An Electron Microscopic Study of the Intestinal Villus

I. The Fasting Animal

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ABSTRACT

The structure of the intestinal villus of the rat was studied in thin sections of tissue fixed in buffered osmium tetroxide and embedded in methacrylate. The simple columnar epithelium investing the villus is surmounted by a striated border consisting of slender projections of the cell surface. These microvilli are arranged in almost crystalline, hexagonal array, and increase the apical surface area of the cell by a factor of 24. The core of each microvillus is filled with fine fibrils which arise from the filamentous substance of the terminal web underlying the striated border. Each microvillus is covered by a tubular extension of the plasma membrane of the epithelial cell. Pinocytotic vesicles originating from the plasma membrane occur at the bases of the intermicrovillous spaces. The nucleus, mitochondria, and the endoplasmic reticulum of the epithelial cell display no unusual features. Small bits of ergastoplasm occur in the apical cytoplasm.

A thin basement membrane separates the epithelium from the lamina propria which consists of vessels, nerves, and numerous lymphocytes, eosinophiles, mast cells, plasma cells, smooth muscle fibers, and macrophages suspended in a delicate stroma of fibroblasts and collagen fibers. Intercellular fat droplets often occur in this stroma, even in animals fasted for 40 hours. The blood capillaries are distinguished by their extremely attenuated, fenestrated endothelial cells. The lacteal has a thicker endothelium which, although not fenestrated, appears to have significant interruptions, especially at the margins between neighboring lining cells. Strands of smooth muscle always accompany the lacteal but do not form an integral part of its wall. Unmyelinated nerves, many of which are too small to be distinguished with the light microscope, course through the lamina propria in association with the vessels. The nerve fibers evidently do not cross the basement membrane into the epithelium. Neuromuscular junctions or other terminal apparatus were not found.

INTRODUCTION

As the principal absorbing structure of the alimentary tract, the intestinal villus provides a favorable subject for investigations designed to elucidate morphological correlates of function. Indeed, since the beginning of modern histology, the study of the intestinal villus has been directed by attempts to substantiate contemporary physiological hypotheses by means of morphological observations (23). Still, with the advent of electron microscopy and thin-sectioning techniques, morphologists have tended to restrict their attention to the fascinating structure of the epithelial cell and its striated border (4, 5, 9, 29, 33). Except for the work of Weiss (31), no report has appeared which treats the villus as a unit. This situation is unfortunate because some physiologists seem inclined to regard the intestinal wall as a simple boundary be-

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tween the contents of the lumen and the blood and to neglect the complex structures which comprise that boundary (2).

The present study was initiated by the chance observation of fat droplets within and between the intestinal epithelial cells in rats that had recently eaten a fatty meal. As a basis for detailed examination of the absorptive process, an investigation of the fine structure of the villus in fasted animals was undertaken.

Materials and Methods

Blocks of tissue including the full thickness of the intestinal wall were obtained from the jejunums of fifteen young adult Sprague-Dawley rats. The animals were placed in individual cages and deprived of food, but allowed access to water, for at least 24 hours before fixation. The rats were then lightly anesthetized with ether and given an intraperitoneal injection of sodium pentobarbital (3.5 mg. per 100 gm. of body weight). They almost immediately wakened from the effects of the ether and became unconscious again in 12 to 15 minutes. When the rats had become unresponsive to painful stimuli, the abdominal cavity was opened by means of a midline ventral incision. In most instances the upper end of the jejunum lay coiled just beneath the incision. If not, it was quickly located with minimal handling.

The jejunum was fixed *in situ* by injecting the fixative into the lumen through a hypodermic needle and simultaneously dripping fixative onto the serosa. An incision across the ileum prevented accumulation of excessive pressure. A segment of jejunum that had not been touched by instruments during the previous maneuvers was now isolated (with the fixative still in the lumen) by means of a curved hemostat, quickly detached with a scissors, and removed to a plate of dental wax. At the end of this part of the procedure the rat was still alive and had lost very little blood. The segment of jejunum was then trimmed and cut under a drop of fresh fixative into pieces 1 mm. square. Only the pieces from the central part of the segment were retained. The fragments were then transferred to weighing bottles containing 2 to 3 ml. of chilled fixative and left in a refrigerator at about 5°C. for 1 to 2 hours.

All tissues were fixed in chilled 2 per cent osmium tetroxide in acetate-veronal buffer (pH 7.3–7.5). After fixation the fragments were rinsed with buffer solution of the same pH and rapidly dehydrated by passage through an ascending series of methanol concentrations. They were then embedded in a prepolymerized 6:1 mixture of butyl and methyl methacrylates at 60°C. Thin sections cut by means of glass knives on a Porter-Blum microtome were picked up on 150 mesh copper grids coated with a thin film of formvar or carbon The sections were examined in either the RCA 2E or 3B electron microscope. Electron micrographs were taken at original magnifications of 4000 to 12000 and enlarged photographically as desired.

Thicker sections of the same blocks were spread on glass slides, mounted in glycerol, and examined with phase contrast optics. These were useful for orientation. Most of the observations reported here were made on sections through the apical halves of the villi.

Tissues used for observations on fat absorption were obtained according to the methods given in the second paper of this series (22).

OBSERVATIONS

The intestinal villus in the rat is a leaf-like projection of the mucous membrane of the small intestine. Covered by a simple columnar epithelium, the villus contains an extension of the lamina propria with its delicate connective tissue, capillary plexus, central lacteal, and strands of smooth muscle. It is a contractile structure capable of altering its volume considerably by reducing its length without change in width (30). In the following description all of the components of the villus will be considered, except for the goblet cells of the epithelium which have been treated in another communication (21).

The Surface of the Intestinal Epithelial Cell.— The columnar epithelial cells investing the intestinal villi have smooth, flat basal surfaces which are applied against a thin homogeneous basement membrane (Figs. 1 and 2). The lateral surfaces are thrown into compressed interlocking folds generally lying in a plane parallel to the long axis of the cells (Figs. 1 and 9). The plication usually appears in two sets: a small one just below the terminal bar and about 1 to 2 micra beneath the free surface of the epithelium, and a second, more extensive, and more elaborate set at the level of the nuclei. At other levels, the apposing membranes of neighboring cells present smooth, shallow undulations. At their bases the cells also exhibit small, broad, foot-like processes that abut one against the other or invaginate adjacent cells (Fig. 2).

The distance between plasma membranes of adjacent cells is usually uniformly 95 to 120 A, but even in specimens apparently well preserved according to other criteria, there are sites where the two membranes diverge as much as 50 to 200 mμ. The plasma membrane measured at different points along the lateral and basal surfaces of the cell is 32 to 42 A think. Zetterqvist (33) gives this dimension as 70 A in the mouse. In our preparations it appears to consist of a single layer, although for

very short distances a suggestive doubling of the profile can be seen, and here the membrane appears twice as thick.

From ¼ to 1 micron beneath the apical junction of adjacent cells the opposing plasma membranes display a characteristic modification which corresponds to the terminal bar of light microscopy (Figs. 5 and 6). At these positions the adjacent surfaces are more widely separated than usual (about 40 mμ) and appear thicker and denser. The membrane frequently appears as a double adielectronic[1] line with a thin, light intervening line (Figs. 5 and 6). Numerous extremely fine filaments in the matrix of the cell insert into the cytoplasmic surface of the membrane (Fig. 6), so that even at low magnifications the region of the terminal bar is recognizable as a dark spot below the surface of the cell. This characteristic formation evidently encircles the cell, like the hoop of a cask, and in this respect is different from the intercellular bridges of stratified squamous epithelium (6), in which the apparently corresponding formation is discontinuous and restricted to well defined patches on the surface of the cells.

The free surface of the intestinal epithelial cell is provided with a remarkable elaboration of the plasmalemma and underlying matrix (ectoplasm) which is known to light microscopists as the striated border (see Baker, 1, and Zetterqvist, 33, for reviews of the light microscopic interpretations of the striated border). As is well known, the striated border is a brush-like formation consisting of myriad microvilli: smooth, straight, regularly spaced, cylindrical projections with rounded tips (4, 5, 9, 33). The dimensions of the microvilli seem to vary from animal to animal, depending upon the incidence of the sections and success of fixation, among other factors. When, however, the microvilli are measured in sections passing parallel to their long axes, their length is always close to 1 μ and their width, 0.07 μ. The microvilli on cells at the apex of a villus are taller and thinner than those at the base. The microvilli are arranged in close packed array, each unit equidistant from the six others surrounding it, as can be seen in sections passing through a plane transverse to their long axes (Figs. 4 and 5). Some impression of the close packing can be gained by calculations based upon estimates of the size of the microvilli and of the free surface they cover. If the length of the average microvillus is taken as 1 micron and its diameter 0.1 μ, then each microvillus has a cross-sectional area of 0.0078 μ^2 and a surface area of 0.3 μ^2. For the purpose of this calculation, the microvillus is considered as an open cylinder and the closed rounded tip is neglected. By counting the microvilli in a sample area of Fig. 4, the population density is found to be about 75 per μ^2 of cross-sectional cell surface. From these figures and an average cross-sectional area of the whole cell of 15 μ^2 (9), one may calculate that the microvilli occupy approximately 60 per cent of the free surface of the cell and increase this free surface by a factor of 24. Values of the same order were obtained by Granger and Baker (9). Zetterqvist (33) calculated that in the mouse the cross-sectional surface area of the cell is increased by a factor of 14 and that only 35 per cent of the cell surface is covered by microvilli. He found an average of 47 microvilli per square micron of free surface area. Granger and Baker calculated that, if each cell has a cross-sectional free surface area of 15 μ^2, a single cell must bear nearly 3000 microvilli. Zetterqvist, however, attributed these high figures to thick sections, and estimated that, in the mouse, there are only 650 to 700 projections per cell. The sample figures in the present study indicate that about 1000 microvilli project from the surface of each cell (15 x 75 = 1125), a number close to Zetterqvist's value.

The plasma membrane covering or limiting the profiles of the microvilli appears in high-resolution electron micrographs as a pair of thin adielectronic lines, 40 A in thickness, separated by a finer light line, 25 A wide. Zetterqvist has published excellent pictures of this detail, and it is visible in Fig. 8 of the present paper. According to Sjöstrand and Zetterqvist (29), the double dense lines become a single line, 100 A thick, in specimens obtained from animals absorbing protein. At the junction between cells the plasma membrane fuses into a thin single line which then divides again in the zone of the terminal bar (Figs. 5 and 6).

