AN ELECTRON MICROSCOPIC STUDY OF FILAMENTS AND MICROTUBULES IN THE CHIEF CELLS OF THE EPITHELIUM OF THE RAT INTESTINE

by

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TABLE OF CONTENTS

INTRODUCTION

Within the short history of the application of electron microscopy to cytological and histological problems, most of the emphasis has fallen on the membraneous components of the cell. Mitochondria, the Golgi apparatus, granular and agranular endoplasmic reticulum, and the nuclear envelope have all been studied intensively. There have also been many studies on specializations of the cell membrane (Fawcett, 1962). Only recently have intracellular filaments been given some attention by electron microscopists, although they still have not gained acceptance as an ubiquitous organelle in epithelia. Most descriptions of cytoplasmic filaments have been made in conjunction with studies on terminal bars or desmosomes.

Concerning filaments, there are several major problems other than the strictly morphological one of where they are located and how they are attached to the plasma membrane and its terminal bar and desmosome specializations. One such problem is the role of filaments in the formation of the microvillus border. The intestine is a particularly good tissue for the elucidation of this problem since there is an orderly development both in size and number of microvilli of absorptive cells from the crypt to the tip of the villus (Brown, 1962).

A second problem is the role of filaments and their attachment sites in the desquamation of the epithelium at the tip of the villus. A study of the cells at the apex of the villus shows that the base of the cell, the area of the cell with the least filamentous support, degenerates first. How in fact, do the cells fall into the lumen while maintaining the integrity of the epithelial border? Study of this problem may reveal how desmosomes and terminal bars are formed and broken down.

This thesis is concerned principally with a morphological analysis at the fine structural level of the appearance, orientation, distribution, attachment sites, and possible functions of filaments in the intestinal absorptive cells.

This work will also describe the morphology, connections, and possible physiological functions of cytoplasmic microtubules, which are seen in abundance in the intestinal epithelium fixed with aldehydes. These are structures which have been observed only recently and this work will serve to document their presence in the intestine.

HISTORICAL REVIEW OF LIGHT MICROSCOPE LITERATURE ON FIBRILS AND TERMINAL BARS

Schmidt (1943) was the first to find evidence for a fibrillar component in the apex of the intestinal cells of the tadpole. Using

polarization optics, he was able to show that the apical cytoplasm was highly birefringent, suggesting a fibrillar network orientated parallel to the striated border. Earlier M. Heindenhain (1892) and Cohn (1895) had seen terminal bars using the mordant stain iron-hematoxylin but were unable to visualize any web structures in the cells. Puchtler (1956) reported on a new mordant-staining technique, the tannic acid, phosohomolybdic acid, amido black technique (TPA technique). The Carnoy-fixed tissue is treated with the two mordants and then stained with amido black. Using this stain Puchtler and Leblond (1958) were able to demonstrate a delicate web extending completely across the apex of intestinal cells immediately beneath the striated border. They also noted the close association of this web with the terminal bar, which stained more intensely with TPA. (See Kallenbach, 1963). This web was named the terminal web, although the term had been introduced earlier by Sauer (1937) for a similar structure in the neural tube of the chick. Leblond, Puchtler and Clermont (1960) were able to show many configurations of cell web in various cell types and tissue.

Kallenbach (1963) in a continuation of this work showed that there are two webs in the intestinal absorptive cells, a more densely-stained distal web and a lighter-stained proximal web. In a section transverse to the axis of the cell through the apical cytoplasm the

distal terminal web was not seen to be homogenous but to be composed of a "plate with holes in it." He also comments that the distal web attaches to the solid terminal bar, while the proximal web attached to the granular-appearing row of proximal desmosomes. The cells of the crypt of Lieberkühn have a more obvious double web. It should be noted that both Puchtler and Leblond, and Kallenbach found that the striated border was invariably unstained with TPA. The possible significance of this will be discussed below.

REVIEW OF ELECTRON MICROSCOPE LITERATURE

ON FILAMENTS

Granger and Baker (1950) in the first electron microscopic study of intestine showed that the striated border was made up of densely packed microvilli, each of which was a pseudopod-like extension of the apical cytoplasm, and each covered by a plasma membrane. However, it remained for Zetterqvist (1956) and Palay and Karlin (1959) to describe in some detail the filamentous components in the apex of intestinal cells. Zetterqvist showed that the microvilli that make up the striated border have a "longitudinal fibrillar structure" which extends straight into the apical cytoplasm as a rootlet for 0.5 u. He also noted a thickening of the cell border in the terminal bar region with associated dense material. He observed that the apical cytoplasm just below

the microvillus border was free of organelles, although he suggested no explanation for this. Palay and Karlin (1959) published a more complete description of the filamentous components. They observed that the microvillous cores merged with a dense felt-work of filaments approximately 0.25 u thick, mainly oriented parallel to the striated border, and further that this web attached to the terminal bars on all sides. (They included the apical desmosome with the terminal bar: see their Fig. 5). They described an ectoplasmic zone completely surrounding the cell characterized by 1) the presence of filaments and 2) the absence of other cell organelles. Dispersed fine filaments were noted throughout the cytoplasm. Beneath the dense terminal web appeared a zone devoid of any structure save a few widely-scattered filaments. Subsequent electron microscope studies have served to confirm the findings of Zetterqvist, and Palay and Karlin. Zamboni (1961) made the observation that the scattered zone of filaments was associated with desmosomes.

There has been some disagreement in the literature concerning the arrangement of the filaments of the core. Some authors (Zetterqvist, 1956; Trier, 1961) have described them as straight, while Zamboni (1961) described them as having a "chicken-wire-like" arrangement. Millington and Finean (1962) suggested that the cores might be composed of small tubules instead of filaments. The resolution

obtained by the previous studies did not allow the structure or arrangement of the filaments to be demonstrated.

Palay and Karlin, and Millington and Finean thought that rootlets were the result of faulty fixation while Zetterqvist thought they were true structures.

The disposition and appearance of the endings of these filaments in the apical cytoplasm have also been the subject of observations which are apparently in conflict.

ON TERMINAL BARS

Zetterqvist (1956) was the first to describe the terminal bar in the intestine, although his findings are difficult to interpret in the light of more recent findings. The region which corresponds to the zonula occludans (Farquhar and Palade, 1963) is described as two apposed membranes separated by a 100 A space, each membrane being a single opaque layer 50 A wide, which extends for ¼ to ½ u from the brush border. In his zone 3 (zonula adherans) each membrane again appears as a trilaminar-arrangement and the membranes are separated by a distance of 200 A. The desmosomes along the lateral surface of the cell are also called terminal bars. Palay and Karlin (1959) describe the terminal bar as consisting of two segments, the upper with its aggregation of terminal web filaments and lower part into

which insert finer filaments. Their figures 5 and 6, however, do not resolve the details of these structures. Farquhar and Palade (1963) elucidated the terminal bar problem in the intestine as well as in other epithelia. They have described the terminal bar as consisting of two parts: the tight junction or the zonula occludans which occurs closest to the lumen and extends down the lateral surface for a distance which varies with the epithelium (0.2-0.5 u in the intestine) and the intermediate junction or zonula adherans. The zonula occludans is a quintuple-layered structure due to the fusion of the outer leaflet (OL) of the apposed membranes. Thus in this region there is an obliteration of the intercellular space.

The zonula adherans is characterized by a true intercellular space 200 A wide which separates the unit membranes of the adjacent cells. The membranes in this zone have the same dimensions as the rest of the lateral cell membrane (30-25-25 A = 80 A, inner leaflet-outer leaflet). In this region the cytoplasm has a higher density which on higher magnification can be shown to consist of a mat of fine fibrils oriented generally parallel to the junction. "Occasionally the mat is condensed further into a plate which runs parallel to the cell membrane from which it is separated by a narrow light zone."

ON DESMOSOMES

Desmosomes, originally discovered in stratified squamous epithelia with the light microscope, have now been described in many epithelia with the electron microscope. Although there have been a considerable number of papers on their morphology (Odland, 1958; Karrer, 1960; Hama, 1960; Hay, 1961; Tapp, 1962; Fawcett, 1962) there still remained a great deal of confusion between terminal bars and desmosomes; many investigators use the terms interchangeably. Farquhar and Palade (1963) in their survey of attachment sites have attempted to alleviate some of this confusion.

A desmosome is an oval-shaped structure in face view (Tamarin and Sreebny, 1963). In cross-section the apposed membranes are separated by a regular intercellular space 250 A in thickness. This space is bisected by an intermediate dense plaque 50 A thick. A dense plaque slightly separated from the membrane appears on the cytoplasmic side. Attached to the cytoplasmic plaque are the desmosomal filaments. In their description of the attachment of filaments to the desmosome, Farquhar and Palade (1963) say that the "fibrils, coarser and more distinct than those of the terminal web, converge on the inner aspect of each desmosomal plaque... (and) approach the plaque at a high angle, but occasionally the bundles run parallel to it and fray out into the cytoplasmic matrix at its margins."

ON MEMBRANES

Zetterqvist (1956) was the first to study the thickness and structure of the membranes of intestinal cells at high resolution. He found the apical membrane covering the microvilli to be 105 ± 3 A. This was composed of a 25 A light space surrounded on both sides by dense lamina 40 A in thickness. Large gaps in the membranes were not observed, although pores smaller than 50 A could not be ruled out. No mention of either intracellular or extracellular material attaching to the membrane was made. Several earlier papers (Sjostrand, 1953; Rhodin, 1954) had shown a trilaminar structure of certain membranes and attempts were made to correlate this structure with the model developed on physiological evidence by Danielli (1936). Zetterqvist found the lateral plasma membrane to be 70 A thick, the inner dense leaflet (IDL) being 20 A, the intermediate space (IS) as 25 A, and the outer dense leaflet (ODL) as 25 A (as calculated from his data). Palay and Karlin (1959) agreed with Zetterqvist's measurements of the apical membrane but found the lateral membrane much thinner, appearing as a single dense layer approximately 32 - 42 A. However, they say that a "doubling of the membrane can be seen, and here the membrane appears twice as thick." Farquhar and Palade (1963) measured the apical membrane as 110 A (40-30-40) which is, although, well within experimental error. Their lateral membrane, also slightly

thicker, measured approximately 80 A (30-25-25 ODL). Thus the lateral cell membrane is thinner and more asymmetrical than the apical membrane (the apical membrane being only slightly asymmetrical following OsO_4 and symmetrical after $KMnO_4$). The apical membrane corresponds to Farquhar and Palade's Type 1, a thick (-110 A) nearly symmetrical membrane, while the lateral membrane corresponds to their Type 2, a thin (-80 A) asymmetrical membrane, which is by far the most common plasma membrane in most cell types. Elfvin (1963) using freeze-dried myelin preparations also found the 75 A unit membrane of the Schwann cells to be asymmetric. Robertson (1960) postulated that the bimolecular lipid leaflet was sandwiched between dissimilar layers of protein, or protein polysaccharide complexes. Brandt (1962) and others have shown that membranes have the property of concentrating certain substances on their surfaces. The striated border has long been known to be PAS positive and presumably this neutral polysaccharide material is visualized in electron micrographs as the flocculent material attaching to the outer leaflet of the apical membrane. No one, to date, has discussed the possibility of material attaching to the cytoplasmic side of the membrane, except at the specialized attachment sites, terminal bars and desmosomes.

ON MICROTUBULES

The earliest reports of structures which are probably microtubular were in the achromatic spindle of both invertebrates (Porter, 1957; Ruthman, 1959; Roth and Daniels, 1962; Roth, Obetz and Daniels, 1960; Harris, 1959; Mazia, 1961; and Yasazumi, et.al., 1961) and vertebrates (Bernhard and DeHarven, 1958; Buck, 1963; and Selby, 1953). Microtubules have also been described in neurones (Schmitt and Geren, 1950; Schultz, 1957; Gray, 1959; Palay, 1960; Whitear, 1960; DeRobertis, 1961; Elfvin, 1961). In addition to these reports on specialized organelles and cell types, there have been numerous reports on microtubules free in the cytoplasm of unicellular organisms (Rudizinska, 1957; Rouiller, 1956; Roth, 1957, 1958, 1959; Randall, 1956, 1957; and Noirot-Tomithee, 1958, 1959). Fawcett (1959) showed microtubules in the margin of toadfish erythrocytes. Philpott (1962) described microtubules in the chloride cells of the fish. Various authors have described microtubules in spermatozoa (Burgos and Fawcett, 1955, 1956; Christensen, 1961; Christensen and Fawcett, 1961; and Manton, 1959). Microtubules have also been reported in the nervous and pigment segments of the retina (Eakin and Westfall, 1959; Porter and Yamada, 1960; and Ladman, 1961).

In all these reports these structures were reported as either

microtubular or as "paired filaments", depending on the quality of fixation. The diameter or interfilament width ranges from 140 to 270 A. It has been observed by numerous authors that the tubules are characterized by a constant diameter (within the same tissue) and a generally straight course which can be followed for several micra within the plane of the section. Sandborn has found microtubules to be widespread in mammalian tissue, including nerve and ganglion cells, thyroid, pancreas, and mammary gland (Burton Society of Electron Microscopists, October, 1963; Sandborn,et.al., 1964).

ON FIXATION

Osmium tetroxide has been known for decades to be an excellent cytological fixative at the level of the light microscope. Its use was limited somewhat by its slow penetration rate. In the first attempts at fixation for the electron microscope, (Porter,1953) used OsO_4 vapors to fix the thin cytoplasmic sheets of tissue culture fibroblasts. In attempts to fix tissue blocks for sectioning, Palade (1953) found that unbuffered OsO_4 caused the same disturbances as the fixative buffered at pH 5. From this, he concluded that the penetration of OsO_4 was preceded by a wave of acidification. His best fixation, i.e., that which most closely resembled tissue culture fibroblasts fixed with osmium vapors, occured when the veronal-acetate buffer was pH 7.5.

It was soon appreciated that Palade's solution was hypotonic. Zetterqvist (1956) raised the osmolarity of his fixative solution to that of blood by adding sodium, potassium and calcium chloride. Caulfield (1957) raised the osmolarity by adding sucrose. Palay et.al. (1962) decided that the most important added component was an excess of calcium ions. Luft and Wood (1963) studied the extraction of protein during fixation and dehydration. Of the six buffers systems used (s-collidine, phosphate, arsenate-bicarbonate, veronal-acetate, chromate-dichromate), only the s-collidine showed significantly greater extraction (30% vs 8%). Sabatini et.al. (1963) studied a wide variety of aldehydes with respect to their fine structural preservation, and their preservation of enzymatic activity for histochemistry. Both glutaraldehyde and acrolein were judged excellent in preserving fine structure. Aldehydes do not preserve the lipid components of the cell, which include membranes. Thus it was necessary to post-fix the tissue with OsO_4 in order to visualize membranes and lipid inclusions. Sandborn et.al. (1964) mixed glutaraldehyde and acrolein to try to combine the best features of each, followed by a post-fixation with OsO_4.

MATERIALS AND METHODS

The results of different procedures for fixation were studied both with respect to fine structural preservation of the whole mucosa, and to the preservation of cellular details, such as filaments and microtubules. Two percent osmium tetroxide in a veronal-acetate buffer (ph 7.5; Palade, 1953) did not give optimal preservation with excised tissue. In this procedure, a small piece of tissue was excised, removed to a drop of fixative, diced, and fixed in the cold solution for two hours. To improve the fixation an attempt was made to perfuse the cold osmium fluid through the lumen of the gut for fifteen minutes prior to excising the tissue. The results were equal to those in published material. On account of defects found in tissues prefixed with either glutaraldehyde or acrolein, Sandborn employed a combination of these fixatives preceding osmium post-fixation. This method, by combining the rapid penetration of acrolein with the better protein preservation of glutaraldehyde, resulted in an increase in secretory materials as well as an improved preservation of filaments and microtubules in all cell types examined to date.

PERFUSION FIXATION

Four young adult rats (Sprague-Dawley) of both sexes were used for perfusion. No attempt was made to fast the animals prior to fixation. The perfusion mixture was freshly prepared and consisted of 6.5% glutaraldehyde and 2% acrolein in Sorenson's phosphate buffer (ph 7.5; Sandborn, 1964). The animals were anaesthetized with ether.

Following the perfusion, a small piece of the duodenum was taken at a distance of 1 cm from the pyloric sphincter and transferred to a drop of fresh fixative where it was diced into blocks. These blocks were put into a vial of the aldehyde mixture and fixed for one and one half hours at room temperature. The tissue was then post-fixed in 2% veronal-acetate buffered OsO_4 at 4^{o} for an additional two hours.

INTRALUMINAL PERFUSION

Duodenum also was fixed from four animals using an intraluminal perfusion of the aldehyde mixture. Again no effort was made to fast these animals. The animals were anaesthetized with ether. The abdominal cavity was opened and the proximal part of the jejunum was snipped open. Twenty cc's of the fixative were perfused into the stomach for fifteen minutes. Following this, the tissue was removed, diced, and placed in the aldehyde for one and one half hours, and post-fixed as above.

PREPARATIVE PROCEDURE

Following fixation, the tissue was dehydrated in graded alcohols consisting of 15 minute changes of 30, 50, 70, 90, 95% ethanol and three changes of anhydrous alcohol. Embedding followed the procedure of Luft (1961) with the added precaution of leaving the blocks in the second infiltration mixture overnight.

For orientation, thick sections 0.5 to 1 u were cut, mounted on glass slides and stained with 1% toluidine blue made alkaline by saturating with $Na_2B_2O_4.8H_2O$ to be examined with the light microscope. For electron microscopy, sections having grey or silver, and gold interference colors were cut on a Porter-Blum ultramicrotome, and picked up on uncoated grids or grids with a formvar-carbon substrate. Grids were stained with lead according to Karnovsky's method A (1961) and/or uranyl acetate. Survey micrographs were taken with an RCA EMU 3E electron microscope using an accelerating voltage of 50 kv and an objective aperature of 50 u. High resolution micrographs were taken with a Siemen's Elmiskop 1 using accelerating voltages of 60 and 80 kv, and an objective aperature of 50 and 25 u. Both Kodak Contrast Projector slide plates and Ilford N-40 were used, and developed with Kodak D-11. Prints were routinely enlarged 3½ times, although some plates were enlarged up to 14 times. Mensuration was done on both the enlargements and on the plates using a 10x magnifier with a millameter reticle.

OBSERVATIONS

VILLUS

FILAMENTS IN THE MICROVILLOUS BORDER

Seen with the light microscope, villous epithelial cells are covered with a homogenous or faintly striated layer. The electron microscope has shown that this striated border is composed of a large number of thin cylindrical projections, each covered by a fold of the plasma membrane. These projections, or microvilli, are approximately 1.0 u in length and 0.1 u in diameter.

Longitudinal sections of the apical portion of absorptive cells show delicate filaments which extend downwards from the apex of the microvillus into the cytoplasm where they end as rootlets (Fig. 1). Each filament is approximately 60 A in diameter and as many as eight can be seen in a longitudinal section of a microvillus. The filaments are quite straight and can be followed for considerable distances in the section in contrast to the filaments of the terminal web below. Within the microvillus, branching of the filaments is not seen, although cross-linking of the filaments cannot be ruled out. In some places, the filaments are quite uniform in diameter, while in other places, they are seen to have a nodular appearance. The plasma membrane of the cell is separated from the core of filaments by a filament-free zone approximately 200-300 A in thickness, except

at the apex of each microvillus.

At the apex, the filaments appear to end in the accumulation of dense material adjacent to the inner leaflet of the plasma membrane (Fig. 3). The lower end of the filaments terminate in the apical cytoplasm as rootlets (Fig. 1 and 2). The rootlets average 1.0 u in length, thus making them approximately equal in length to their respective microvilli.

In a transverse section of the microvillous border and the apical cytoplasm, filaments are observed in cross and oblique section (Fig. 4). Each microvillus contains a central core of filaments surrounded by a homogenous zone of medium density. The filaments have an average diameter of 60A. There are approximately 50 filaments per core, although the number varies depending on the size of the microvillus. In several locations, the filaments are arranged in a hexagonal packing with a center to center spacing of approximately 100-150 A. The appearance of the filaments in rows is probably due to a slight tilting of the core or rootlet.

TERMINAL AND DESMOSOME WEBS

Immediately below the microvillous border lies the terminal web, a transversely oriented meshwork of filaments which attach to the zonula adherans of the terminal bar. In at least one instance

(Fig. 5) filaments of the terminal web have contributions from the apical desmosomes. Below the dense terminal web lies the desmosome web, a loose zone of filaments mainly derived from the apical desmosomes. The desmosome web can have two appearances: one in which the filaments are disorganized and loosely woven (Fig. 6) and one where the filaments are straight and parallel (Fig. 7).

CONNECTIONS BETWEEN ROOTLETS AND TERMINAL WEB

As the rootlets extend down into the apical cytoplasm, they meet the dense felt-work of the terminal web. In some places, right angle bends of the rootlet filaments can be seen (Fig. 1 and 6). In other sites, filaments lying at a 45^{o} angle are seen, sometimes forming a cone-shaped structure. Other filaments continue through the terminal web to end in the desmosomal web. Another appearance of the rootlets mimics the microscopic appearance of the villi and the crypts, i.e., one crypt in between several villi. Here the base of a rootlet would not correspond to any core but would supply filaments to several at once (two in a plane of section) (Fig. 8). Six or more rootlets are sometimes seen joining at their lower ends forming a cone-shaped structure with the apex toward the nucleus (Fig. 9).

CRYPT

FILAMENTS IN THE MICROVILLOUS BORDER

The crypt epithelium has many fewer microvilli per cell compared to the villus. At the base of the crypt, one might find two or less microvilli in any one section of a cell (Figs. 10 and 11). At the level of the upper crypt, or the junction between crypt and villus, the number increases to six or eight per cell (Fig. 12). The fewer the microvilli per section, the more irregular is the angle of projection of the microvillus into the crypt lumen. At the base of the crypt, angles of 45^{o} or less between the microvillus and the surface of the cell are common. Upper crypt microvilli rarely tilt more than 15^{o} from the perpendicular. In many cells at the base of the crypt the two microvilli at opposite ends, adjacent to the terminal bar, point outwards giving the appearance of horns (Fig. 11). Crypt microvilli are smaller than those on the villus. In the lower crypt, they average 0.3 u in length and 0.1 u in diameter (Fig. 11). In the upper region of the crypt, they are 0.37 u long and 0.11 u in diameter (Fig. 12).

A cross section of a crypt of Lieberkühn shows an oval-shaped lumen into which project the irregular microvilli of the crypt cells (Figs. 11, 12, 13 and 20). Due to their irregularity, one can expect

longitudinal, oblique and cross sections of microvilli within the same field. In longitudinal section, the microvilli of the crypt cell show essentially the same structure that was described for microvilli on the villus: parallel filaments extending from the apex into the apical cytoplasm. The surrounding plasma membrane is separated from the filamentous core by a filament-free zone approximately 200-300 A (Fig. 13 and 14). The filaments measure 60 A in diameter. A maximum number of six filaments has been counted in longitudinal section. Clear demonstrations of their attachment to the apical end of the microvillus has not been possible.

There are approximately 20 to 25 filaments per microvillus seen in cross section (Fig. 13) although again this number varies with the size of the microvillus.

The rootlet is a direct continuation of the core filaments into the apical cytoplasm; the rootlet filaments retain their grouped configuration. The rootlets are approximately twice the length of their respective microvilli.

TERMINAL AND DESMOSOME WEBS

The terminal web is not a regular structure in crypt cells. This is readily apparent in comparing the various plates of the crypt. In some cells, it extends across the apex of the cell joining

the opposing terminal bars (Fig. 10). In other cells, the terminal web seems not to cross the apex, at least not in the plane of section (Fig. 14). In either case, the terminal web of crypt cells is not the regular structure seen in villus cells. The same is true of the apical desmosome web. In some cells the desmosomal filaments can be traced across the cell (Fig. 14) while in others it appears very disorganized, sometimes joining a rootlet and running basally.

CONNECTIONS BETWEEN ROOTLETS AND TERMINAL WEB

It is much more difficult to develop a precise picture of the connections between the rootlets and web structures in the crypt. The rootlets of strongly-tilted microvilli merge with the terminal web filaments forming an acute angle between them (Fig. 11). More perpendicular rootlets (Fig. 11) form a cross junction similar to those of the mature microvillous border.

SUMMARY OF THE DIFFERENCES BETWEEN VILLUS AND CRYPT MICROVILLI

VILLUS: Microvilli as 1.0 u in length; 0.1 u in diameter; and numerous (closely packed); vertically disposed; containing approximately 50 filaments per core; ratio of the length of core to rootlet, approximately 1:1.

CRYPT: Microvilli as short (0.35 u); stout; few in number; strongly angulated; ratio of the length of core to rootlet, approximately 1:2.

LATERAL AND BASAL WEBS

In the apex of the cell, the desmosome web is a loose zone of dispersed filaments. At the level of the apical desmosome, these filaments become grouped into bundles. These bundles course down the lateral wall of the cell, traveling from desmosome to desmosome generally deeping within 0.2 mu of the lateral membrane (Figs. 15, 20 and 22). Within a single longitudinal section, the lateral web is seen in cross, oblique, and longitudinal section (Figs. 17 and 20). The three-dimensional arrangement of the lateral and basal web resembles a coarse net. Occassionally, sworls of filaments enter the endoplasmic region of the cell. Desmosomes are located at intervals along the lateral membrane down to the level of the nucleus. From this level, the lateral web continues down the side and across the base of the cell. They retain a close proximity to the basal cell membrane, without the aid of desmosomes (Figs. 16, 17 and 18). No structure corresponding to a "half-desmosome" has been seen in this study. In addition to the lateral and basal webs, there are filaments throughout the cytoplasm which occur singly or in bunches. These appear as straight tracts or as sworls in the sections.

