A FINE STRUCTURAL ANALYSIS OF THE EPIDERMIS OF THE EARTHWORM, *LUMBRICUS TERRESTRIS* L.

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ABSTRACT

A fine structural analysis of the cuticle, epidermal epithelium, and underlying fibrous tissue of the earthworm is presented. The extreme scarcity or absence of fibroblasts in this animal is pointed out. This finding is further evidence for the epithelial origin of the cuticular fibers, and suggests that at least some of the collagenous connective tissue fibers in the interior of this animal are epithelial in origin. The junctional specializations that unite epithelial cells in the epidermis and intestine are described. Of special interest is the fact that the septate desmosome rather than the tight junction is found in these epithelia. It is shown that the septa are not extensions of the plasma membrane across the intercellular gap. Finally, the nature of the small ellipsoidal bodies that are embedded in the outer layer of the cuticle is discussed.

INTRODUCTION

The epidermis of the earthworm, *Lumbricus terrestris*, consists of an epidermal epithelium and an overlying fibrous cuticle (14, 4, 13). Underlying the epidermis are connective and muscular tissues that form the bulk of the body wall. The cuticle is of particular interest in that it is a collagenous tissue (cf. references 1 and 18) that is separated from the rest of the organism by the epidermal epithelium. For this reason, it has been suggested that the cuticular collagen is produced and aligned by the epidermal epithelial cells (25, 2) rather than by cells of mesenchymal origin as is thought to be the case in vertebrates (11, 21). Previous electron microscope studies of this epidermis demonstrated the general shape and dimensions of the cuticular fibers and some of the surface specializations of the associated epithelial cells (23, 24, 9, 22, 19, 26, 2). The present study extends these observations with a more complete description of the fine structure of the underlying epidermal epithelium and subjacent connective tissue, and adds further detail about the structural organization of the cuticle. Further evidence supporting the hypothesis that the epithelium is responsible for the origin and arrangement of the cuticular collagen will be presented, and this concept will be extended by proposing that connective tissue fibers in *Lumbricus*, as well as cuticular fibers, may be produced by epithelial cells.

Also of comparative interest are the specialized septate intercellular junctions that unite epithelial cells in the epidermis and intestine in the earthworm, for they differ from the junctions that unite homologous cells in the vertebrate. In a previous study on the fine structure of the nervous system in *Lumbricus* (5), the absence of "tight junctions" between glial cells, endothelial cells, coelomic lining cells, and segments of the dorsal giant axons led the author to consider the possibility that tight junctions may not occur anywhere in

Lumbricus. The fact that septate desmosomes rather than tight junctions were found, in the present study, joining epithelial cells in earthworm epidermis, and intestine, provides further evidence to support this suggestion.

MATERIALS AND METHODS

Specimens labeled *Lumbricus terrestris* L. were obtained from a biological supply house, and this identification was kindly confirmed by Dr. Gordon E. Gates of the Zoology Department of Harvard University. Pieces of epidermis and intestine were carefully removed and placed in fixative. The fixatives used were (*a*) osmium tetroxide buffered with collidine (3), (*b*) glutaraldehyde buffered with cacodylate followed by postfixation in osmium tetroxide (10), and (*c*) 0.6% potassium permanganate with sodium chloride added to make a 250 milliosmolar solution. The tissue was dehydrated with increasing concentrations of ethanol, then placed in propylene oxide and embedded in Araldite. Thick sections for light microscopy were cut at ½ μ and stained with 1.0% toluidine blue and 1.0% sodium borate in distilled water. Some thick sections were also stained by the PAS method. Thin sections, ranging from gold to gray in color, were cut for electron microscopy and mounted on bare grids. The thin sections were stained with an 0.2% lead citrate solution (28) or with a saturated solution of uranyl acetate in distilled water. Occasionally, the uranyl acetate was followed by the lead citrate. The thin sections were examined in RCA EMU-3E, F, or G electron microscopes.

