THE ROLE OF THE GOLGI COMPLEX IN FAT ABSORPTION AS STUDIED WITH THE ELECTRON MICROSCOPE WITH OBSERVATIONS ON THE CYTOLOGY OF DUODENAL ABSORPTIVE CELLS

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Plates 85 to 90

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Biscossi (1) and later Cramer and Ludford (2) beautifully illustrated their contention that the Golgi complex plays an important role in fat absorption by intestinal cells. Unfortunately, their clear observations have been obscured by the controversy which has raged over the structure, and indeed the very existence of this organelle. Electron microscopy has clarified the confusion regarding the Golgi complex (3, 5, 15), demonstrating both its existence and its main structural features. Taking advantage of this technique, we have reinvestigated the process of fat absorption, and have confirmed the earlier observations, succinctly stated by Cramer and Ludford, that " . . . during fat absorption, the Golgi apparatus is the cell structure mainly concerned."

Materials and Methods

Eight to ten week old male Swiss albino mice were used in this study. The animals were killed by decapitation. A 2 to 3 mm. segment of the second centimeter of the duodenum was removed within 1 minute of the time of death, opened by a longutudinal slit, and placed into a small amount of the pH 7.2 osmic acid–dichromate fixative recently devised by Dalton (4). After fixing for 1 hour, the tissue was washed for a total of 30 minutes using six changes of tap water, and then dehydrated by successive transfers lasting 10 minutes each through three changes of 95 per cent ethanol and three changes of absolute ethanol. The tissue was next transferred to a half-and-half mixture of absolute ethanol and methacrylate. The methacrylate was composed of three parts *n*-butyl methacrylate to one part methyl methacrylate. After three changes of methacrylate the tissue was embedded in methacrylate to which the catalyst, benzoyl peroxide, had been added. Polymerization was carried out at 37°C. for 24 hours. Sections were cut with a Minot microtome modified as described by Dempsey and Lansing (6) and equipped with a glass knife. The sections were examined without removing the plastic in an RCA model EMU2E microscope with a 40 μ aperture in the objective lense. Electron micrographs were taken at an original magnification of from 1,000 to 10,000 times and enlarged photographically as desired.

The fine structure of duodenal cells from control and fat-fed animals was studied. Control mice received regular Purina lab chow and water *ad lib*. In the fat-feeding experiment seven mice were deprived of food and water for a 24 hour period, starting at 6 a.m. At 6 p.m. on

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the 2nd day two controls were killed immediately, and five mice were given whipping cream *ad lib.*, of which each took about 0.3 cc. Mice were killed at 9 minutes, ½ hour, 1 hour, 1½ hours, and 3 hours after first drinking cream. In the animal killed at 9 minutes the cream was confined to the upper half of the stomach, but in all other animals cream was present in the duodenum.

The duodenal cells of many animals were studied during the course of experiments designed to determine the mechanism of cation transport across these cells. These experiments and their results will be described in the following paper (16). Observations made during the course of these studies were used in the present paper when they bore upon normal cell structure or upon fat absorption.

OBSERVATIONS

The Normal Duodenal Absorptive Cell.—The velvety mucosal surface of the duodenum is lined by innumerable small, closely packed villi, each of which possesses a core of vascular connective tissue and a covering sheet of closely applied absorptive epithelial cells. The structure of these cells as revealed by the light microscope is well known (9). They are columnar, roughly polygonal in cross-section, rest upon a basement membrane associated with the contiguous connective tissue of the villus core, and possess at their apical margins a striated cuticular border. The oval cell nucleus is centrally placed, its long axis parallel to that of the cell. Light microscopic studies reveal three principal cytoplasmic organelles: the prominent Golgi complex caps the nucleus apically and laterally; the ergastoplasm, scanty in amount, is scattered throughout the cytoplasm; and numerous mitochondria are widely distributed in both apical and basal cytoplasm.

Plasma Membrane.—Electron microscopy reveals the presence of a fourth cytoplasmic organelle, the plasma membrane. This smooth, osmiophilic structure, approximately 90 A thick, forms a boundary around the cell, basally, laterally, and apically. In animals deprived of water the basal plasma membrane extends straight across the bottom of the cell, occasionally sending a slit-like invagination into the basal cytoplasm (Fig. 1). The basal plasma membrane is separated by a variable interval from its basement membrane, which appears to be a condensation product of extracellular connective tissue material, and which forms a band of variable thickness and density upon which the absorptive cell rests (Figs. 1 and 6). The thickness and density of the basement membrane are variable.

Occasional discontinuities appear to occur in the lateral plasma membrane (Figs. 1 and 3). For the most part, however, it is clearly defined. The membranes of adjacent cells are ordinarily separated from one another by an electron-lucent interval of about 90 A. Dilatations of the lower part of this intercellular space occur which are filled with electron-lucent material and/or electron dense globules (Figs. 1, 2, 6, 10).

The configuration of the lateral plasma membranes is complicated by interdigitations of neighboring cells (Figs. 1, 2, 3, 5, 8). For example, a simple finger-

like projection of one cell into another, cut perpendicular to its long axis some distance from its point of origin, will appear as a double membraned loop some distance from the main course of the lateral cell boundary. The loop may be circular or elliptical depending upon the shape of the invagination and the plane of section. The outer membrane of the loop represents the plasma membrane of the cell in which the loop is found; the inner membrane represents the plasma membrane of the neighboring cell; the electron-lucent interval between membranes is the intercellular space; and the cytoplasm within the inner membrane is that of the neighboring cell. This simple case may be compounded by branched invaginations, invaginations into invaginations, or combinations of these situations. Characteristically, one complex interdigitation occurs in the basal cytoplasm, and another occurs in the apical cytoplasm just below the level of the terminal bar. The basal interdigitation may be obliterated by wide dilatation of the intercellular space in this region.