Toward the apex of the villus, the epithelial cells become

separated from each other below the level of the nucleus, creating large intercellular spaces (Fig. 18). Often these intercellular spaces are penetrated by macrophages, lymphocytes and other migratory cells from the lamina propria (Figs. 18 and 19). Although some filamentous material is still noticed in the intranuclear region of these cells, very few desmosomes can be demonstrated.

Occassionally one finds a peculiar type of web structure in which slightly undulating filaments are well separated by a light density substance. These filaments are coarser than desmosome filaments measuring approximately 95 A in diameter. This has been seen in the apical desmosome web (Fig. 20) and in the lateral web (Fig. 21). The width of these structures is fairly constant, averaging 0.4 u. On one side, darker stained filaments of the desmosome web are seen. These darker filaments can be seen branching out into the cytoplasm (Fig. 21) or attaching to a lateral desmosome (Fig. 22). They are also seen in Figs. 14 and 15.

TERMINAL BARS

A longitudinal section through the terminal bar region (Figs. 23 and 24) shows the same features as described by Farquhar and Palade (1963). The zonula occludans extends from the junction of the apical membranes to a distance 0.3 u along the lateral membrane. This zone

is characterized by the fusion of the outer leaflets, thus obliterating the intercellular space. The thickness of the membrane is characteristic of the lateral membrane. In longitudinal section the fusion line is not continuous, but consists of a sequence of fusions and separations. The fusion line is seen to be continuous for a considerable distance in cross section (Fig. 4). The crypt terminal bar is characterized by a longer and possibly more curved zonula occludans than the villus (Fig. 12). Rootlet filaments are sometimes seen parallel to the zonula occludans, but do not appear to attach to it. Small accumulations of filamentous material are seen in the adjacent cytoplasm. The zonula occludans is straight or gently curved.

The zonula adherans in longitudinal section (Fig. 23) starts immediately under the zonula occludans and continues basally for a distance of 0.3-0.5 u. An intercellular space of approximately 150 A separates the outer leaflets. Dense accumulations of filaments are associated with this zone on the cytoplasmic side. They can sometimes be seen to condense to form a plaque which is slightly separated from the unit membrane (Fig. 9). The filaments lie parallel to the zonula adherans and parallel to the axis of the cell (Fig. 25). In longitudinal section, this zone often has an "S" or reverse "S" shape (Figs. 24 and 27). The point of contact between

the two zonulae is sometimes marked by a bend in the lateral membrane (Fig. 14).

DESMOSOMES

Desmosomes are localized structures which appear at intervals along the lateral membrane from the zonula adherans to the level of the nucleus. They are ovoid in face view with the major axis generally parallel to the cell axis. The most apical desmosomes seem more irregular in their direction than the lower ones. The maximum and minimum lengths of desmosomes in the intestine are measured as 0.4 u and 0.15 u. The intercellular space within this region is a regular 200 to 230 A and is bisected longitudinally by an intermediate dense plaque approximately 50 A wide (Fig. 26). The unit membrane of the desmosome has the same dimensions as the lateral membrane. At high magnification, short bridges can be seen connecting the intermediate dense plaque and the outer leaflet. A dense cytoplasmic plaque approximately 150 A wide is applied to the cytoplasmic side, separated from the membrane by a slight space approximately 50 A wide. In this light space, short bridges can be seen connecting the inner leaflet and the cytoplasmic plaque. These bridges are regularly spaced every 100 A.

Desmosome filaments are larger than core-rootlet filaments;

they average 75 A in diameter. The desmosome filaments approach the plaques at a shallow angle, i.e., almost parallel to the axis of the desmosome, and run parallel to the plaque thus exposing for attachment the length of the filament adjacent to the plaque (Fig. 15). The filaments are directed in different angles with respect to the axis. In most desmosomes, filaments on both sides run in the same direction. In the midpart of the cell, most of the filaments are longitudinally or transversely oriented filaments at the plaque, sometimes showing a longitudinal arrangement on one side and a transverse arrangement on the other side of the same desmosome, (Fig. 14).

PLASMA MEMBRANES

Both the apical and latero-basal membranes are resolved into their trilaminar unit membrane structure (Fig. 3,4,17,23 and 26). The apical membrane measures 105 $\pm$ 5 A in total thickness and is symmetrical (40-25-40). An irregular coat of amorphous material can be seen adherant to the outer leaflet (Figs. 2,8,23,24 and 26). This material is especially prominent at the tips of the microvilli where it appears as a "cap" (Fig. 3). It is also present in lesser amounts along the sides of the microvilli (Fig. 3). An inner density

has been described for the apex of mature microvilli. Crypt microvilli have greater (Fig. 34) and lesser (Fig. 14) amounts of the outer fuzzy material. Here it is not confined to the microvillous tip. It can occur anywhere along the microvillus and on the apical membrane where no microvilli are present.

The lateral and basal membrane of both the villus and the crypt have a 75 $\pm$ 5 A total thickness. They have a distinct asymmetry, due to the thinness of the outer leaflet. The intercellular space is irregular, but remains close to 200 A wide except at the villous tip where the base of the cells become widely separated from each other.

CYTOPLASMIC MICROTUBULES

Cytoplasmic microtubules (or more simply microtubules) are an ubiquitous cell organelle when aldehyde fixation is used. They are most obvious when seen in longitudinal section as a pair of dense lines separated by a light interspace. They appear in almost every micrograph taken, regardless of the cell type or the area of the cell. They are even seen in the apical ectoplasm of the columnar cells, a region in which every cellular inclusion except filaments and occasional vesicles are excluded (Fig. 1,5,6 and 13). They are seen bunched and singly close to the lateral membrane, in the supra-

nuclear region, and in the basal region. One is impressed with the straightness of these structures. Often they can be followed for several micro within the plane of the section running straight or curving only slightly.

In oblique section, they still appear as paired dense lines, although their length is much shorter. In cross section they appear as a small, round profile with a dense periphery and a light core (Fig. 13). Their outside diameter measures 230 A (210-250 A; 8 measurements), while the lumen is approximately 115 A wide. The wall is therefore estimated at 50-60 A. A trilaminar structure is not visible.

Microtubules are seen in proximity to every cellular organelle. They are often seen within the Golgi region (Figs. 28 and 29). They are also notably present near centrioles, where they seem to radiate from them (Figs. 30 and 31).

Tubules morphologically similar to microtubules are seen in the spindle apparatus of mitotic cells (Fig. 32). They attach to the kinetochore region of the chromosomes. They are straight and have a diameter of 230 A. These tubules are the spindle fibers of light microscopy. Large numbers of free ribosomes are seen throughout the spindle region.

SPECIAL CELLS

Occassionally cells are found within the intestinal epithelium which have a unique morphology. To date, one has been found on the villus and one in the crypt. The unique villous cell is unusual because of the extensive development of its filamentous and microtubular components (Fig. 33). There is no evidence of any autolysis or degeneration. Its microvilli are 1.5 times the size of microvilli in the adjacent cells. The rootlets are concommitantly long, reaching 3 u in length. The terminal web can be seen adjacent to each terminal bar but does not extend across the apex of the cell. It seems to turn basally and merge with the rootlet closest to the terminal bar. The desmosome web follows the same pattern. The cell has an extraordinary amount of endoplasmic filamentous material, obvious even at a low magnification. Most of the filaments are oriented parallel to the cell's axis. In addition, the cell has an abundance of microtubules, again having a generally longitudinal orientation. Many of them can be seen extending between the rootlets, coming as close as 1/3 of a micron to the apical membrane.

The crypt special cell has a completely different morphology (Fig. 34). It is located toward the base of the crypt as seen by the few microvilli in the adjacent cells. It, itself, has microvilli

characteristic of the upper crypt. Rootlets are also highly developed, reaching 1.3 u. As is the villous special cell, the terminal and desmosome webs do not cross the apex of the cell. Large numbers of microtubules are present. Toward the right side they can be seen diverging upon the lateral membrane at a 45^{o} angle. The cytoplasm is also characterized by an absence of mitochondria normally found in this area. There are many vesicles which contain varying amounts of dense material.

DISCUSSION

FILAMENTS

Brown (1962) has shown that microvilli increase in number, length, and diameter from the crypt to the villous tip. To these three criteria several more can be added. 1. The number of filaments per core increases from approximately 25 to approximately 50. 2. The ratio of core to rootlet length changes in the crypt from 1:2 to 1:1 on the villus. In addition, crypt microvilli are often strongly angulated. This effect is probably merely a steric one; in that a solitary microvillus would have potentially more space to occupy than one which is packed in on all sides by adjacent microvilli.

Most studies of the intestinal epithelium have shown only a suggestion of an organized structure within microvilli (Brown, 1962; Ladman, Padykula and Strauss, 1963; Sjostrand, 1963; Strauss, 1963; Trier, 1962).

Zetterqvist (1956) in his study of the small intestine, showed that jejunal microvilli contain a "longitudinal fibrillar component". He also described a continuation of this component into the apical cytoplasm which he called a rootlet. Palay and Karlin (1959) in a study of the rat jejunum described the microvillous core as composed

of a "dense fibrillar meshwork" and that each fibrillar core was continuous with the "filamentous substance of the terminal web underlying the striated border". However, they attributed the presence of rootlets to faulty fixation. Millington and Finean (1962) observed that the rootlets only become distinct in villous cells after small changes of tonicity, pH or early post-mortem autolysis. It is possible that the accentuation of the rootlets in this material was due to the decrease in density of the background cytoplasm which obscured them in the cells fixed in isotonic solutions. The rootlets can be seen in all of their micrographs but less clearly in those fixed in isotonic solutions. Farquhar and Palade (1963) have tacitly approved of the existence of rootlets by labeling them in their figures. Zamboni (1961) has described the appearance of the core filaments as "chicken-wire-like". This appearance is probably due to suboptimal preservation. Trier (1963) has documented the presence of fine straight filaments in microvilli of crypt cells in the human jejunum.

None of the aforementioned studies have presented conclusive evidence that these structures are truly filamentous. The suggestion that the cores might be composed of small tubules instead of filaments (Millington and Finean, 1962) seems to be negated by a comparison

with the cytoplasmic microtubules seen in the same section as the core filaments (Fig. 1). From the evidence of the cross and longitudinal sections and from the comparison of microtubules with the structures in the core of the microvillus, it is suggested that the core structures are filamentous.

The variety of appearances of the junction of rootlets and the terminal web is partially the result of the angle of the section and whether the apex was stretched or compressed. It is probable that a regular arrangement exists between the rootlets and the terminal web on the villus. Two general appearances suggest themselves: one in which the outer filaments of the rootlet branch out and unite with adjacent rootlets like the hand guard of a fencing sword, as the center filaments continue straight to meet the desmosome web, and one in which the rootlets would be like the intracellular analogue of the crypt, i.e., not related to any one villus but supplying epithelium to the several villi around it. Thus a rootlet at the level of the desmosome web would not correspond to any one microvillus but to several. In either model, the terminal web and rootlet filaments are continuous.

From the evidence presented in this work, it seems likely that the filaments of the rootlet and core represent an extension of

filaments from the terminal web. In light microscopy, Kallenbach (1963) found the terminal and desmosome webs TPA positive while the striated border is negative. Since the cell web is partially defined in terms of stainability with TPA, one must conclude that the filamentous component of the microvilli is not a part of the cell web. This evidence is not in agreement with the results presented here. Both core and terminal web filaments have the same dimensions and there seem to be ample evidence of their interconnections. It seems incautions to define web structures in terms of stainability with TPA in as much as TPA is known to stain filaments (terminal web) tubules (spindle fibers), Golgi zone (ameloblasts) and mitochondria (proximal convoluted tubule) (Kallenbach, 1963).

The desmosome web is seen as both a loosely-woven, disorganized net, and as tightly stretched filaments. This could be interpreted as a contraction of the filaments, or that the cell had been stretched at the apex, thus putting tension on the filaments. Most investigators have assumed a purely structural or supportive role for intracellular filaments. In the intestine, the striated border is remarkably resistant to mechanical distortion (Chambers and De Renyi, 1925). This seems logical when it is remembered that the entire villus contracts and expands, putting considerable strain on the epithelium. Intracellular filaments in other epithelia have been correlated with

tensile strength. Stratified squamous epithelium has considerable frictional forces applied to it; it also has a large amount of intracellular filamentous material. These filaments have poor mechanical strength but considerable tensile strength. No one has postulated a contractile role for intracellular filaments to the author's knowledge.

In conclusion, the filaments of the terminal web are continuous with the filaments of the rootlets and cores of the microvilli. These filaments probably function in the mechanical support of the microvillous border.

TERMINAL BARS

Light microscope studies employing the stains iron-hematoxylin and TPA, as well as Farquhar and Palade's electron microscope study (1963) have shown that the terminal bar complex extends completely around the apex of various epithelial cells. Farquhar and Palade have shown that this complex, or more specifically the zonula occludans presents an impenetrable barrier to the passage of material, both electrolytes (Farquhar and Palade, 1963, abstract) and large molecules such as panereatic zymogen and hemoglobin. In most epithelia, the zonula occludans is the most luminar junction between cells. The second zone, the zonula adherans, having the bulk of

the filamentous support, is probably responsible for most of the adhering properties of the terminal bar. Brightman and Palay (1963) have shown that the ependymal canal represents an unusual arrangement of these two zones. In the ependymal canal the zonula adherans is most commonly at the luminal boundary, while segments of both are seen in random arrangement along the lateral border. Ferritin has been shown to pass through the zonula adherans down to the level of the zonula occludans (Brightman, 1962). It has not been ascertained in this tissue if the zonula occludans represents a complete functional barrier, or whether it is incomplete. In the latter case, material might be able to transverse the maze thus formed.

Zonulae occludans have been found in a wide variety of epithelial and mesodermal cell types. They have been described in cardiac muscle (Sjostrand, Anderson-Cedergren and Dewey, 1958), human cervical epithelium (Karrer, 1960), toad bladder (Peachey and Rasmussen, 1961), smooth muscle (Dewey and Barr, 1962), glial cells (Gray, 1961; Peters, 1962), the mesaxon of Schwann cells (Robertson, 1960; Rosenbluth and Palay, 1961), and blood capillaries (Muir and Peters, 1962). Farquhar and Palade, extended these observations to include renal tubules, pancreatic acini, liver bile capillaries, blood capillaries, intestinal and gastric epithelia. These junctions

have been variously called "quintiple-layered junctions", "external compound membranes", and other names. Since these structures are probably homologous, it is sensible to restrict the terminology to the term zonula occludans.

Farquhar and Palade have described an increase in density in the cytoplasm immediately adjacent to the zonula occludans. This thin zone of condensation is slightly more in evidence in glutaraldehyde-acrolein fixed material, although it is trivial compared to the mass of filamentous material bordering the zonula adherans. Since little is known about the mechanism of intercellular adhesion, one cannot evaluate the relative importance of these two zones in this function.

There are several morphological aspects of the zonula adherans which require substantiation. Both Farquhar and Palade (1963) and Brightman and Palay (1963) have reported a thin median density in the intercellular space. Although this feature may be visible on their micrographs, it has not survived the photographic reproduction. This could not be confirmed by this work, possibly because of insufficient resolution. The other point, concerns the insertion of filaments into this junction. Farquhar and Palade describe the filaments as parallel to cell surface junction, sometimes condensing

into a plate separated from the membrane by a light zone. Brightman and Palay disagree somewhat, stating that the zonula adherans "has no adjacent fascicle of longitudinal fibrils running perpendicularly to the plaque. Instead, tufts of cytoplasmic filaments insert directly into the plaque at an angle." Neither study described the orientation of the filaments, whether parallel or perpendicular to the longitudinal axis of the cell. Although the plaque is not a obvious feature, it is sometimes seen. From this study, it is tentatively concluded that the filaments, while immediately adjacent to the zonula, are oriented vertically. From this position they must curve away from the zonula and assume the transverse orientation characteristic of the free terminal web.

DESMOSOMES

Desmosomes were described originally in the intercellular bridges of stratified squamous epithelium (Odland, 1958). Subsequent electron microscope studies have shown them to be a common, if not a universal feature of epithelia. They have been shown in frog mesothelium (Hama, 1960), human cervical epithelium (Karrer, 1960), blood endothelium (Fawcett, 1961), skin (Selby, 1955; Porter, 1956; Odland, 1958; Horstmann and Knoop, 1958), embryonic blastoderm (Overton, 1962) and in various epithelia by Farquhar and Palade (1963).

They have all agreed on the spot or button-like character of the desmosome, in contrast to the terminal bar. Farquhar and Palade have combined its structure and function in a new name for this specialization-the macula adherans.

Tamarin and Sreebny (1963) described the three-dimensional appearance of the desmosomes in the submaxillary gland, as ellipsoidal in planer view, and oriented with their major axis parallel to the longitudinal axis of the cell. The major axis measured 0.41 u and the minor axis measured 0.25 u. This parameter was not studied thoroughly in this work. However, in a longitudinal section of an absorptive cell, the length of desmosomes ranged between 0.15 u to 0.4 u. This would indicate a more random orientation of desmosomes in the intestine. They did not correlate the filamentous direction with the orientation of the desmosome. The present study indicates that the orientation of the desmosome has no relation to the direction of the filaments. The orientation of the filaments along the middle third of the lateral membrane is roughly longitudinal, correlating with the major axis of the desmosomes. This might be expected since the filament bundles project more or less longitudinally along the lateral borders. Toward the apex of the cell, the filament orientation becomes more irregular, sometimes

resulting in vertical filaments on one side of the desmosome and horizontal filaments on the other.

There has been little attempt to describe the attachment of filaments to the desmosome. Farquhar and Palade describe the filaments as approaching the desmosomal plaque at a "high angle", i.e., almost perpendicular to the desmosome. When considered for a few moments it seems logical that the angle of filament approach is dependent upon the angle of the desmosome and the tension applied to the filaments. Along the mid-point of the lateral membrane in intestinal cells, filaments approach parallel or nearly parallel to the plaque. In fact, filaments need not even change direction in approaching, transversing or leaving the desmosome. Here the filaments would have a longitudinal tension. At the apex of the cell, the desmosomal filaments leave the lateral border to become parallel with the free surface of the cell. At the apex, tension would be applied nearly perpendicularly at the apical desmosome, resulting in filaments which approach the desmosome perpendicularly.

Farquhar and Palade made the observation, confirmed in this study, that parallel to the main plaque were "one or two plate-like zones of further densification". In favorable sections, it is possible to trace a single filament completely across the desmosome without it coming within 400 A of the cytoplasmic plaque. Two

significant conclusions can be drawn from this. It seems likely that the desmosome does not represent the end of a filament, but rather that it is the lateral attachment for a length of filament equal to the length of the desmosome. In other words, the filament does not insert into the plaque only to have another filament continue from a separate locus on the plaque. The filament passes in close proximity to the plaque, and in doing so is bound sideways within the region of the plaque. Teleologically, a lengthwise attachment of the filaments would offer more strength than merely exposing the tip of the filament for attachment. A good analogy might be the many converging railroad tracks entering a city, running parallel through the freight yards and diverging on the other side of town.

If the filaments do not embed themselves in the cytoplasmic plaque, how can they be structurally and functionally adherant to the plasma membrane? Since there are continuous light zones between the filaments and the plaque, there must be an unstained or extracted substance which is responsible for the binding. Pannese (1960) studied the birefringence of fibrillar material in the stellate reticulum of the enamel organ. His findings showed a positive intrinsic birefringence with respect to the fibril axis due to a non-collagenous

protein, and a negative birefringence due to a lipid moeity. Thus the protein molecules are oriented parallel to the fibril and the lipid perpendicular to it. It is tempting to speculate that this lipid material is responsible for the lateral bonding observed in the desmosome and zonula adherans. Obtaining a pure fraction of intracellular fibrils and analysing them biochemically might solve this problem.

Desmosomes have long been considered as regions of strong intercellular contact. There are many lines of evidence to support this. Skin, the epithelium most subject to abrasive forces, has a large number of desmosomes; the desmosomes are almost the only place where adjacent cells are in close apposition to each other. Most of the cells in the intestinal epithelium are closely apposed, even along the extensive interdigitated processes. Cell shrinkage due to fixation or storage in hypertonic media causes the lateral membranes to withdraw from one another, resulting in large intercellular spaces and microvillous-like processes. In such shrunken tissue, the lateral membranes are only in close apposition within the range of the desmosome, again indicating its functional role in cell adhesion (Fig. 37).

The short bridges seen between the intermediate dense plaque and the outer leaflet, and between the inner leaflet and cytoplasmic

plaque are probably in some measure responsible for the adhesive properties of the desmosome.

Berwick and Coman (1962) showed that calcium ions are extremely important in intercellular adhesiveness, less so in cell-to-surface adhesiveness. Dissociation studies show that these two systems have different modes. A threshold of tension is required to separate two cells: once passed, the cells separate easily. This contrasts with the cell-to-surface bond which can be separated by a constant tension. They postulate a "glue" substance which they suspect to be a complex mucopolysaccharide. It seems likely that the all-or-nothing effect of cell-to-cell contact is due to the breakage of desmosomes.

If a stress were applied to a contact site, it is apparant that without internal support the site would tear off the membrane. With a system of internal filaments, external tension would be distributed to adjacent desmosomes and desmosomes on the other side of the cell. Distribution of stress would insure that no one point of the cell would be ruptured from excess forces.

In conclusion, desmosomes are randomly oriented structures localized at intervals along the lateral membrane. Their major axis does not necessarily correspond with the long axis of the cell, or with the direction of the filaments attaching to it. The filaments lie

parallel to, but not contiguous with the cytoplasmic plaque, thus exposing a length of filament for attachment.

MEMBRANES

The varying appearance of the plasma membrane using different fixatives and stains has been reviewed by Farquhar and Palade (1963).

The present model of membrane structure was postulated by Danielli (1936) on the basis of physiological and biochemical data. Electron microscopic studies of myelin, combined with x-ray diffraction data, have confirmed and elaborated upon this theory. The present concept of the unit membrane as proposed by Robertson (1960) consists of a bimolecular leaflet of phospholipid flanked on either side by dissimilar layers of protein or protein-carbohydrate complexes. The lipid is oriented perpendicular to the membrane, while the protein is parallel to the membrane.

Robertson's original concept of the unit membrane (1960) assumed that all membranes were approximately 75 A thick which appeared in electron micrographs as a trilaminar structure, a light zone bordered by two dense layers. In his effort to emphasize the universality of this structure, he overlooked some reports of size differences. Zetterqvist (1956) had shown that the apical membrane of intestinal cells measured 105 A. This measurement has been confirmed repeatedly

(Palay and Karlin, 1959; Sjostrand, 1963; Farquhar and Palade, 1963). Farquhar and Palade have shown this thick membrane at the apex of a variety of epithelia, including stomach, colon, gall bladder and parts of the nephron. On the basis of their measurements, Farquhar and Palade have classified plasma membranes into two groups: a Type 1, a thick (110 A) and symmetrical structure; and Type 2, a thin (70-80 A) and asymmetrical membrane. The fine, less-dense outer leaflet is responsible for the asymmetry. Robertson has agreed that the increased thickness of the Type 1 must "reflect underlying chemical differences in the components of the membrane" (Robertson, 1961 B). The fact that the Type 1 is only seen in the apex of certain epithelia, and that most of the increased thickness occurs in the outer leaflet indicates that this type of membrane is a result of special environmental conditions facing the cell.

This study has confirmed in glutaraldehyde-acrolein fixed tissue the measurements of 105-110 A for the thickness of the apical membrane, and 70-80 A for the lateral and basal membrane.

Brandt (1963) has shown that amebae are capable of concentrating proteins at their plasma membrane. Schumaker (1958) has estimated that the cell can accumulate on its surface protein up to 50 times its own volume in five minutes. With the electron microscope extraneous amorphous material can be seen adherant to the plasma membrane.

This extraneous material is divided into two zones, an inner structureless zone approximately 200 A thick, and an outer filamentous zone. Protein conjugated with thorotrast can be seen attached to the outer filamentous material. Once concentrated on the membrane, the material could be taken in by micropinocytosis or diffusion. The plasmalemma of amebae has been shown to be PAS positive.

Intestinal microvilli are seen to have a similar hazy material adherant to the apical membrane, although it is not divided into an amorphous layer and a filamentous layer. Presumably this material is responsible for the PAS staining of the striated border in light microscopy. This could be shown definitively in electron microscopy by the use of the PA-Silver test. Pierce, et.al. (1962) showed that the basement membrane of epithelial tissue, also PAS positive, is a product of the epithelial cells and not of the connective tissue underlying it. This finding suggests that the secretion of neutral mucopolysaccharide is a general property of epithelial tissue. This could also be confirmed by the PA-Silver technique.

In addition to the neutral mucopolysaccharide component of the adherant material, it also appears that acid and alkaline phosphatases are localized in this material (Sheldon, et.al., 1955; Brandes, et. al., 1956). Higher resolution studies are necessary to determine whether the reaction product is localized on the membrane itself or

in the adherant coat.

This amorphous material is preserved with glutaraldehyde-acrolein. Seen with this fixative, it is concentrated at the apex of the microvilli forming a dense "cap".

MICROTUBULES

Microtubules are difficult to preserve using standard OsO_4 fixatives unless Ca and Mg ions are added (Roth, 1963). They are also preserved with aldehyde fixatives. Since these methods have been developed, microtubules have been seen in a wide variety of organisms. In longitudinal section, microtubules are difficult to recognize from paired filaments. Even today there are authors (Roth, et.al., 1963) which conservatively refer to microtubules as paired filaments. They have been described in several orders of higher plants (Ledbetter and Porter, 1963), in invertebrates (Slautterback, 1963), and in a variety of mammalian cells (Sandborn, et.al., 1964). The fact that these structures have been seen in such divers species make it likely that they are a universal cellular organelle. The plethora of microtubules seen in every section of intestine makes this impression even stronger.