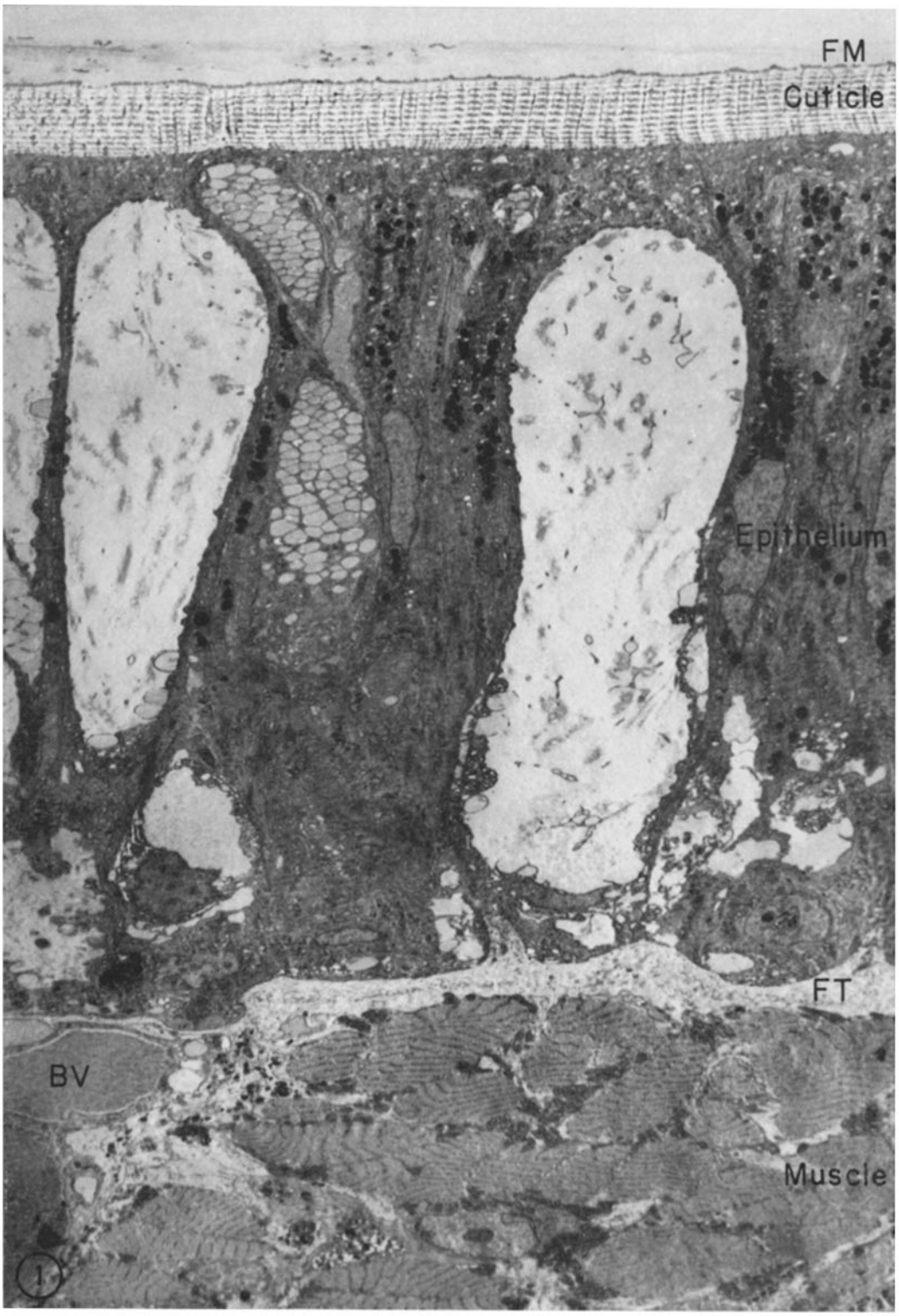
OBSERVATIONS

The Cuticle

The cuticle of the earthworm consists of collagenous fibers embedded in an amorphous matrix (Fig. 1). As previous investigators have noted (23, 24, 9, 22, 19, 26, 2), the fibers are arranged in layers, each layer consisting of a single row of parallel fibers, and the main fiber axis of each layer making an angle of approximately 90° with respect to the fiber axis in the layers above and below (Figs. 2 and 3). In the specimens examined here, the number of layers varies from 10 for the cuticle that lines the mouth (Fig. 2) to 24 or more for the cuticle of the outer body surface. The usual cross-sectional outline of a fiber is roughly square, although rectangular and circular profiles are also seen (Fig. 2). The fibers in the center of the cuticle are thickest, measuring 0.1 to 0.2 μ on a side, while the outermost and innermost are considerably thinner (Figs. 2 and 3) (9, 22, 19). In longitudinal section, the fibers appear to be indefinitely long and have a beaded appearance (Figs. 2 and 3). The fiber is darker at the beads which are the points of maximum diameter, but it is not clear whether this is just a reflection of increased thickness or whether it represents an actual periodic increase in density.

The outer surface of the cuticle, called the epicuticle by Astbury (2) and Ruska and Ruska (26), consists of a layer of homogeneous, electron-opaque material in which are embedded myriad small ellipsoidal bodies (projections) (Figs. 2, 3, 5, and 6). The homogeneous, electron-opaque layer resembles a basal or external lamina (basement membrane), except that it is not directly associated with the surface of any cell. The embedded ellipsoidal bodies are approximately 400 A wide and 800 A long (Fig. 10). Their usual center-to-center distance is 1000 A, but there is considerable variation in this value (Figs. 2, 3, 5, and 6). With collidine-buffered osmium tetroxide fixation, most of them have a homogeneous content of low density, but some appear to have a distal cap of higher electron opacity (Fig. 5). With permanganate fixation, their content is dense, and they are seen to be enclosed by a unit membrane, the inner leaflet of which is difficult to resolve, owing to the matching density in the interior of the body (Fig. 10). In appropriately stained histological sections, the outer surface of

FIGURE 1 A low power electron micrograph of the earthworm epidermis, which consists of a cuticle and underlying epithelium, and the fibrous (*FT*) and muscular tissues that lie below. On the outer surface is a row of small ellipsoidal bodies to which are attached a meshwork of filaments (*FM*). The large pale regions in the epithelium are the mucous cavities described in the text. The numerous dark bodies seen in the cytoplasm of the columnar epithelial cells are presumed to be pigment. A small blood vessel (*BV*) is seen in the muscular layer adjacent to the epithelium. × 1850.


FM
Cuticle
Epithelium
FT
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Muscle
1

the cuticle is intensely PAS-positive, but the resolution of the light microscope does not distinguish between the presumed external lamina and the ellipsoidal bodies. Radiating from the tips of the ellipsoidal bodies are fine filaments which form a layer approximately 1 μ thick (Figs. 1 and 5). This filamentous layer is not mucus, as evidenced by its distinctive fine structure and the fact that it is PAS-negative. It bears a striking, fine structural resemblance to the filamentous nap on epithelial cells of the gall bladder (31) and on intestinal microvilli (12), except that these latter are PAS-positive.