At the bases of the intermicrovillous spaces the plasma membrane occasionally dips into the underlying cytoplasm to form shallow furrows or short tubules (Figs. 3 to 5). In almost every section,

[1] *Adielectronic*, meaning scattering or not transmitting the electron beam. This term is suggested for its simple descriptiveness without any reference to the particular reason (often unknown) for scattering of electrons by a given specimen. The positive form, *dielectronic*, corresponds to the term *diactinic*, which means, transmitting the actinic rays of light.

even in the fasted animal, at least one of these dips appears to end in a small vesicle, 50 to 65 mμ in diameter. As will be described in the succeeding paper (22) these structures are interpreted as evidence of pinocytosis (15).

Internal Structure of the Intestinal Epithelial Cell.—The cytoplasmic matrix of the intestinal epithelial cell contains an exceedingly fine filamentous material which extends in all directions among the mitochondria and other organelles. Throughout most of the cytoplasm it forms a loose meshwork of individual fibrils similar to that seen in many other cells, *e.g.* the neuron. These fibrils are probably responsible for the weak birefringence of the cytoplasm reported by Schmidt (28). In the superficial portions of the cytoplasm, however, the fibrils are condensed into a close feltwork lying just within the plasmalemma. Since this cortical zone is clearly distinguished by the presence of this feltwork and by the virtual absence of other cytoplasmic structures, it may be properly designated as *ectoplasm.* It is thickest in the apical region of the cell, where it occupies a zone about 0.25 μ deep under the striated border. It is thinnest laterally, except at the terminal bar, and of intermediate thickness basally.

Of all these ectoplasmic zones, the apical one is undoubtedly the most interesting, because of its relation to the striated border and the terminal bars. The core of each microvillus is occupied by a dense fibrillar meshwork composed of fine filaments that are imperfectly resolved in the present study (Figs. 3 to 5). In general, the filaments are oriented in the long axis of the microvillus, but many extend in other directions as well (Fig. 8). The fibrillar meshwork passes into the superficial apical cytoplasm of the cell where it merges with the dense feltwork of filaments just beneath the striated border (Figs. 1, 3 to 5, and 8). In light micrographs (Figs. 1 to 3 in the succeeding paper, 22), this superficial feltwork (the terminal web) is represented by a thin refringent line coursing across the tops of the cells immediately under the striated border (1). Although recognized by many previous writers, this line was first named by Sauer (26, 27) as the terminal web. Puchtler and Leblond (24) have shown by histochemical tests that it contains protein and little or no lipide or carbohydrate. The electron micrographs (Figs. 3, 5, 6, and 8) reveal that the fine filaments in this region of the cytoplasm are predominantly oriented in a plane parallel with the free surface of the cell and appear to insert into the lateral surfaces of the cell at the terminal bars. This orientation is consistent with the negative birefringence of the terminal web with respect to the long axis of the cell (28).

When the fixation has been unsatisfactory, the striated border retracts from the apical cytoplasm, leaving an empty strip between the border and the terminal web. In such preparations the cores of the microvilli remain with the plasma membrane. Imperfect preservation may also result in the appearance of tufts or rootlets beneath the microvilli, as described by Zetterqvist (33). These discontinuities in the arrangement of the terminal web do not appear in well preserved specimens.

Beneath the ectoplasm is another zone, usually about equally deep, that is sparsely populated with small vesicles, fine granules, and mitochondria. In this zone the fibrillar component of the matrix is also sparse so that here the cytoplasmic matrix appears lighter in electron micrographs than it does in other parts of the cytoplasm (Figs. 3 and 5).

The great majority of the cytoplasmic organelles are clustered in the perinuclear endoplasm of the cell. Mitochondria are most numerous and largest in the apical endoplasm, but they are also found below the nucleus and sometimes beside it. Their size, shape, and number are independent of whether the animal had recently been fasted or fed. In general, the mitochondria are filamentous or rod-shaped, but some of them are branched and some are nearly spherical. The supranuclear mitochondria are typically oriented parallel with the long axis of the cell, whereas the infranuclear ones are more haphazardly disposed. As Zetterqvist (33) has pointed out, this difference in orientation results in a larger proportion of circular mitochondrial profiles in longitudinal sections through the basal cytoplasm. The fine structure of the mitochondria corresponds to previous descriptions (18, 33). Most of the cristae mitochondriales are arranged transversely, and their origin from the inner mitochondrial membrane is clearly demonstrated in numerous sections (Fig. 7). The matrix of the mitochondria is denser than the surrounding cytoplasmic matrix. Frequent, small, dense, rounded bodies are located in the mitochondrial matrix. No relationship between their size and number and the absorptive state of the cell was noted (32).

The Endoplasmic Reticulum.—In the following description the various configurations of the endoplasmic reticulum are classified according to two

characteristics: (1) the association with the fine particulate component of the cytoplasm, and (2) the degree of order manifested by the membrane-bound components. Thus, endoplasmic reticulum encrusted with fine ribonucleoprotein particles is designated "granular reticulum," and that devoid of particles is called "agranular reticulum." Granular reticulum exhibiting a high degree of order, that is, arranged as stacks or piles of cisternae, is termed "ergastoplasm." Agranular reticulum in highly ordered arrays is equated with the Golgi complex of light microscopy. All of these terms will be used in the following description with the understanding that they designate parts of a generalized cytoplasmic vacuolar system dynamically interrelated and interconnected (20).

In the intestinal epithelial cell the granular reticulum is relatively restricted in amount, as compared with that in a protein-secreting cell like the pancreatic acinar cell. This observation is consistent with the slight basophilia noted in stained sections viewed with the light microscope. In electron micrographs, the granular reticulum (Figs. 1, 2, 7, and 10) appears as narrow, extended profiles studded with granules and sparsely distributed in the supranuclear cytoplasm. Branching and anastomosis of these cisternae are frequent. Usually the profiles are oriented in the long axis of the cell, often in sets of three or four parallel members which form small masses of ergastoplasm (Figs. 1 and 2). The close association of some of these profiles with mitochondria is notable (Fig. 10). In addition to the cisternae, numerous small vesicles studded with granules are dispersed through the supra- and infranuclear cytoplasm.

The arrangement and quantity of the granular endoplasmic reticulum varies with the absorptive state of the cell (22). Epithelial cells from animals that have absorbed much fat contain large quantities of fat droplets and few granule-covered cisternae. The granular reticulum in such cells is largely in the form of small vesicles. The cells from animals that have been fasted for 40 hours before fixation contain few fat droplets and greater quantities of granular reticulum, which is largely in the form of ordered arrays (ergastoplasm).

The supranuclear cytoplasm usually contains large numbers of small vesicles devoid of encrusting granules. These vesicles of agranular reticulum are about 30 mμ in diameter and contain a moderately adielectronic, homogeneous substance. The vesicles occur in small numbers in the most superficial cytoplasm just beneath the apical ectoplasm and increase markedly in number deeper in the cell. Here they are frequently joined together in short chains and clusters. The quantity of these vesicles bears no obvious relation to the amount of absorbed fat droplets.

The Golgi complex in the intestinal epithelial cell is composed of one or more groups of four to six stacked agranular cisternae located immediately above the nucleus (Fig. 10). These aggregates are often small, spherical arrays of closely imbricated cisternae and associated swarms of small vesicles. In all animals, even those that have been fasted for 40 hours before fixation, some of the cisternae are dilated and contain small, dense, rounded particles. The size, number, and frequency of these particles vary directly with the amount of fat absorbed in the cell, but even in the epithelial cells from animals that have been absorbing fat for 3 or more hours before fixation and whose cytoplasm is filled with fat droplets, there are always some completely empty and collapsed cisternae among the fat-filled ones. Very large vacuoles such as those described by Weiss (31) appeared in our material only together with other signs of faulty fixation.

The Core of the Intestinal Villus.—The lamina propria comprising the core of the intestinal villus is separated from the epithelium by a thin basement membrane, which appears in electron micrographs as a moderately adielectronic, continuous line, about 6 mμ thick, applied to the bases of the epithelial cells. This basement membrane is composed of a condensation of fine fibrils, and where these fibrils are less closely packed it appears thicker and its fibrillar nature is more evident. Between the basement membrane and the epithelial cells is a light space about 8 mμ thick. Another basement membrane of similar composition also coats the outer surfaces of the endothelial cells of the capillaries. Between these two basement membranes lies a tenuous stroma of thin unit collagen fibrils, and extended flat cells (probably fibroblasts). In the villi of well preserved specimens there is no empty space between the epithelium and the capillaries of the lamina propria (Fig. 2).

The central part of the lamina propria is occupied by an assemblage of various cells: round lymphocytes, macrophages, plasma cells, eosinophiles, mast cells, and strands of smooth muscle. Through this mass, course lymphatics, blood capillaries, slender unmyelinated nerves, and fine col-

lagen fibers. The macrophages appear as large, elaborately ruffled cells with the majority of their mitochondria and endoplasmic reticulum clustered densely around the nucleus. The peripheral regions of these cells are filled with exceedingly fine fibrils, marked off into sectors by chains of small vesicles that extend from deep indentations of the cell surface (Fig. 15). Even in fasted animals these indentations contain numerous dense, round bodies, presumably absorbed fat, and occasionally the vesicles are also occupied by such particles.

Rarely, a lymphocyte or an eosinophile is found which appears to be in the process of entering the epithelium from the lamina propria (Fig. 14). Such cells are dumbbell-shaped, lying partly in the lamina propria and partly in the epithelium. The shape of the lymphocyte shown in Fig. 14 is characteristic of this cell during its migratory phase (16). The close contact between the wandering cell and the epithelial cells, without the usual basement membrane between them, indicates that the invader has penetrated the basement membrane and intruded between the epithelial cells.

Blood capillaries in the lamina propria consist of a single layer of flattened, delicate endothelial cells that overlap at their edges, leaving a crevice 10 to 15 mμ wide between neighboring cells. The endothelial wall itself is fenestrated by pores about 20 to 50 mμ wide that run through its more attenuated portions (Fig. 13). Furthermore, small cytoplasmic vesicles like those described by Palade (19) are numerous in both the thin and thick regions of the endothelium.

The lymphatics are most readily distinguished from blood capillaries in the villi of fat-fed animals, where the former usually contain large quantities of fat droplets, whereas the blood capillaries only rarely contain any. However, study of electron micrographs provides simple criteria for distinguishing between these two types of vessel even when their characteristic contents of fat droplets or erythrocytes are absent. Like the blood capillary, the lacteal is lined by a flattened endothelium, but the cells of which it is composed appear more substantial and less compactly joined than those of the blood capillary. As shown in Fig. 11, the wall of the lacteal is deeply fluted on both inner and outer surfaces. Despite these irregularities the minimal thickness of the lacteal endothelium is five or six times that of the capillary endothelium (Figs. 12 and 13). Pinocytotic vesicles are associated with both the inner and outer surface membranes of the lacteal endothelium, but it does not display the small fenestrations characteristic of blood capillaries in the villus. Instead, as is shown in Fig. 11, the endothelial cells of the lacteal are occasionally disjointed. The resulting interruption in the coherence of the wall leaves a passage of variable dimensions through which interstitial fluid and lymph may communicate. Moreover, a definite basement membrane is not always discernible (Figs. 11 and 12). The lacteal is accompanied by strands of smooth muscle (Fig. 11), which do not form an integral part of its wall but lie at varying distances from the endothelium.