The terminal bar is a modification of the lateral plasma membrane at the very top of the cell. In the light microscope it appears as a thickening in the region of the apical cell boundary (9). In the electron microscope this thickening is seen to be caused by an increased width and density of the plasma membrane (Fig. 3). The intercellular space does not seem to be changed. The length of the cell membrane involved in terminal bar formation is variable, being as short as 0.1 μ and as long as several micra.

The apical plasma membrane covers the microvilli. An occasional invagination of the plasma membrane may extend from the base of a microvillus for a short distance into the apical cytoplasm.

The Cytoplasmic Matrix.—The cytoplasmic matrix is a continuous aqueous phase contained within and bordered by the plasma membrane. The nucleus, and the cytoplasmic organelles and inclusions are embedded in it. Porter first observed that with long periods of fixation with osmic acid the more electron-dense materials of the cytoplasmic matrix leach out (12). As Dalton has pointed out (4), fixation with osmic acid–dichromate mixture preserves the electron-dense material. With this fixative there appears to be little tendency for disruption of the interrelationships among cytoplasmic organelles, probably as a result of the good preservation of the binding cytoplasmic matrix. The electron-dense material of the cytoplasmic matrix presents an entirely homogeneous appearance at low magnifications (Fig. 1). At higher magnifications this homogeneous material can be seen to contain randomly scattered lines, dots and circles, about 40 A in smallest dimensions (Fig. 3).

The apical cytoplasmic matrix is unique in three respects: it is thrown up into microvilli; no organelles or inclusions are present within the microvilli and for a short distance below them; and the cytoplasmic matrix in this region is denser than that found elsewhere (Figs. 1 and 3). The microvilli, first depicted in electron micrographs by Granger and Baker (7), are finger-like pro-

jections which are circular in cross-section, and rarely branch (Figs. 1 to 3). They average 2 μ in length and 1/3 μ in diameter.

The Ergastoplasm.—Scattered throughout the cytoplasm, except at the very apex of the cell, are elements of the ergastoplasmic complex (14) (Figs. 1 to 6). Only a few ergastoplasmic sacs are present, flattened and sheet-like, and these units are widely separated from one another. Their lumina are invariably very narrow, the opposite smooth inner surfaces of their membraneous walls being separated by only about 200 A in most cases. Electron-dense ergastoplasmic granules on the outer surface of the ergastoplasmic membrane are characteristic of these structures (14). Recently Palade has shown that these granules may occur unattached to ergastoplasmic membranes (11). In the duodenal cell the ergastoplasmic granules are arranged in small clusters of varying number and shape. Clusters of free granules may, at times, give the appearance of being transformed into very fine double membranes: very fine osmiophilic lines may be separated by a very thin clear space (Figs. 2 and 5). In addition, fine, straight or curved membranes of varying width may sometimes border a group of ergastoplasmic granules (Figs. 2 and 5). This suggests the possibility that smooth membranes may form in connection with ergastoplasmic granules.

The Mitochondria.—Many rod-shaped mitochondria are present throughout the cytoplasm. They are particularly numerous in the basal region of the cell (Figs. 1 and 6). Their structural features have been described by many investigators (10 and 13).

The Golgi Complex.—The electron microscopic picture of the Golgi complex of duodenal cells has been described by Dalton (3) and by Dalton and Felix (5). Our observations are in essential agreement with their most recent description.

The Golgi complex is a system of sacs and vacuoles enclosed by smooth membranes, which are about 40 to 90 A in thickness. Flattened, pillow case–like Golgi sacs are found in compact groups just above the nucleus (Figs. 2, 4, 5). The lumen of each sac is very narrow, the opposite walls being separated by about 90 A. By dilatation of larger or smaller terminal segments, the sac gives rise to the Golgi vacuoles, which vary in size from several micra to about 500 A in diameter (Figs. 4 and 5). This may not be the only mode of formation of Golgi vacuoles; at times it is difficult to decide whether a smooth membraned vacuole is derived from ergastoplasmic granules or Golgi sacs (Fig. 5).

The largest Golgi vacuoles, most prominent component of the complex, cap the nucleus apically and laterally. They contain electron-dense masses of varying size and shape, which do not fill the vacuole. They are bounded by a membrane which may be discontinuous, leaving regions to be bounded only by a poorly defined edge of cytoplasmic matrix (Figs. 1, 2, 4, 5). The medium sized vacuoles usually have a clearly defined, continuous membrane around them, and contain only one or two electron-dense spherules (Figs. 1, 2, 4). The

smallest Golgi vacuoles are about 500 A in outside diameter and have an electron-lucent lumen (Fig. 5). These structures were originally termed Golgi granules by Dalton and Felix, a terminology which we earlier followed (15). However, we now believe that they represent the smallest of the general class of Golgi vacuoles.

Fat Absorption in the Normal Mouse.—Examination of many normal duodenal absorptive cells has revealed that numerous morphologically similar osmiophilic bodies are present in a variety of locations. Most of these bodies are spherical, and average 0.1 μ in diameter. They are found in the central lacteal, in the extracellular connective tissue space around it, in the spaces between the absorptive cells, within the large Golgi vacuoles, and surrounded by a smooth membrane in the apical cytoplasm (Figs. 1 to 7). Their occurrence within the lacteal and their osmiophilia indicate their lipide nature. A detailed examination of their morphology in different locations yields a very suggestive picture of the sequence of events in fat absorption.

The central lacteal can be identified by the presence within its lumen of small fat droplets (Fig. 7). The lacteal wall is formed by endothelial cells. Except for the bulge of cytoplasm containing the nucleus, the endothelial cell is a thin sheet. A number of these sheets, derived from more than one endothelial cell, overlap one another to form a more or less continuous wall for the lacteal. There is a very poorly defined and discontinuous basement membrane. Lipide droplets similar to those present within the lacteal are scattered freely in the connective tissue spaces, and some are also found in the spaces between overlapping sheets of lacteal endothelium (Fig. 7). Morphologically similar droplets are also found in the lower part of the space between absorptive cells. We have never observed them in the intercellular space above the apical intercellular interdigitation; nor have we seen a separation of the cell membranes at the terminal bar.