The Epidermal Epithelium

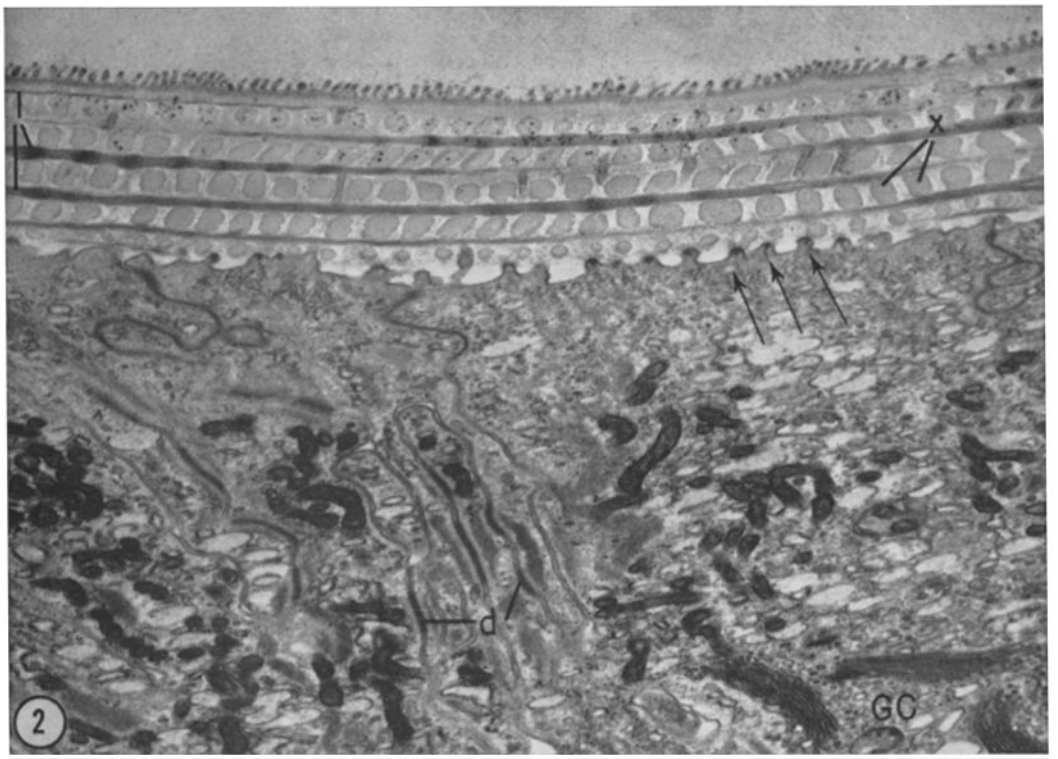
The epidermal epithelium that underlies the cuticle is 50 to 70 μ in height (Fig. 1). There is no external lamina between the cuticle and the epithelium, but the epithelium is underlain by a basal lamina 200 to 1000 A thick (Fig. 7). At certain points, the basal lamina seems to be lacking, but it is not clear whether it is completely absent at these sites or has just become very thin. A fibrous layer beneath the basal lamina separates the epidermal epithelium from the body wall musculature (Figs. 1 and 7). The fibers in this layer, like the cuticular fibers, are collagenous in nature (25, 2). They are approximately 150 A in diameter, are of indefinite length and, in cross-section, have a dark periphery surrounding a lighter core. They are cross-banded with a simple repeating period of about 300 A. These values differ from those reported for the same fibers by Ruska and Ruska (120 to 180 A wide with a period of 540 A, reference 26), by Astbury (no diameter given, a period of 640 A, reference 2), and by Staubesand et al. for similar fibers around the nervous system (350 A in diameter with a period of between 400 and 700 A, reference 27). It is not known whether these discrepancies are the result of technical differences or whether they signify that there are several types of connective tissue fiber in the earthworm.

The connective tissue of the earthworm body wall is largely acellular, but, at extremely rare intervals, cells are found embedded emong the collagen fibers. Some of these scattered cells are filled with large numbers of bacteroides crystals (2, 15); the rest are characterized by large vacuoles which contain material that is interpreted as partially digested foreign matter. No cell which appears morphologically akin either to the vertebrate fibroblast or to the small fibroblasts that are common in the connective tissue around the leech nervous system (6) has been found. This point was specifically checked by preparing 100 serial 1 μ–thick sections of a segment of earthworm body wall that was approximately 1 mm deep and 2 mm wide. No cells of any kind were seen in the fibrous layer directly under the epidermis, and only a few cells filled with bacteroides crystals were seen in deeper parts of the body wall, at least 200 μ from the base of the epidermal epithelium. The same absence of connective tissue cells has been noted in the connective tissue of the gut and nervous system. It is therefore concluded that fibroblasts are either absent or extremely sparse in *Lumbricus*.

The epidermal epithelium consists of three ma-

FIGURE 2 The cuticle of the earthworm consists of an alternating series of fibrous layers embedded in a fibrous meshwork. In this micrograph, one set of fibrous layers is cut almost longitudinally (*l*), the other transversely (*X*). Note the beading in the longitudinal fibers. Most of the transverse profiles are square or rectangular in outline. Note that the fibers in the middle of the cuticle are the thickest. Half-desmosome–like junctions at the tips of short microvilli join the cuticle to the epithelial cells (arrows). Also, numerous desmosomes (*d*) are seen between the epithelial cells. The marked interdigitation of the epithelial cells is shown, and a small part of a Golgi complex (*GC*) can be seen. × 23,000.

FIGURE 3 An almost horizontal section through the cuticle. The pattern of alternating fibrous layers and the density of the cuticular matrix are well shown here. Note the absence of an external lamina between the epithelial cell (*EC*) and the cuticle, and the presence of the outer cuticular layer at the outer edge of the cuticle (arrow). Seen in cross-section are the short microvilli (*sMv*), the long microvilli (*lMv*), and the ellipsoidal bodies (*e*). × 36,000.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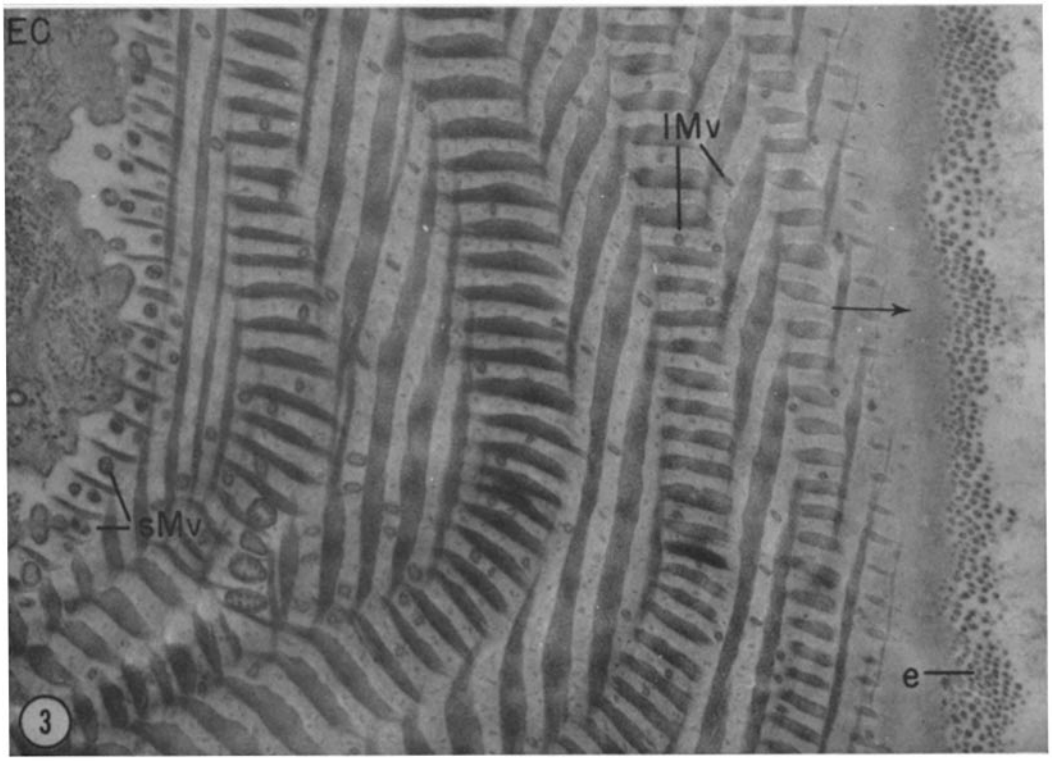
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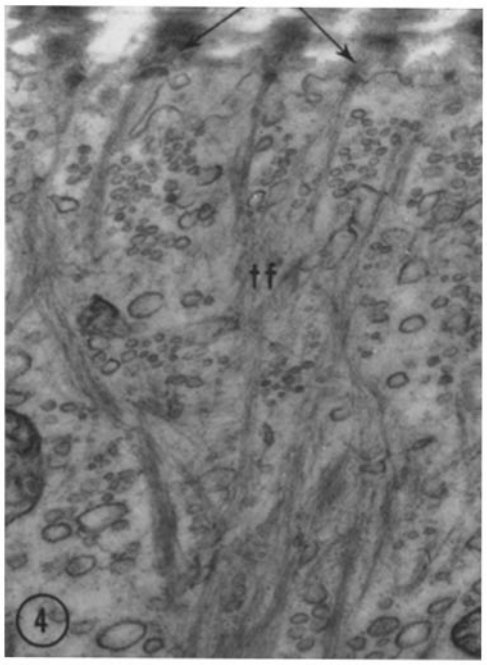