Slautterback (1963) failed to demonstrate a trilaminar structure in his 50 A microtubular wall. He still concluded that the wall was

membraneous, "similar to other complex phospholipid-protein membranes with which it is continuous". He also attributed to the wall the known membraneous ability to concentrate ions at its surface. This property is basic to his hypothesis for microtubular function in ion transport.

From a review of the literature, Slautterback (1963) has classified microtubules, on the basis of size, into two groups: 270 A or 120 to 200 A. "The former microtubules function evidently as elastic bodies, such as in the fish erythrocyte.....whereas the latter microtubules seem to function in the synthetic or metabolic activities of the cell, and they are found related to the Golgi complex, the endoplasmic reticulum, the centrioles or, in the special case of mitosis, to the achromatic spindle." The present author believes that such a classification is premature. The analysis of size differences should be based on cross sectional measurements, and using the same embedding medium. Secondly, the theories concerning microtubular function are unfounded.

Ledbetter and Porter (1963) have reported the microtubular wall as homogeneously dense and indicate that the wall is made up of smaller filaments. "Presumably, these 'smaller units' which appear circular in cross section represent the major macromolecular elements of which the tubules are constructed." In a later presentation,

Ledbetter (1963 abstract) reported the wall of the microtubule to have a pentagonal outline, supposedly representing five filaments.

As confirmed in this study, these microtubules of interplase cells are morphologically similar to the spindle "fibers" of the mitotic apparatus. Microtubules are also related to the "fibrils" of cilia and flagella, centrioles, and basal bodies in having approximately the same dimensions and a fairly straight character.

Relevant to the discussion of the structure and composition of the microtubular wall is the fact that microtubules are preserved only poorly with OsO_4. Lipoprotein membranes whether plasma or organellar are adequately preserved with standard OsO_4 fixatives. If microtubules or spindle tubules have lipoprotein structure, they should also be preserved with equal facility. An even stranger case can be made for permanganate fixation which is a better membrane fixative than OsO_4. Microtubules are absent in permanganate-fixed tissue.

This study was unable to demonstrate a pentagonal outline of a microtubule in cross section; nor was it able to show trilaminar unit membrane structure.

SPINDLE TUBULES

A number of investigators have indicated the similarities

between microtubules and spindle tubules. Ledbetter and Porter (1963) have reported that spindle tubules are slightly smaller than microtubules, averaging approximately 200 A as opposed to 230 to 270 A. In the intestine, spindle tubules have exactly the same diameter as microtubules, approximately 230 A. Without knowing the cell was in mitosis, it would be impossible to differentiate them from microtubules. In addition to sharing the same diameter, both are quite straight. Like microtubules they are best preserved by OsO_4 with Ca and Mg ions, or aldehydes. Mazia (1961) has used various procedures to isolate and redisolve the mitotic apparatus . The mitotic apparatus (chromsomes, spindle and asters) can be isolated in a medium containing Ca and Mg ions, but can not be redisolved. A sucrose or dextrose medium containing dithioglycol, a compound which stabilizes protein by favoring disulphide linkages, is the gentlest medium for isolating the mitotic apparatus and allowing its subsequent solution (Mazia, et.al., 1961). The role of free -SH groups and disulphide bridges have been strongly implicated in the formation and stabilization of the mitotic apparatus, yet no one has a reasonable hypothesis concerning their function. Aldehyde such as acrolein and glutaraldehyde are known to precipitate proteins by cross linkages. Presumably cross linkage is the function of Ca and Mg ions.

Zimmerman (1960) has shown that the isolated mitotic apparatus consists of two proteins separable by electrophoresis and ultra-centrifugation. One protein seemed associated with RNA normally found in the mitotic apparatus. The major protein component has a molecular weight of 315,000 ± 20,000.

Went (1959) has demonstrated with immuno-diffusion techniques the identity of a protein in unfertilized sea urchin eggs and the protein of the mitotic apparatus of fertilized eggs. He concluded that the mitotic apparatus did not contain any protein not found in a premitotic cell. This supports the formed precursor theory of the spindle formation. His work also indicates the relative simplicity of the spindle proteins, suggesting that it contains one or perhaps two protein components.

It is reasonable to assume that spindle tubules are the contractile elements in the mitotic apparatus, yet no one has put forward an acceptable hypothesis for the molecular basis of this contraction. The role of -SH and -S-S- groups is the only clue so far. Even the energy requirements for chromosome movement are poorly understood (Mazia, 1961). Similarily, there are no adequate theories concerning ciliary or flagellar motion (Gibbons and Grimstone, 1960). It seems likely that the acto-myosin system of striated muscle is not involved in tubular contraction. It is noted that microtubules are present

in the cortical zone of amoeboid cells, the region thought responsible for locomotion. Thus contractility seems an attractive hypothesis for the function of microtubules.

Slautterback (1963) has proposed that the microtubules function in the transport of water, ions and small molecules. Central to this theory is the fact that membranes have the "ability to concentrate ions at their surfaces". No one has been able to prove that the microtubular wall is a unit membrane structure. Most of the evidence indicates that the wall is not trilaminar. Therefore, it seems premature to base a theory on a structure that it possibly may not have. The special requirements both in section thickness and orientation for an analyses of the nature of the wall of the microtubule have not been obtainable for this study.

SUMMARY AND CONCLUSIONS

A combination fixative of glutaraldehyde and acrolein followed by post-fixation with osmium tetroxide was used to study intracellular filaments and microtubules in the chief cells of the rat duodenum. Microvilli contain a central core of straight filaments which extend into the apical cytoplasm as a rootlet and connect with the terminal web. Villous microvilli have more filaments (approximately 50) than crypt microvilli (approximately 25). The terminal web stretches across the apex of the cell and attaches on all sides to the zonula adherans. The desmosome web extends throughout the ectoplasm of the cell. In the apex, the filaments are scattered; elsewhere the filaments are grouped into bundles, resembling a coarse fish net. A hitherto unreported filamentous structure is described which consists of coarse undulating filaments separated by light density material. This structure appears in several locations within the cell's apex. Two unusual cells are described which have an overdevelopment of filamentous and microtubular elements.

The findings of Farquhar and Palade (1963) on the size of the apical (105 A) and lateral (70-80 A) membranes, and on the two zonulae of the terminal bar are confirmed. Several additional features of desmosome fine structure are described. Short bridges are seen

within the desmosome. The manner in which filaments attach to the desmosome is described.

Straight microtubules approximately 230 A in diameter are found throughout the cytoplasm of all cells examined. They are common in the ectoplasm. Although no definitive connections were seen, they are in proximity to every organelle, notably the Golgi apparatus and centrioles. Spindle tubules of mitotic cells are indistinguishible from microtubules of interphase cells.

APPENDIX

THE PROBLEM OF FIXATION

Although the problem of fixation is one which electron microscopists must face continually, it is the most difficult to assess of all the facets of the technique. With the light microscope, the artifacts of fixation, dehydration, and staining can be analysed and compared to living tissue viewed with the phase-contract microscope. There is no equivalent for the electron microsoope. Due to the high vacuum and thin sections required, only fixed or dried tissue can be examined. Artifacts of fixation can be judged by comparison with the results of other techniques, or can be assessed wholly within the technique.

At the upper end of the range of the electron microscope, electron microscopic data from myelin, collagen and several other macromolecules have been compared with data derived from biochemical, x-ray diffraction, and ultracentrifugation studies. At the lower end of the scale, the appearance of the whole cell, and the architecture of the tissue can be compared with light microscope findings on living cells and cells treated with various fixatives.

REQUIREMENTS OF A FIXATIVE

1. Resemblance to living tissue. This is the most basic

requirements of a fixative. The fixative must quickly stop all the processes of the cell while preserving the morphology of the living state. In doing so, it should precipitate in situ all the various components, proteins, mucopolysaccharides, lipids, and nucleoproteins so they will not be extracted during the fixation, dehydration, or embedding.

2. Reproducibility. A fixative must give the same result in every case; otherwise, it would be impossible to study the effects of chemical, physical or pathological variables since one could not tell if changes in the appearance of the tissue were due to a real difference or fixation artifact.

3. Speed. The fixative must be able to penetrate the full thickness of the block rapidly in order to prevent post-mortem autolysis in the deeper portions.

CRITERIA OF FIXATION

Artifacts can be created anywhere in the technique from the fixation of the tissue to its examination in the microscope. Artifacts of sectioning, such as knife marks and chatter, are easily recognizable. The fine and coarse precipitation artifacts of staining are also easily identifiable. Embedding and microscopical artifacts are more subtle, requiring the discrimination of an

experienced microscopist. (For these, the reader is referred to general texts, such as Pease, 1960 or Kay, 1961.)

The classical criteria for fixation in electron microscopy as stated by Palade (1956) and Palay et.al. (1962) are defined in negative terms. In other words, the absence of certain characteristics denotes good fixation. These negative characteristics are: precipitation in the cytoplasm and nucleoplasm, large discontinuities in the cell membrane, empty spaces, clumping and retraction, swelling of mitochondria, distension of the endoplasmic reticulum, disarray of orderly structures, thickening of membranes, and vacuolization of lipid inclusions. These criteria serve only to differentiate very poorly fixed tissue from moderately-well fixed tissue. Peachey (1959) states the problem as follows. "A simple and reasonable criterion for good fixation is the continuity of membrane structures and lack of obvious distortion and discontinuity in cytoplasmic detail. While such continuity is not proof that all structures have been fixed exactly as they were in the living cell, the presence of discontinuities and empty spaces is a clear indication of bad fixation." Regarding the above criteria, it has been realized that some distention of the endoplasmic reticulum is probably within the physiological range of the cell and not the result of poor

fixation (Palade, 1959).

With the development of fixatives other than variations of OsO_4, it has become apparent that more sophisticated criteria may be applied. The following might represent a more recent list of criteria of fixation. In this list, some of the classical criteria will be re-emphasized.

1. Density of the cytoplasm and apposition of cells. Both of these phenomena are dependent upon the tonicity of the fixative solution. In tissue fixed in hypertonic solutions, the background cytoplasm shows a high density due to the shrinkage of the cell and condensation of its organelles. Cell shrinkage also causes adjacent cells to separate, leaving irregular intercellular gaps, In tissue fixed in isotonic or hypotonic solutions, epithelial cells are closely apposed to each other. It also reveals a low density of the background cytoplasm with a slight swelling of such organelles as mitochondria and vesicles (Zetterqvist, 1956). Since osmolarity of tissue fluid is largely unknown, determination of the tonicity must be empirical, i.e., by examining the tissue.

2. Chromatin distribution. When seen in its living configuration with phase-contrast optics, the nuclear chromatin is distributed unevenly within the nuclear envelope. In general, it is condensed at the periphery and on the nucleolus. Osmium has

little effect on nucleic acids in vitro (Bahr, 1955). When stained with toluidine blue, osmium-fixed chromatin appears pale and evenly distributed. Aldehyde-fixed chromatin is intensely stained and is clearly seen in clumps. Chromatin is also difficult to stain with lead or uranyl acetate. Chromatin fixed with glutaraldehyde or acrolein is intensely stained with lead or uranyl acetate. Nucleolus, chromatin, and the kinetochore region of mitotic chromosomes are readily identified from each other. Chromatin patterns are clearly seen.

3. Vesiculation. Rosenbluth (1963) has shown in nerve tissue that a single continuous invagination from the cell membrane seen after permanganate fixation is seen as a chain of isolated vesicles following osmium fixation. Osmium that has a tendency to break up a large membraneous structure into smaller vesicles. In serial section studies on the ciliary body, Tormey (1963) has shown what others were interpreting as a chain of vesicles were an unusual highly-contorted form of the agranular endoplasmic reticulum. These two interpretations assume critical importance when discussing the concept of micropinocytosis which is based on the transport of material in small vesicles. Tormey's studies have cast doubt on the previously proposed theory of micropinocytosis in the production of the aqueous humor by the ciliary body. It is clear that more

studies should be done on this problem using different fixatives. In comparing the effects of OsO_4 and the aldehyde mixture on the intestine, it seems that there is more vesiculation following OsO_4 fixation (Fig. 35).

4. Membrane size and symmetry. This is not truly a criterion for fixation, since the appearance of membranes can vary with the fixative used and the stain employed. This criterion, then, is used in a new sense from its classical meaning. Osmium fixation yields a plasma membrane which is asymmetric, due to its outer dense leaflet being much thinner than the inner dense leaflet. Following permanganate fixation or block staining, the plasma membrane is symmetrical. Robertson (1961 A) in his study of myelin structure, found that osmium was a poorer fixative than permanganate, which in turn was poorer than chrome-formalin fixation. Osmium caused the intermediate dense line to break up into a string of globules. This line was intact following permanganate fixation. With the chrome-formalin fixative, this line was resolved into two distinct layers. The appearance of myelin following glutaraldehyde-acrolein is similar to the chrome-formalin preserved myelin (Sandborn, personal communication).

5. Special cytoplasmic inclusions. Recently it has become apparent that there are several cytoplasmic inclusions which are

preserved only poorly or not at all with conventional OsO_4 fixation. Inner mitochondrial subparticles (IMS) have been shown with negative staining technique to be a regular component of mitochondria from a wide variety of tissues and species (Fernandez-Moran, 1963; Parsons, 1963; Parsons and Verboon, 1964; Stoeckenius, 1963). These particles project into the mitochondrial matrix from the inner mitochondrial membranes.

Stoeckenius (1963) exposed a negative stained preparation of mitochondria to OsO_4 vapors and found only naked membranes left in contrast to the particle-studded membranes normally. This would explain their absence in histological sections. If the particles are first stabilized with aldehydes, they can be visualized in embedded and sectioned material even when post-fixed with OsO_4 (Fig. 36).

Cytoplasmic microtubules have recently been proposed as a common, if not universal, cell organelle (Slautterback, 1963; Ledbetter and Porter, 1963; Sandborn, et.al., 1964). They are only preserved with difficulty with the conventional OsO_4 techniques. They can be preserved in readily accessible tissue by lowering the pH of the OsO_4 solution to approximately 5, and adding the divalent cations Ca and Mg (Roth, et.al., 1963). Microtubules are also preserved with glutaraldehyde-acrolein, without adding extra ions and by keeping

the pH at 7.5. This fixative is also excellent at preserving cilia, flagella and the spindle fibers (tubules) of the mitotic apparatus (Fig. 32).

COMPARISON OF GLUTARALDEHYDE-ACROLEIN-OSMIUM AND OSMIUM FIXATION

With the possible exception of the inner mitochondrial subparticles and microtubules, the appearance given by the two fixatives are similar enough to allow direct comparison. Using the above criteria, it is found that glutaraldehyde-acrolein-osmium is a better fixative in every case. As has been demonstrated by Sandborn in several other cell types in the rat, the quality of fixation is reproducible. This study was based on the intestine of six rats. Its greater speed of penetration allows the investigator to study inaccessible organs and tissues, without autolysis or premature manipulation of the tissue.

The classical criteria of Palade show this fixative to be more than adequate in the preservation of tissue. Cells are in close apposition; large, empty spaces are absent between the epithelial cells and the elements of the lamina propria. Discontinuities of the plasma membrane are absent; the background cytoplasm is finely granular and evenly distributed. Mitochondrial or endoplasmic reticular swelling is not in evidence. Chromatin patterns are obvious due to the dense staining of the chromatin. Fewer empty

Fig. 1.

An electron micrograph of a longitudinal section through the striated border of a duodenal villus absorptive cell.

The longitudinally arranged central core (c) is composed of straight filaments. These continue into the apical cytoplasm as the rootlet (r). The terminal bar (tb) and apical desmosome (d) have been sectioned obliquely. The terminal web (tw) is on the same level as the terminal bar. Similarly the desmosomal web (dw) is on the same level as the desmosome. Small arrows indicate angulations of rootlet filaments in the region of the terminal web. Cytoplasmic microtubules (mt) can be seen near the desmosomal web.

Glutaraldehyde-acrolein fixation (x 52,500).


mt
tw
dw
tb
d
r
c
1

Fig. 2

A longitudinal section through the microvillous border and apical cyto-plasm.

The trilaminar apical membrane can be seen surrounding the microvilli. Note the irregular depths of the membrane between the microvilli. Terminal web (tw) filaments are seen between the rootlets (r) and on both sides of the zonula adherans (za). Note broad apical ectoplasm (ect), devoid of all organelles except filaments and few vesicles.

Glutaraldehyde-acrolein fixation (x 52,500).

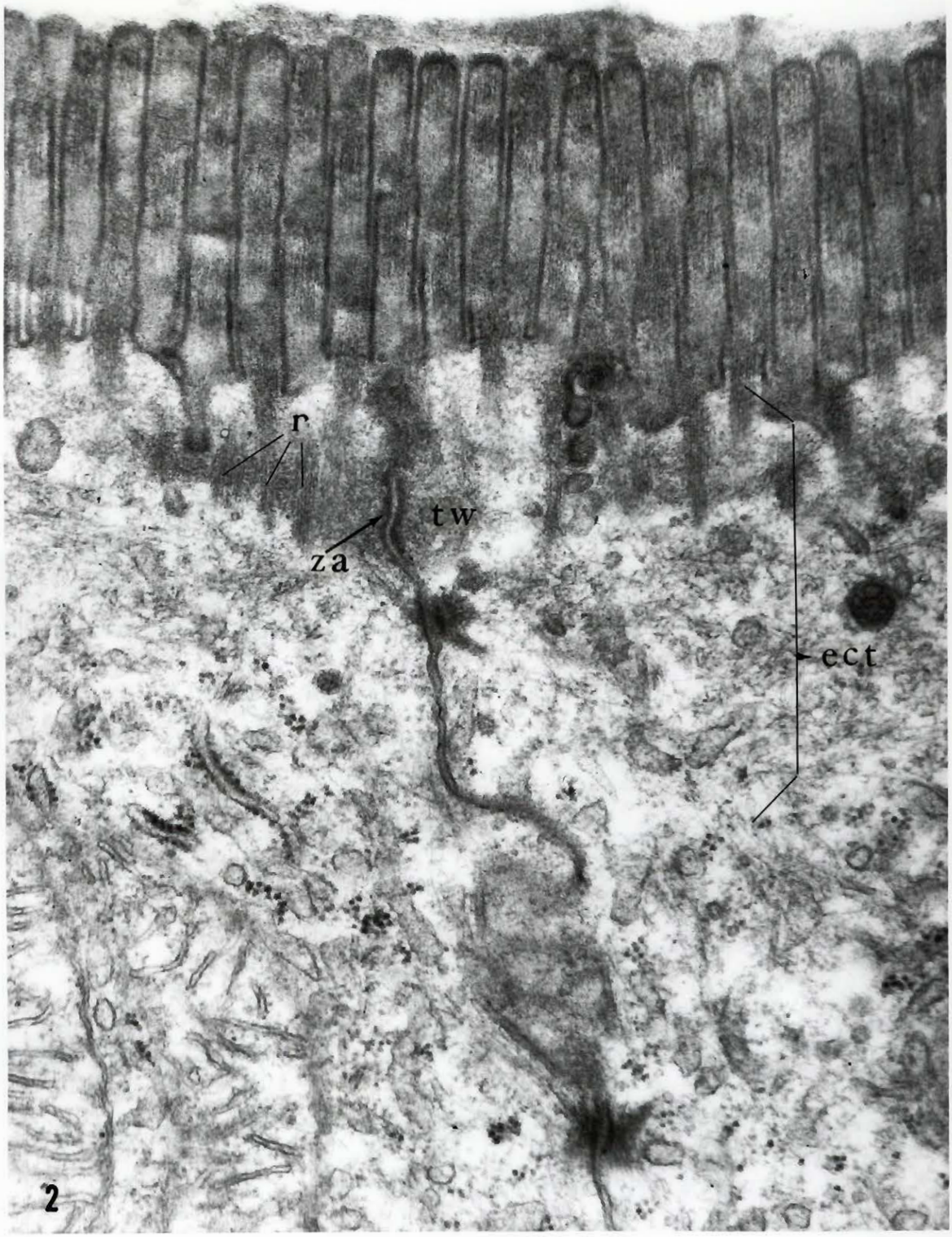

r
tw
za
ect
2

Fig. 3

Higher magnification of the microvilli.

On the outer surface of the apical plasma membrane, irregular accumulations of densely-stained material are seen. This amorphous material is concentrated at the tip of the microvillus forming a "cap". In the cytoplasm within the tip of the microvillus, a zone of increased density is seen. Filaments (fil) are embedded in this inner dense material. Attachment to the membrane itself cannot be resolved. Arrows indicate regions where trilaminar unit membrane can be seen clearly. Glutaraldehyde-acrolein fixation (x 210,000).

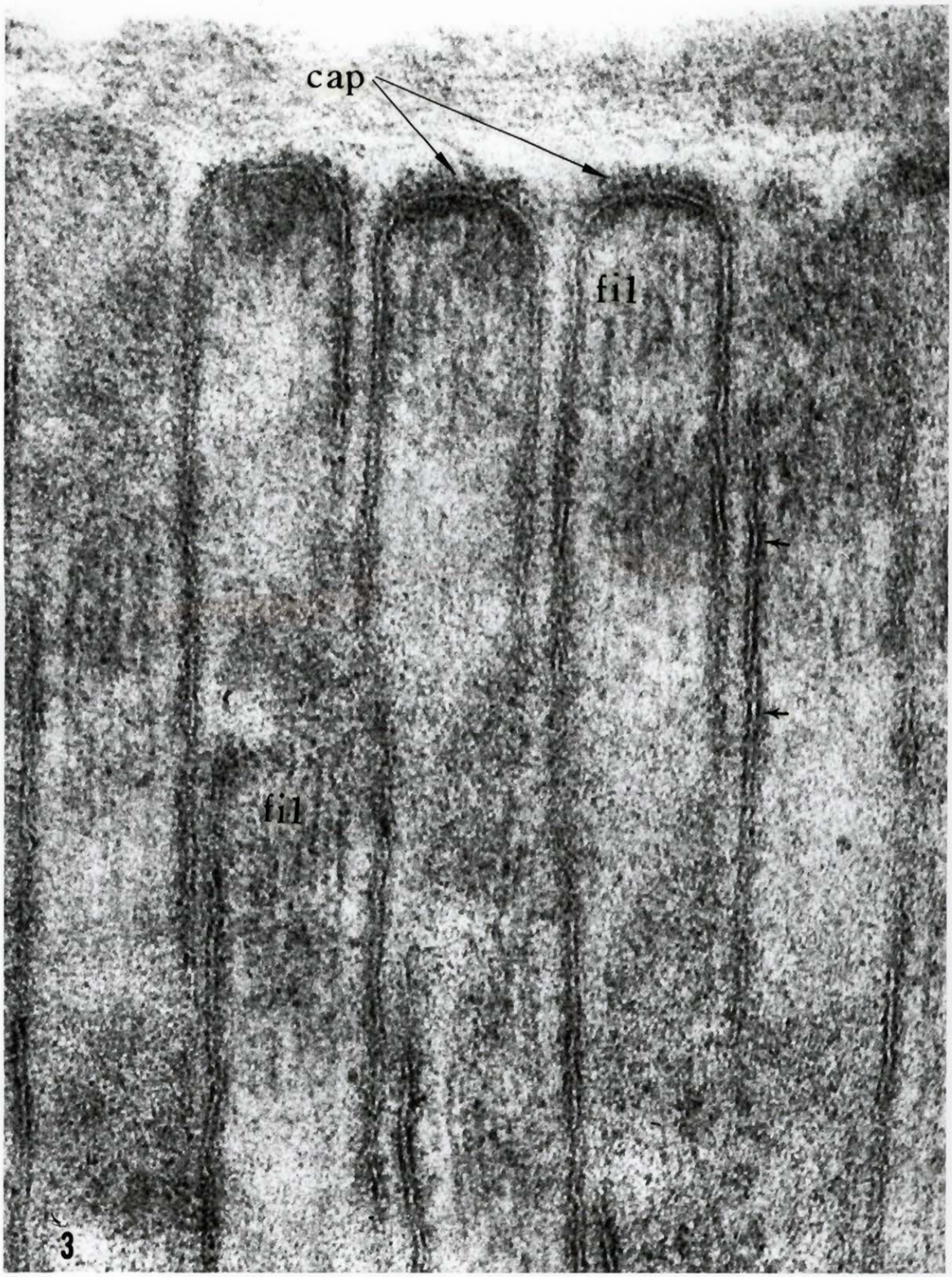

cap
fil
fil
3

Fig. 4

A cross section through microvilli, and rootlets (r) in the apical ectoplasm of a cell of the duodenal villus.

The apical plasma membrane (am) can be seen encircling the microvilli with a zone of lesser density between the membrane and the filamentous core (c). In other areas, the plasma membrane can be seen adjacent to the ectoplasm in which many rootlets can be identified. The zonula occludans (zo) of the terminal bar is illustrated.

Glutaraldehyde-acrolein fixation (x 105,000).