Unmyelinated nerves are frequently found in the lamina propria of the intestinal villus (Figs. 2, 11, 13, 14, 16). Accompanied by their Schwann cell sheath (Figs. 2, 13, 14, and 16), they lie just beneath the epithelium and near the vessels. The axons measure from 75 to 200 mμ in diameter. In the present study no nerves have been seen in the epithelium, and no structures that could be identified as endings have been discovered either in the epithelium or in the lamina propria. In Fig. 16, a Schwann cell enclosing numerous nerve fibers is shown as it approaches the epithelium. Although most of the nerve fibers are completely encircled by the plasmalemma of the Schwann cell, some of them are at least partially exposed to the interstitial space outside, as if they were about to leave their sheath.

DISCUSSION

Integrity of the Intestinal Epithelium.—The simple columnar epithelial cells investing the intestinal villus appear to be fastened together by two morphological contrivances: (1) the elaborate interlocking of apposed lateral surfaces, and (2) the terminal bars encircling the apices of the cells. The latter are thought to be zones of strong adhesion between adjacent cells similar in function, as in structure, to the desmosomes of epidermal cells and to the intercalated discs of cardiac muscle (6).

The two sets of interdigitations observed in the present study might be considered as forming a complex mechanical joint, locking neighboring cells together all round their circumference. But it is clear that this joint, if it operates as a lock at all, must be a transient one at best. Several considerations support this interpretation. Since the epithelial cells of the villus originate near the mouths of

the crypts (13, 14) and migrate to their ultimate positions on the surface of the villus from which they slough off, they must be capable of shifting their relative positions in the epithelium, of gliding upon the basement membrane, and of accommodating their shapes to those of neighboring cells. Furthermore, the variations in the surface area of the villus during contraction require some reciprocal changes in shape of the epithelial cells. The dilatation of the apical ends of developing goblet cells is accompanied by unfolding of the interdigitated surfaces below the terminal bars (21). Finally, the confronted cell surfaces can be separated and pushed aside by invading lymphocytes and eosinophiles from the lamina propria (Fig. 14). Fat droplets also slip between cells, sometimes in large aggregates (22, 31). These observations indicate that probably the plications themselves are not rigid and have little importance in maintaining the integrity of the epithelium, but that, rather, they represent the play of the lateral surfaces of the cell, a limited and confined undulation similar to the superficial vagaries of more isolated cells, such as macrophages and fibroblasts. This interpretation is consistent with the fact that the plications of neighboring epithelial cells do not always follow each other unremittingly (see Fig. 9).

The Striated Border.—In contrast to the folds of the lateral surfaces, the striated border at the apical surface of the cell seems to be a more rigid and persistent organelle. Chambers and de Rényi (3) noted that it appears to be stiff under microdissection. As is well known, the striated border persists even after the epithelial cells have been mechanically separated from one another (8, 11). It even resists the expanding contents of the goblet cell much longer than do the lateral folds (21). However, on morphological grounds alone, the almost crystalline array of the microvilli and the density of the terminal web that underlies and extends into them bespeak a more static construction at the apical surface of the cell than at the sides. The microvilli of the intestinal striated border are distinguished by their uniform dimensions, the parallelism of their long axes, and their hexagonal close packing. It seems probable that their strikingly regular arrangement is imposed by the terminal web of filaments which forms their core and lies subjacent to them. This feltwork evidently represents a firm ectoplasmic gel, which extends from terminal bar to terminal bar and stiffens and stabilizes the entire apical surface of the cell (24, 27).[2] The terminal web and its substituent microvilli may then be considered as an effective arrangement for maintaining a free surface of maximal area on a cell which faces upon a moving stream of semisolid material, which lies upon a contractile substratum, and which absorbs and transports relatively large volumes of water, salts, and foodstuffs over short periods of time.

Significance of Basement Membranes.—The vessels of the intestinal villus are separated from the epithelium by an intervening connective tissue space packed with collagen fibrils and numerous cells, among which the most prominent are eosinophiles, plasma cells, mast cells, macrophages, and smooth muscle fibers. The walls of the vessels display structural characteristics that may be of functional importance. The endothelium of the blood capillaries is extremely tenuous and is permeated by numerous small vesicles, which have been described as characteristic of capillary endothelia in many organs (17, 19). These vesicles have been interpreted as evidence of pinocytosis, a process which may be significant in the transport of substances, especially fluids, across the capillary wall. The blood capillaries of the intestinal villus have an additional characteristic: their endothelium is fenestrated. In this respect they resemble the capillaries of endocrine glands and the kidney, and differ from those of striated muscle and lung. The fenestrae are apparently stopped by an uninterrupted basement membrane that envelops the endothelium.

It is interesting that the terminal lymphatic capillaries or lacteals in the intestinal villi have a structure different from that of the blood capillaries. The endothelial wall is noticeably thicker (compare Figs. 12 and 13), and the enveloping basement membrane is much less definite or even absent. Although pinocytotic vesicles abound, fenestrations are absent. This difference, too, is surprising because the lacteals are the principal route for conveying fat droplets out of the villus, and pores would appear to be more pertinent to their function than to that of the blood capillaries. The wall of the lacteal, however, displays occasional

[2] Sauer (27) proposed that, by limiting the free surface of the epithelial cells, the terminal web determines their columnar shape, and that, together with the network of terminal bars, it maintains the integrity of the epithelium.

endothelial separations (Fig. 11), enlarged intercellular spaces through which fat droplets may pass (22, 31). The slips of smooth muscle that form a discontinuous coat around the lacteal are applied closely in some places and in others lie at a distance with intervening collagen fibrils. When these muscles contract they shorten the whole vilus and incidentally empty the lacteal into the lymphatic plexus at the base of the villus.

Since the vessels and the superficial epithelium of the villus are each bounded by a thin independent basement membrane intervening between them and the connective tissue elements, substances absorbed from the lumen of the intestine must traverse at least nine successive phases in their journey from the lumen to the blood or lymph: (1) the apical surface and striated border of the epithelium, (2) the cytoplasm of the epithelium, (3) the basal or lateral surfaces of the epithelial cells, (4) the basement membrane underlying the epithelium, (5) the loose connective tissue of the lamina propria, (6) the basement membrane surrounding the capillaries and lacteals, (7) the outer surface of endothelial cells, (8) the cytoplasm of the endothelium, and (9) the inner surface of the endothelium. As will be seen in the second paper of this series (22), for certain substances like particulate fat the first three phases are probably by-passed, and for most substances the last three phases may also be by-passed by way of pinocytosis and the fenestrations or stomata in the endothelium. Therefore, the most important barriers to transport of substances between the intestinal lumen and the capillary lumen may be the two independent basement membranes. Little is known about the properties of these membranes, although they are presumed to consist of a mucoprotein film probably secreted by the endothelium and the epithelium. The chemical and physical characteristics of these basement membranes may well be significant factors in controlling absorption, selective permeability, and transport. These membranes deserve systematic and experimental study.

Innervation of the Villus.—Ramón y Cajal (25) described a delicate feltwork of unmyelinated nerve fibers, which ramify in the lamina propria of the villus and terminate as small varicosites associated with the vessels and smooth muscle cells. Although he emphatically denied that these fibers are sensory or that they have anything to do with the epithelium, Kuntz (12), Hill (10), and others found immediately beneath the epithelium, a dense plexus which they believed to be sensory. Fibers from this plexus were described as penetrating between epithelial cells and forming a terminal network around their basal halves. Ramón y Cajal (25) also referred to stellate and fusiform neurons which were very difficult to impregnate with silver and which he believed to form a plexus additional to the one issuing from the submucous plexus of Meissner.

The electron micrographs of the present study demonstrate small bundles of unmyelinated nerve fibers coursing through the lamina propria of the villus in association with blood vessels. They come close to the epithelium (Figs. 2, 14), but do not cross the basement membrane. Many of these fibers are too small to be distinguished with the light microscope, unless it is assumed that silver staining enlarges them. However, bundles of the smallest fibers would undoubtedly be visible, and it seems likely that these are what appear in methylene blue preparations, often as single beaded fibers. The small size of the axons and their incomplete enclosure by the Schwann cell sheath are features that recall the nerve fibers recently described by Fawcett and Selby (7) in the atrial musculature of the turtle's heart. Although these nerves probably innervate the smooth muscle of the villus, we have not recognized any neuromuscular junctions or other terminal apparatus. A more intensive search, however, is required before the existence of specific terminations can be excluded. We have also discovered no neurons in the lamina propria of the villus.

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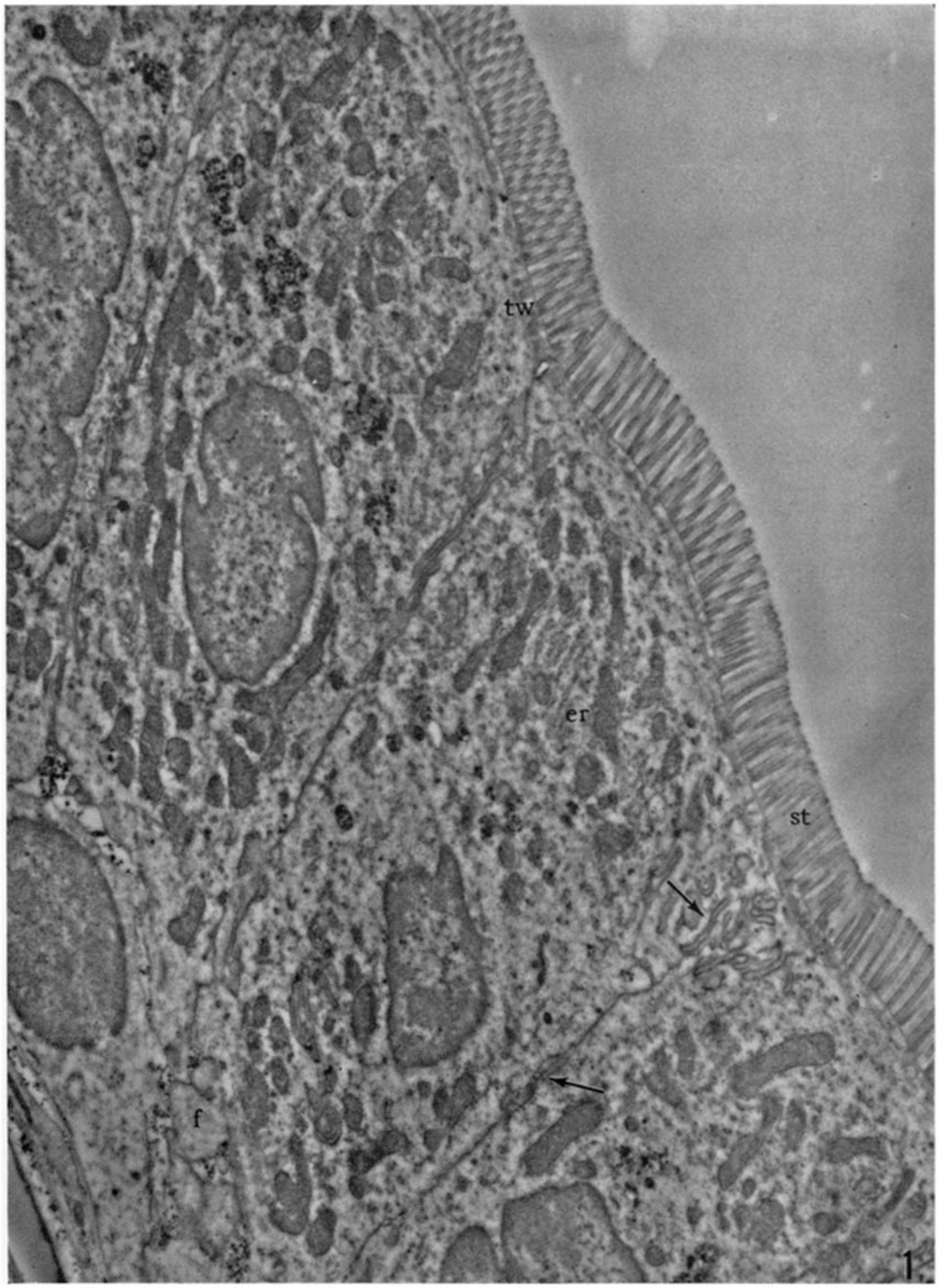
EXPLANATION OF PLATES