FIGURE 4 A columnar cell fixed with the epidermis under stretch. Note the tonofilaments (*tf*) that insert into dense plaques of cytoplasm adjacent to the cuticular plasmalemma. Also note the tufts of extracellular filaments (arrows) radiating into the base of the cuticle. × 40,000.

jor cell types: the columnar cell (frequently called the supportive cell) and the mucous cell, both of which extend from the cuticle to the basal lamina, and the basal cell which is confined to basal regions. If the animal is relaxed before fixation, numerous, short, blunt, regularly-spaced microvilli are seen at the cuticular surface of the columnar cells (Fig. 2). These short microvilli were first seen by Rudall in an electron microscope analysis of the surface of the epithelial cell from which the cuticle had been stripped (24). At that time, they were inappropriately termed granules, but their precise orthogonal arrangement was well demonstrated. A plaque of electron-opaque cytoplasm is found immediately adjacent to the apical plasmalemma of each short microvillus, and cytoplasmic tonofilaments insert themselves into the plaque. A tuft of fine extracellular filaments radiates from the tip of the microvillus. If the animal is stretched before fixation, the microvilli disappear and the inserting tonofilaments, plaques of electron-opaque cytoplasm, and associated extracellular filaments are now connected to relatively flat patches of the plasmalemma (Fig. 4). These special structures at the cuticular surface of the columnar cell resemble half-desmosomes and presumably help maintain the attachment of the epidermal epithelium to the amorphous material of the cuticle.

In addition to the short microvilli described above, there are less numerous long microvilli that penetrate between the fibers of the cuticle so that their tips are directly exposed to the environment at the upper surface of the cuticle (Figs. 5 and 6). These long microvilli are not regularly spaced (Fig. 3). Specializations of their surface which might serve to attach them to the cuticle have not been observed. It has been suggested that the long microvilli may be partially responsible for the orthogonal pattern of the cuticular fibers (22, 9, 19). However, the studies referred to have confused the short and the long microvilli, and it is not easy to understand how the irregularly spaced, long microvilli could be responsible for the precise arrangement of the cuticular fibers.

At the basal surface of the epithelial cell are half-desmosomes that presumably attach the cells to the underlying fibrous tissue. These consist of a localized region of electron-opaque cytoplasm closely applied to the basal plasmalemma of the cell. Tonofilaments insert themselves into these dense patches of cytoplasm, and there is a corresponding extracellular density in the basal lamina that underlies the cell.

The nuclei of the three types of epidermal epithelial cells are irregularly round or oval in outline (Fig. 1). The interphase chromatin is in the form of dense clumps randomly dispersed in a less dense matrix. The nucleolus is usually round and consists of clusters of dark, 150 A–wide particles embedded in a more finely granular material. In a previous study (5), a similar fine structure was described for the nuclei of cells associated with the earthworm nervous system. Intestinal epithelial cells also possess similar nuclei. Thus epidermal epithelial cells, intestinal epithelial cells, connective tissue cells, muscle cells, endothelial cells, amebocytes, ganglion cells, and glial cells all possess nuclei strikingly similar in appearance, and differ only in size and shape.

The cytoplasm of the columnar cell is abundantly provided with organelles (Fig. 1). The Golgi apparatus is large and is situated between the nucleus and the apical pole of the cell. It con-

FIGURE 5 In the upper part of the micrograph, the filamentous meshwork can be seen attached to the outer ends of the small ellipsoidal bodies, the lower ends of which are embedded in the outer cuticular layer. Two long microvilli end among the small projections (upper arrows). The bases of other long microvilli are seen in the lower parts of the cuticle (lower arrows). × 56,000.