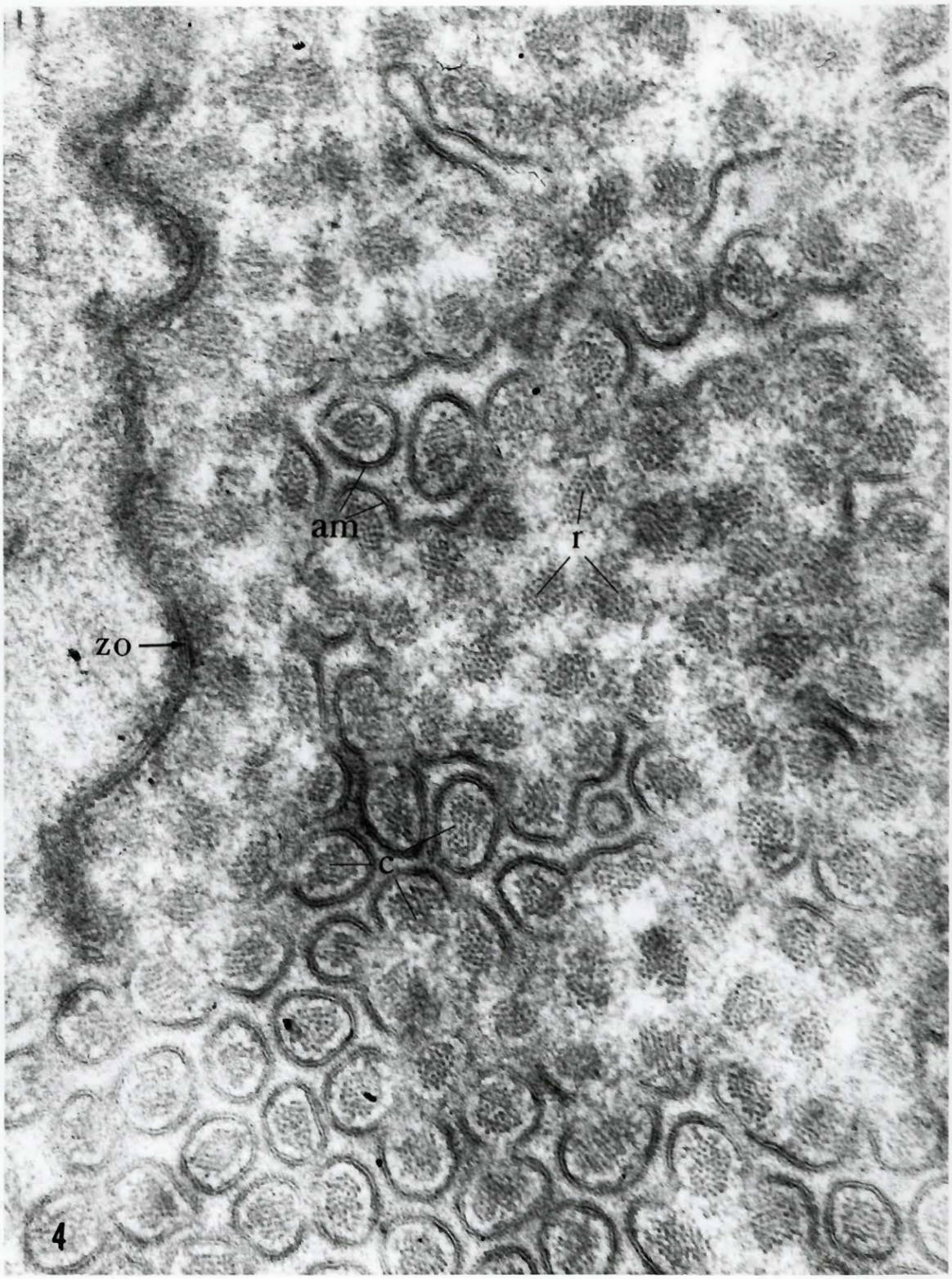

am
r
zo
c
4

Fig. 5

Longitudinal section of the microvillous border and apical cytoplasm. The terminal web (tw) is seen adjacent to the zonula adherans (za). Filaments from the desmosome can be seen merging with the terminal web. Note the parallelism of the zonula adherans and the right adjacent rootlet. Microtubules (mt) are seen in cross, oblique and longitudinal section in the desmosomal web and elsewhere in the cytoplasm. Glutaraldehyde-acrolein fixation (x 52,500).


tw
za
dw
mt
5

Fig. 6

A cell from the mid point of a villus.

The number of microvilli has reached the mature value although the microvilli are shorter (0.8 u) than mature microvilli (1 u). The cores have their full complement of filaments. The terminal web (tw) is fully developed and can be seen in between the rootlets. Angulations of the rootlet filaments are seen (arrows). A microtubule (mt) is seen between the desmosome and terminal webs. Desmosome web filaments (dw) are dispersed and disorganized.

Granular and agranular ER, free ribosomes and mitochondria can be seen in the lower left.

Glutaraldehyde-acrolein fixation (x 52,500).

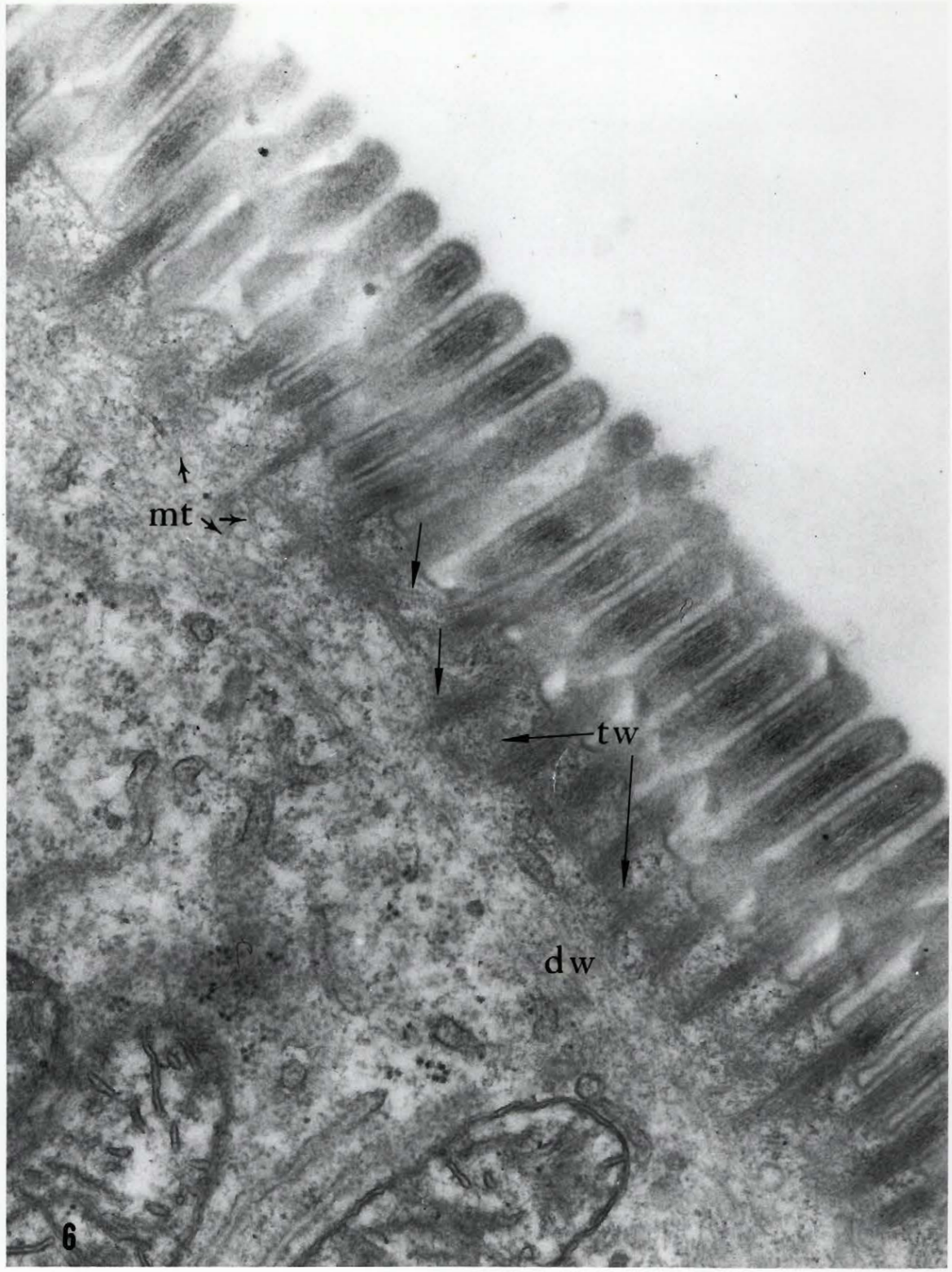

mt
tw
dw
6

Fig. 7

An oblique section through the striated border.

The terminal web (tw) can be seen connecting the rootlets. The desmosomal filaments (dw) are straight.

Glutaraldehyde-acrolein fixation.

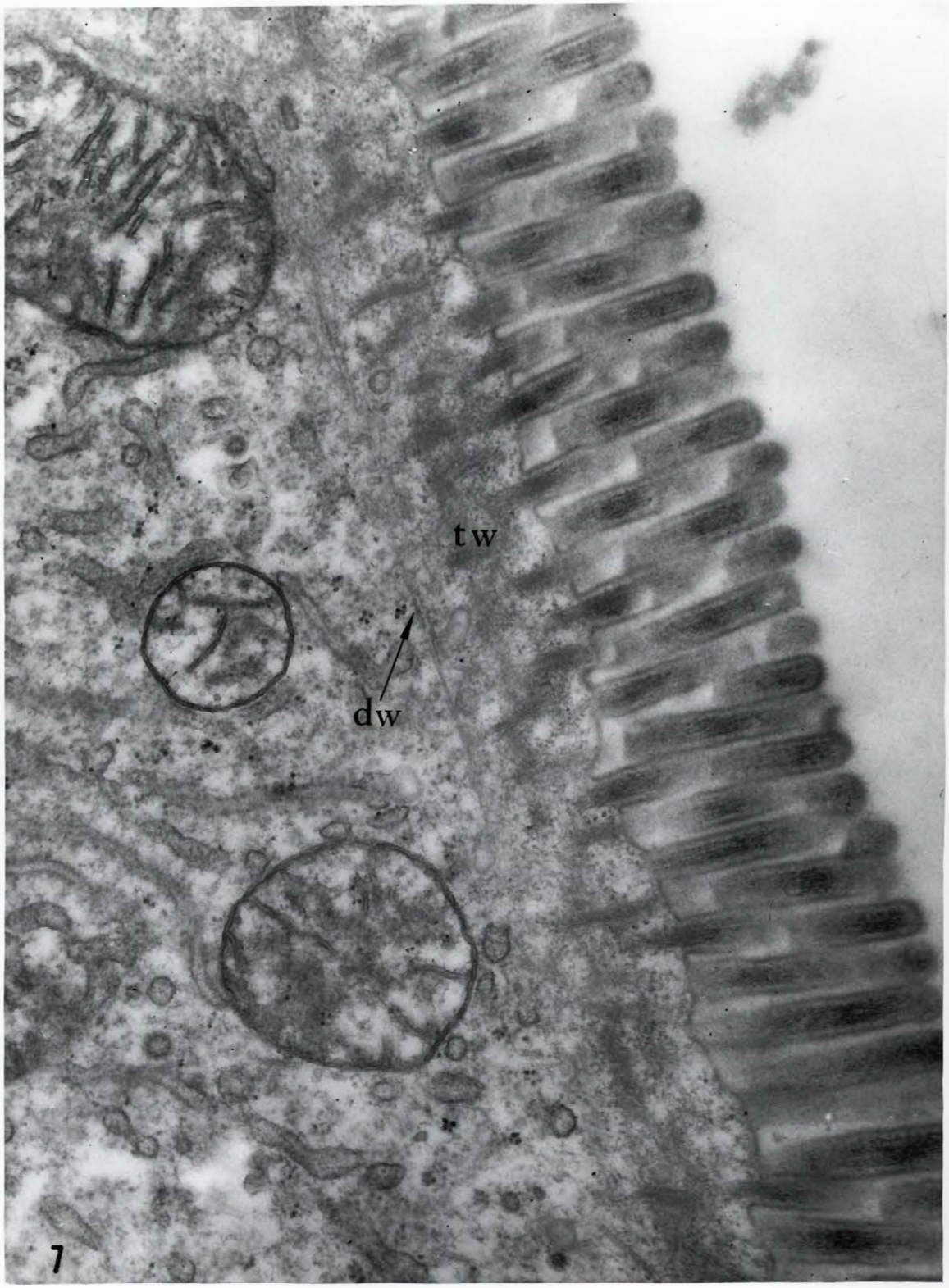

tw
dw
7

Fig. 8

Longitudinal section through the striated border of a mature villus cell.

The rootlets (r) are clearly shown as extensions of the cores (c). Rootlets can be seen to bend and join with adjacent rootlets. A multivesicular body (mvb) lies in the disoriented desmosome web. Cross sections of filaments can be seen in the desmosome web. Microtubules are seen (small arrows).

Glutaraldehyde-acrolein fixation (x 47,200).


c
r
mvb
8

Fig. 9

A longitudinal section of a villous cell.

The bases of several rootlets (r) can be seen merging to form a cone-shaped structure. One group of filaments is directed toward the right terminal bar. The desmosome web is hardly in evidence. A multivesicular body (mvb) is seen in the apex. Microtubules (mt) are seen in cross and longitudinal section.

Glutaraldehyde-acrolein fixation (x 52,500).

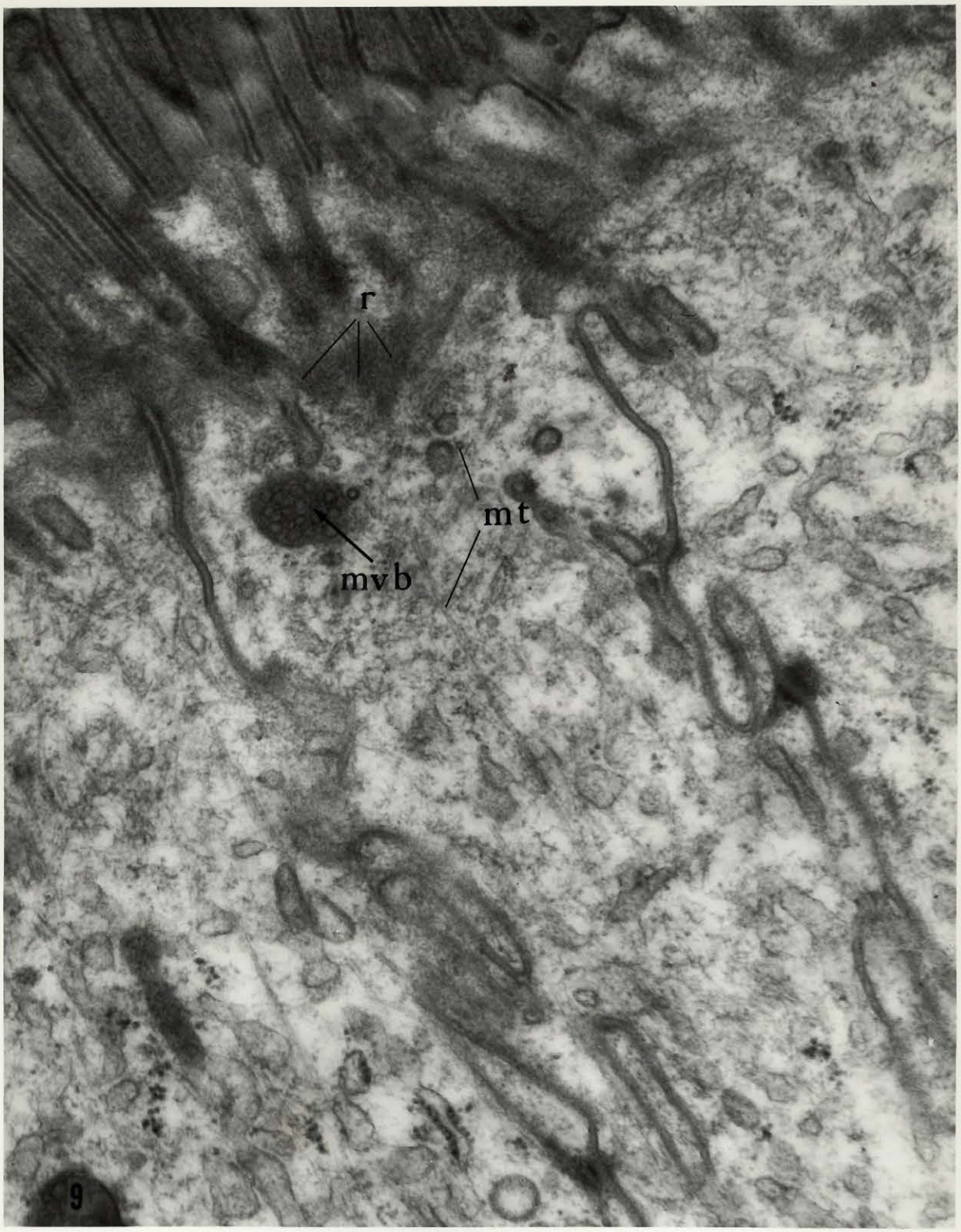

r
mt
mvb
9

Fig. 10

A section of a lower crypt cell.

The terminal web (tw) is seen stretching across the apical margin of the cell. A single microvillus projects from the cell while others appear in oblique section. Amorphous material is adherant to the apical membrane. No apical desmosomes are present, yet desmosomal filaments (dw) can be seen to the left. Many free ribosomes characteristic of undifferentiated cells are illustrated. Nucleus (N) is seen in lower right.

Glutaraldehyde-acrolein fixation (x 49,000).

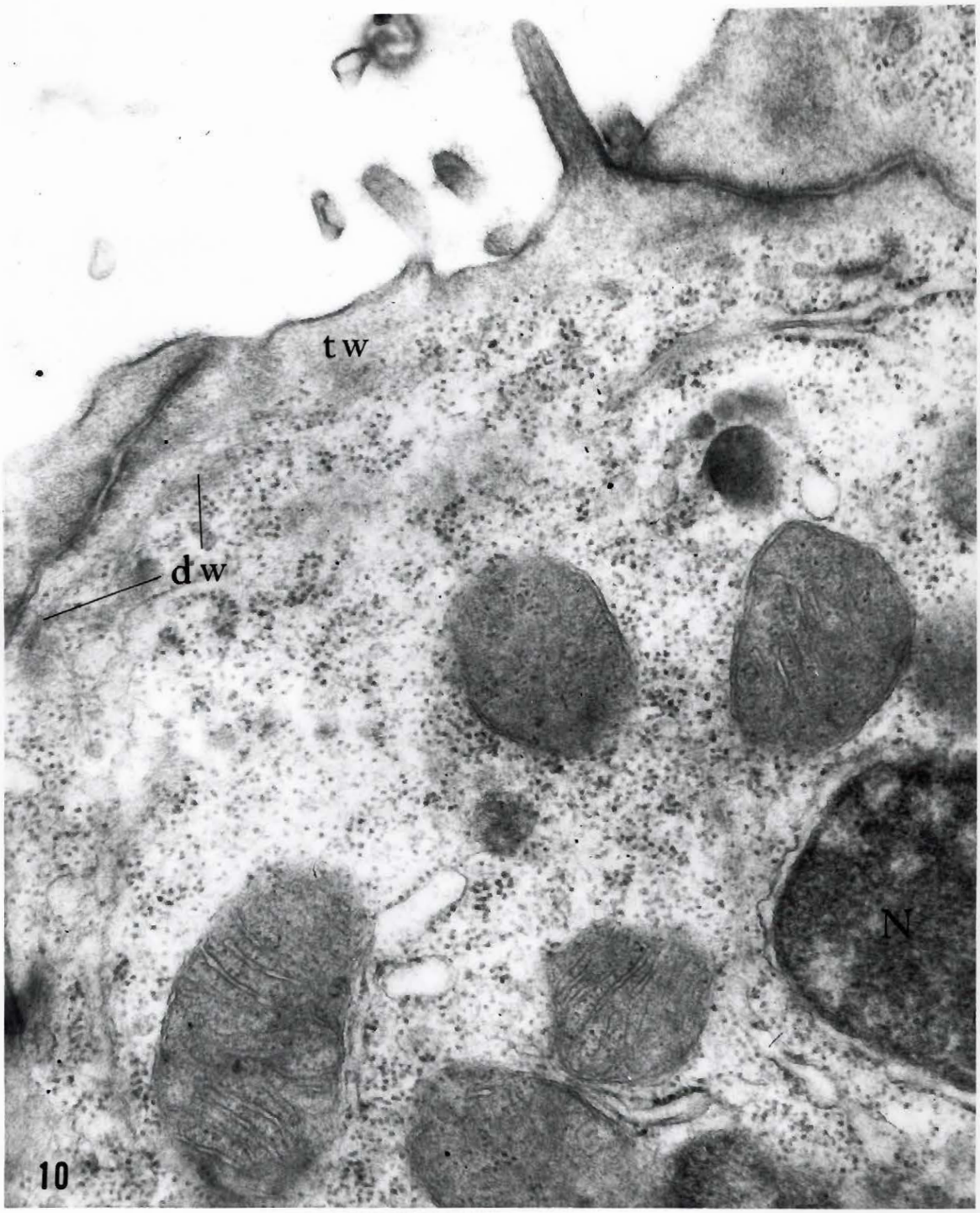

tw
dw
N
10

Fig. 11

Longitudinal section through the apex of crypt undifferentiated cells. Two microvilli from adjacent cells can be seen at the upper left. The outward projecting microvilli near the terminal bar is a common feature in the crypt. A rootlet (r) can be seen merging with the terminal web (tw) at an acute angle. Apical desmosomes web filaments (dw) are continous with the lateral web. Vacuoles (v) containing absorbed material are seen in apex and near lateral membrane and elsewhere. Two desmosomes are seen.

Glutaraldehyde-acrolein fixation (x 52,500).

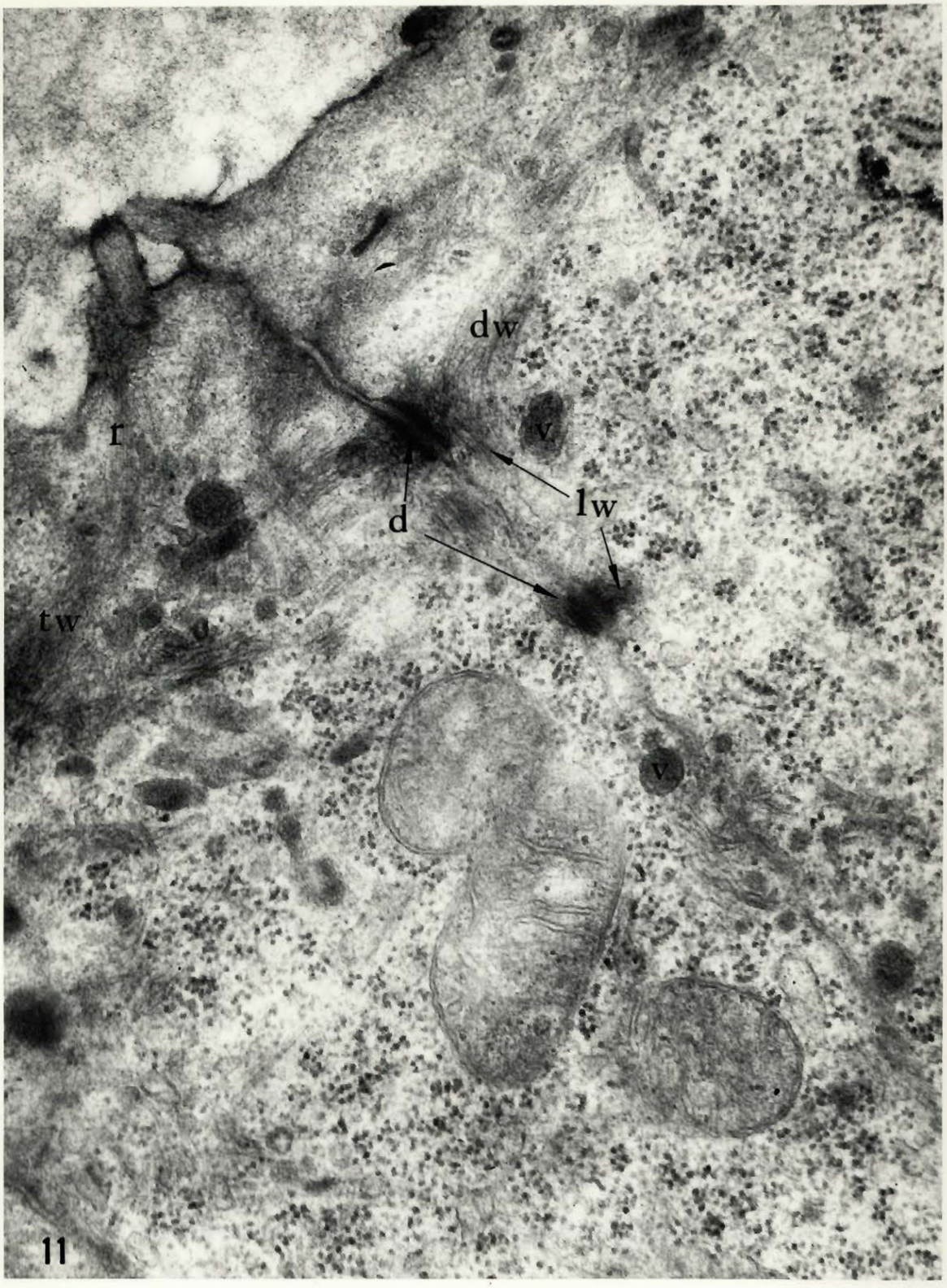

dw
r
v
lw
d
tw
v
11

Fig. 12

Survey micrograph of a cross section of portion of a crypt close to the crypt-villus junction.