Fat-Fed Mice.—The duodenal epithelium of animals deprived of food and water for 24 hours presents striking differences when compared with that of normal animals. Lipide droplets are scarce, and the Golgi complex is greatly reduced in size, consisting of only a few small sacs, and a number of 400 A vacuoles in the supranuclear region (Fig. 8).

Nine minutes after cream feeding no changes can be observed, but 30 minutes post prandial the apical cytoplasm contains many vacuoles (Fig. 9). Although of widely varying size, the vacuoles are similar in appearance. They are bounded, sometimes incompletely, by a smooth membrane. Very small, extremely osmiophilic particles cluster at the periphery of the vacuoles; the remainder of its contents are electron-lucent. The closely packed, large vacuoles in the supranuclear region can be recognized as part of the Golgi complex by their location and typical configuration. Three hours after fat feeding the picture has not changed except for the appearance of fine lipide droplets in the

intercellular space (Fig. 10), the extracellular connective tissue space, and the central lacteal.

DISCUSSION

Our observations suggest that the following sequence of events occurs during fat absorption:—

Lipide passes through the apical plasma membrane in particles which are very small, probably less than 40 A in diameter. These small particles are transformed into larger ones and deposited within vacuoles to form fat droplets. The droplets pass through the cytoplasm to the lateral plasma membrane, which they somehow penetrate, to enter the intercellular space somewhere below the terminal bar region. The droplets pass downward between the cells, penetrate or pass through a gap in the basement membrane, and enter the extracellular connective tissue space. Finally they dissect between overlapping layers of the lacteal wall to enter the lacteal.

Although Hewitt (8) and others have suggested that fat may be absorbed extracellularly, our failure to observe fat droplets in the upper part of the intercellular space leads us to believe that fat must pass through the apical cytoplasm of the absorptive cell. The presence of numerous fat droplets in the apical cytoplasm supports this view. In addition fat droplets have not been observed in the region of the brush border. The largest particles seen in the cytoplasmic matrix of the microvilli and of the region just below them are less than 40 A in diameter, suggesting that fat penetrates this region in particles at least that small.

All the intracellular fat droplets appear to be enclosed within vacuoles bounded by smooth membranes. The recently formed vacuoles found in absorptive cells after cream feeding to a fasted animal are morphologically different from those found under other circumstances (*cf.* Figs. 2 and 6 to Figs. 9 and 10). In any given animal, however, the vacuoles scattered throughout the apical cytoplasm are morphologically similar to those just above the nucleus. The large supranuclear vacuoles are part of the main Golgi complex. It seems reasonable that the smaller, similar vacuoles scattered throughout the apical cytoplasm should also be considered Golgi vacuoles.

SUMMARY

An electron microscopic study of the morphology of fat absorption in the duodenum of normal and fat-fed mice supports the view that all absorbed fat passes through the cytoplasm of absorptive cells. The Golgi complex plays an important role in the transport of fat across these cells.

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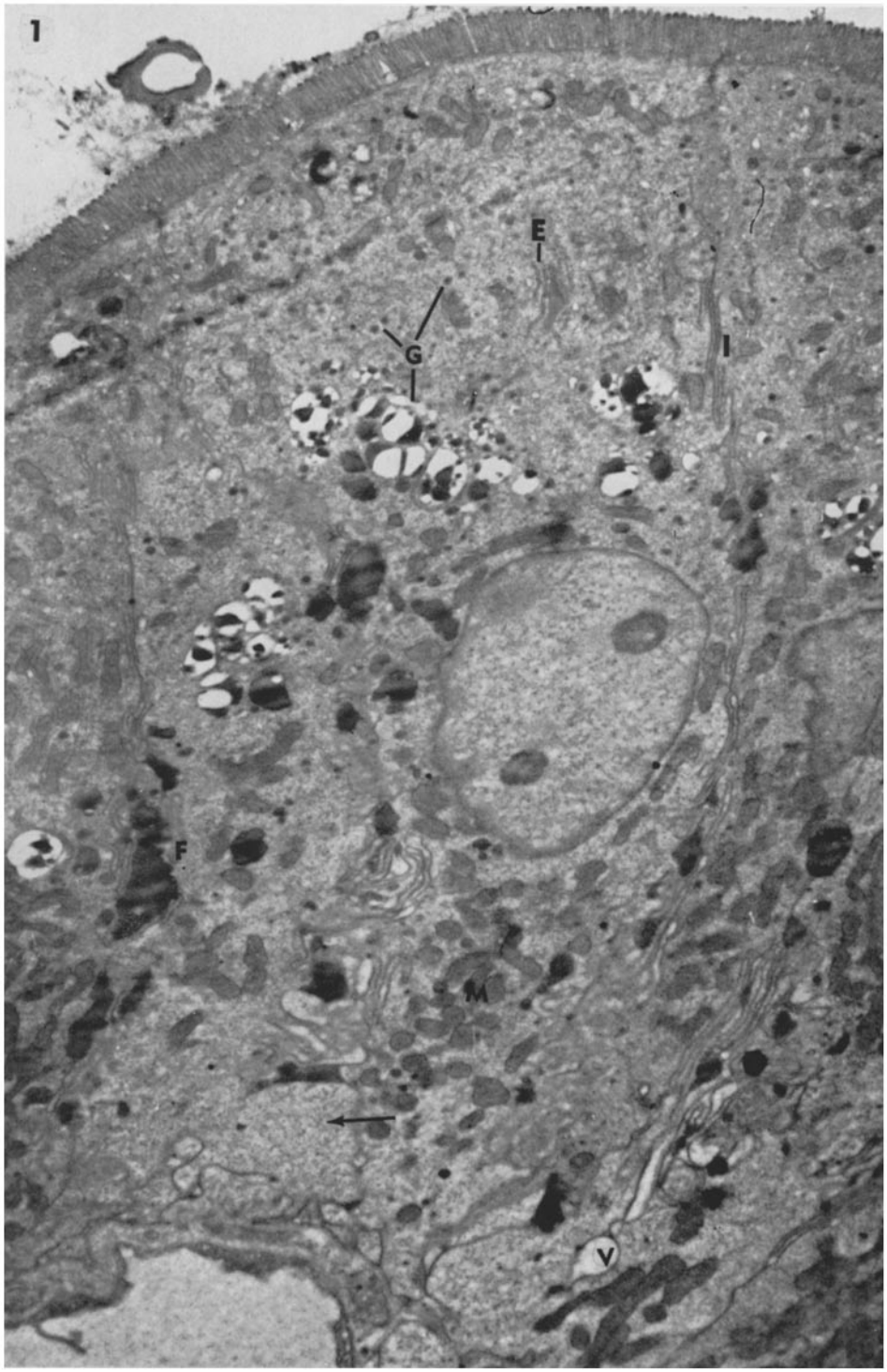
EXPLANATION OF PLATES