Unless otherwise noted all figures are electron micrographs of tissues taken from rats that had been fasted for 24 hours.

PLATE 148

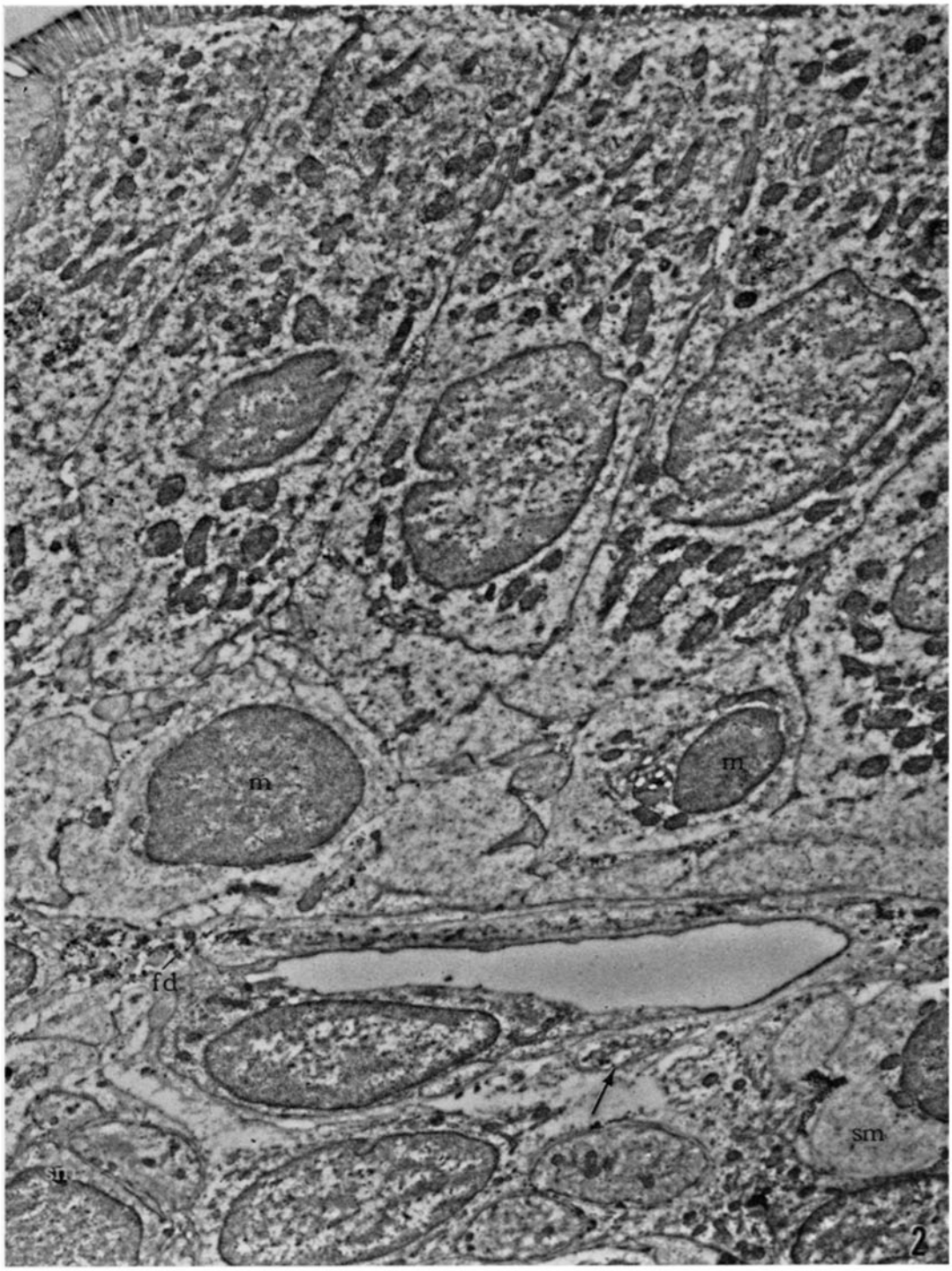
FIG. 1. General view of the epithelium of an intestinal villus near its summit. The microvilli of the striated border (*st*) are sectioned at various degrees of obliquity. The micrograph shows the two sets of elaborate plications (arrows) of the lateral cell surface and the broad basal protrusions or feet (*f*). The terminal web (*tw*) occupies the narrow zone of the cytoplasm lying just below the striated border and clear of other organelles. The supranuclear cytoplasm contains profiles of mitochondria and granular endoplasmic reticulum (*er*) often arrayed parallel to the long axis of the cell. Lying just above the nuclei are numerous small clusters of extremely adielectronic droplets (probably residual absorbed fat) which mark the sites of the Golgi complex. Similar droplets are dispersed through the intercellular spaces, particularly at the bases of the epithelial cells. Beside and beneath the nuclei are more mitochondria and assorted profiles of the endoplasmic reticulum. Throughout the cytoplasm is a fluffy, fine fibrillar material which contributes to the background density of the micrograph. The irregular nuclear profiles are due to folds and invaginations in the surface of the nuclei. × 7,000.



(Palay and Karlin: Intestinal villus in fasting animal)

PLATE 149

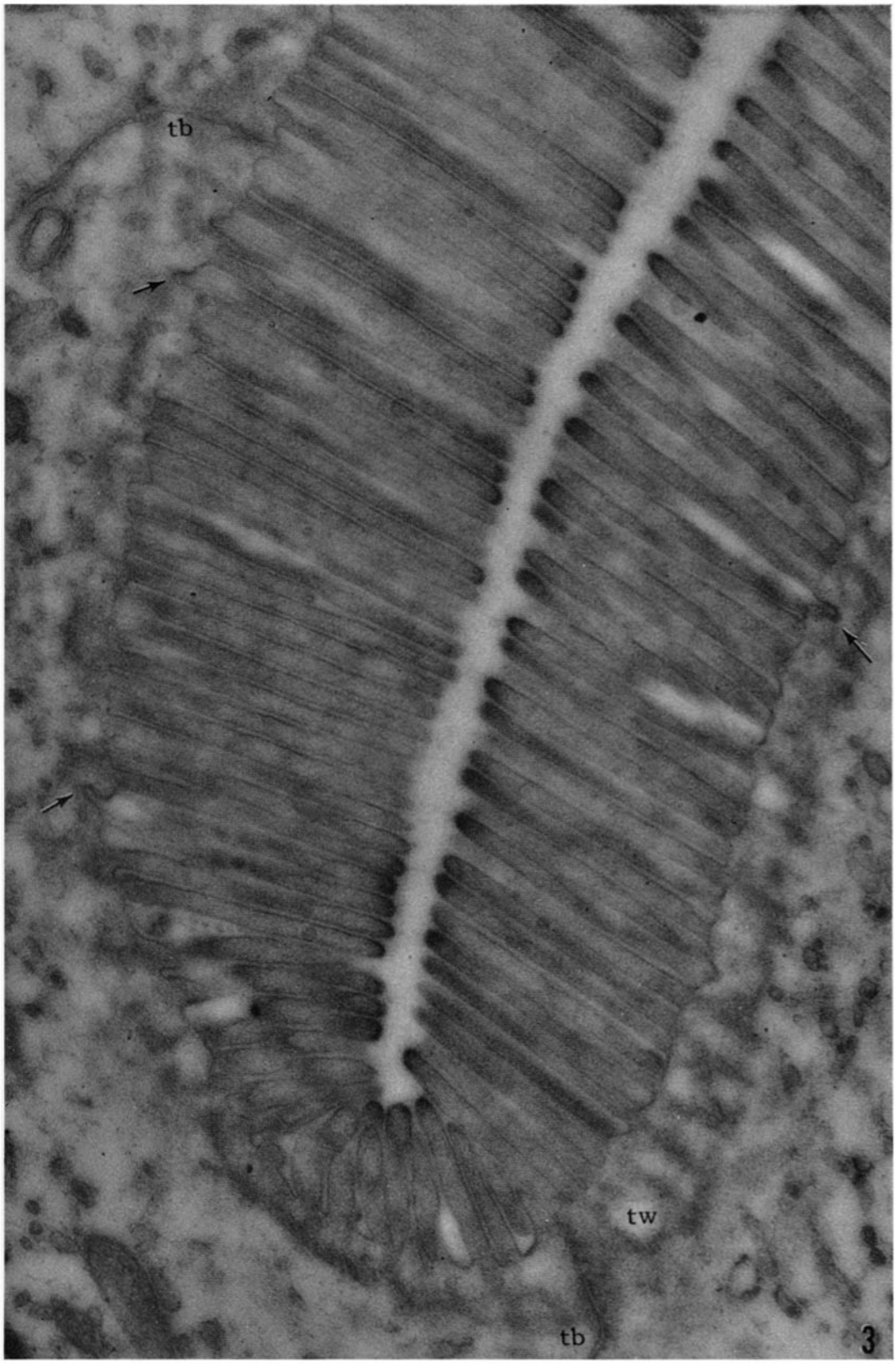
FIG. 2. General view of the intestinal epithelium and the subjacent lamina propria of the villus. In addition to the features illustrated in Fig. 1, the epithelium contains two intrusive cells (*m*), probably small macrophages, which lie among the basal protrusions of the epithelial cells. A blood capillary appears just beneath the epithelium, and alongside it is an unmyelinated nerve (arrow). Smooth muscle cells (*sm*) are visible at the right and left margins of the figure. Numerous extracellular fat droplets (*fd*) are distributed through the connective tissue. × 7,000.



