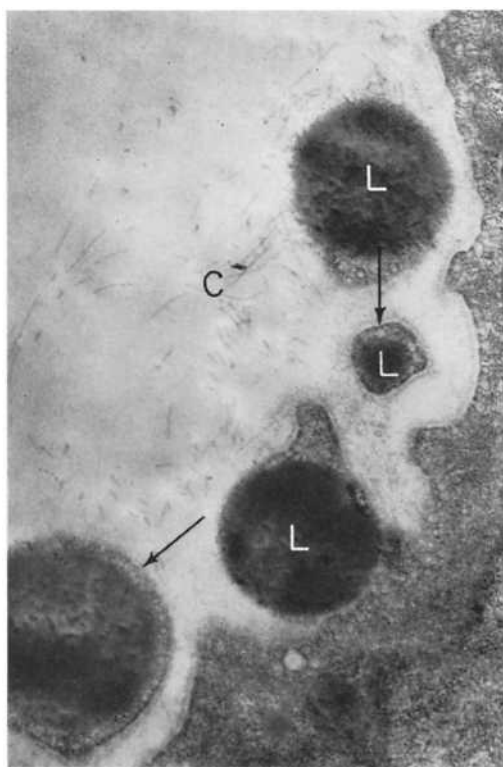
FIGURE 17 Portion of a lipid-depleted fat cell from an animal fasted 6 days and then refed a diet rich in unsaturated fats (corn oil) 18 hours prior to death. Several small lipid droplets (L) are present in cytoplasmic processes. The thin rim of cytoplasm surrounding these droplets is lined almost continuously by microvesicles which abut directly against underlying lipid (arrows). Collagen (C). × 19,500.

of hydrolysis at the surface of endothelium or in transit through it.

Studies by Rodbell (57) demonstrating incorporation of labeled triglyceride fatty acids *in vitro* do not eliminate the possibility that triglycerides are normally hydrolyzed by endothelium prior to uptake.

Fig. 20 is a schematic representation of the hypothetical processes postulated for the transport of lipid through endothelium and its uptake by fat cells. Obviously, many questions concerning the precise manner in which these various processes are accomplished cannot be answered at the present time. Intra- and extracellular differences in pH, ionic constitution, and proteins are un-

doubtedly important, since relatively minute changes in these factors have profound effects upon lipid and protein interactions (19, 21). Studies of the behavior of interfacial films (33, 34) and of interactions between surface-active lipids (soaps and detergents) and protein (58–60) indicate that elucidation of these problems may be amenable to electron microscope studies of *in vitro* models.

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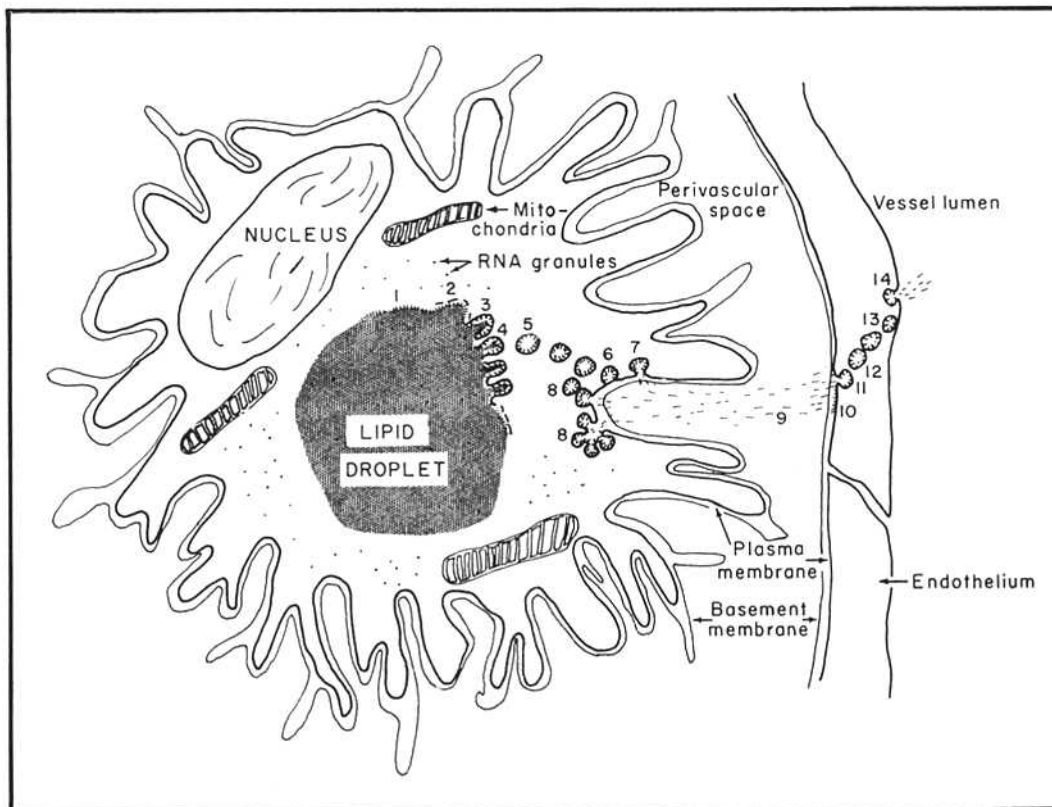5. CASLEY-SMITH, J. R., The identification of