PLATE 85

FIG. 1. Electron micrograph of a small part of a duodenal villus from a control animal. Portions of several duodenal absorptive cells are cut in logitudinal section. The oval, centrally placed nucleus contains two nucleoli. Just above the nucleus is the main part of the Golgi complex, composed of large Golgi vacuoles, which are easily visible, as well as Golgi sacs and 400 A vacuoles, which cannot be seen at this magnification. The large vacuoles contain peripherally placed masses of fat; electron-lucent material fills most of the lumen. Throughout the apical cytoplasm 0.2 μ lipide droplets are present, each of which is surrounded by a smooth membrane. These are also a part of the Golgi complex (*G*). Mitochondria (*M*) are present throughout the cytoplasm, but are especially numerous in the basal region. A few ergastoplasmic sacs (*E*) are found in the apical and basal cytoplasm. Most of the ergastoplasmic complex, however, is present in the form of clusters of ergastoplasmic granules (lower arrow) not associated with an ergastoplasmic membrane. The cytoplasmic matrix is light gray, and appears homogeneous at this magnification. It is denser in the brush border, both within the microvilli and for a short distance below them. The separate layers of the plasma membrane cannot be resolved (along the lateral cell margins). The complex course of the lateral plasma membranes is due to intercellular interdigitations (*I*). Below the level of the Golgi complex, the intercellular space is dilated by masses of fat (*F*) and by electron-lucent material (*V*). Near the brush border the plasma membranes are denser, forming the terminal bar.

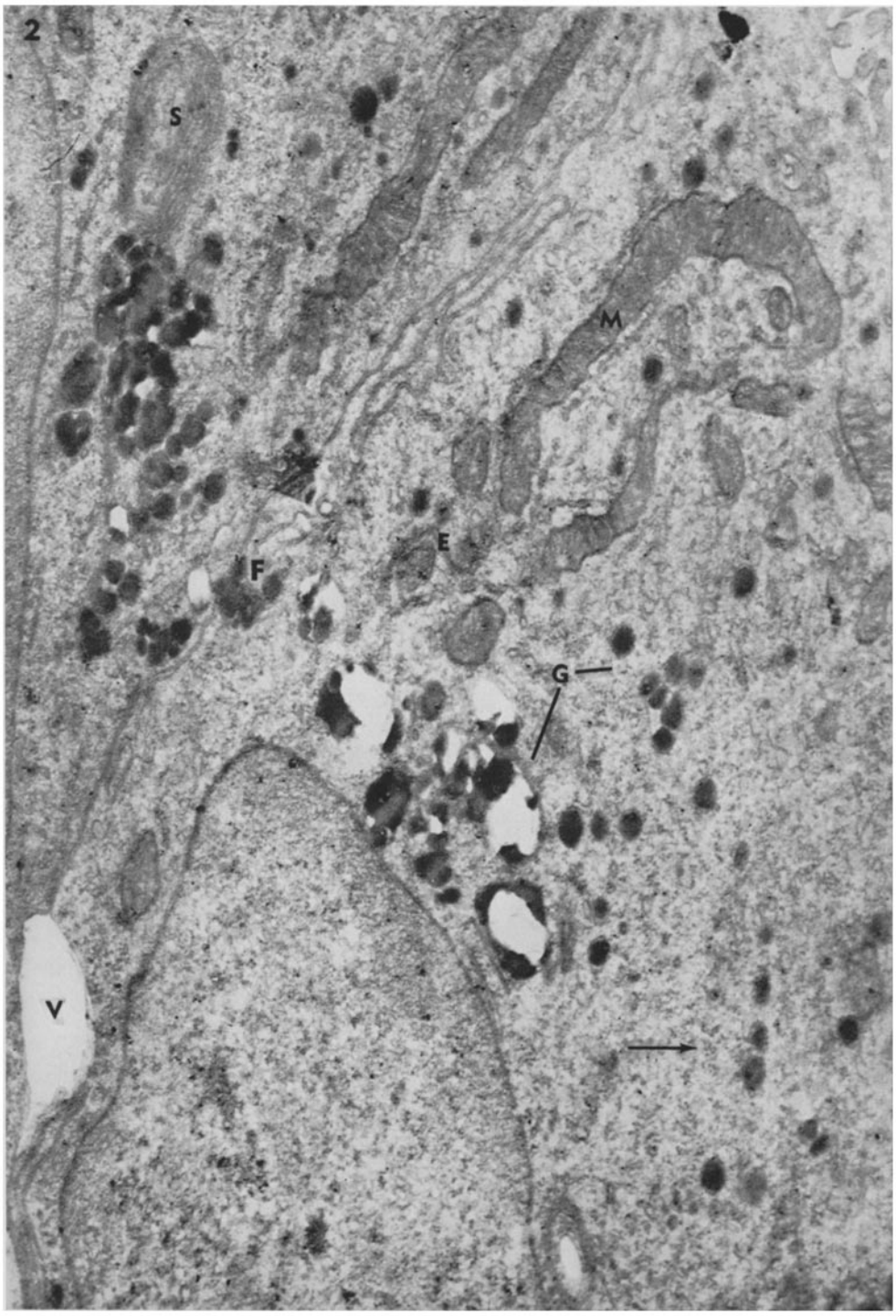
A lacteal is present in the lower left-hand corner of the micrograph. Approximate magnification, 5,500.