(Palay and Karlin: Intestinal villus in fasting animal)

PLATE 150

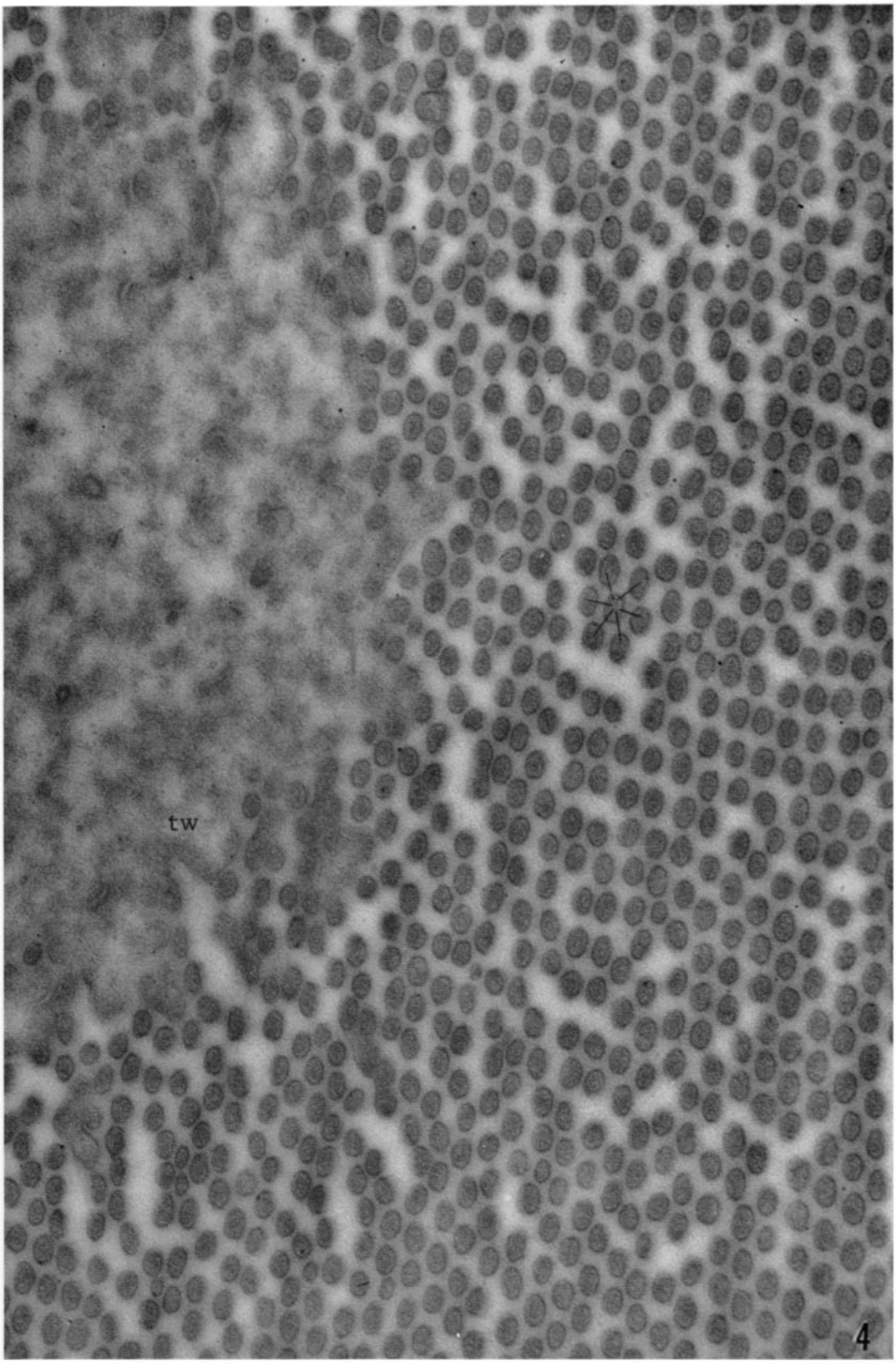
FIG. 3. The striated border of two neighboring epithelial cells in the depths of a fold in the surface of a villus. The microvilli are generally disposed longitudinally in a plane nearly parallel with the plane of the section. The figure displays the nearly uniform height of the microvilli, the continuity of the surface membrane over them, and the continuity of the terminal web substance (*tw*) into their cores. Terminal bar formations (*tb*) may be seen at the left and lower margins of the figure. In a few places (arrows) the intermicrovillous space dips into the terminal web, forming a furrow or open vesicle bounded by the plasmalemma. Note that the terminal web contains no mitochondria or other organelles, which first appear in the cytoplasm beneath it. × 47,000.



(Palay and Karlin: Intestinal villus in fasting animal)

PLATE 151

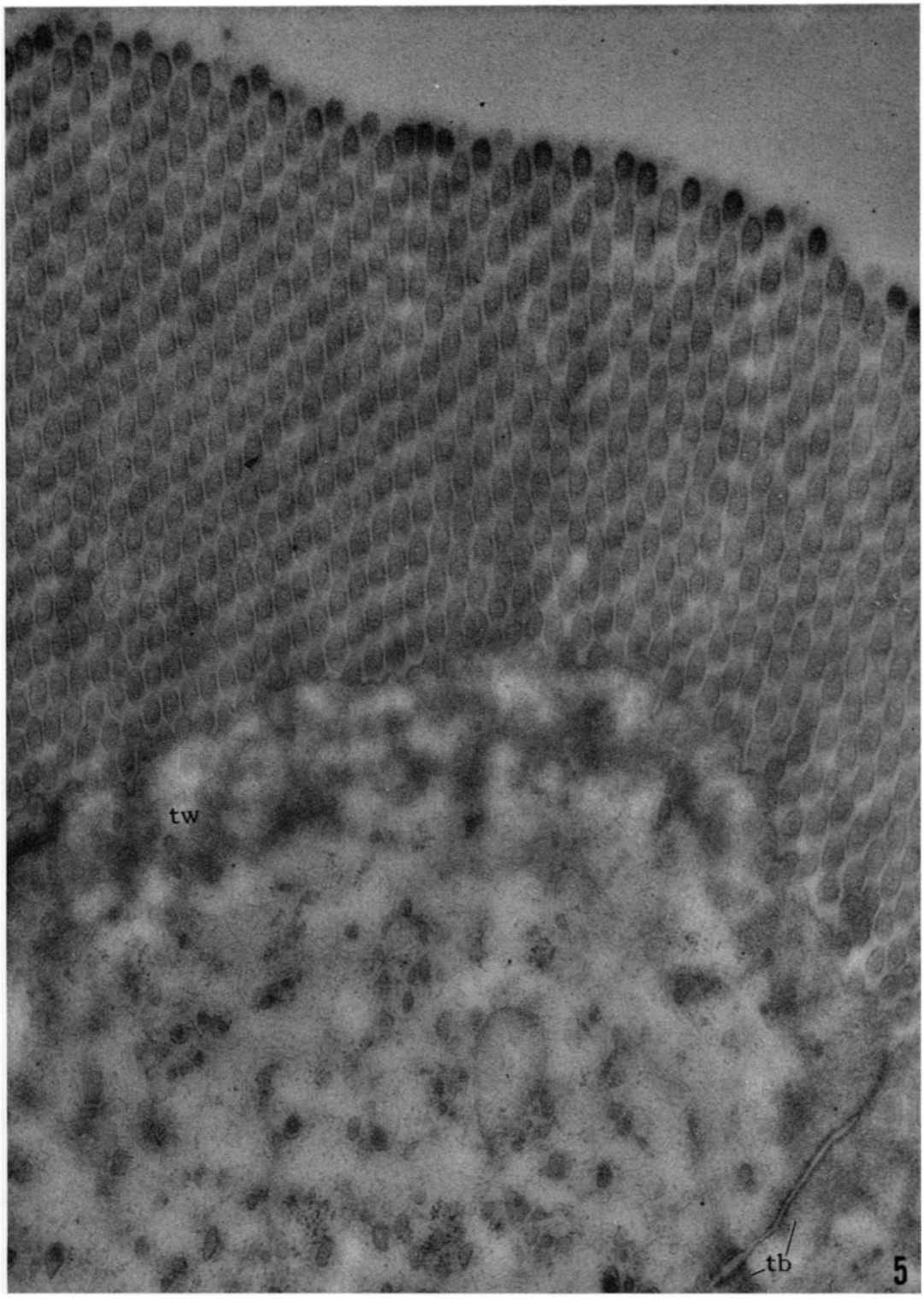
FIG. 4. Transverse section of the apex of an intestinal epithelial cell which passes through the terminal web and the striated border. The figure displays the nearly uniform cross-sections of the microvilli and their ordered packing in hexagonal array. Occasional deviations from this order are probably due to defects in sectioning. The microvilli are embedded in a homogeneous, moderately adielectronic, extracellular material.The irregular profile of the apex of the cell (left side of figure) is produced by extensions of the intermicrovillous spaces to form furrows and pits in the cell surface. Such appearances suggest that the surface is active in pinocytosis at these sites. The terminal web substance (*tw*) consists of exceedingly fine fibrils. × 46,000.



(Palay and Karlin: Intestinal villus in fasting animal)

PLATE 152

FIG. 5. Oblique section through the striated border and the apical cytoplasm of an intestinal epithelial cell, showing the almost crystalline hexagonal array of the microvilli. Beneath the terminal web (*tw*) a fine filamentous material permeates the apical cytoplasm. A terminal bar (*tb*) is visible near the right lower margin of the figure. In this instance it is a bipartite structure. In the upper segment, the terminal web substance is aggregated against the doubled opposing cell membranes, and below, the fine cytoplasmic filaments insert into the lower segment. Between the two segments the cell membrane is single and very thin. × 42,000.