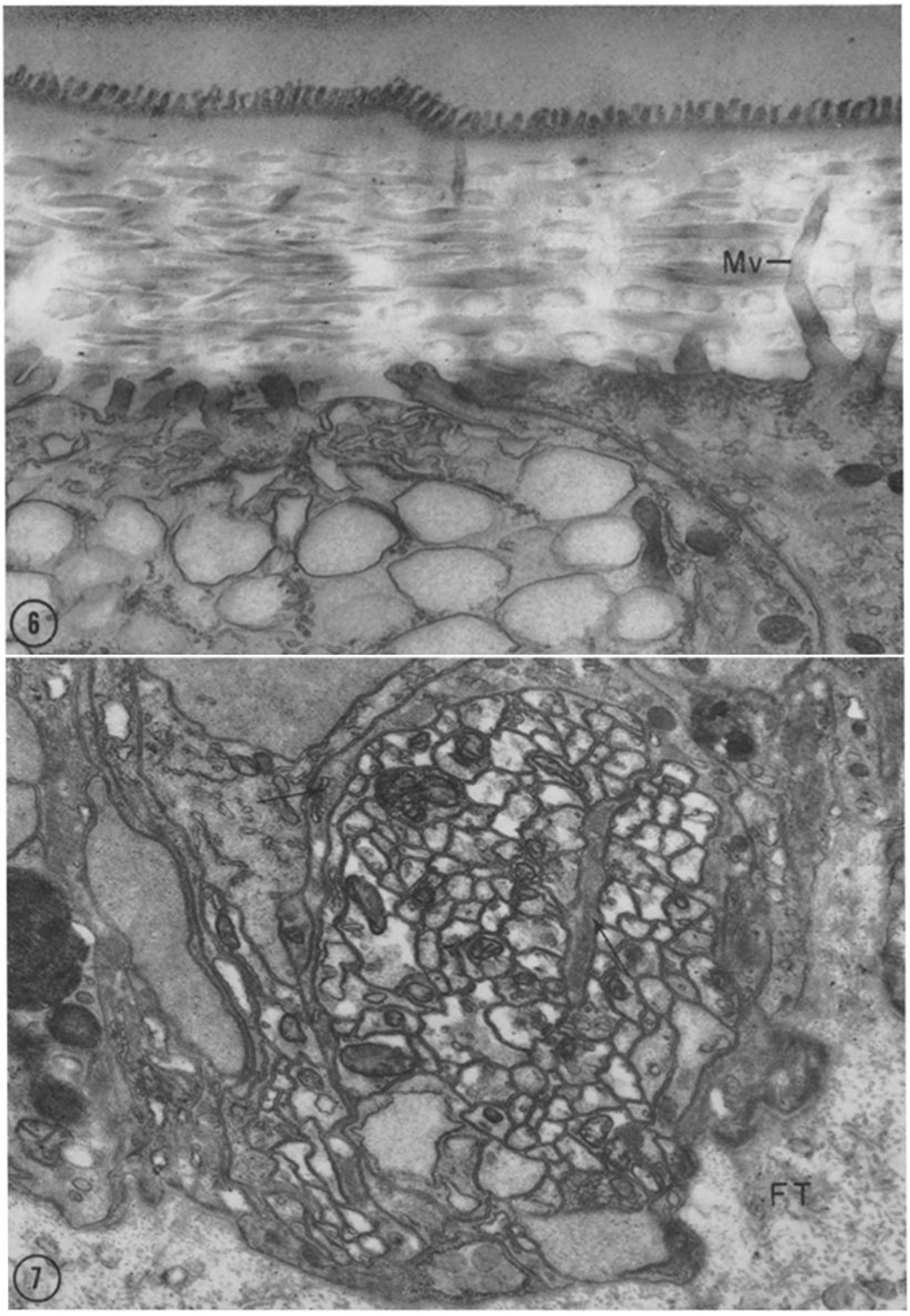

FIGURE 6 The apex of an immature mucous cell. The large vacuoles are filled with mucus and many of the vacuolar membranes are ruptured. A long microvillus (*Mv*) can be followed most of the way through the cuticle. × 52,000.

FIGURE 7 A relatively large nerve at the base of the epidermal epithelium. The nerve is not separated from the rest of the epithelium by an external lamina. A process of a presumed satellite cell is indicated by the arrows. Note the characteristic fibers of the earthworm connective tissue (*FT*). × 48,000.