(Weiss: Golgi complex in fat absorption)

PLATE 86

FIG. 2. Electron micrograph of apical portions of two duodenal absorptive cells from a control mouse. Portions of the nuclei are along the left margin, and in the lower left-hand portions of the micrograph. At the upper right-hand corner are cross-sections through a number of microvilli. The Golgi complex (*G*) is very prominent in the supranuclear cytoplasm. It is composed of Golgi vacuoles, partially or completely filled with osmiophilic material, and Golgi sacs (*S*). A fine Golgi membrane completely surrounds the medium sized and small Golgi vacuoles, but may be discontinuous around the large vacuoles. Several mitochondria (*M*) are also present; they are characterized by a double outer membrane, and internal double membraned folds. Clusters of ergastoplasmic granules, some of which may be bordered by a fine membrane, are scattered throughout the cytoplasm (arrow). A few ergastoplasmic sacs (*E*) are present. The lateral plasma membranes are separated by about 90 A, except where fat droplets (*F*) or electron-lucent material (*V*) cause dilatations in the intercellular space. Approximate magnification, 23,000.



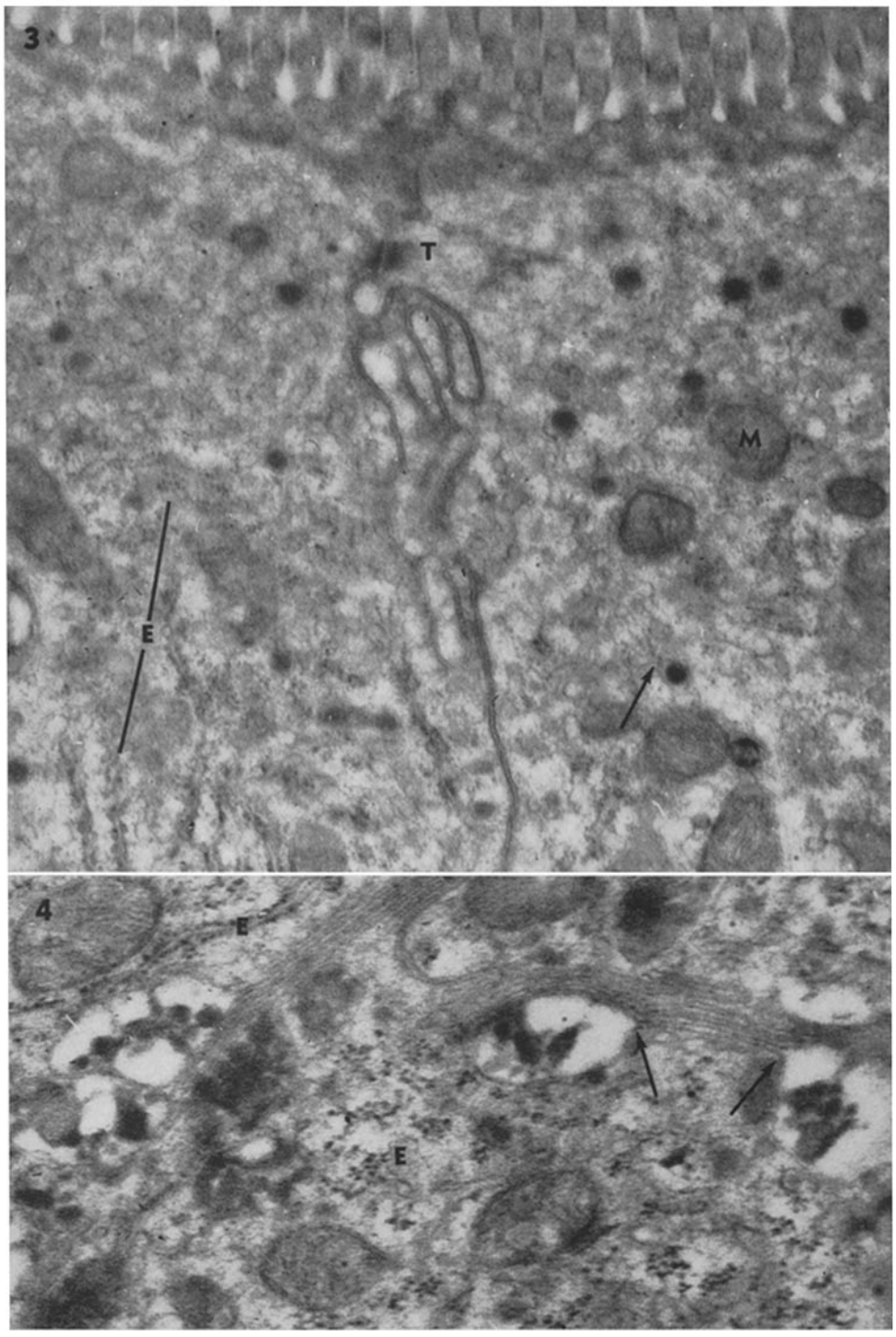
(Weiss: Golgi complex in fat absorption)