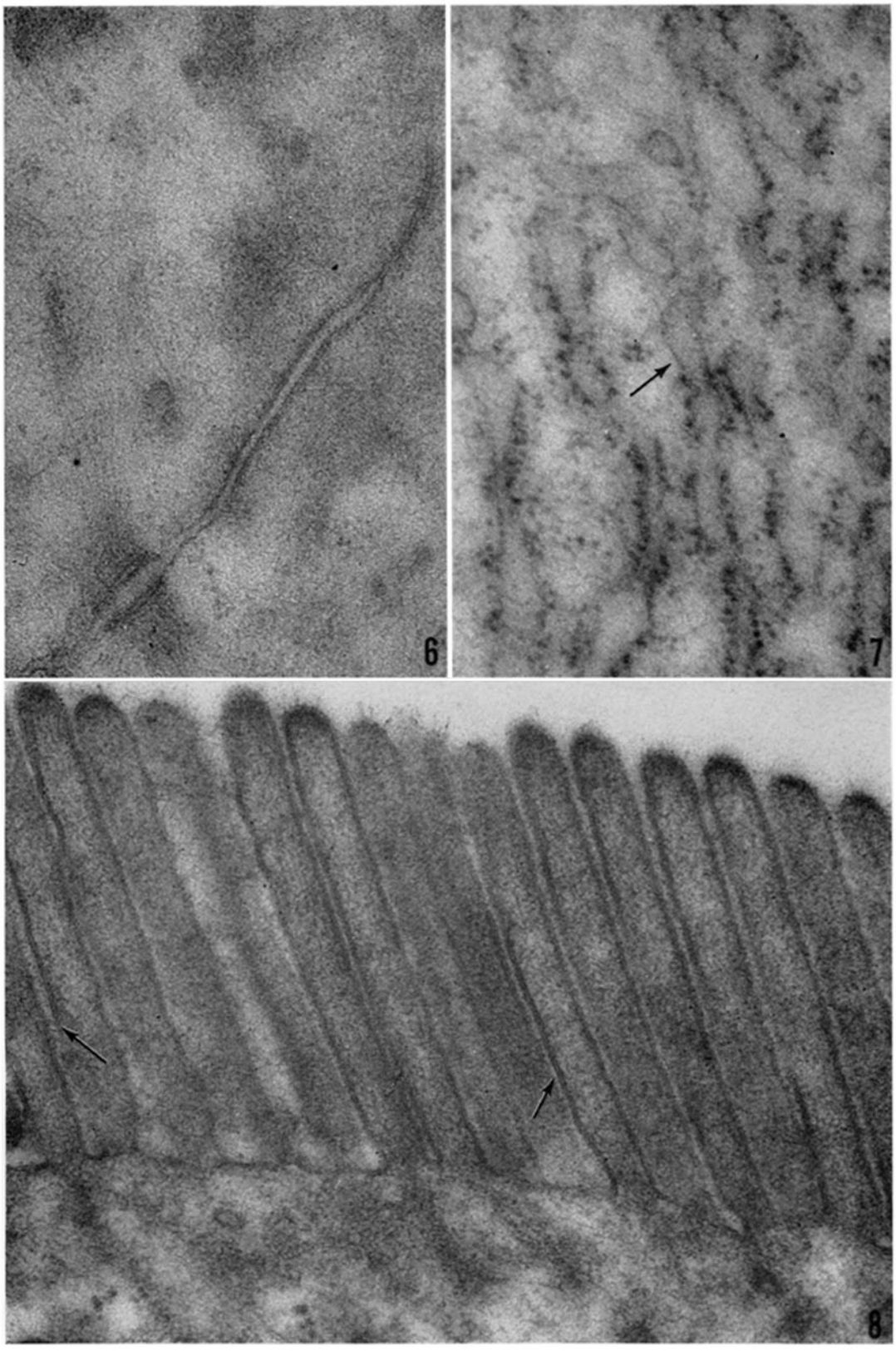
(Palay and Karlin: Intestinal villus in fasting animal)

PLATE 153

FIG. 6. Enlargement of the terminal bar shown in Fig. 5. × 90,000.

FIG. 7. Portion of the apical cytoplasm of an intestinal epithelial cell from a rat that had received corn oil 22 minutes before fixation. The figure shows a zone of continuity between granular and agranular portions of the endoplasmic reticulum (arrow). × 93,000.

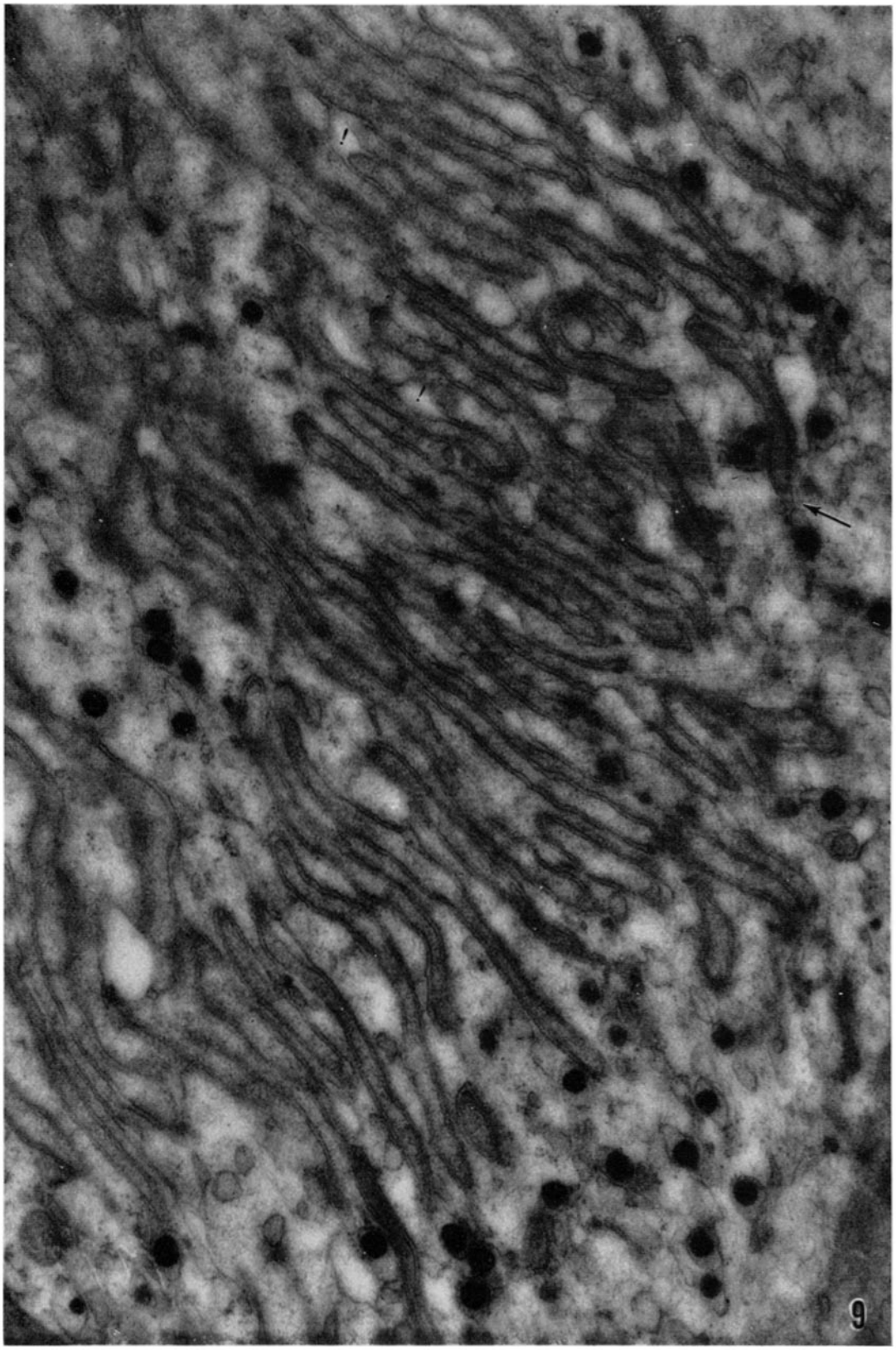
FIG. 8. The striated border of an intestinal epithelial cell from a rat that had received corn oil 25 minutes before fixation. The plasma membrane bounding each microvillus appears as a pair of thin dark lines separated by a fine light line (arrows). The fibrillar substance of the terminal web extends into the cores of the microvilli and fills them with filaments predominantly oriented in the longitudinal direction. × 94,000.



(Palay and Karlin: Intestinal villus in fasting animal)

PLATE 154

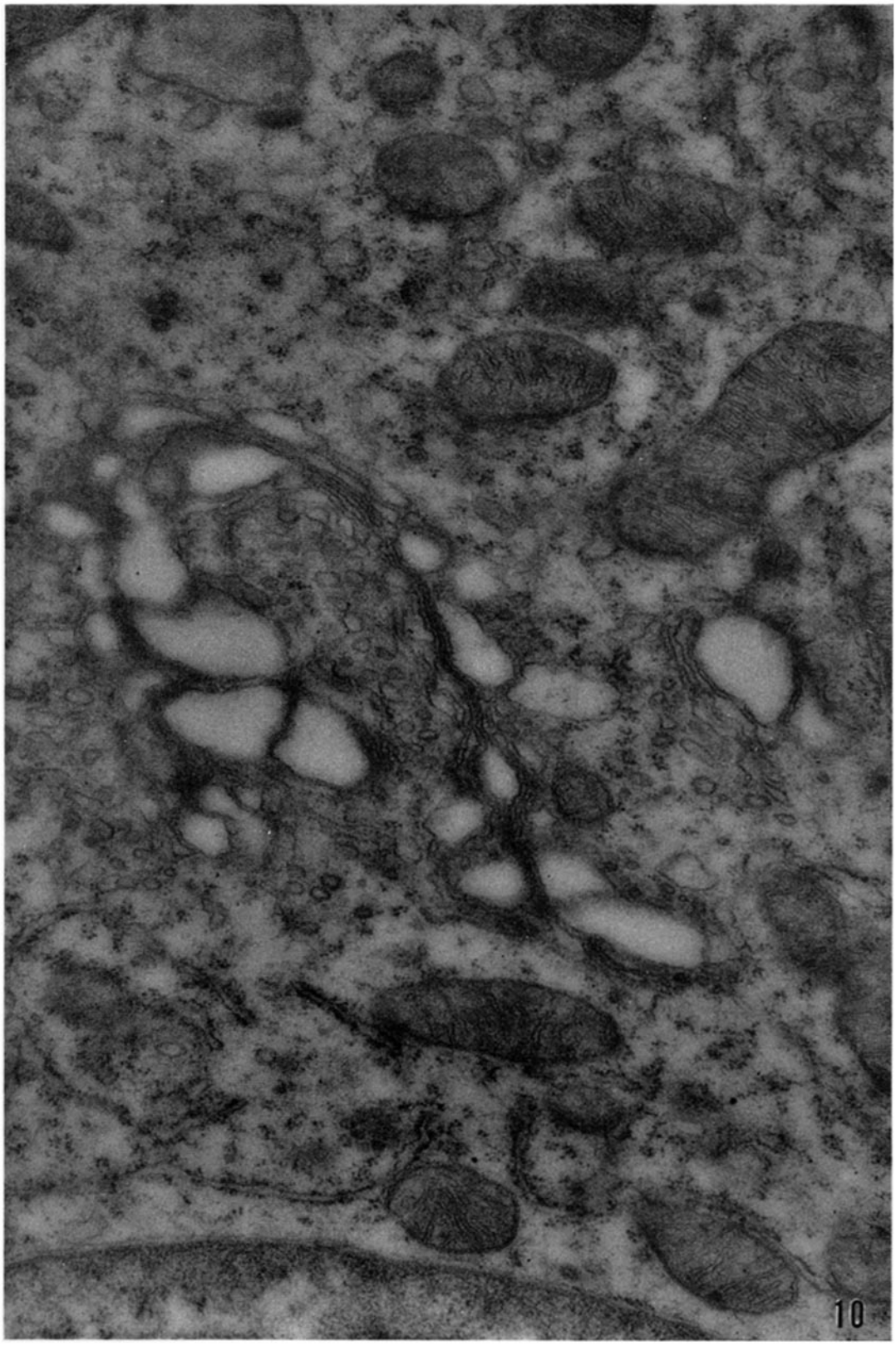
FIG. 9. Confronted cell membranes in the upper set of interdigitations between neighboring epithelial cells. This section comes from the jejunum of a rat that had been given corn oil 35 minutes before fixation. The plane of the section is nearly perpendicular to the free surface of the villus and passes obliquely through the plasma membranes of adjacent cells. In general, the folds of the respective cell surfaces follow each other and match almost perfectly. Here and there, however, they diverge somewhat (as indicated by *!*). The small, round, extremely adielectronic bodies are droplets of absorbed fat, most of which are still intracellular, each enclosed in a membranous envelope (see second paper in this series, reference 22). The arrow indicates an apparent junction of a fat-containing vesicle with the intercellular space between the plasma membranes. × 39,000.