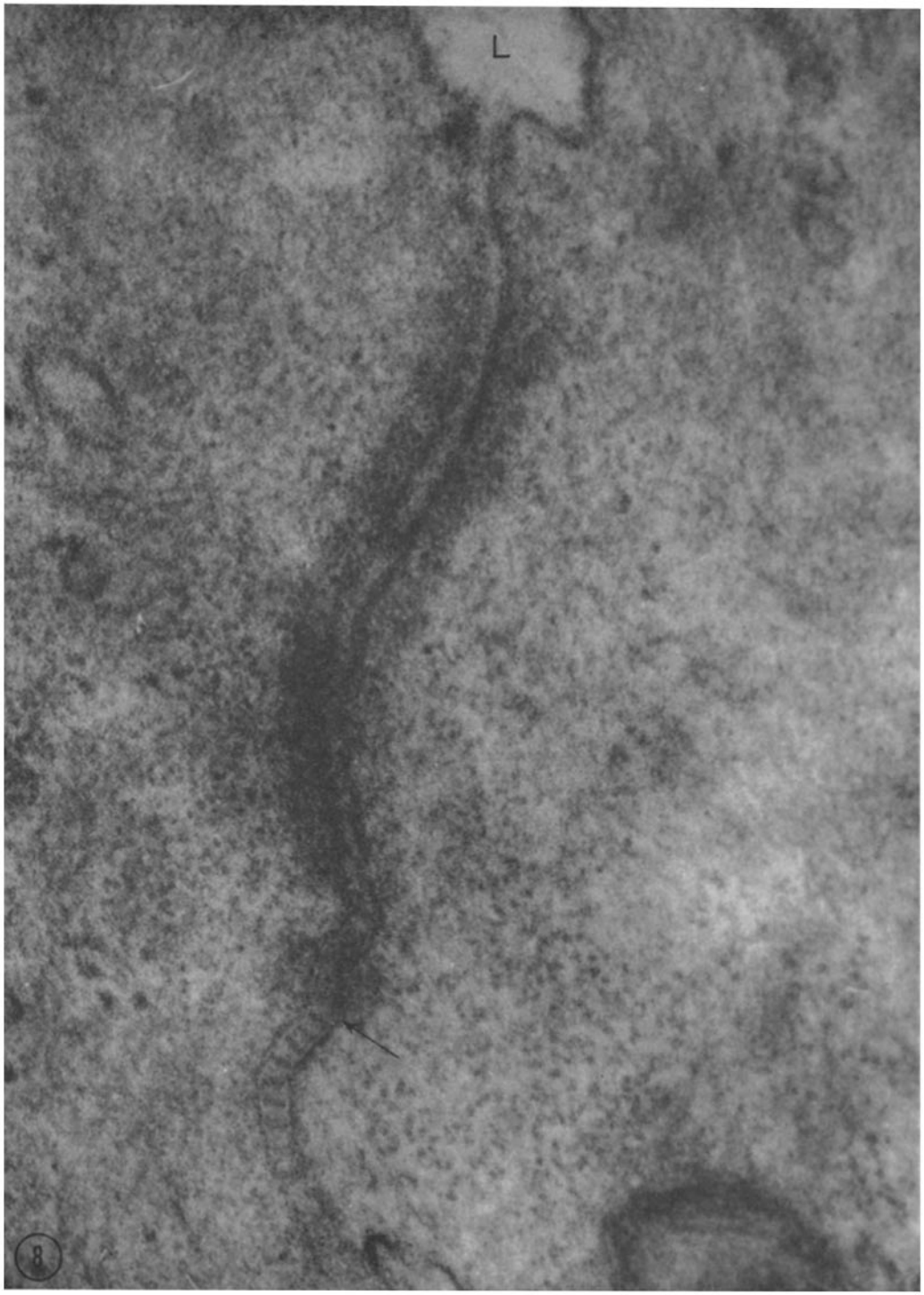

FIGURE 8 The apical part of an intercellular junction between two intestinal epithelial cells. A zonula adhaerens, characterized by a 200 A intercellular cleft, and a layer of electron-opaque cytoplasm closely apposed to the plasma membranes extends from the lumen (*L*) to the arrow. Below the arrow the intercellular septa are seen. The epidermal intercellular junctions are similar, except that the zonula adhaerens is not so extensive. × 218,000.

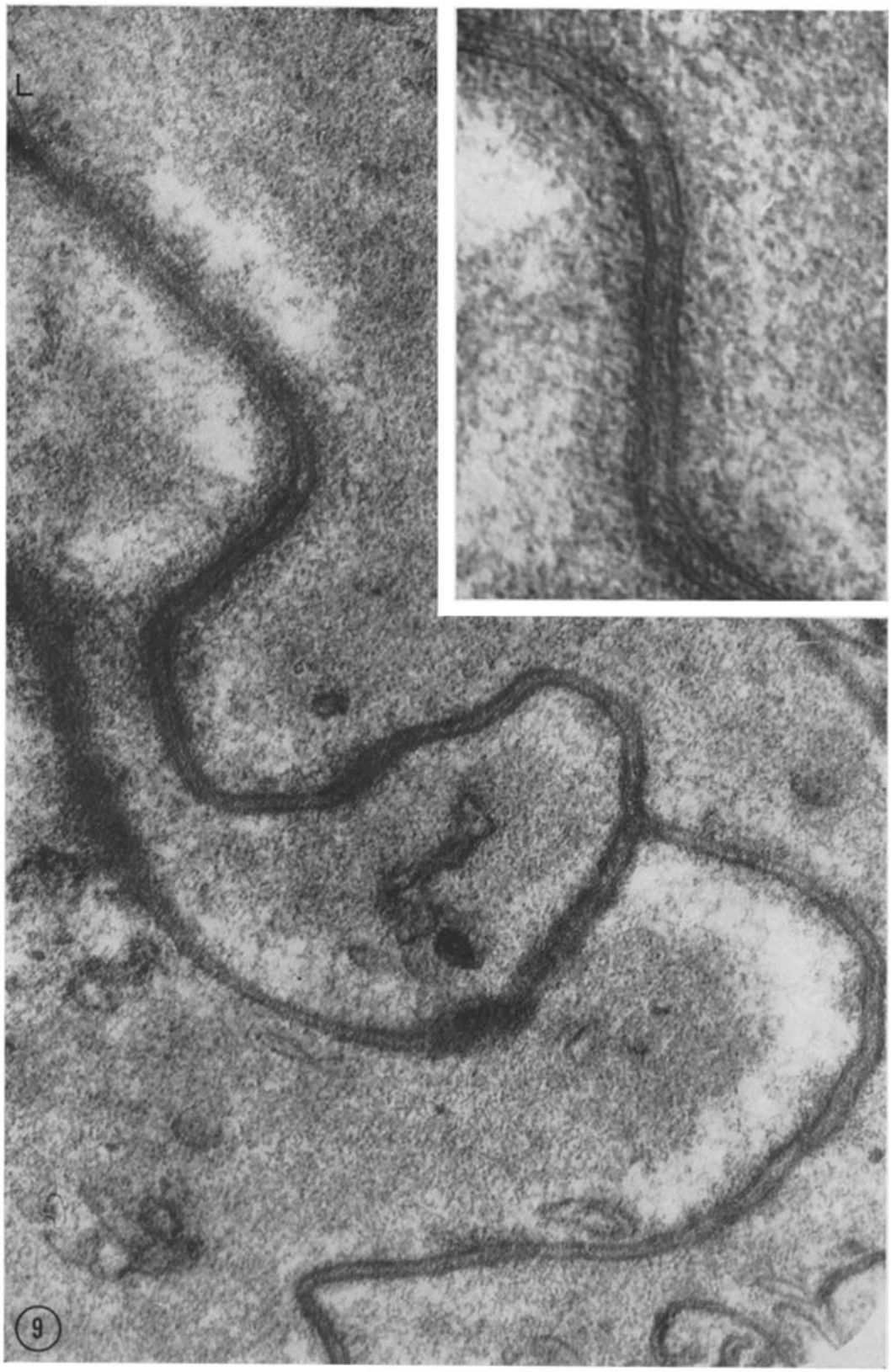

FIGURE 9 The apical part of an intercellular junction after permanganate fixation (*L*, lumen). Note the lack of septa in the intercellular gap. The inset shows the trilaminar nature of the plasma membranes and the granular material in the intercellular gap. Fig. 9, × 96,000; inset, × 220,000.