PLATE 87

FIG. 3. Electron micrograph of a part of the apical regions of two duodenal absorptive cells from a control animal. The microvilli, at the top of the micrograph, are cut obliquely. Within the microvilli, and for a short space below them, the cytoplasmic matrix is denser than elsewhere in the cell; it is not homogeneous, but the particles which it contains are no larger than 40 A in diameter. The plasma membranes of the two cells are separated by about 90 A. The terminal bar (*T*), which results from a thickening of the plasma membranes, is just below the striated cuticular border. The devious course of the plasma membrane below the terminal bar is a result of an intercellular interdigitation. The loss of clarity for short intervals of plasma membrane may be due to an actual disappearance of the membrane, or to a tangential section through it.

In the cytoplasm osmiophilic droplets, surrounded by a thin, smooth membrane, are present, as are a number of mitochondria (*M*), ergastoplasmic sacs (*E*), and clusters of ergastoplasmic granules (*E*). Thin, smooth membranes, possibly part of the Golgi complex, and apparently at times continuous with the membranes around fat droplets (arrow), may represent precursors for the Golgi vacuoles which contain fat. Approximate magnification, 28,000.

FIG. 4. Electron micrograph of a part of the supranuclear Golgi complex in a duodenal absorptive cell from a control mouse. The Golgi sacs, which have membraneous walls and narrow lumens, lie parallel to one another. They give rise to Golgi vacuoles by terminal dilatations (arrows). The Golgi vacuoles contain peripherally located osmiophilic droplets and varying amounts of electron-lucent material. Several mitochondria, an ergastoplasmic sac (*E*), and a large number of clusters of ergastoplasmic granules (*E*) are also present. Approximate magnification, 23,000.

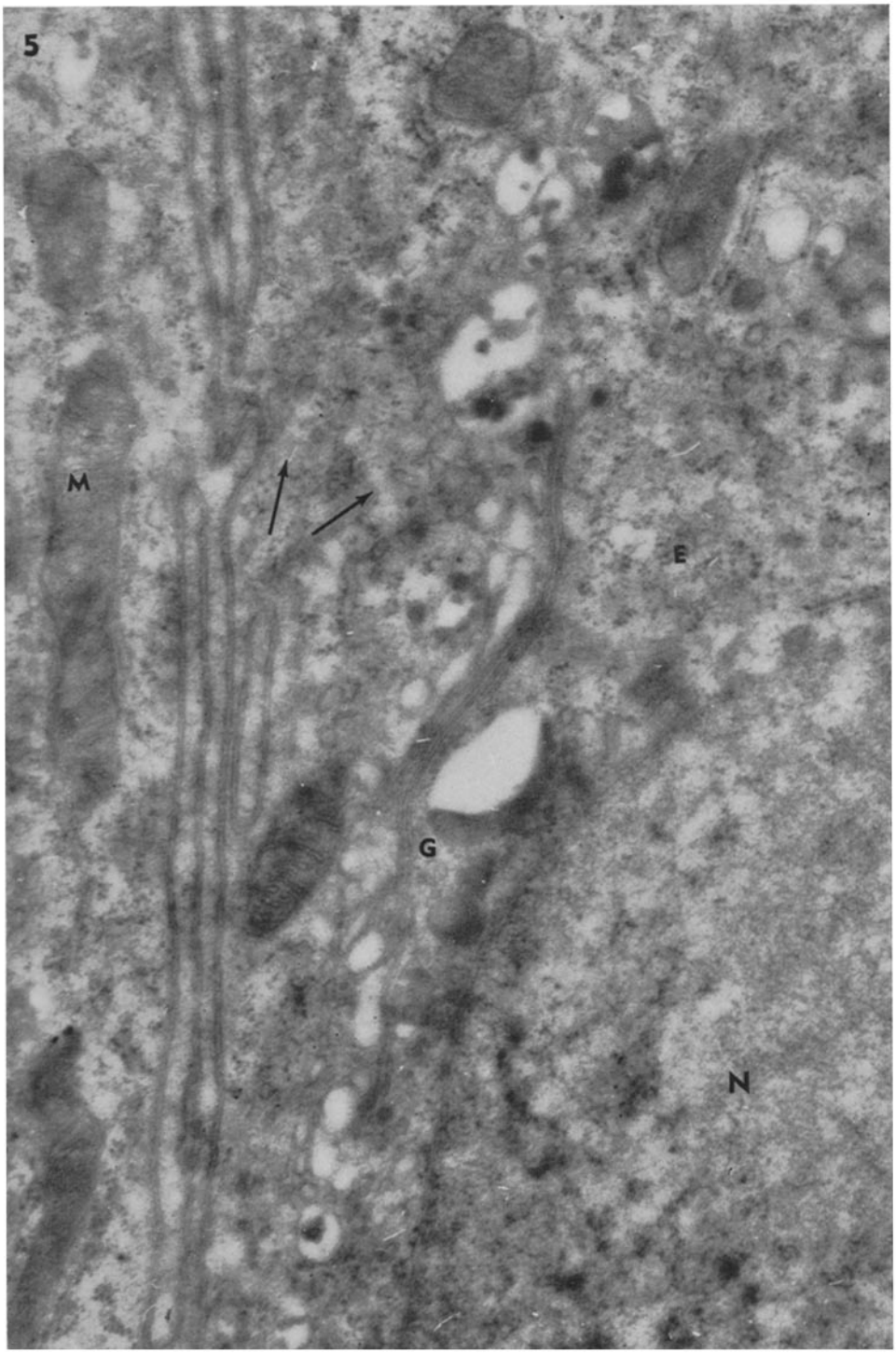


(Weiss: Golgi complex in fat absorption)

PLATE 88

FIG. 5. Electron micrograph of a part of the supranuclear Golgi complex (*G*) in a duodenal cell from a control animal. The Golgi sacs are large and flat. Terminal dilatations give rise to Golgi vacuoles of varying size, the lumina of which are filled with electron-lucent material and an occasional peripheral osmiophilic droplet or mass. It is not clear whether some of the smooth membraned vacuoles are derived from the ergastoplasm or from the Golgi (arrows indicate this general region).