(Palay and Karlin: Intestinal villus in fasting animal)

PLATE 155

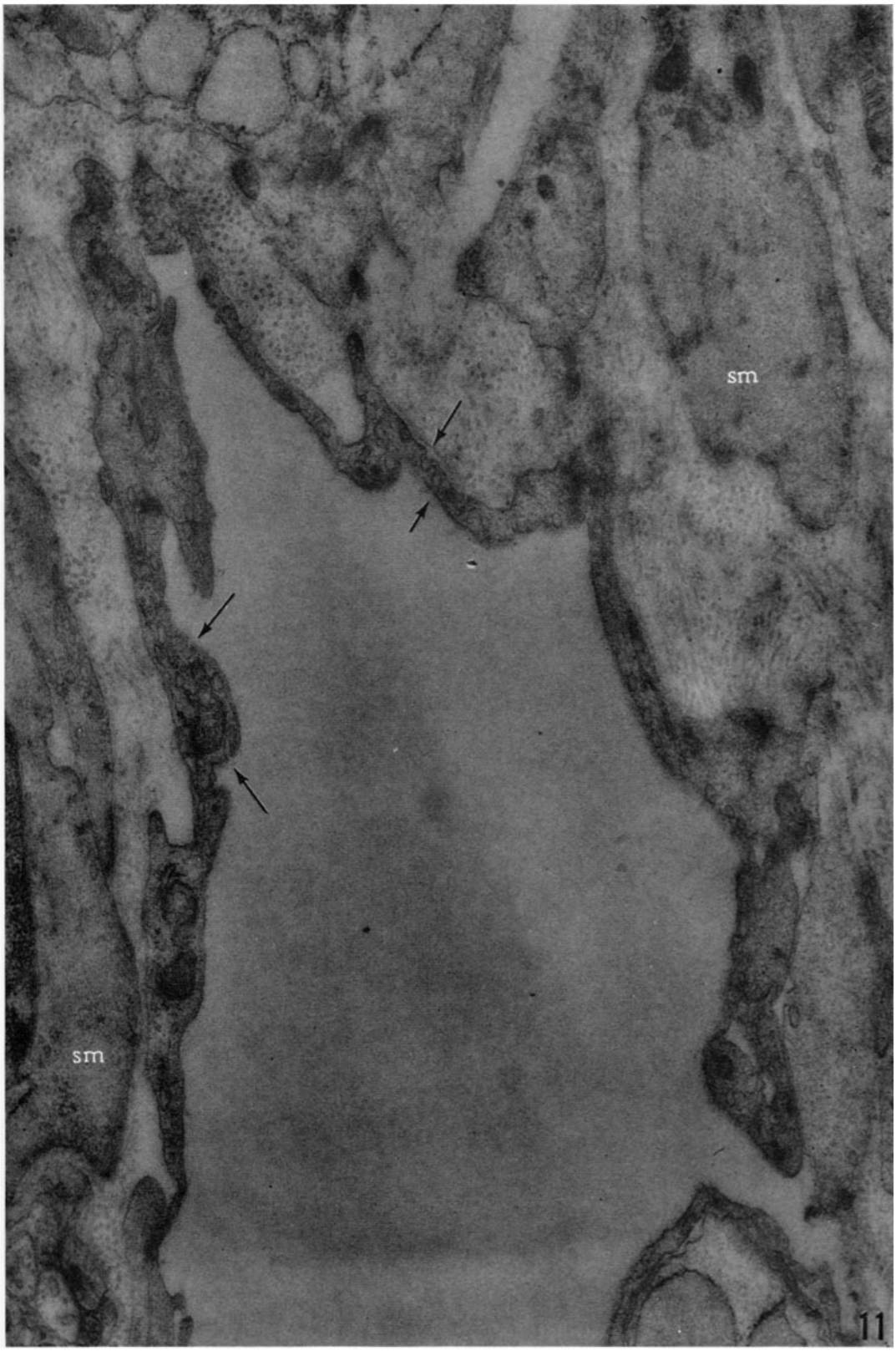
FIG. 10. Supranuclear cytoplasm of an intestinal epithelial cell showing the Golgi complex, mitochondria, and elements of granular endoplasmic reticulum. The nucleus and its envelope are barely included at the lower margin of the figure. The Golgi complex consists of imbricated cisternae and small and large vesicles arrayed in a fairly dense elliptical mass. The larger vesicles (or dilated cisternae) usually contain clusters of fat droplets even in fasted animals. This micrograph was selected for illustration because the absence of fat droplets permits one to see the architecture of the Golgi complex more clearly. This figure should be compared with Fig. 8 in the succeeding paper (22). × 47,000.



(Palay and Karlin: Intestinal villus in fasting animal)

PLATE 156

FIG. 11. A lacteal, or terminal lymphatic capillary, in the center of an intestinal villus. The endothelium of this vessel consists of flattened, overlapping, and fluted cells, which display many pinocytotic vesicles opening on either the luminal or connective tissue surface (arrows). The basement membrane appears to be absent, and at the right lower margin the endothelium is clearly interrupted, allowing the lumen to communicate directly with the connective tissue spaces outside the vessel. This interruption cannot be an artifact because the endothelial cells are turned away from each other. Surrounding the lacteal is a layer of collagen fibrils. The smooth muscle cells (*sm*) that accompany the lacteal are visible to the right and left of the vessel. In the left upper and right lower corners of the figure, portions of two mature plasma cells may be seen. A small bundle of unmyelinated nerves lies in the left lower corner of the figure, near a smooth muscle cell. × 27,000.



(Palay and Karlin: Intestinal villus in fasting animal)

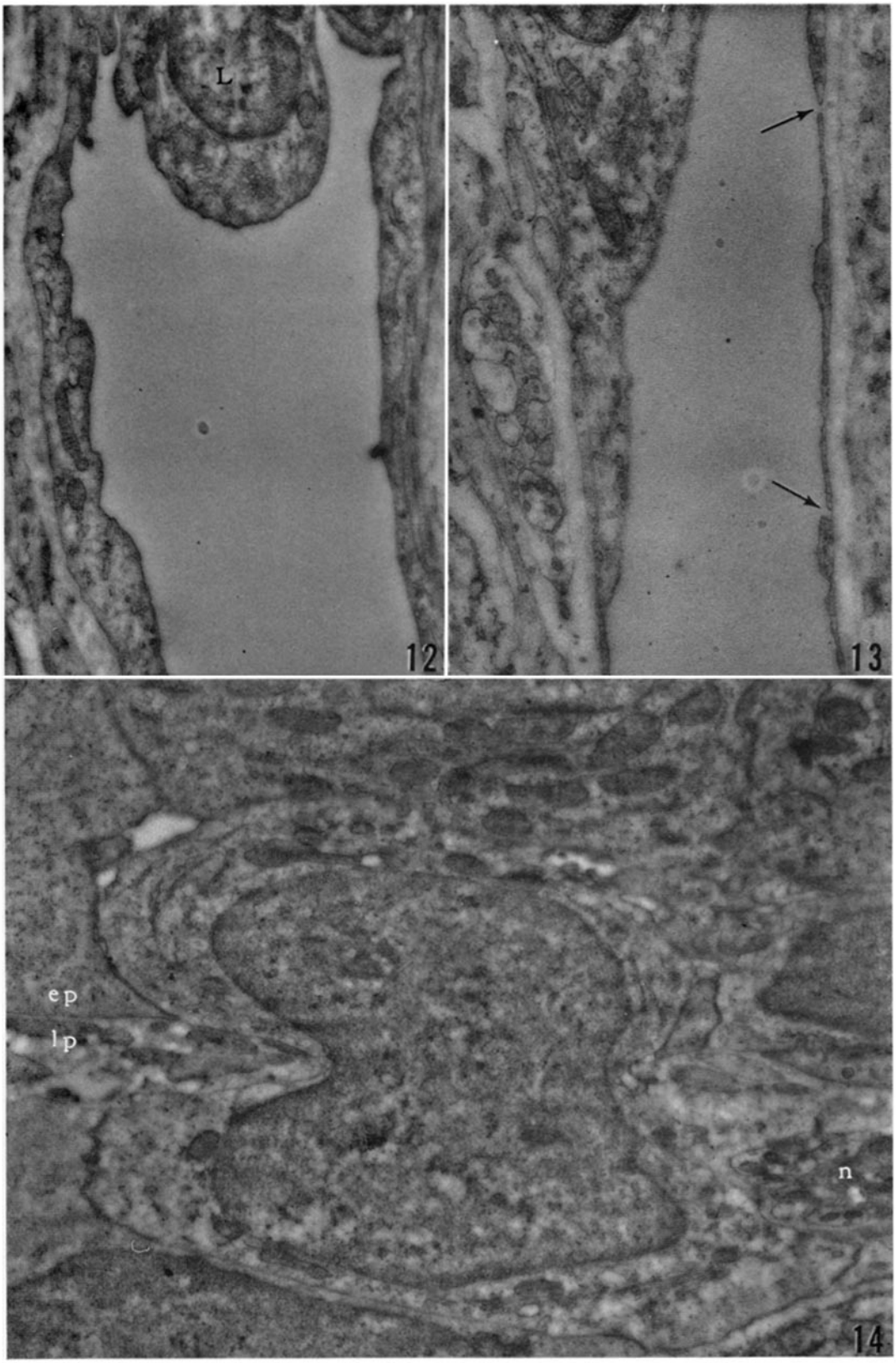
PLATE 157

FIGS. 12 and 13. Comparison of portions of lacteal and blood capillary from an intestinal villus.