FIGURE 18 Schematic representation of proposed mechanism of egress of fatty acids from fat cells. Triglycerides at the surface of droplets are hydrolyzed to free fatty acids which are oriented at the interface with their non-polar tails in the lipid phase and their polar carboxyl groups in the aqueous phase (*1*). Intracytoplasmic proteins associate with the free fatty acids by ionic bonds, producing membranes (*2*, *3*) which form vesicles (*4*, *5*). The latter are transported to the surface of the cell where they fuse with the plasma membrane (*6*) and burst (*7*). Vesicles fusing with plasma membrane invaginations form rosettes (*8*). The fatty acids are then released into extracellular fluid, transported through the basement membrane of the fat cell, through the perivascular space (*9*), and through the perivascular basement membrane to the plasma membrane of endothelial cells. They induce vesicle formation of endothelial plasma membrane (*10*, *11*) and are transported through endothelium (*12*) to the lumen of the vessel (*13*, *14*).
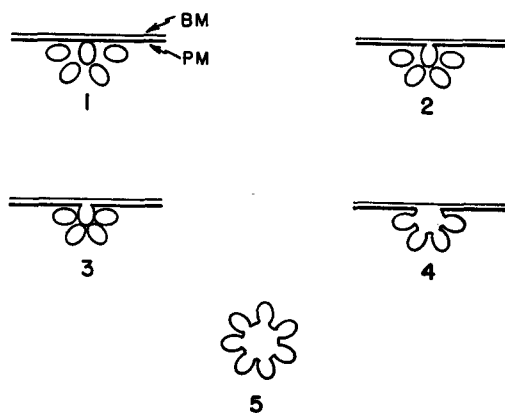

FIGURE 19 Schematic representation of proposed mode of formation of microvesicular rosettes. When the limiting membrane of a microvesicle fuses with the plasma membrane (*PM*) and bursts (*1*, *2*), the lining of the vesicle remains in the form of a flask-like invagination of plasma membrane. If this flask-like invagination possesses the properties of the plasma membrane, it is reasonable to expect that microvesicles in the immediate vicinity should fuse with (*3*) and open into it (*4*), forming the rosette-like structures observed. The rosette-like appearance (*5*) is produced when these structures are viewed in a plane of section parallel to the cell surface. Basement membrane (*BM*).
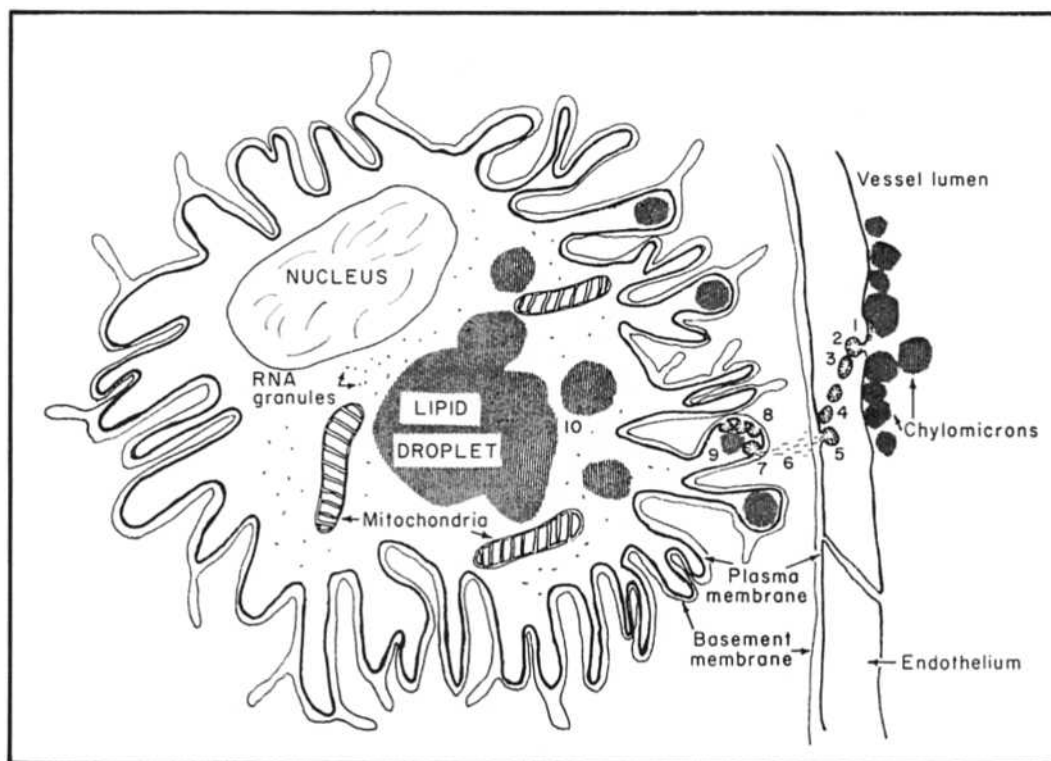
FIGURE 20 Schematic representation of proposed mechanism of lipid ingress into fat cells. Chylomicron triglycerides in contact with endothelial plasma membranes are hydrolyzed by lipoprotein lipase. Free fatty acids derived from lipolysis induce vesicle formation in plasma membrane (1, 2) and are transported through endothelium as the inner lining and/or in the lumen of vesicles (3). At the opposite side, the vesicles fuse with the plasma membrane (4) and burst (5), releasing free fatty acids into the perivascular space. The fatty acids, probably bound to albumin, are then transported to the fat cell (6), where again they induce vesicle formation of plasma membrane and enter the cell (7, 8). Newly acquired fatty acids are rapidly esterified (9), and the resulting triglycerides coalesce to form the larger central aggregates of lipid (10).

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