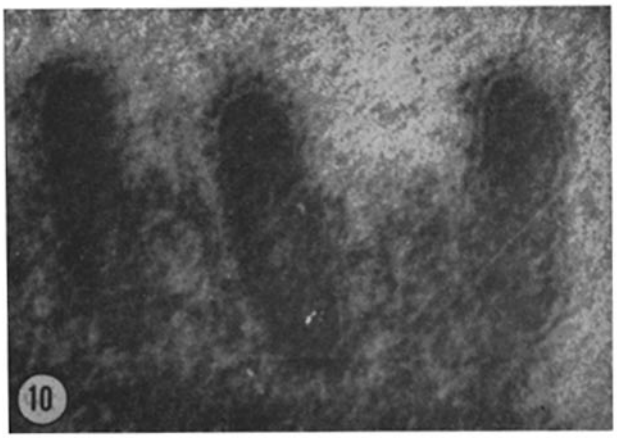

FIGURE 10 A permanganate-fixed cuticle showing the small ellipsoidal bodies embedded in the outer cuticular layer. Note that there is a trilaminar membrane around the projections, the inner layer of which blends in many places with dark material that makes up the core of the projections. × 220,000.

sists of 10 to 20 cisternae, each group surrounded by a cloud of vesicles (Fig. 2). A serial reconstruction of a cell would probably reveal that these cisternal groups form a single extensive network. The cisternal and vesicular contents are amorphous and appear slightly more dense than the cytoplasmic matrix. Innumerable small mitochondria are found throughout the cytoplasm (Figs. 2 and 5), but they, too, are most abundant in the apical region of the cell. Solitary cisternae of the granular endoplasmic reticulum are numerous. The agranular endoplasmic reticulum is relatively poorly represented.

Many dark, membrane-bounded granules, 1000 to 2000 A in diameter, are found throughout the cytoplasm of the columnar cell, but they are most abundant in the perinuclear region (Fig. 1). These granules appear to be melanin or lipofuscin, but the possibility that they are lysosomes cannot be ruled out here. Numerous bundles of fine tonofilaments course throughout the cytoplasm (Figs. 2 and 4). Some of them terminate in the desmosomes at the lateral cell surface, others into the half-desmosomes that attach the cell to its basal substrate, and still others into the microvillar connections with the cuticle, but the majority appear to end free within the cytoplasm. They are presumably cytoskeletal in function.

The cytoplasmic organization of the basal cells is similar to that of the columnar cells, except that the Golgi complex and mitochondria are more evenly distributed throughout the cell.

The mature mucous cell is distended with mucus (Fig. 1) so that, with the light microscope, it appears as a globular cavity filled with PAS-positive material and enclosed by a tenuous layer of cytoplasm. The cavity communicates to the outside via a channel in the cuticle. Other cells may also contain a single mucous cavity, but they are not so distended with mucus, and the cytoplasm is thicker and less attenuated. Still others possess large vacuoles, 0.1 to 1.6 μ in diameter, filled with mucus, and the electron microscope reveals that the membranes around the larger vacuoles are ruptured, allowing the contents of several vacuoles to coalesce (Fig. 6). These stages presumably represent a cycle of mucus production and release.

Intercellular Junctions

Three junctional specializations unite epithelial cells in epidermis and intestine in the earthworm. They are the zonula adhaerens, the macula adhaerens, and the septate desmosome. Each will now be considered in more detail.

THE ZONULA ADHAERENS: Typically, at the cuticular surface of the epidermal epithelium or the luminal surface of the gut, the membranes of apposed epithelial cells are relatively straight and are separated by a distance of approximately 200 A (Fig. 8). After osmium tetroxide or glutaraldehyde fixation, a narrow band of electron-opaque cytoplasm can be seen closely applied to the inner aspect of the apposed plasmalemma (Fig. 8). Favorable tangential sections allow this junction to be followed for some distance around the periphery of the cell, thus establishing it as a belt or zonula. This configuration identifies it as a zonula adhaerens (8). In the earthworm epidermis, the zonula adhaerens is usually 300 to 500 A deep (rarely, 1000 A), whereas in the intestine, it usually extends the full width of the terminal web, which is sometimes more than 1 μ.

THE MACULA ADHAERENS OR DESMOSOME: Desmosomes are found anywhere from the zonula adhaerens to the basal lamina that underlies the epithelium (Fig. 2). These junctions consist of symmetrical plaques of electron-opaque cytoplasm into which cytoplasmic tonofilaments are inserted. The plaques are apposed to facing patches of the lateral cell membranes of two epithelial cells. In these regions, the intercellular gap is approximately 150 A, and the cell membranes are relatively straight. These junctions differ somewhat from their vertebrate counterparts which have a widened intercellular gap bisected by an intermediate line, but the functional correlates of these differences are not known. Both the zonula adhaerens and the desmosome seem to be attachment points between epithelial cells, since, when markedly hypertonic fixatives are used, the epithelial cells are separated except at these points.

THE SEPTATE INTERCELLULAR JUNCTION: The septa that traverse the cleft between earthworm epithelial cells are most numerous at the apical end of the intercellular junction, just under the zonula adhaerens (Fig. 8). In section they appear as bars 60 A thick that span the intercellular gap (Fig. 8). They blend with the outer dark lines of the apposed unit membranes, but they themselves are not trilaminar (Fig. 8). In tangential sections, parallel to the cell surface, they can be followed for some distance. It is possible that they completely girdle the cell. The distance between septa is not constant. For example, septa may occur singly but, more commonly, many are found spaced approximately 200 A apart (Fig. 8). In septate regions, the intercellular gap varies somewhat in its dimensions, but it is usually about 150 A across. The cell membranes are as likely to curve in septate as in nonseptate regions of the intercellular junction, differing in this respect from the cell membranes at the zonula adhaerens or desmosome, which are straighter. In material fixed in osmium tetroxide or glutaraldehyde, the thickness of the plasma membrane is approximately 65 A, and its trilaminar nature is only revealed in very thin sections. With permanganate fixation, the plasma membrane is 75 A wide and its trilaminar nature is more obvious, but the septa are not preserved by this fixative (Fig. 9). Instead, the intercellular gap appears to be occupied by a granular substance which, on occasion, appears aligned to form an indistinct intermediate line.