FIG. 12. Lacteal containing a small lymphocyte (*L*). Although the endothelial wall varies in thickness it is never so attenuated as the capillary wall. Pinocytotic vesicles are present in both endothelia but small fenestrations are absent from the lacteal. The nucleus of an endothelial cell lies at the upper right beside the lymphocyte. × 14,000.

FIG. 13. Blood capillary beneath the epithelium. This micrograph is from a section adjacent to that used for Fig. 2. The endothelial wall is thick in the perinuclear region but elsewhere it is highly attenuated. Fenestrations are indicated by arrows. The basement membrane is not very distinct. A bundle of unmyelinated nerves lies next to the capillary. × 20,000.

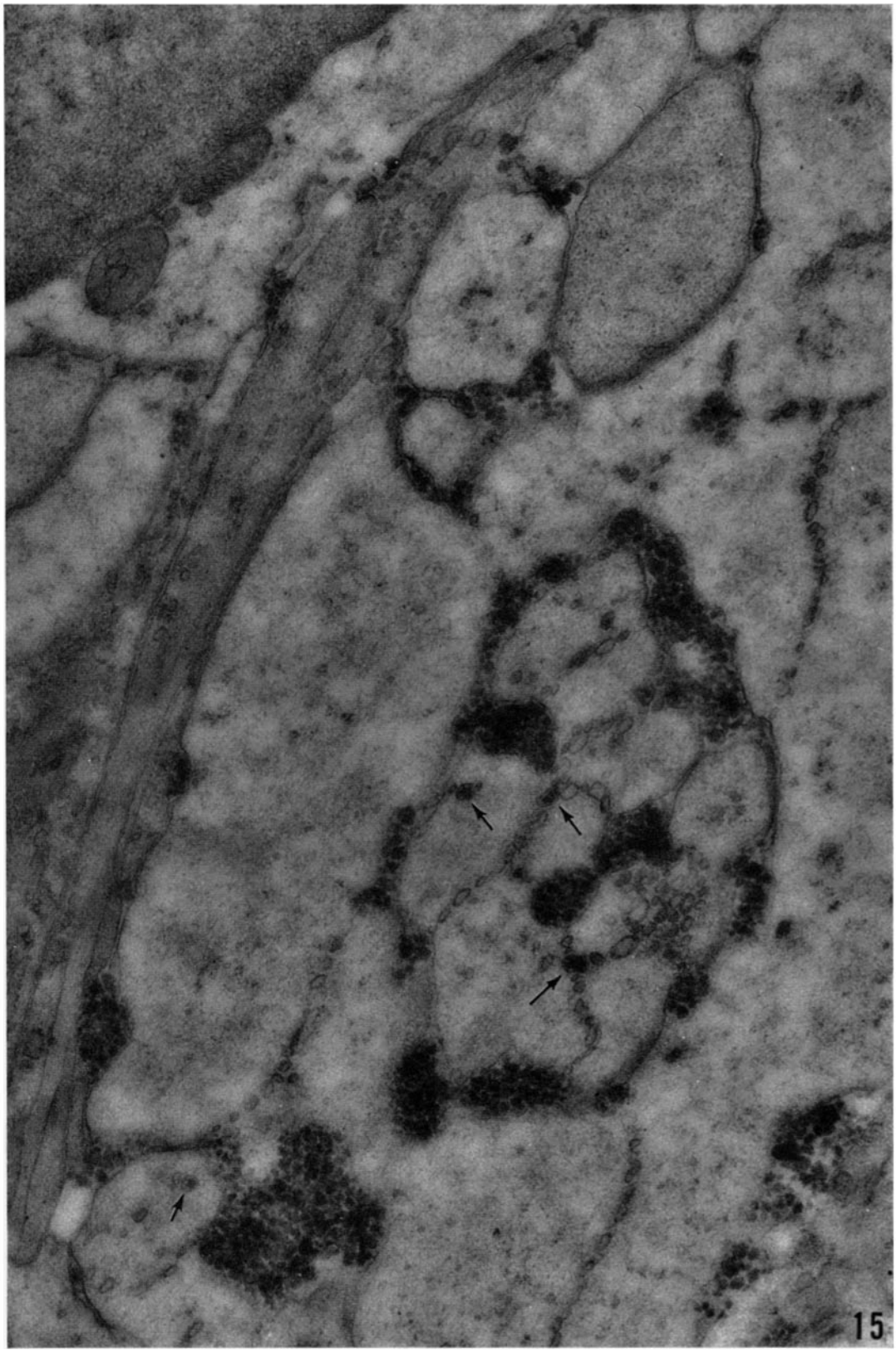
FIG. 14. A lymphocyte apparently entering the epithelium (*ep*) from the lamina propria (*lp*). Its dumbbell shape, with constricted waist, is typical of migrating lymphocytes. Basement membrane material is absent from the surfaces of the epithelial cells adjacent to the lymphocyte. A small unmyelinated nerve (*n*) is visible at the right in the lamina propria. The slender profiles in the lamina propria just beneath the epithelial basement membrane represent fragments of the extended processes of fibroblasts. × 13,000.



(Palay and Karlin: Intestinal villus in fasting animal)

PLATE 158

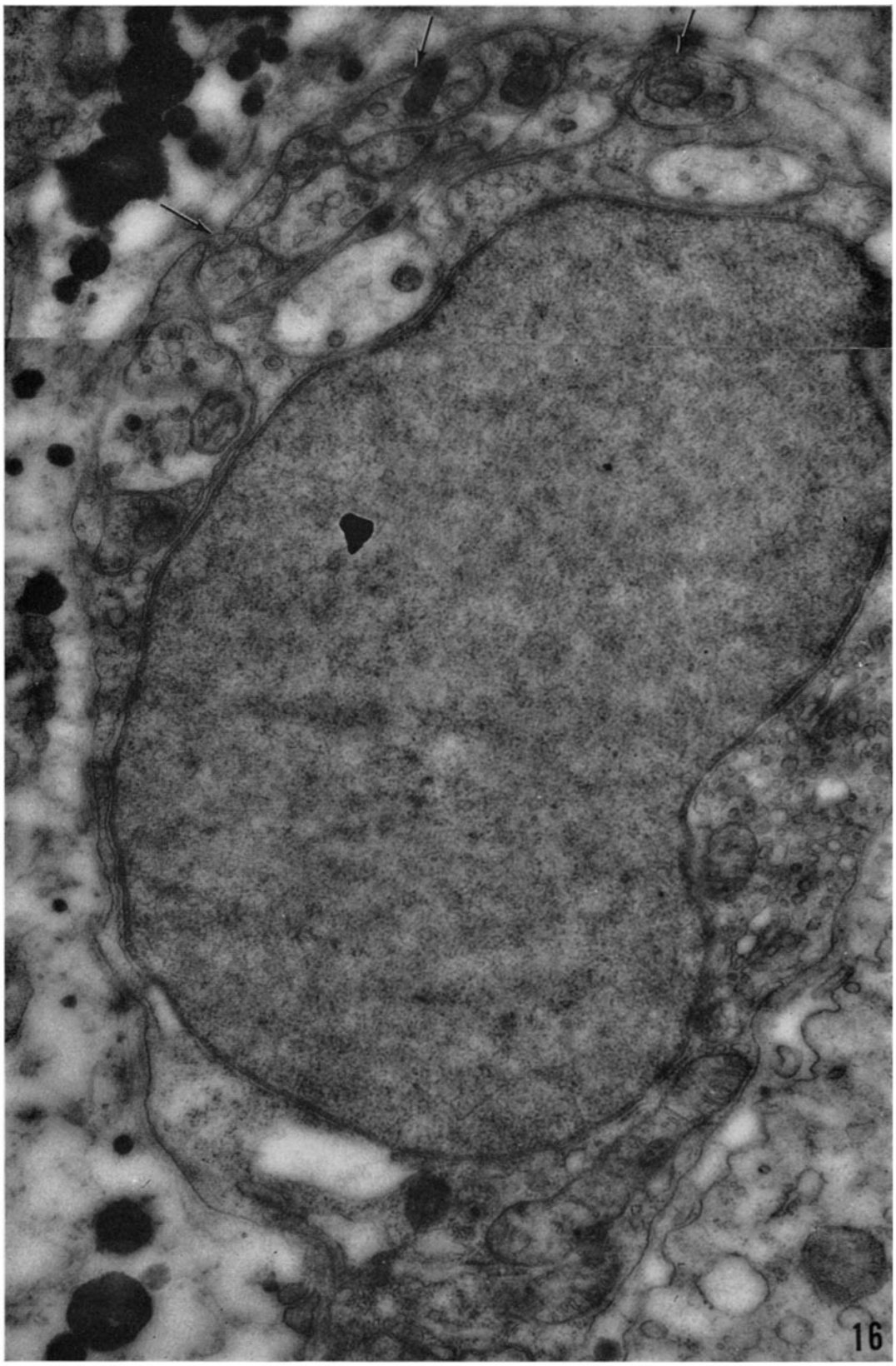
FIG. 15. Peripheral cytoplasm of a macrophage in the lamina propria of an intestinal villus. The micrograph shows the ruffled ectoplasm of this cell, which is free of all organelles except for chains and clusters of vesicles, probably derived from pinocytotic activity of the cell surface. Numerous fat droplets are entrapped in the folds of the surface and some are visible inside vesicles (arrows). × 28,500.



(Palay and Karlin: Intestinal villus in fasting animal)

PLATE 159

FIG. 16. Schwann cell and associated nerve fibers in the subepithelial plexus of an intestinal villus, from the jejunum of a rat that had been given corn oil 150 minutes before fixation. The epithelium is just visible in the left upper corner, and below it are some extracellular fat droplets in the lamina propria. The Schwann cell is almost filled by its kidney-shaped nucleus. Its cytoplasm contains mitochondria, granular endoplasmic reticulum, free granules, and a largely vesicular Golgi complex. The nerve fibers, of various sizes, are collected at one end of the cell (upper part of the figure), where they lie in grooves in its surface. Some of the fibers (arrows) are only partially enclosed. × 35,000.



(Palay and Karlin: Intestinal villus in fasting animal)