An intermediate number of microvilli are seen. The length of the rootlets is twice the height of the microvilli. Note also that the length of the zonula occludans (zo) is much longer. All the organelles of the apical cytoplasm are seen here: multivesicular bodies (mvb), centriole (ct), microtubules (mt), mitochondria, granular ER, free ribosomes, and vesicles (v) with moderately dense material. Glutaraldehyde-acrolein fixation (35,000).

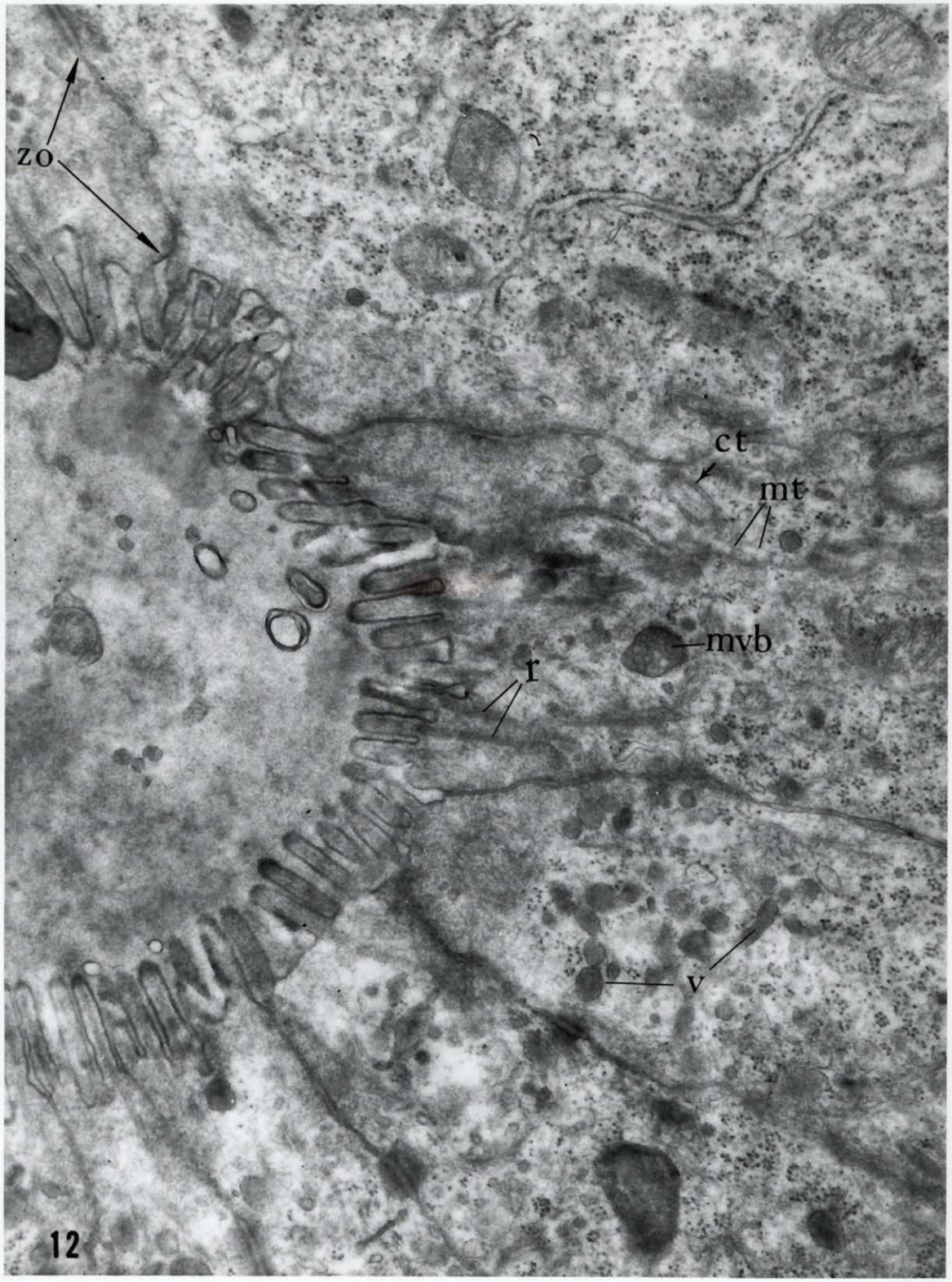

zo
ct
mt
mvb
r
v
12

Fig. 13

An oblique section through a crypt cell.

Microvilli are seen in longitudinal, oblique and cross section. There seems to be less than 20 filaments per core (c). A large group of microtubules (mt) are seen in cross section and oblique section in the terminal web. These tubules measure approximately 230 A in outside diameter and have approximately 120 A lumen. Thus the wall is approximately 60 A thick.

Glutaraldehyde-acrolein fixation (x 52,500).

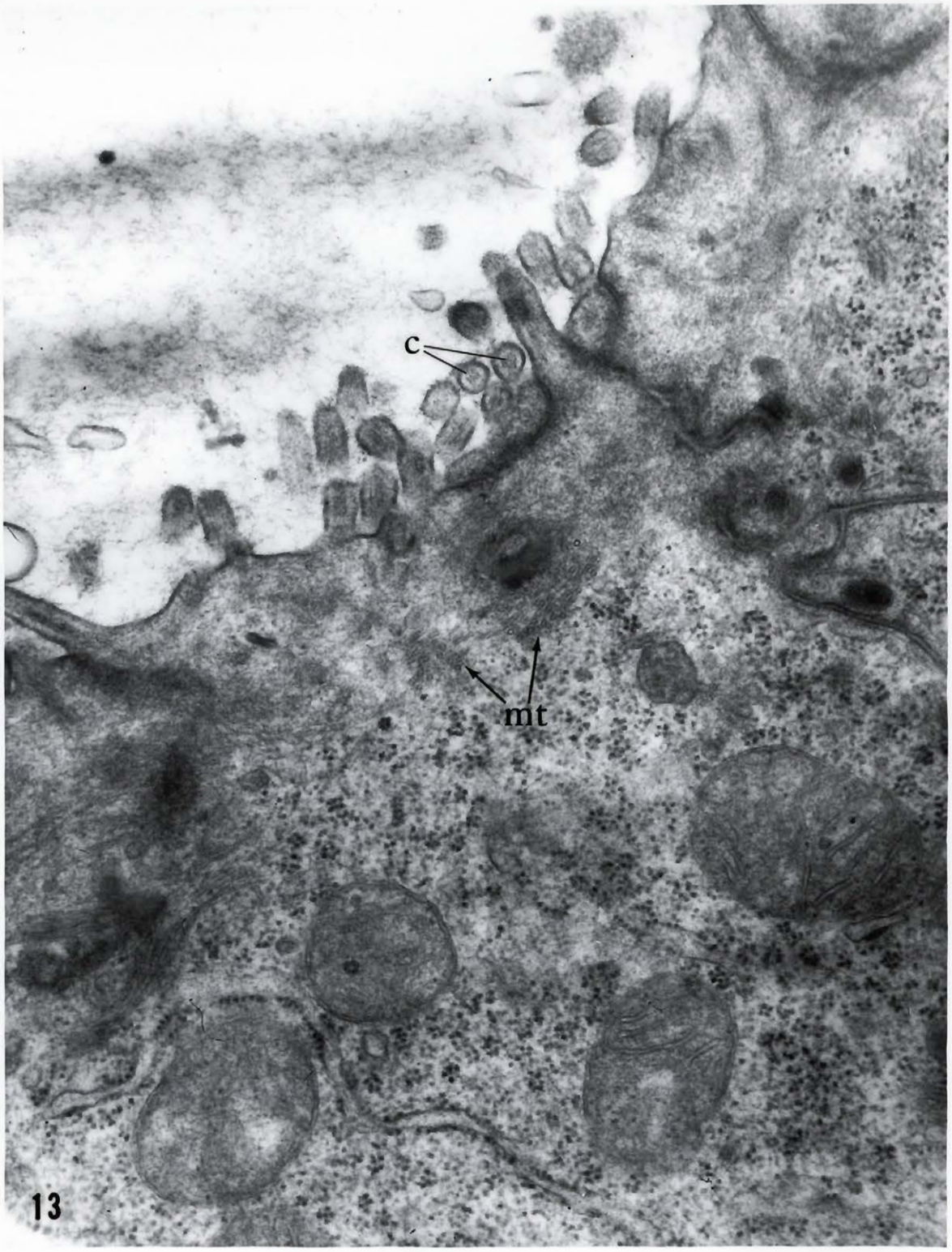

c
mt
13

Fig. 14

The apex of three crypt cells.

The two zonulae of the terminal bar (zo, za) are illustrated. Filamentous material adjacent to the zonula adherans (za) seems to form a plaque. The desmosome web (dw) is seen connecting the desmosomes (d) on opposite sides of the cell. Filaments attach to the cytoplasmic plaque (cp). Filaments on opposite sides of the apical desmosome are running in perpendicular directions. Filaments to the left are parallel to the cell axis, while those on the right are transversely oriented.

Microtubules are seen in long and cross section (small arrows).

Glutaraldehyde-acrolein fixation (x 52,500).

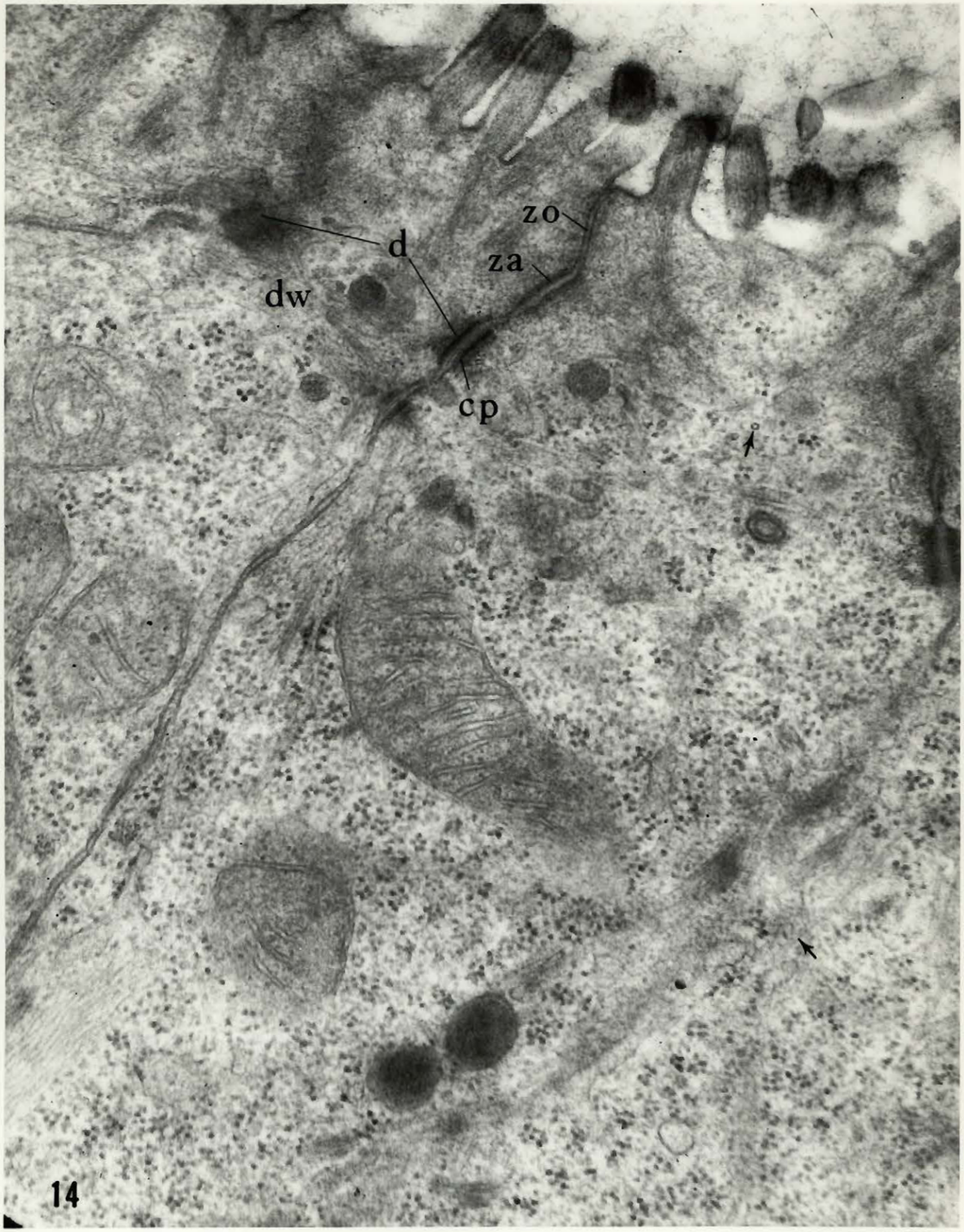

zo
d
za
dw
cp
14

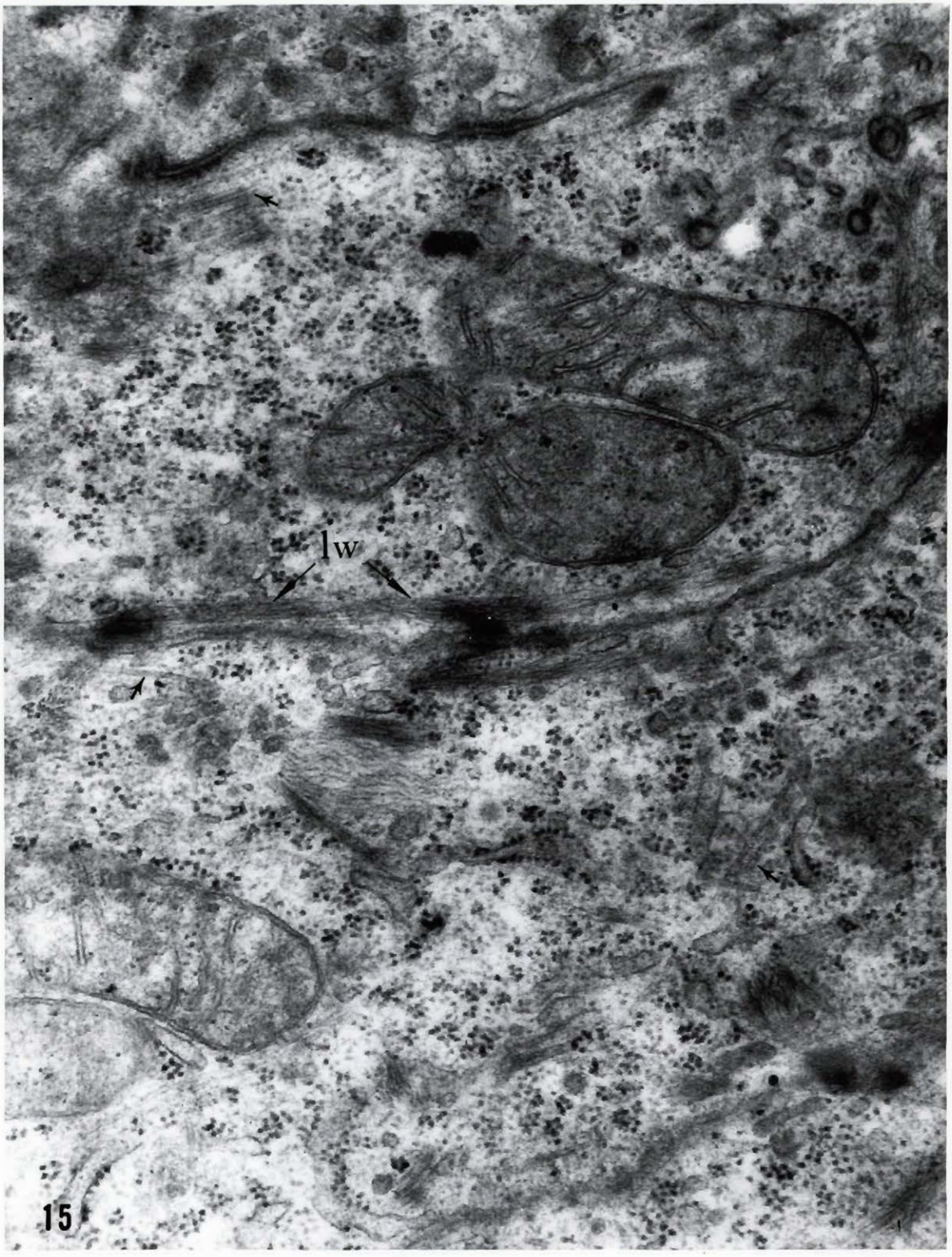
Fig. 15

The supranuclear region of three crypt cells.

The lateral web (lw) is illustrated stretching between desmosomes.

Numerous microtubules are seen (small arrows).

Glutaraldehyde-acrolein fixation (x 52,500).



15

Fig. 16

The basal region of two crypt cells.

Filaments of the basal web (bw) can be identified along the basal membrane. Microtubules are seen (small arrows).

Glutaraldehyde-acrolein fixation (x 52,500).

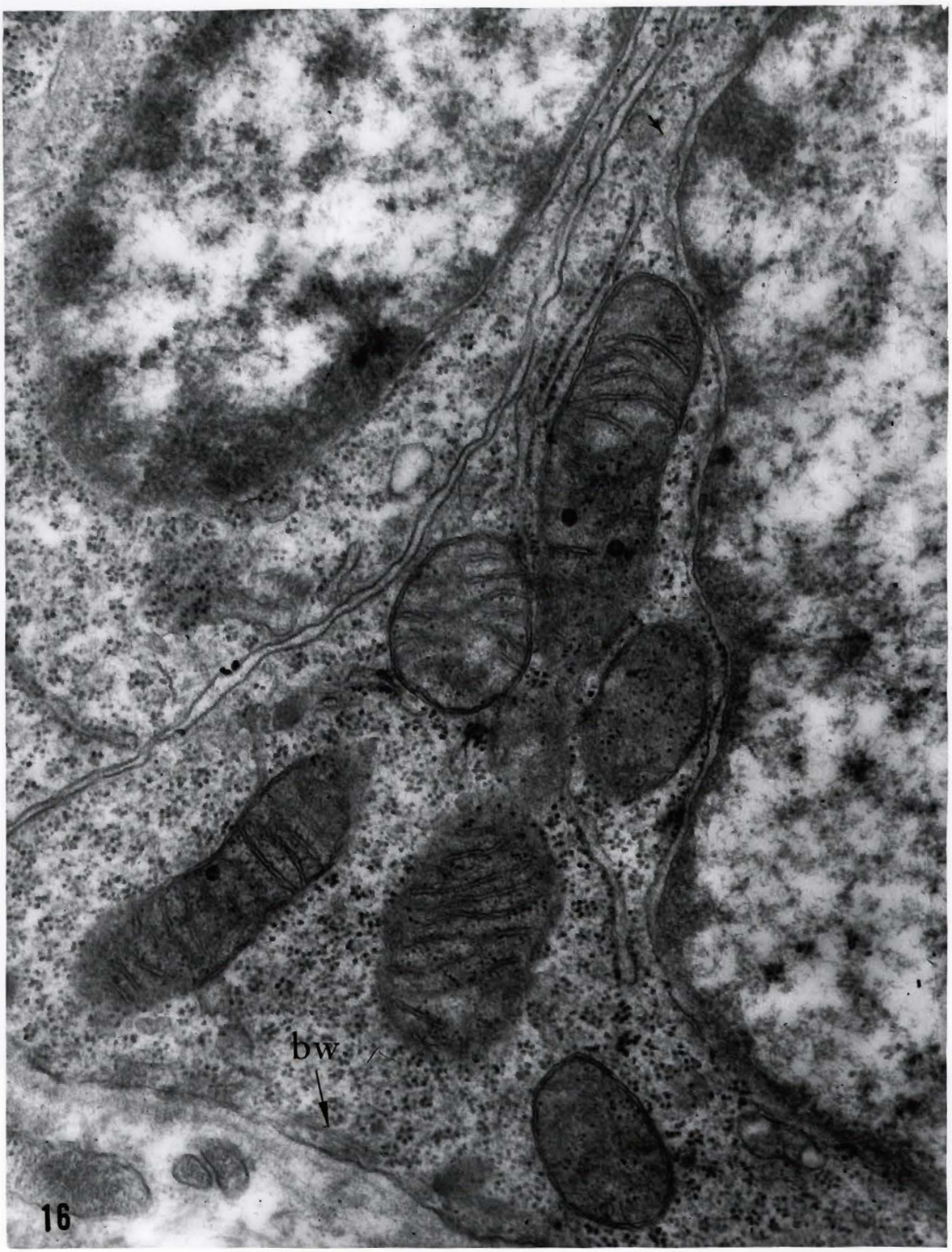

bw
16

Fig. 17

The basal (infranuclear) region of two crypt cells.

A bundle of filaments of the lateral web (lw) can be seen entering a cytoplasmic extension. Basal web (bw) is seen in cross and longitudinal section. Filaments can even be seen in the very end of the process.

A microtubule on the right merges with the web filaments (arrow). Note the absence of any specialized junction or terminal bar at the basal intercellular region. The basement membrane (bm) can be seen between the fibrocytes (fc) and the epithelial cells.

Glutaraldehyde-acrolein fixation (x 52,500).

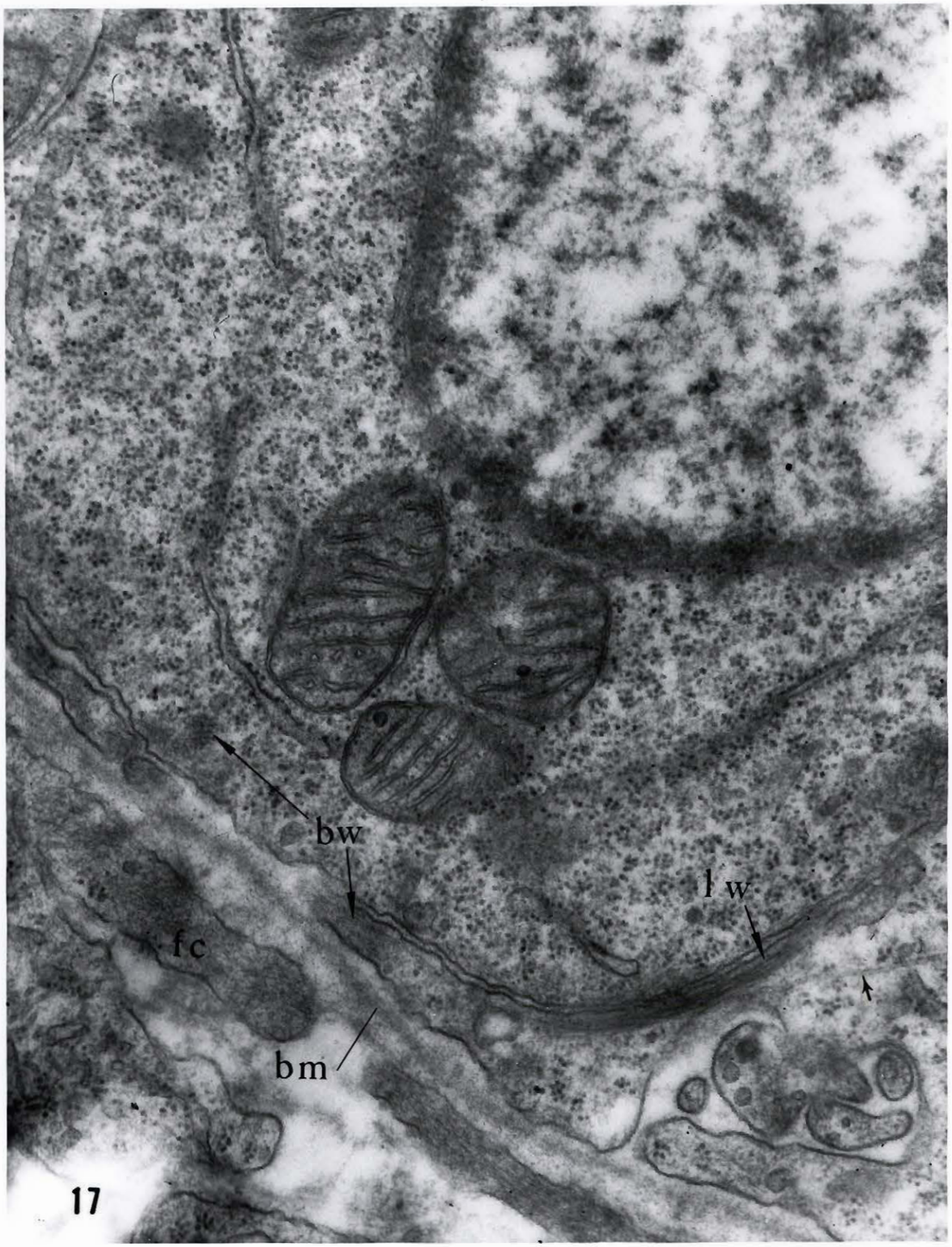

bw
lw
fc
bm
17

Fig. 18

A longitudinal section through the basal region of epithelial cells toward the apex of the villus.

A macrophage (mg) can be seen inserting itself between the epithelial cells. Goblet cells can be recognized in having mucigen granules, more elaborate endoplasmic reticulum with a moderately-dense staining cisternal content, a more prominent Golgi zone, a denser background cytoplasm, and distinctive mitochondria. Toward the lower left, the intercellular space (is) has become prominent. Lacterals (l) and lamina propria elements can be seen in lower right. The basal web (bw) can be seen running along basal part of cells. The basement membrane (bm) is continuous.

Glutaraldehyde-acrolein fixation (x 7,800).