The Innervation of the Epithelium

The epidermal epithelium is richly innervated by large numbers of unmyelinated axons. The axons are minute, measuring 0.05 to 0.4 μ in diameter, and nerves consisting of 5 to 50 axons can be found in almost every electron micrograph of basal parts of this epithelium (Fig. 7). There is no doubt about the identity of these processes, for in favorable sections they can be traced back into the large nerves that course through the underlying fibrous tissue. Considering the small size of the neuronal processes in comparison to the silver and methylene blue pictures of these nerves, it seems certain that under the light microscope the nerve bundles were misinterpreted as single fibers. Small satellite cells are scattered along the nerves but they do not enfold the axons as mammalian Schwann cells do. Instead, the most axons are directly contiguous with one another and the peripheral axons are contiguous with satellite cells, with epithelial cells, or with the basement lamina that underlies the epithelium (Fig. 7). These axons are presumably sensory in nature, although some may innervate mucous cells. A more complete cytologic analysis of the earthworm epidermis would include a description of the structure and innervation of the sense organs. Because of the complexity of this subject, however, it will not be considered here.

DISCUSSION

The earthworm cuticle is an ordered arrangement of collagenous fibers embedded in an amorphous matrix. Since epidermal cells are contiguous with the cuticle and intervene between it and the rest of the organism, it has been stated that the collagenous precursors of the cuticular fibers originate from the epithelial cells (25, 2). Further evidence for the epithelial origin of the cuticular fibers comes from a consideration of the connective tissue cells in the earthworm. If connective tissue cells produce the collagenous precursors of the cuticular fibers, one would expect to find large numbers of mesenchymal cells in the connective tissue. It would also be reasonable to expect that these cells would have a fine structure indicative of a considerable amount of protein synthesis; namely, a well developed granular endoplasmic reticulum and Golgi apparatus. This is not the case, however, for cells in the subepithelial layer are widely scattered and, when found, are full of bacteroides crys-

tals or appear to be scavenging, wandering cells. If more refined studies confirm the morphological observation that fibroblasts are extremely scarce or totally absent in *Lumbricus*, then another cell type must be responsible for the collagen of the cuticle. If this is the case, epithelial cells are the most likely candidates, for (*a*) they are closest to the cuticle and separate it from the rest of the organism, and (*b*) they possess the largest and most highly developed Golgi apparatus and granular endoplasmic reticulum.

The connective tissue fibers in the interior of the organism are also collagen (25, 2), even though they differ morphologically from the collagenous cuticular fibers. Because an epithelium does not separate these deep fibers from the rest of the organism, there has been no compelling reason to suggest that they are formed or aligned by epithelial cells. Nevertheless, if the observation that there are essentially no fibroblasts in the connective tissue of the adult earthworm be accepted, one must assume that the collagenous precursors of the connective tissue fibers, as well as the precursors of the cuticular fibers, are elaborated by another cell type, most probably epithelial cells. The participation of muscle and other body wall cells in this process, however, cannot be ruled out here.

Septate intercellular junctions have been reported in epithelia of various invertebrates (cf. references 29, 19, and 15). Wood, who first drew attention to these junctions (30), described the septa as trilaminar lamellae that appear to be continuations of the plasma membrane from one cell to the next. These conclusions were accepted by Locke (16), who added the finding that, in insect epidermis, the septa form hexagonal arrays best seen when the section is tangent to the intercellular junction. A different septal structure was proposed by Overton (20), who maintained that the septa in the epithelium of *Cordylophora* consist of two parallel, electron-opaque bodies that are not attached to the adjacent plasma membranes. The septa between earthworm epithelial cells do not appear to be similar to any described above. After osmium tetroxide or glutaraldehyde fixation, the septa appear as solid lamellae which, in cross-section, are seen as 60 A–wide bars that span the intercellular gap. They are clearly not extensions of the plasma membrane across the intercellular gap, for they are not preserved by permanganate fixation which is particularly favorable for demonstration of the trilaminar structure of plasma membranes. Although the above descriptions seem to indicate that several distinct junctional devices are grouped under the term septate desmosome, it is possible that the differences reflect different interpretations of a single structure. A reexamination of these junctions using several common fixatives, embedding media, etc., will be necessary to resolve this uncertainty. Such a resolution is particularly pertinent in view of recent evidence indicating that a septate desmosome is probably the low resistance pathway between epithelial cells in the salivary gland of *Drosophila flavorepleta* (17, 29).

The small ellipsoidal bodies embedded in the outer cuticular layer were first visualized in electron micrographs of the isolated, flattened, and shadowed cuticle, and it was then speculated that they might be viruses or bacteriophages (23). Although these bodies are in the size range of viruses or bacteriophages, it would be difficult to account for their constant occurrence and highly ordered arrangement. Moreover, such organisms are usually obligatory intracellular parasites and presumably could not survive on the surface of an extracellular matrix. Another possibility is that these structures are minute bacteria or fungi, but their fine structure differs from that of all known bacteria or fungi. The possibility that these bodies are some form of microorganism cannot be ruled out on morphological grounds alone, but if they are microorganisms, they are different from any previously described.

When these ellipsoidal bodies were first seen in sections, they were identified as microvilli (9, 22). This is an incorrect interpretation of these structures, however, for they are isolated bodies and are separated from the epithelial cell body by the width of the cuticle. The tips of the long microvilli do come to rest among the ellipsoidal bodies, but these microvillar tips are greatly outnumbered by the ellipsoidal bodies. Although the ellipsoidal bodies are not microvilli, it is possible that they are pieces of cytoplasm that have separated from the underlying epithelial cells. This interpretation is not attractive, however, for it is difficult to understand how small, isolated pieces of cytoplasm could become vertically oriented and retain their integrity and viability when separated by up to 20 μ from their parent cell.

A third possibility is that the ellipsoidal bodies are not living protoplasm. This, too, is an unattractive alternative since it is again hard to understand

how they could be aligned vertically. Moreover, nonliving material is not usually surrounded by a unit membrane. Thus the nature of these small ellipsoidal bodies remains obscure. They are not unique, however, for apparently similar small bodies form the outer border layer of the egg of the annelid, *Hydroides hexagonus*, and these, too, are separated from the egg proper by a thick layer of extracellular material (7).

I would like to thank Doctors Don W. Fawcett, Elizabeth D. Hay, and Jean Paul Revel for helpful advice and criticism. This study was partially supported by grant GB 3595 from the National Science Foundation.

Received for publication 15 July 1965.

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