Part of the lateral cell boundary, marked by an intercellular interdigitation, runs up and down the micrograph on the left. A part of the nucleus (*N*) is present in the lower right of the field. Several mitochondria (*M*) are present, as well as clusters of ergastoplasmic granules (*E*), some of them associated with ergastoplasmic membranes. Approximate magnification, 32,000.

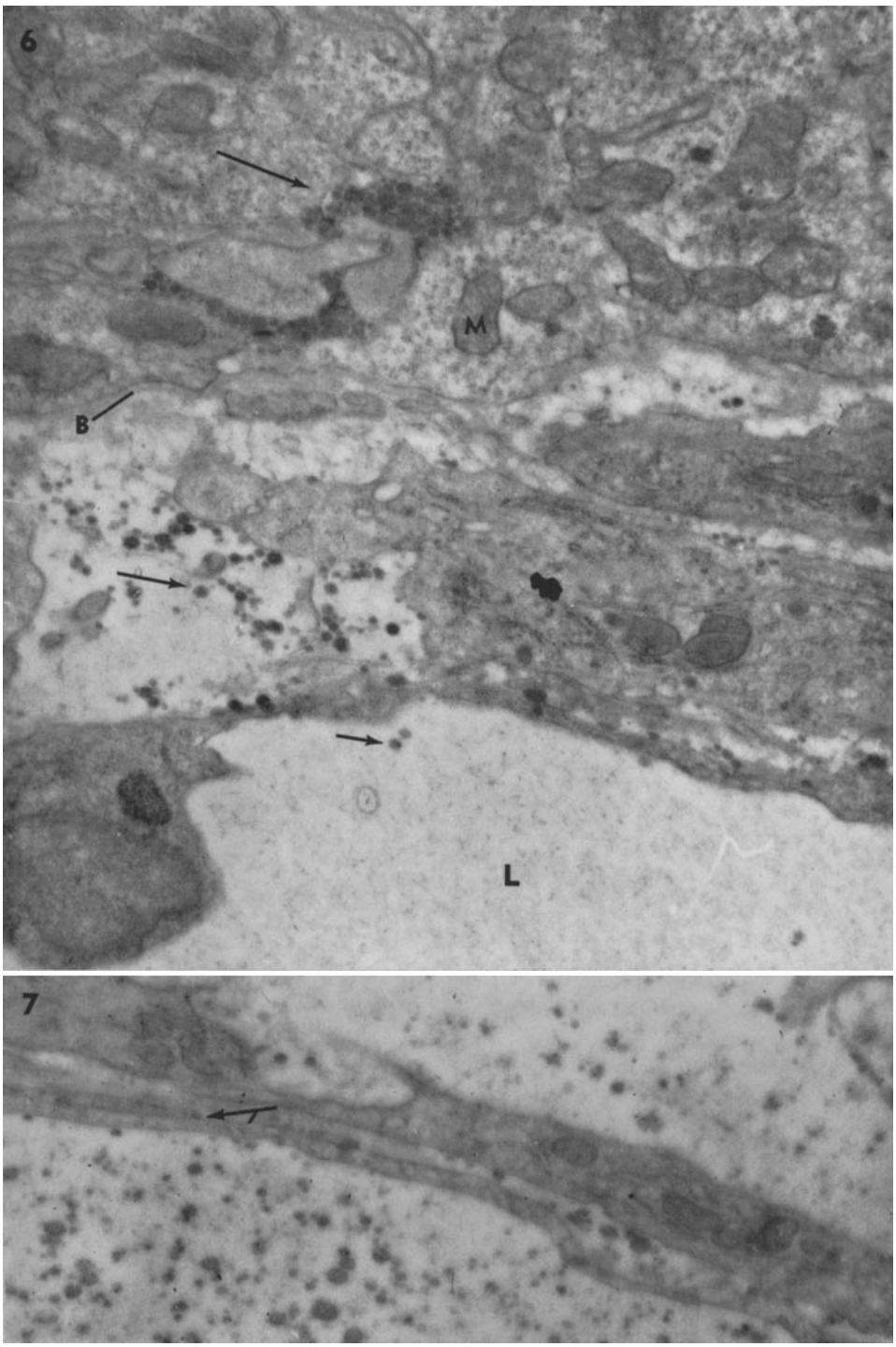


(Weiss: Golgi complex in fat absorption)

PLATE 89

FIG. 6. Electron micrograph of part of a duodenal villus from a control animal. The bottom of several absorptive cells rest upon a poorly defined basement membrane (*B*). Fat droplets (arrows) are present in dilatations of the intercellular space, in the extracellular connective tissue space, and in a lacteal (*L*). Between the lacteal's thin wall and the absorptive cells are two cytoplasmic processes, belonging to connective tissue cells. Mitochondria (*M*) and elements of ergastoplasm are present in the cytoplasm of the absorptive cells, the connective tissue cells and the lacteal endothelium. Approximate magnification, 18,000.

FIG. 7. Electron micrograph of a small part of a lacteal wall in the core of a duodenal villus from a control mouse. The thin endothelial processes overlap (arrow) to form a continuous wall. Outside the lacteal (above the wall in the micrograph) are processes of connective tissue cells. Fat droplets are present in the extracellular connective tissue space and within the lacteal. Approximate magnification, 26,000.