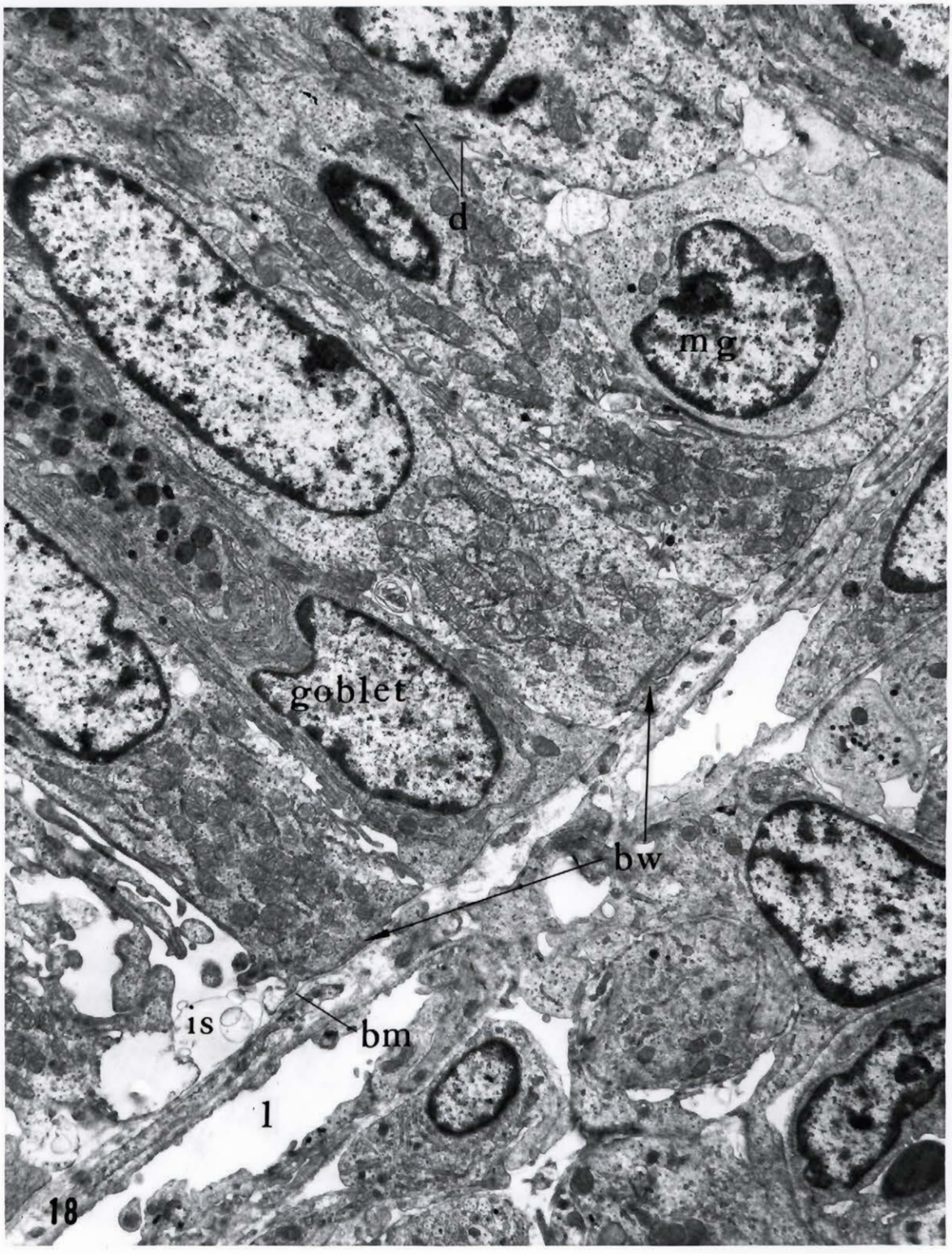

d
mg
goblet
bw
is
bm
l
18

Fig. 19

A longitudinal section through the bases of several cells toward the apex of the villus.

Although the cells are still closely fitted together, evidence for the breakdown of intercellular adhesion can be seen by the insertion into the epithelium of a long pseudopod of a wandering macrophage (mg). The basement membrane (bm), still relatively intact at this level, is broken where the macrophage (mg) has penetrated. Notice the infranuclear clump of mitochondria. A lacteal (l) is seen in the lamina propria. Glutaraldehyde-acrolein fixation (x 7,000).

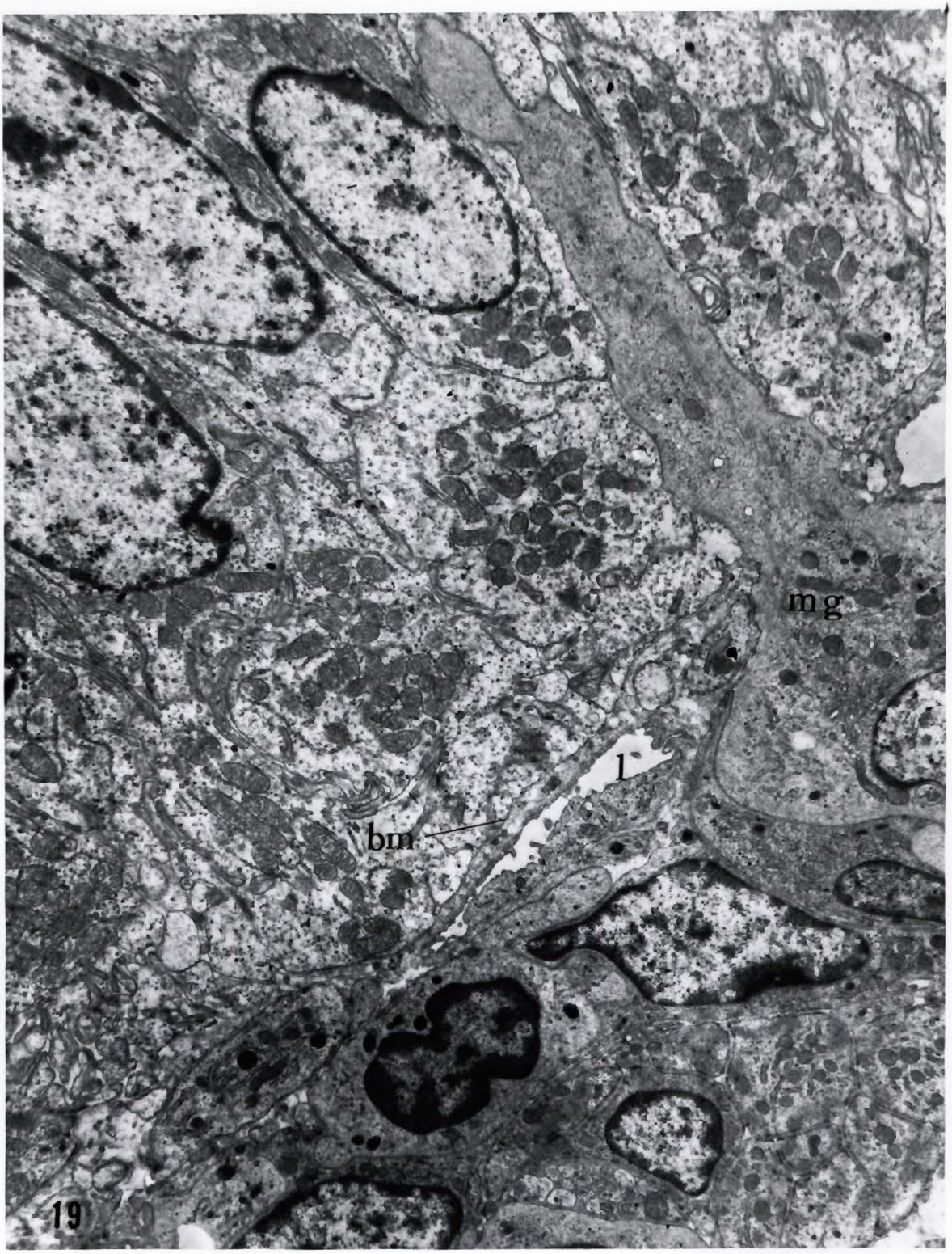

mg
l
bm
19

Fig. 20

A longitudinal section of a crypt cell.

Strongly angulated microvilli, terminal web (tw), desmosome web (dw) and lateral web (lw) are illustrated. Immediately beneath the desmosome web and partially attached to it appears an unusual filamentous structure (large arrow). The filaments, coarser than the regular desmosome filaments, are well separated by a material of light density. The filaments have a slightly wavy configuration. The more densely packed filaments of the desmosome web are seen adherant to the upper aspect. This structure is 0.4 u wide. Microtubules (mt) are seen in longitudinal and cross section.

Glutaraldehyde-acrolein fixation (35,000).

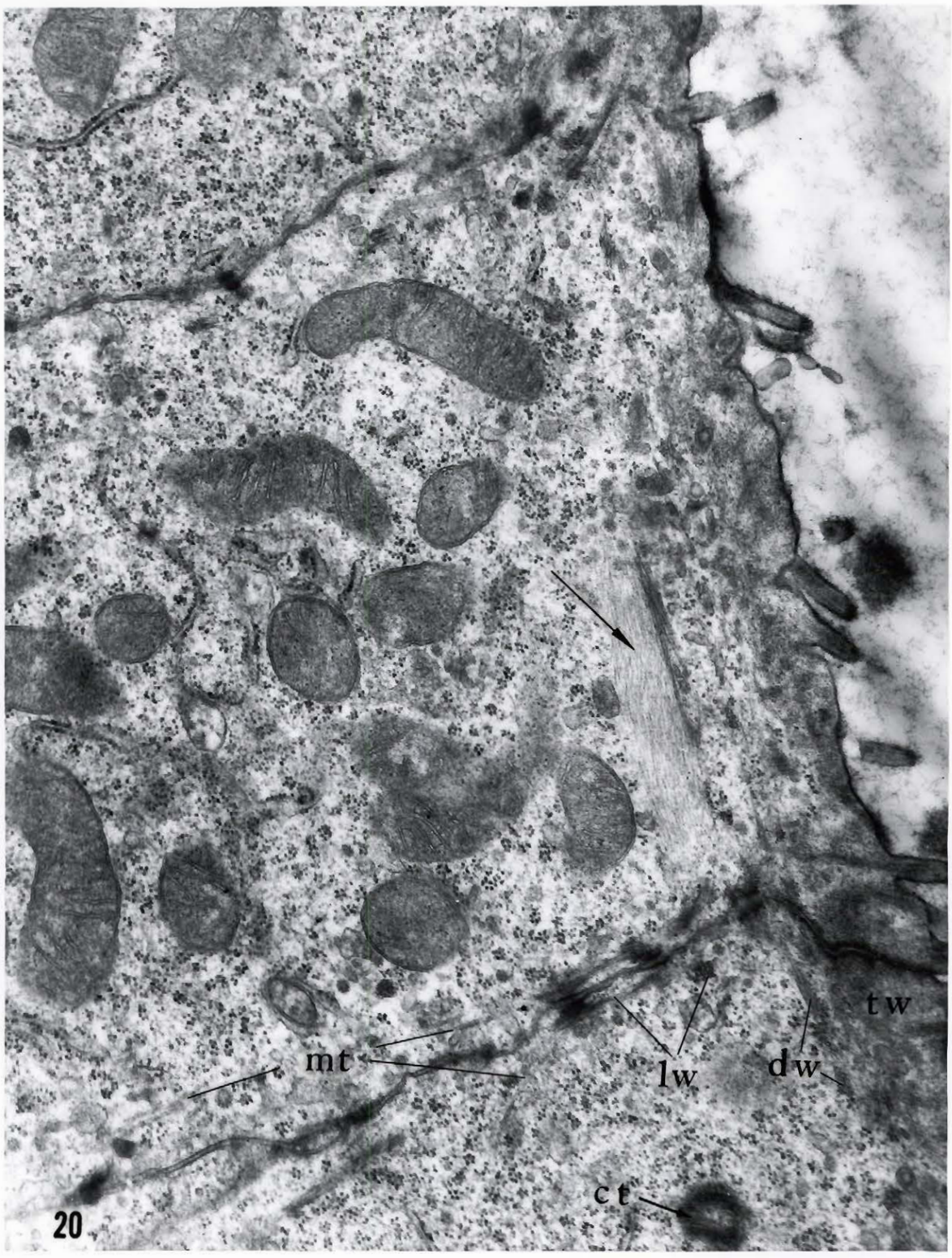

tw
mt
lw
dw
ct
20

Fig. 21

The supranuclear region of a crypt cell.

Another wavy filamentous structure is illustrated (large arrows). On one side, regular lateral web filaments (lw) can be seen branching into three groups. Microtubules (small arrows) are seen in the vicinity. The wavy filaments within this structure are coarser than the regular web filaments.

Glutaraldehyde-acrolein fixation (x 52,500).

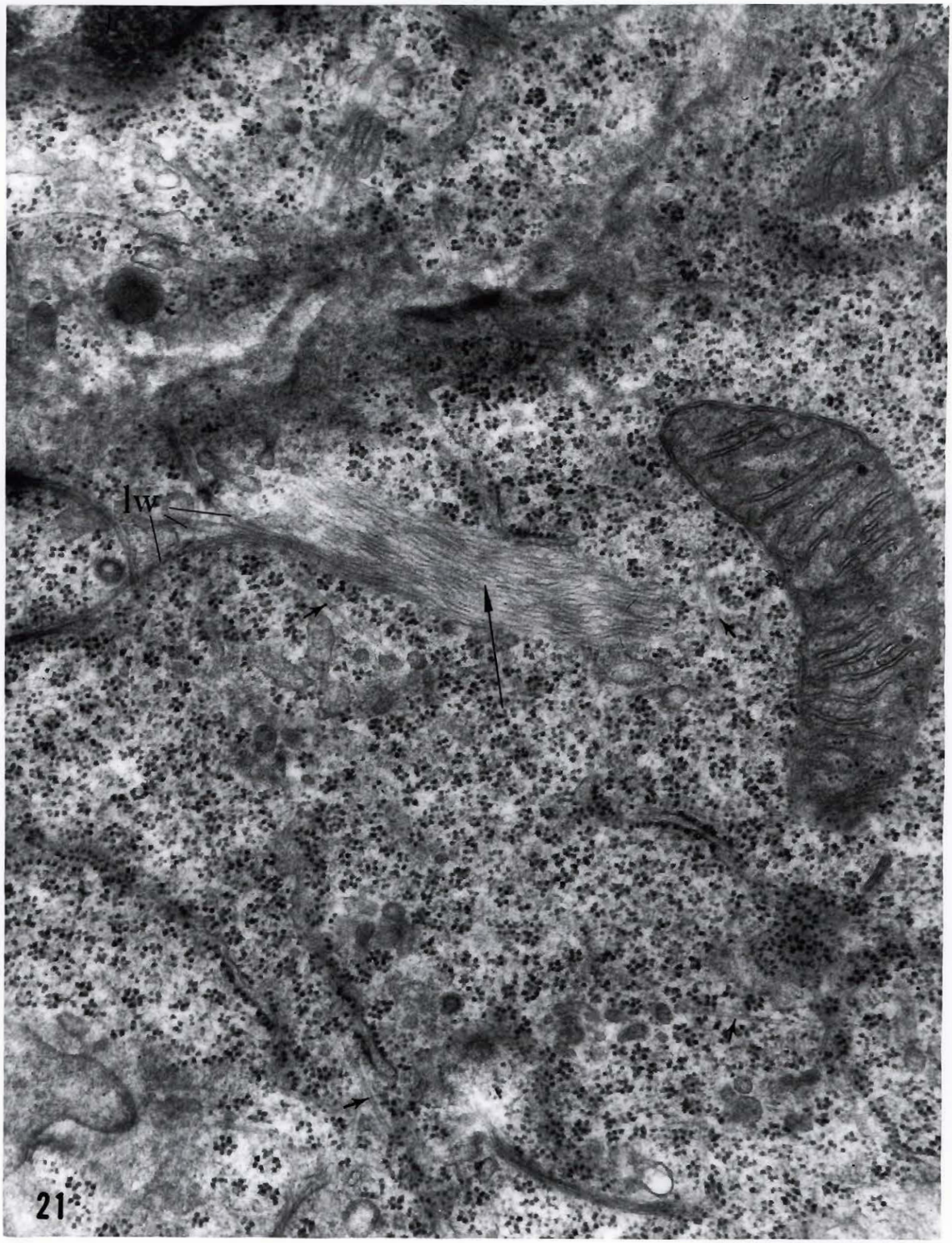

lw
21

Fig. 22

A wavy structure near the lateral membrane.

Filaments coming directly from the desmosome (d) can be seen merging with the structure. It is the same width as the previous structures, 0.4 u. Several microtubules (mt) are seen in the adjacent cell.

Glutaraldehyde-acrolein fixation (x 52,500).

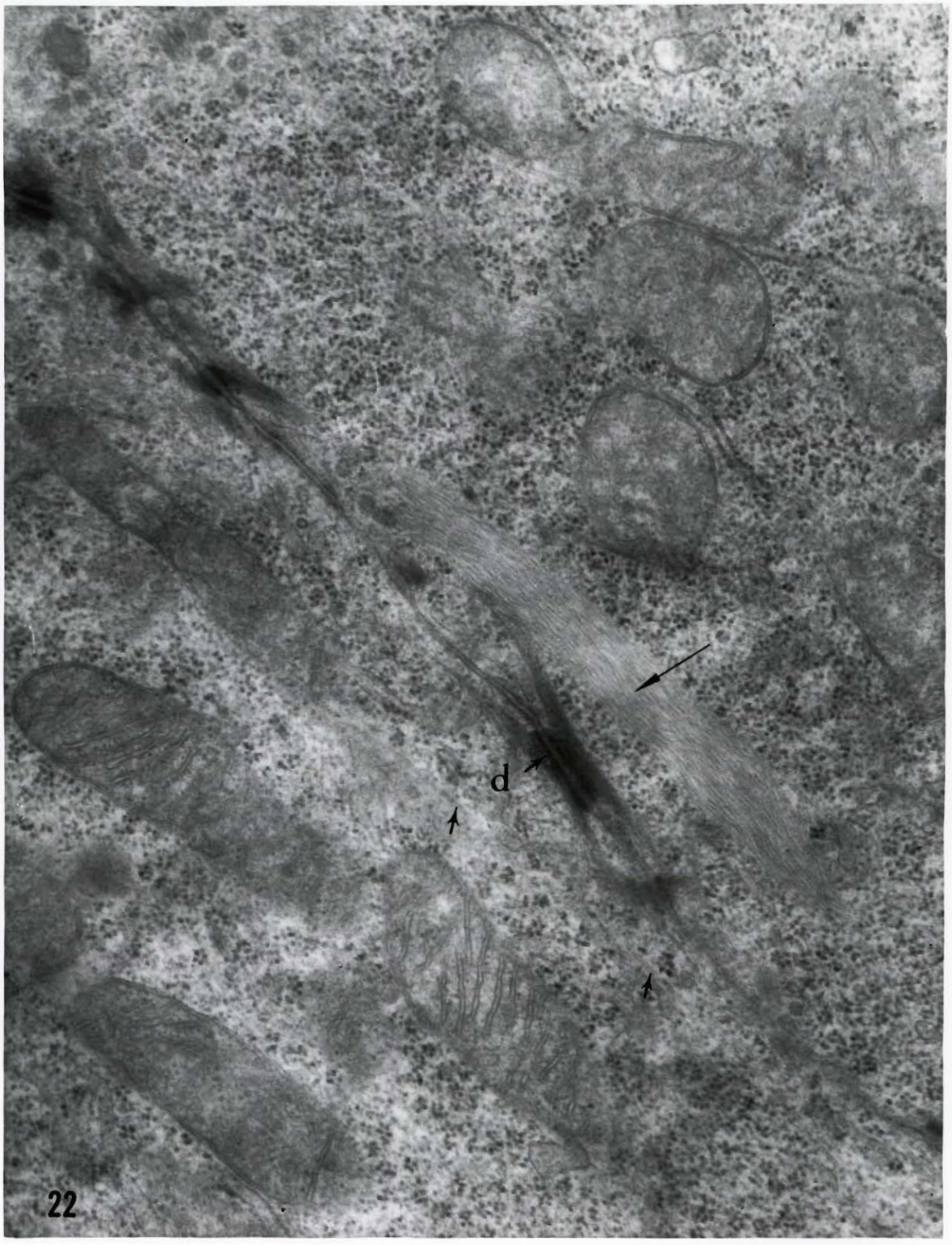

d
22

Fig. 23

A high resolution micrograph of the terminal bar complex.

The zonula occludans (zo) is the most apical junction. The outer leaflets of the membrane within the zone can be seen to fuse and separate, thus obliterating the intercellular space. A thin layer of filaments is applied to the inner leaflet. The zonula adherans (za) has a true intercellular space of approximately 200 A. More extensive filamentous material (tw) is associated with this junction. Occassionally these filaments will condense to form a dense plaque parallel to but separated from the inner leaflet. A short segment of apposed membranes separates the zonula adherans from the desmosome. The desmosome has an intercellular space of approximately 230 A, bisected longitudinally by an intermediate dense plaque -50 A thick (idp). The inner and outer leaflets are denser in this zone. Applied to the cytoplasmic surface of the desmosome is a dense plaque (cp) slightly separated from the inner leaflet. Filaments attach to this plaque.

Glutaraldehyde-acrolein fixation (x 105,000).


zo
r
za
t w
d
idp
cp
23

Fig. 24

The terminal bar complex in longitudinal section.

Within the zonula occludans (zo) the two outer dense leaflets can be seen to merge to form the fusion line. The zonula adherans (za) immediately below is not sectioned perfectly normal to the membrane. Filamentous material is more extensive adjacent to the zonula adherans. The desmosomal web (dw) can be seen to the right.

Glutaraldehyde-acrolein fixation (x 52,500).

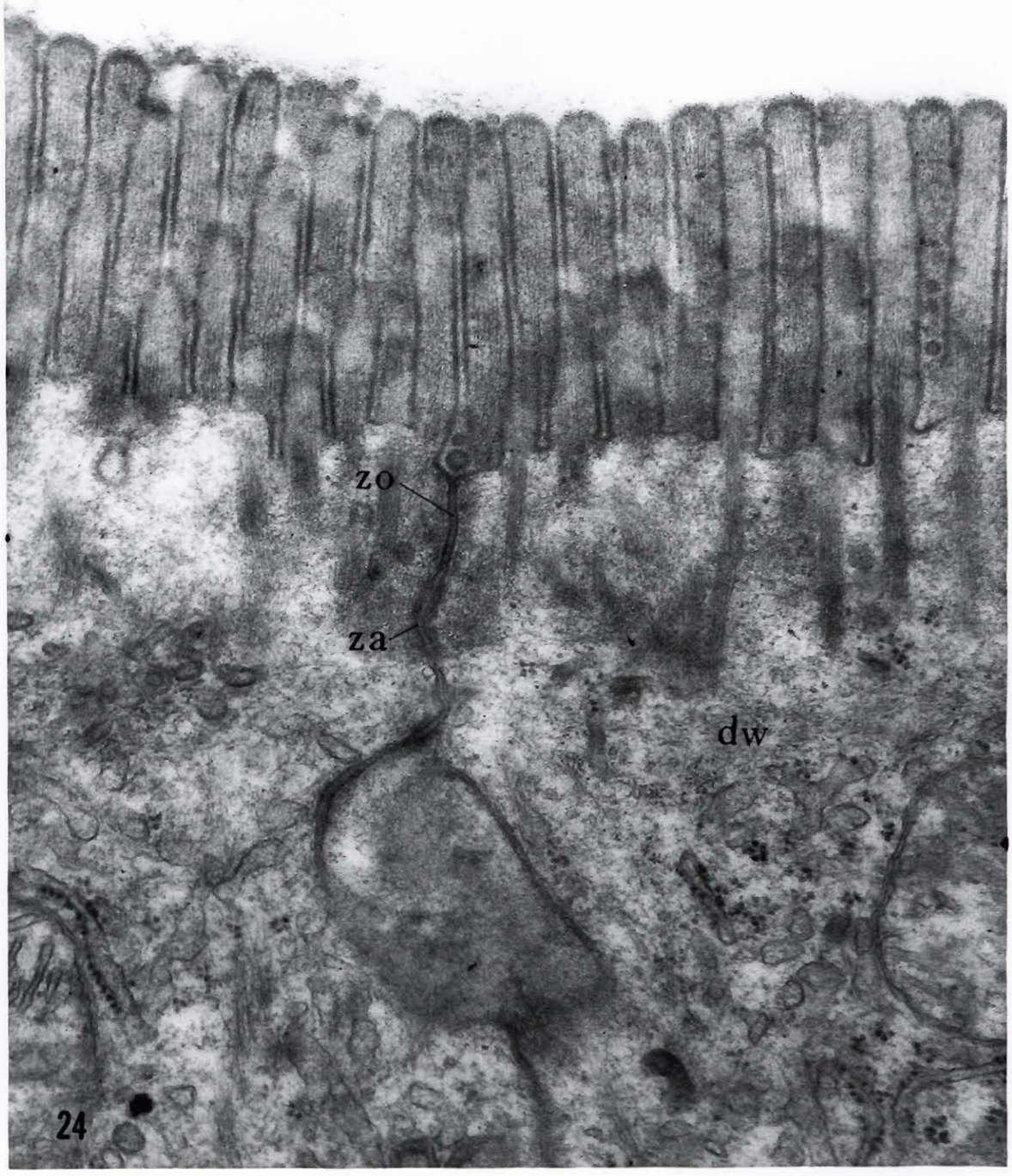

zo
za
dw
24

Fig. 25

An oblique section of an intercellular junction in the crypt is illustrated.