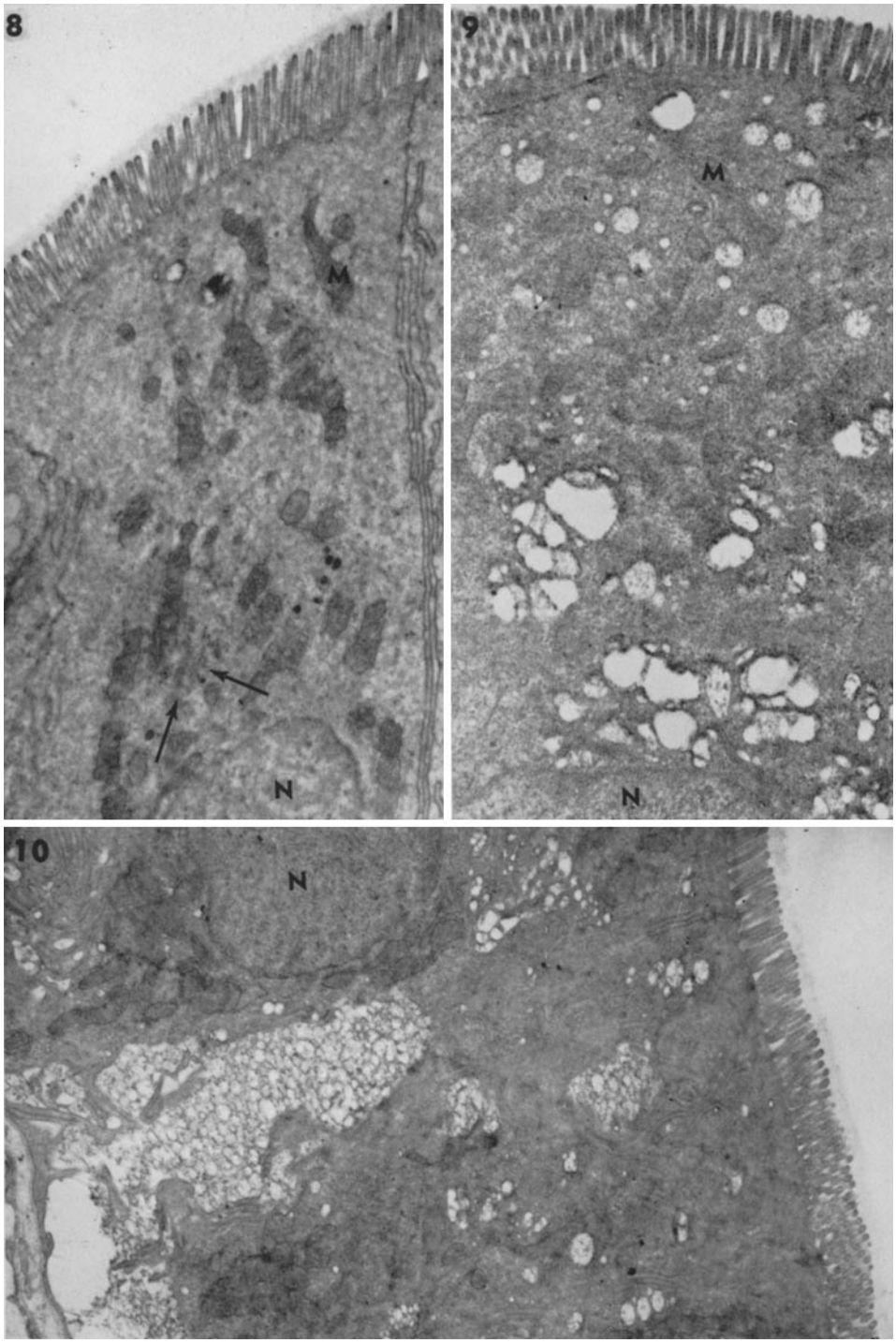
(Weiss: Golgi complex in fat absorption)

PLATE 90

FIG. 8. Electron micrograph of the apex of a duodenal absorptive cell from an animal which had been deprived of food and water for 24 hours. Microvilli, mitochondria (*M*), ergastoplasm, cytoplasmic matrix, plasma membrane and nucleus (*N*) are not much affected. The Golgi complex (arrows), however, is strikingly reduced in extent. It is composed only of a number of small sacs, tightly packed together, and some 400 A vacuoles; no larger vacuoles are present. Compare this to Fig. 1 and to Fig. 9. Approximate magnification, 5,500.

FIG. 9. Electron micrograph of the apex of a duodenal cell from an animal which had been deprived of food and water for 24 hours, and then been given cream 30 minutes before death. Many large Golgi vacuoles have appeared in the supranuclear region, and morphologically similar vacuoles are scattered throughout the apical cytoplasm. No vacuoles are found in the region of the striated cuticular border. A fine, granular, osmiophilic material is present in all vacuoles, mostly at their peripheries. The density of the cytoplasmic matrix is greatly increased, apparently due to the presence of very small osmiophilic particles. The nucleus (*N*) is situated at the bottom of the micrograph. (This micrograph is printed lighter than Fig. 8, as can be seen by comparing the density of the mitochondria (*M*), which should be the same in both pictures.) Approximate magnification, 4,400.

FIG. 10. Electron micrograph of portions of several duodenal absorptive cells from an animal which had been fasted and thirsted for 24 hours, then given about 0.2 cc. of cream, and killed 2½ hours later. Vacuoles containing a finely granular osmiophilic material are present in the apices of the cells; similar vacuoles, packed closely together, are present in the space between absorptive cells, in the left-hand, middle part of the micrograph. The density of the cytoplasmic matrix is greatly increased. The nucleus (*N*) is located at the top of the micrograph. Approximate magnification, 3,500.



(Weiss: Golgi complex in fat absorption)