The zonula occludans (zo) can be identified. The dense, striated material indicated by arrows to the right of the zonula occludans is interpreted as a grazing section of the filamentous material adjacent to the two zonulae. This would indicate that the filaments adjacent to the terminal bar do not form an homogenous, thick mat but are separated by light zones of fairly constant width. The striations are parallel to the axis of the cell. Thus the filaments would attach a short segment of their length of the zonula adherans; the segments touching the terminal bar being roughly parallel to the cell's axis.

Glutaraldehyde-acrolein fixation (52,500).

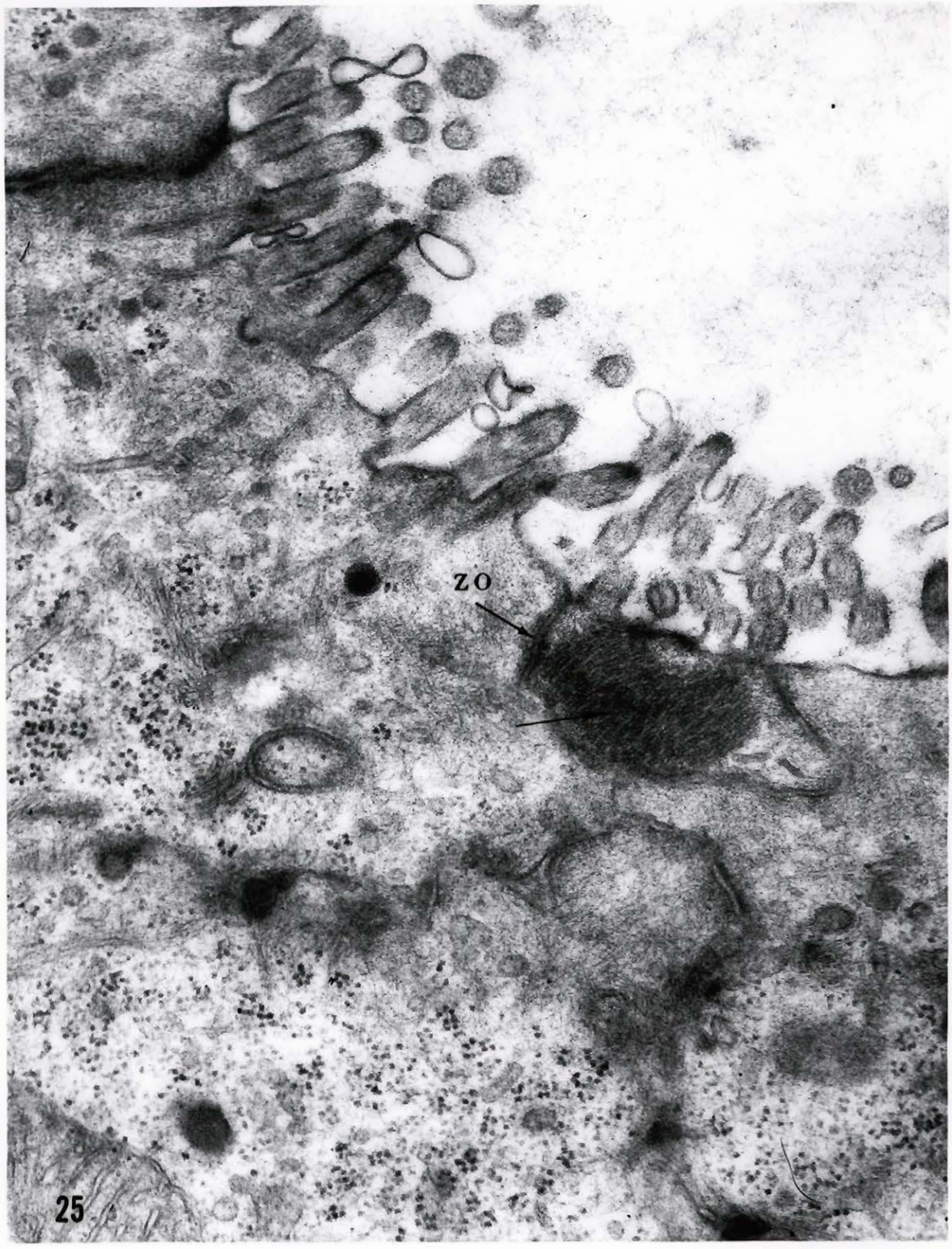

ZO
25

Fig. 26

A high resolution micrograph of a desmosome.

The desmosome has a regular intercellular space of 230 A. The intermediate dense plaque (idp), approximately 50 A wide, is situated midway between the two outer leaflets. The three lamina of the unit membrane are clearly seen within the desmosome. Bridges can sometimes be seen connecting the intermediate dense plaque and the outer leaflets (d). Note that no dense material traverses the central light space of the membrane. The dense cytoplasmic plaque (cp), approximately 150 A thick, is separated from the inner leaflet (il). It is connected to the inner leaflet by means of short bridges placed at regular intervals. Filaments seen here in longitudinal section are arranged parallel to the cytoplasmic plaque. Note the innermost filaments do not touch the cytoplasmic plaque.

Glutaraldehyde-acrolein fixation (x 105,000).

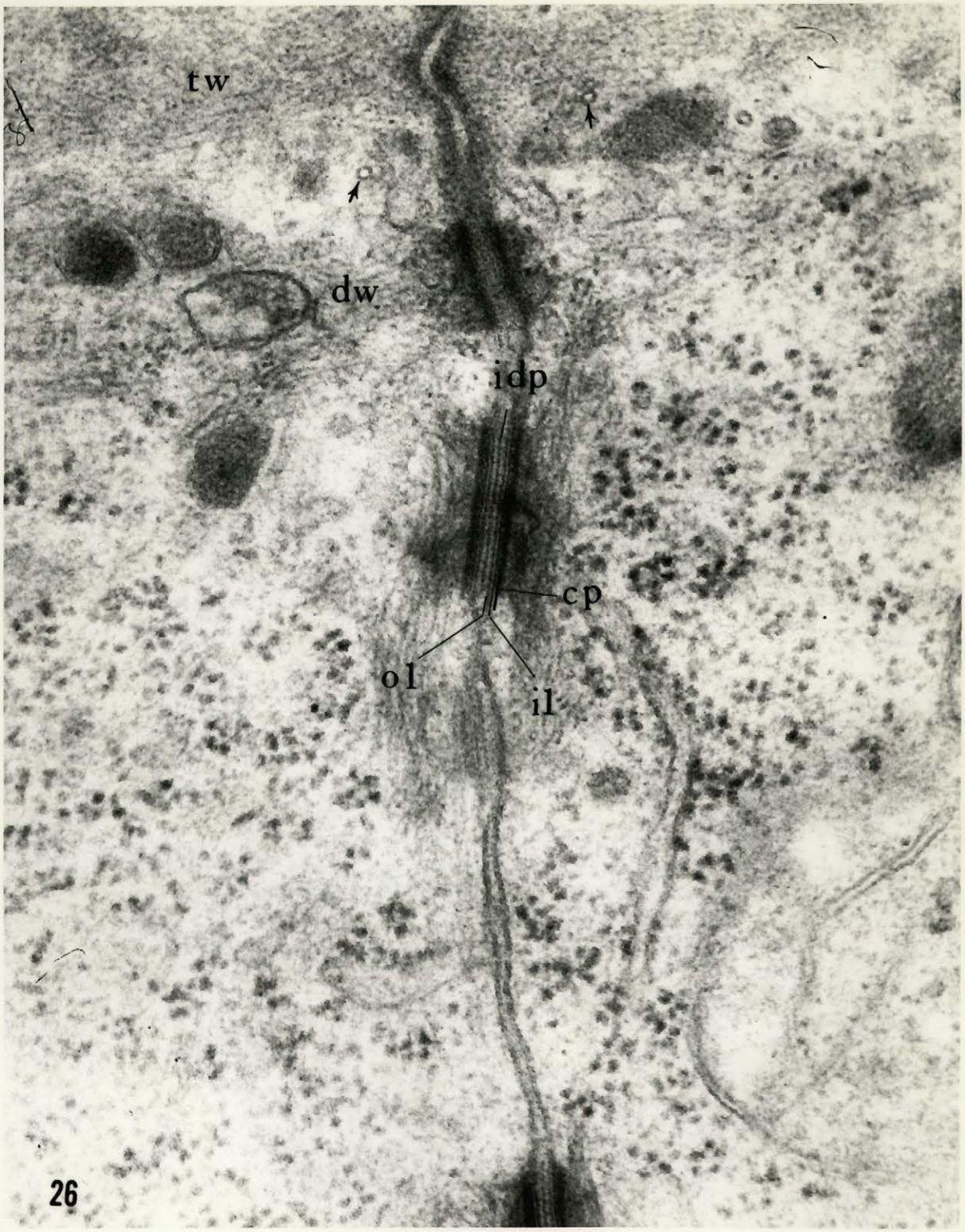

tw
dw
idp
cp
ol
il
26

Fig. 27

Longitudinal section of the terminal bar and desmosome in the villus. Here the relationships between the terminal web (tw) and zonula adherans (za), and the desmosome web (dw) and desmosome (d) are clearly demonstrated. Individual desmosomal filaments can be followed. A vesicle (v) is attaching or separating itself to the lateral membrane, just below the desmosome. Microtubule in cross-section is indicated by arrow.

Glutaraldehyde-acrolein fixation (x 52,500).


za
tw
d
dw
v
27

Fig. 28

A longitudinal section of the supranuclear region of two crypt cells. Several microtubules (large arrows) are seen in close proximity to the Golgi zone. A group of microtubules are seen close to the lateral membrane (small arrows). Lateral web (lw) is also seen. Glutaraldehyde-acrolein fixation (x 35,000).

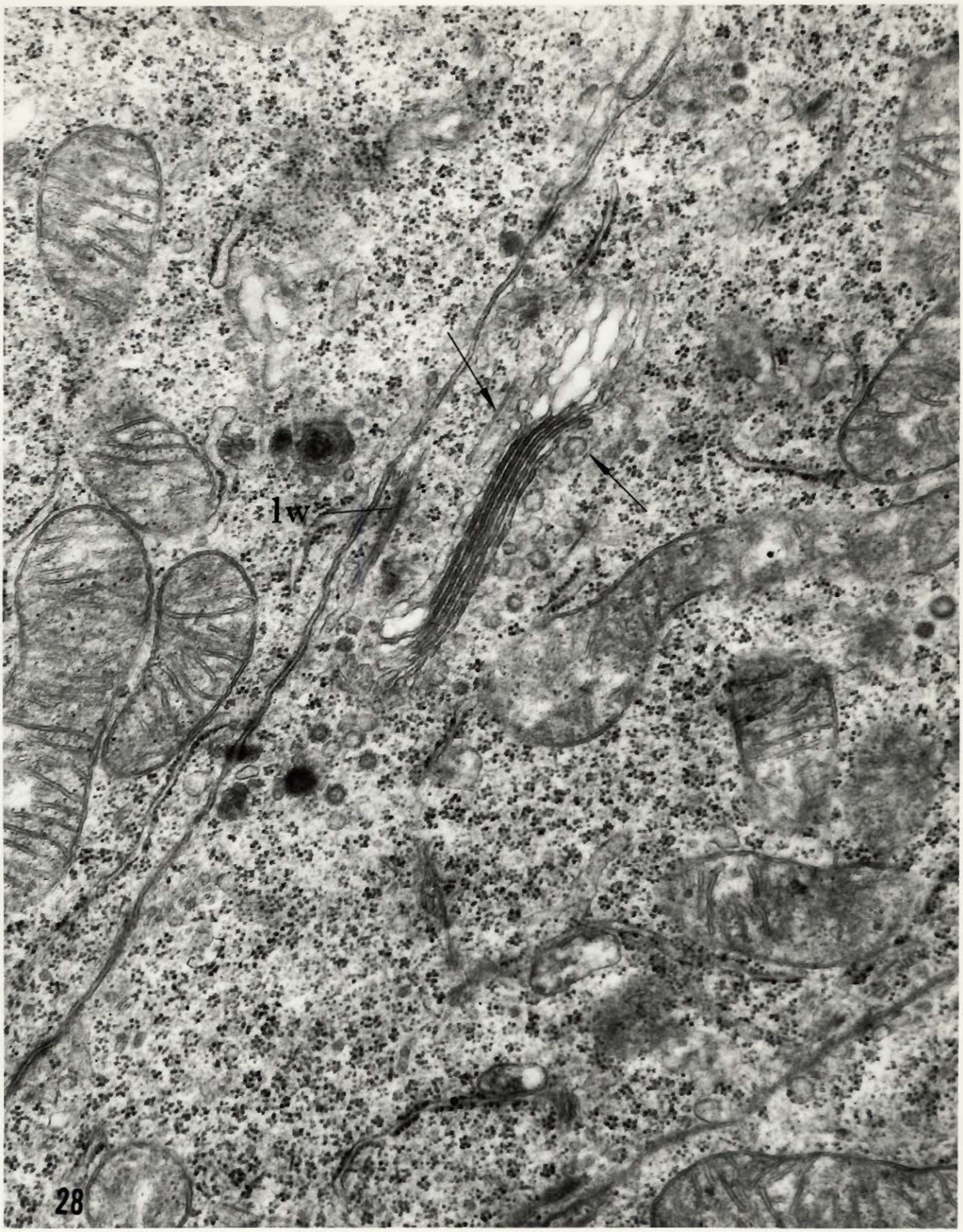

lw
28

Fig. 29

The Golgi zone (G) of a crypt cell.

Microtubules (arrows) can be seen in the cytoplasm in close proximity to the Golgi.

Glutaraldehyde-acrolein fixation (x 52,500).

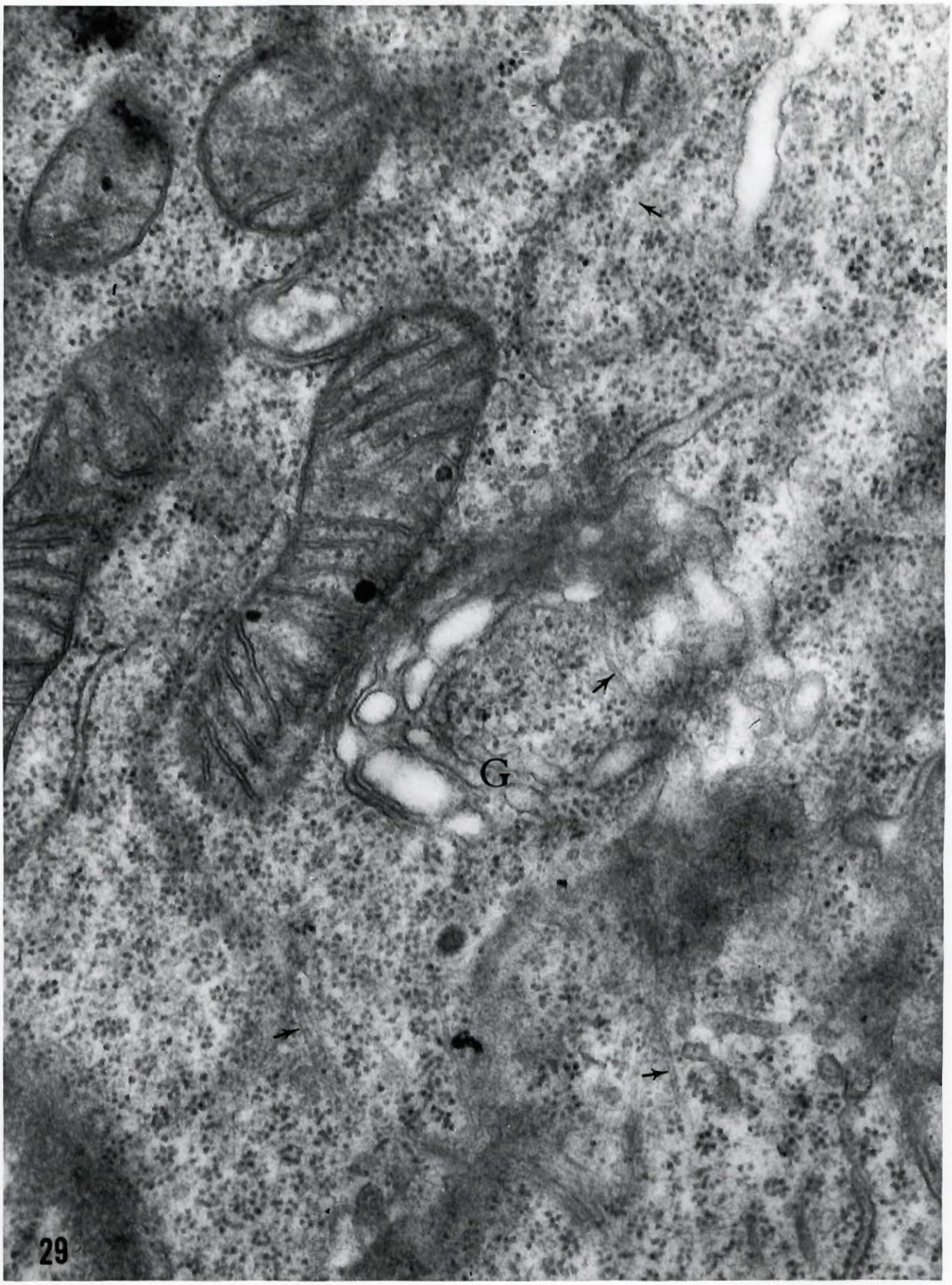

G
29

Fig. 30

Apical cytoplasm of crypt cells showing abundance of filaments and microtubules (small arrows).

The pair of lateral membranes with desmosomes in the center of the figure is obliquely cut. One centriole (ct) is seen in each cell. The centriole to the left shows suggestions of radiating microtubules (mt) to its right.

Glutaraldehyde-acrolein fixation (x 52,500).

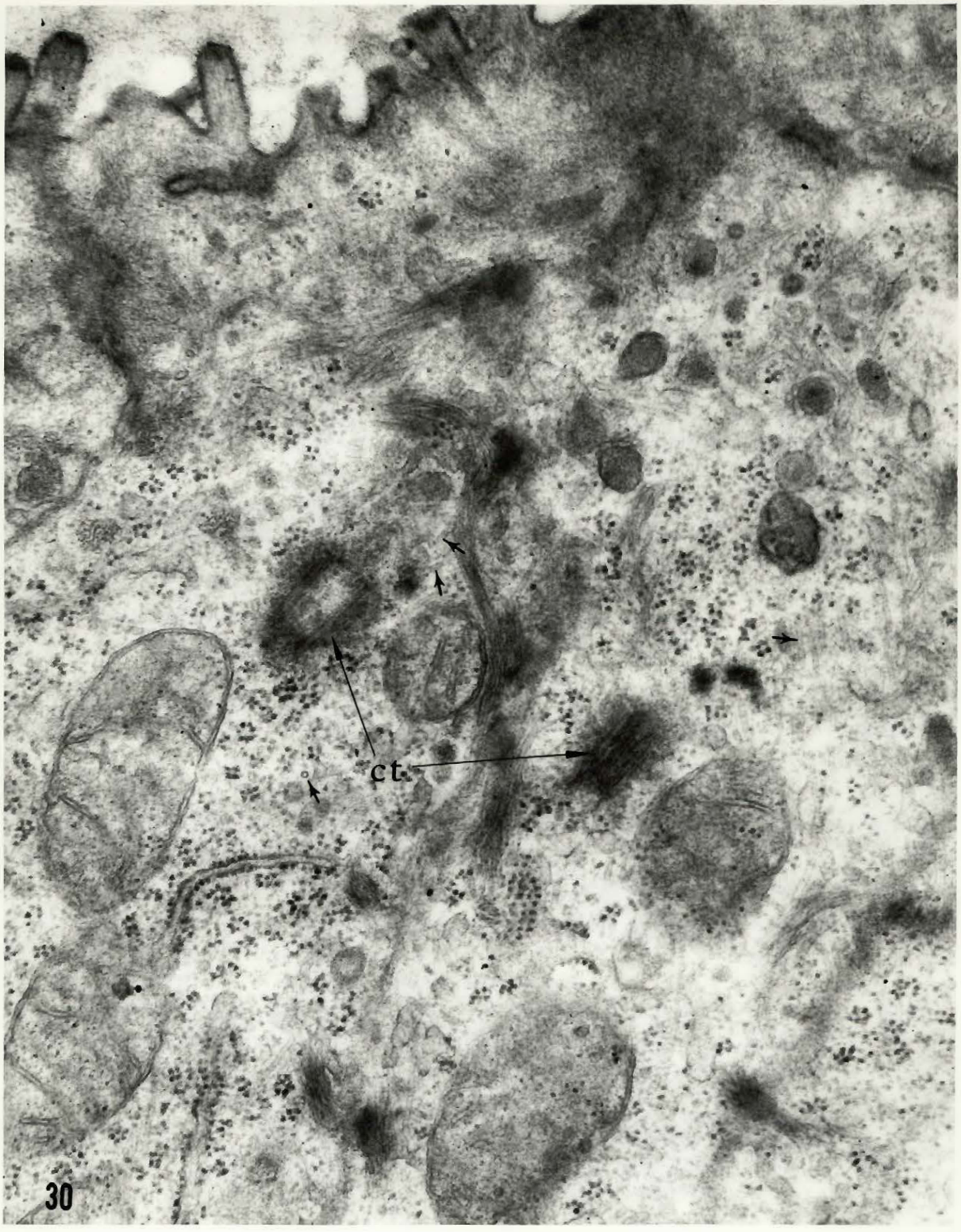

ct
30

Fig. 31

A longitudinal section of a crypt cell.

Note the wealth of both filamentous and microtubular elements . An obliquely-sectioned centriole (ct) is seen. A multivesicular body (mvb) is located just below the apical centriole. The microtubules (arrows) are seen through the cytoplasm.

Glutaraldehyde-acrolein fixation (x 50,000).

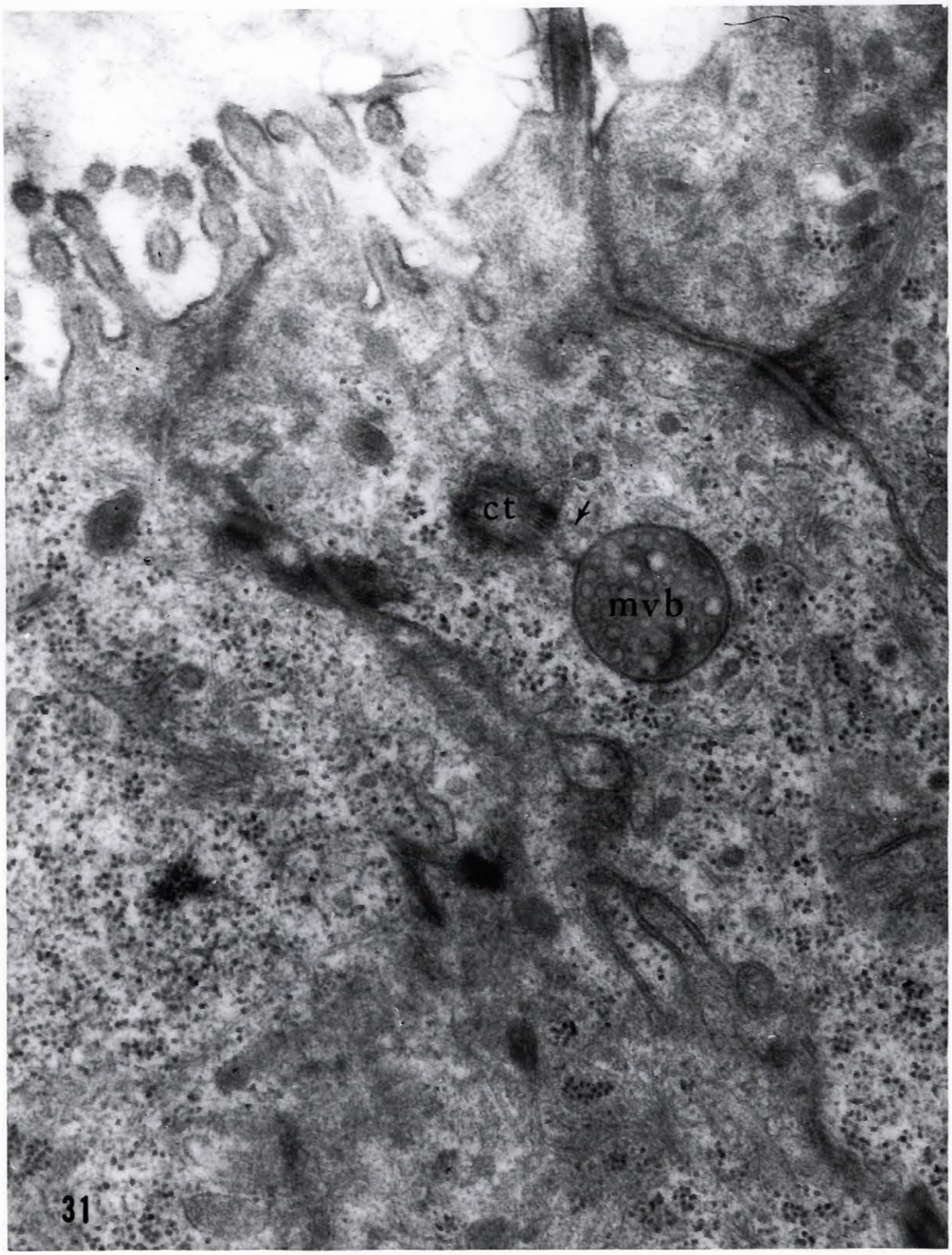

ct
mvb
31

Fig. 32

The spindle region of a crypt cell in metaphase.

Dense metaphase chromosomes (ch) can be seen in the upper part of the figure. Longitudinal and oblique sections of spindle tubules (st) are seen. The kinetochore, the region of the chromosome for the spindle tubule attachment, is seen as a region of lighter density on the chromosome. Several spindle tubules are seen radiating from the kinetochore. The diameter of these tubules, approximately 230 A, is the same as interphase microtubules. The diplosome is not seen in this section. Glutaraldehyde-acrolein fixation (x 52,000).

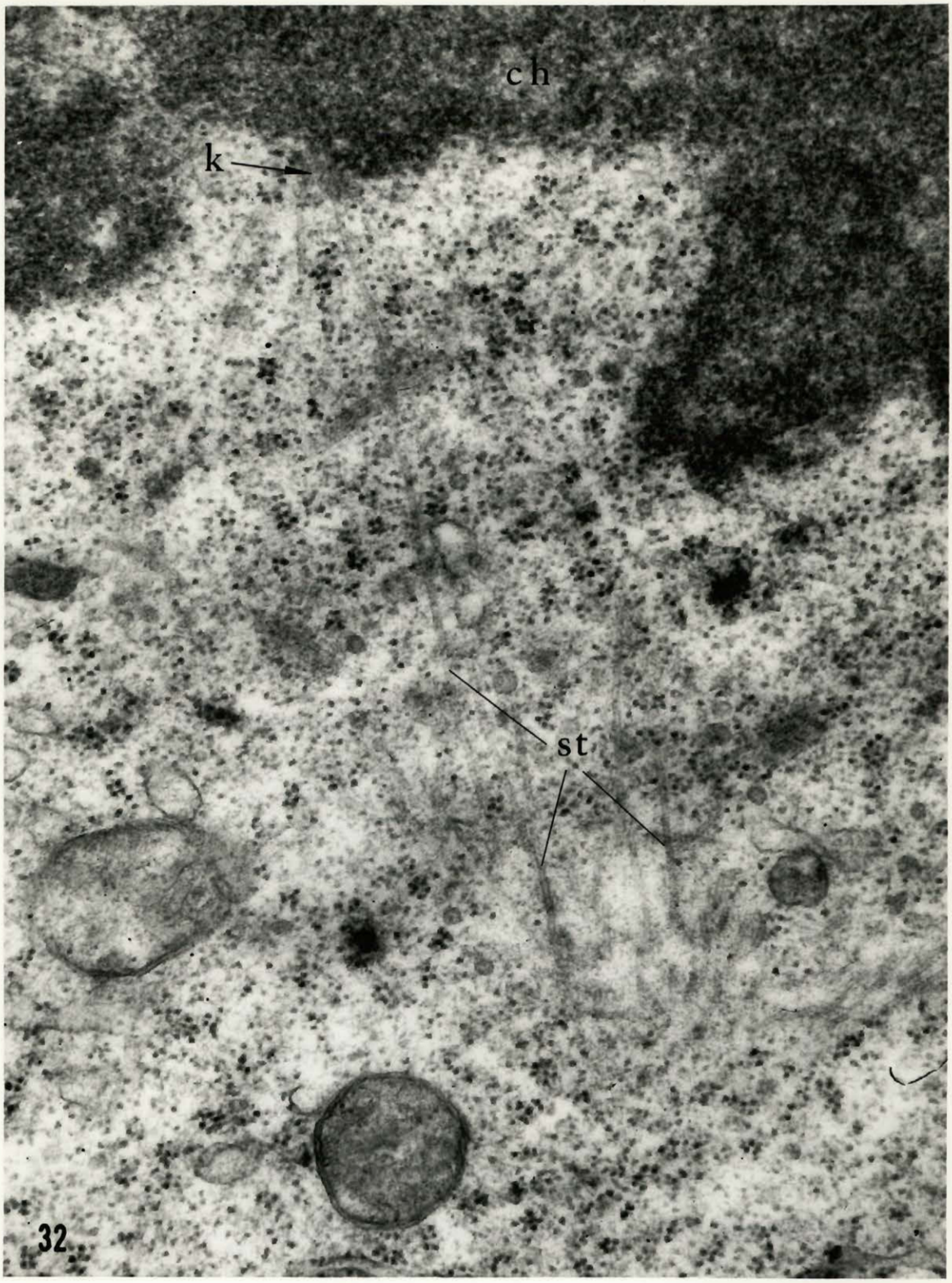

ch
k
st
32

Fig. 33

A longitudinal section of an unusual cell on the villus.

The microvillous border is 1.5 times larger than adjacent cells, both in height and width of microvilli. The rootlets (r) also show an over-development, extending 3 u into the cytoplasm. The terminal (tw) and desmosomal webs (dw) follow the rootlets down into the cytoplasm rather than stretching across the apex. Large numbers of microtubules (arrows) are present throughout the cytoplasm, oriented mainly parallel to the cell's axis.

Glutaraldehyde-acrolein fixation (x 35,000).

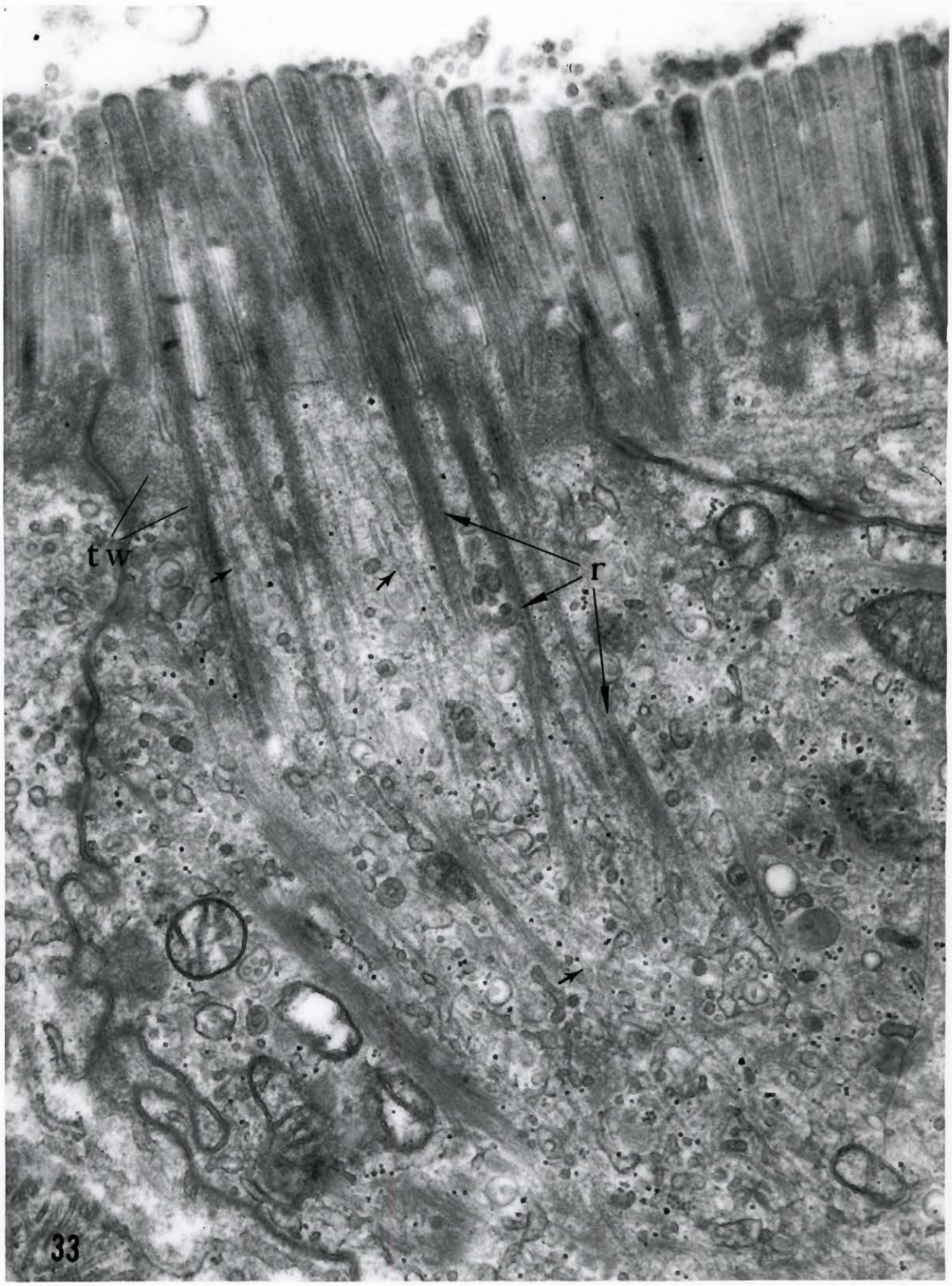

t w
r
33

Fig. 34

A longitudinal section through an unusual cell of the crypt. The microvilli of this cell are well developed compared to neighboring cells. The rootlets extend well into the apical cytoplasm. Microtubules (small arrows) seem prominent, possibly because of the absence of ribosomes and mitochondria. Many vesicles (v) are present. Glutaraldehyde-acrolein fixation (x 52,500).

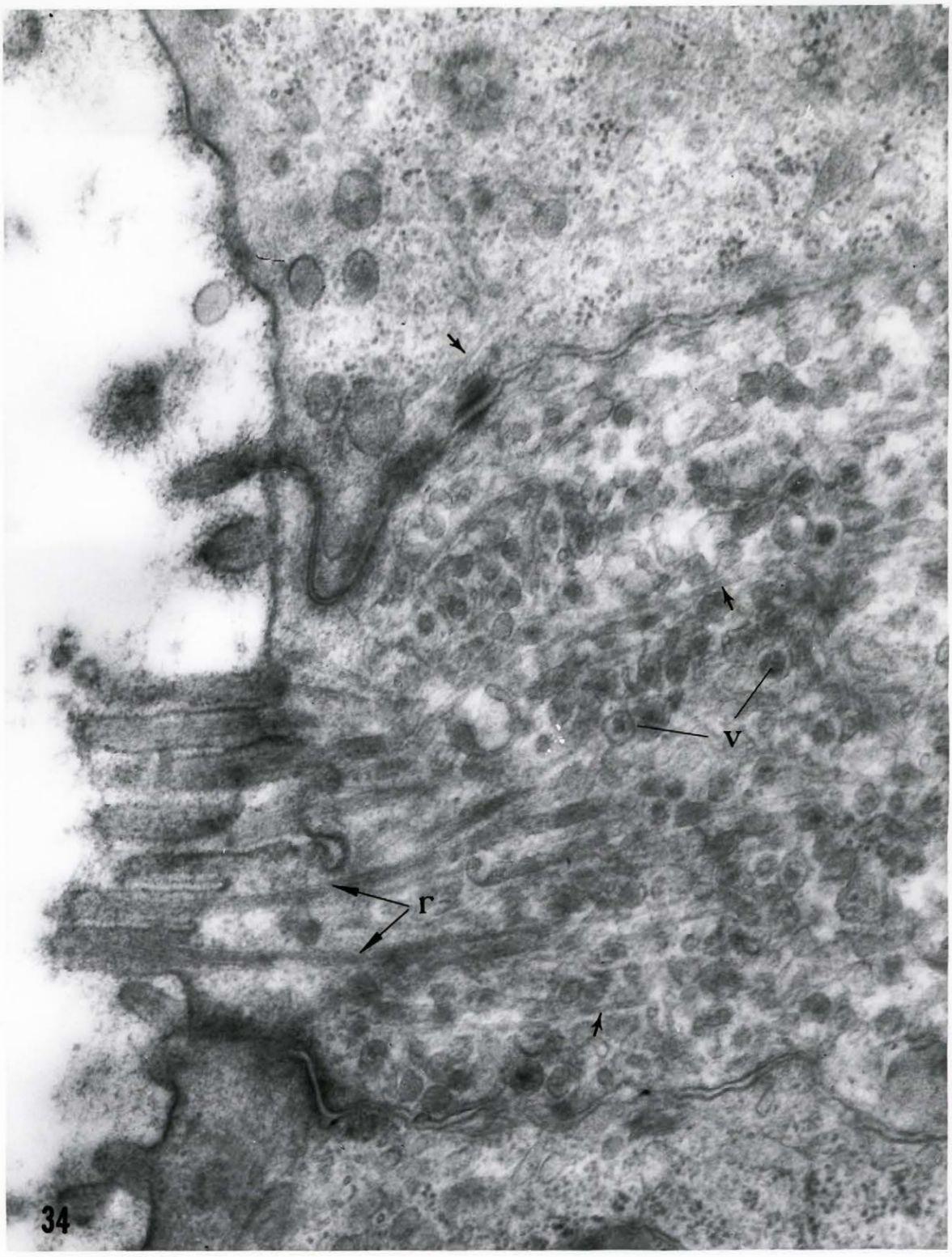

v
r
34

Fig. 35

Intestinal cells fixed with buffered OsO_4.

The apices of three absorptive cells are cut in oblique section. The lighter density apical ectoplasm (ect) is seen separating the cross sectioned microvilli (mv) and the cytoplasm below. The microvilli are devoid of an organized central core. Other than the round densities corresponding to rootlets (r) and occasional vesicles (v), little structure can be seen in the apical ectoplasm. Note the apical ectoplasm continuing down the lateral membrane. Mitochondria appear well-fixed. Granular endoplasmic reticulum and multivesicular bodies are seen in the endoplasm, together with a large number of vesicles. Buffered OsO_4 fixation (x 21,000).

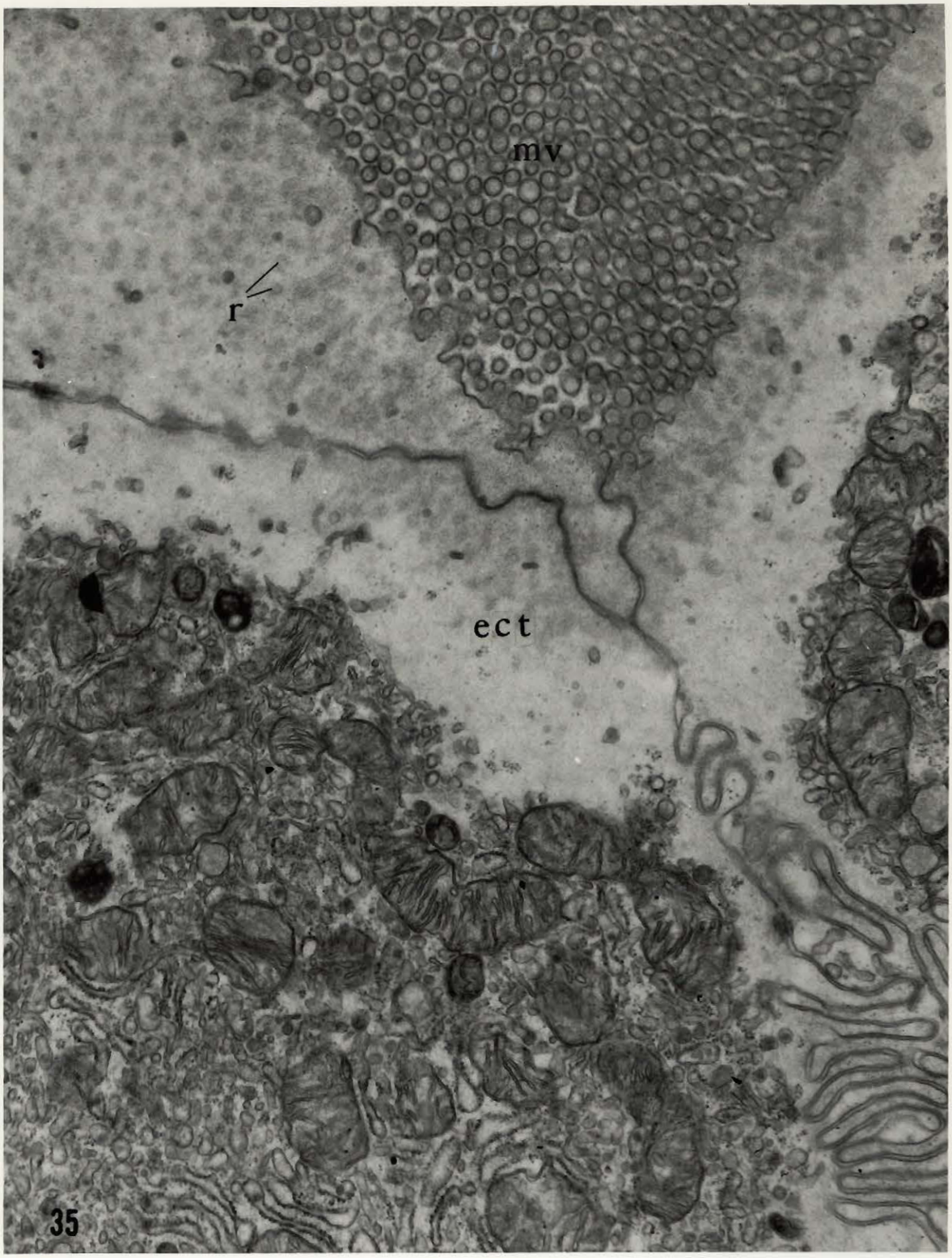

mv
r
ect
35

Fig. 36

A mitochondrion from crypt absorptive cells illustrating several inner mitochondrial supparticles (ims) within the matrix.

One inner mitochondrial supparticle can be seen attached to the inner membrane (arrow). The head, 120 A in diameter, is attached to the membrane by means of a short stem 50 A wide by 70 A long. The head has a roughly polygonal shape and has a center of lighter density.

Glutaraldehyde-acrolein fixation

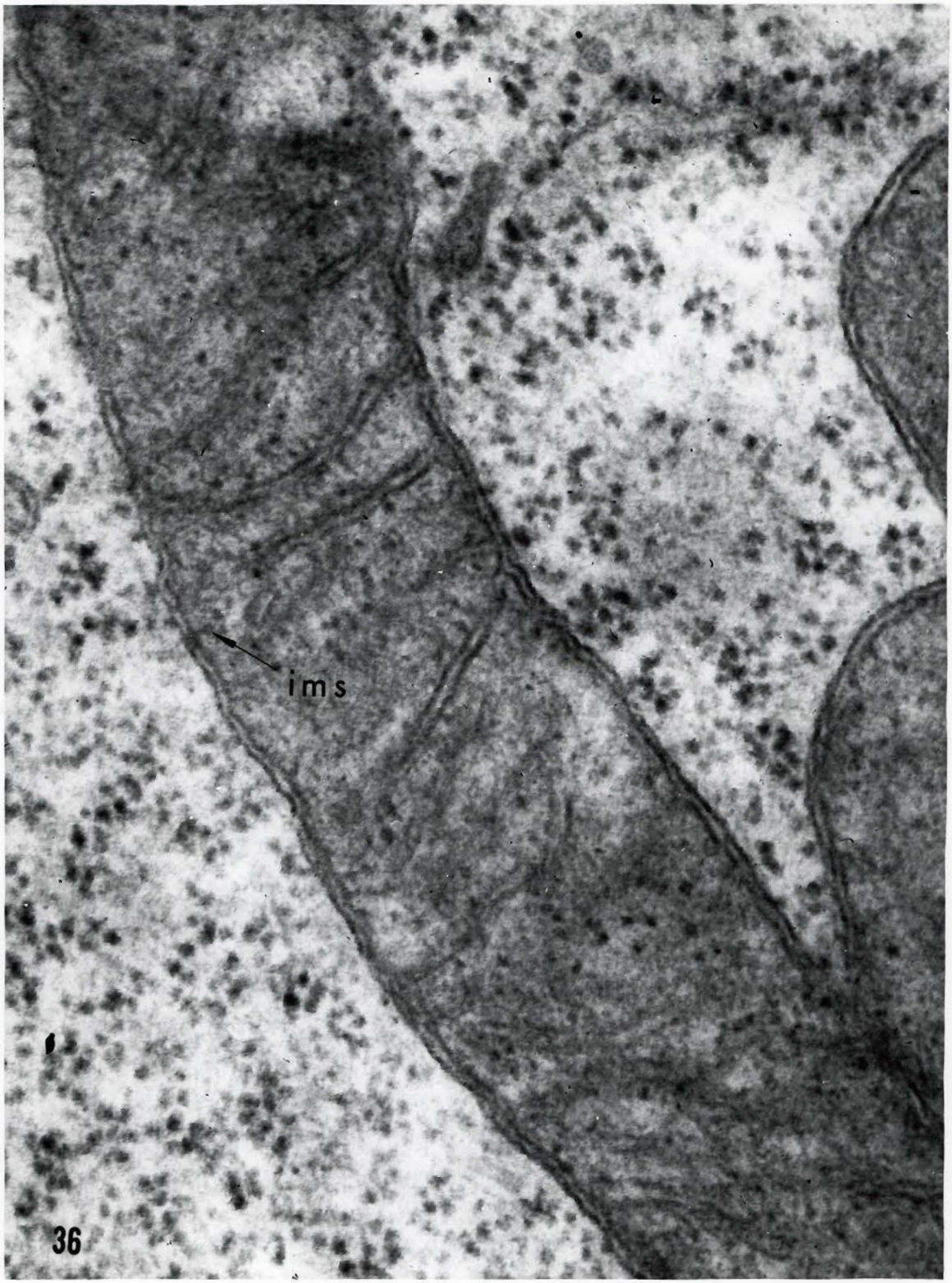
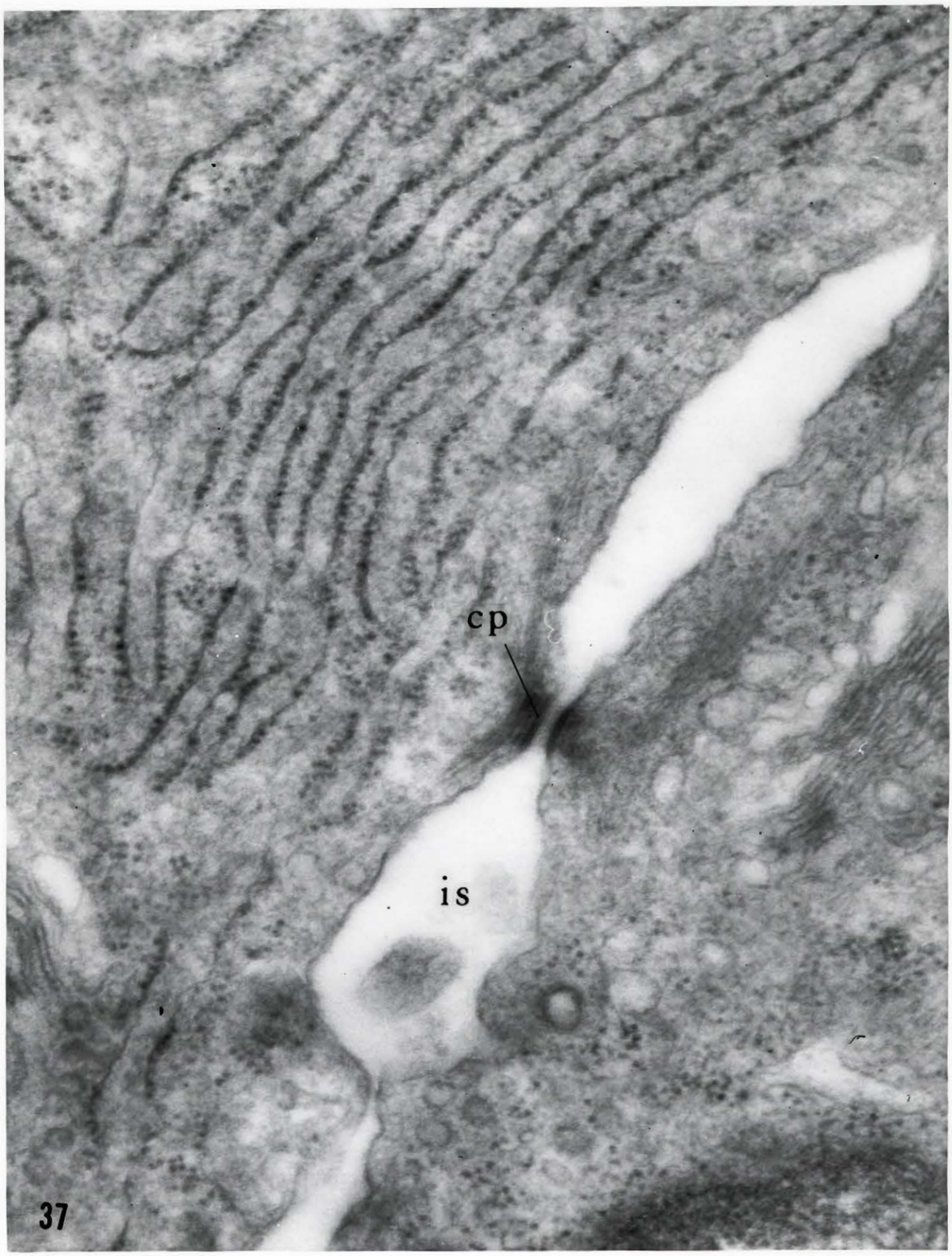

ims
36

Fig. 37

Intestinal epithelium fixed in hypertonic medium.

The lateral membranes have separated leaving an extensive intercellular space (is). Note the cells are still closely apposed in the region of the desmosomes. The intercellular space in this region is the same in isotonically-fixed desmosomes. Note also the layering of the filaments parallel to the cytoplasmic plaque (cp).

Glutaraldehyde-fixation (x 70,000).


cp
is
37

vesicles are found. Membranes are easily resolved into their trilaminar structure; myelin layers are well defined. In addition, inner mitochondrial subparticles, microtubules and spindle tubules are abundantly present in tissue fixed with glutaraldehyde-acrolein-osmium.

If repeated studies prove this fixative to be as good as these initial studies have indicated, glutaraldehyde-acrolein will allow a greater range of tissues to be studied, as well as allowing inexperienced investigators to achieve acceptable if not excellent fixation.

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