PIGMENT EPITHELIAL ENSHEATHMENT AND PHAGOCYTOSIS OF EXTRAFOVEAL CONES IN HUMAN RETINA

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[Plates 1–14]

CONTENTS

The association between extrafoveal cone outer segments and pigment epithelial cells was studied by transmission electron microscopy in three human retinas; ages 5, 45 and 60. The pigment epithelial apical surface from a fourth human retina, age 38, was viewed in the scanning electron microscope. Multiple villous-like apical processes protrude from the pigment epithelium into the space above each cone. Sometimes one or more of these processes is sheet-like in form and contains a wealth of intracellular organelles, including mitochondria. One or more of the villous-like processes reaches the cone and expands to ensheath the upper one-third of the outer segment. Like vertebrate rods, extrafoveal human cones shed their terminal disks in packets and these packets are phagocytosed by the ensheathing apical processes. The phagosomes then ascend in the processes toward the pigment epithelia soma. Digestion of phagosomes appears to begin in the apical processes.

† Deceased.

Introduction

Another study from this laboratory, in cat retina, showed that cone outer segments are ensheathed by long leaf-like apical processes from the pigment epithelium (Steinberg & Wood 1974). Human extrafoveal cones also are closely associated with pigment epithelial processes, as first described with the light microscope by Walls (1934), and later confirmed by Eichner (1958), and recently by electron microscopy (Fine & Yanoff 1972; Hogan & Wood 1973). This relationship is still not widely known and its detailed anatomy has not been previously described.

Phagocytosis of cone disks by the pigment epithelium was suggested by Hogan's (1972) finding of phagosomes in pigment epithelial cells from human fovea and foveola. The existence of a close relationship to the epithelium suggested that it also occurred in extrafoveal cones (Steinberg & Wood 1974) and in an earlier report we demonstrated phagocytosis of extrafoveal cone disks in the retina of a 5 year old boy (Hogan, Wood & Steinberg 1974). At the same time, Anderson & Fisher (1975, 1976) described disk shedding and phagocytosis in squirrel cones (arboreal and terrestrial species). This process appears *not* to occur, however, in the non-mammalian vertebrate retinas so far examined (Young 1971 *a*; Bok & Young, in press).

In the present report we describe the ultrastructure of the relationship between human extrafoveal cone outer segments and their pigment epithelial apical processes. We also describe how these processes phagocytose packets of terminal disks. Finally, we present evidence for the normalcy of this process by showing it in three human retinas that span a wide age range.

Methods

Transmission electron microscopy was performed on three human retinas obtained from eyes that had been surgically removed. The pathological diagnoses were as follows: 5 year old male – rhabdomyosarcoma of the orbit; 45 year old female – malignant melanoma of ciliary body; 60 year old male – adenocarcinoma, arising from ductal epithelium of lacrimal gland. In none of these retinas was there any indication of invasion by the disease or of secondary pathological changes attributable to the disease. The retinas were fixed with 3 % paraformaldehyde-glutaraldehyde in 0.2 M sodium cacodylate buffer with the addition of 1 % sucrose, 0·2 % calcium chloride and 0.5 % potassium chloride for 3–4 h at 20 °C. Specimens then were taken from the central region, washed in 0·2 M sodium cacodylate, 0·5 M sucrose and post-fixed in 2 % osmium-tetroxide in sodium veronal acetate buffer plus sucrose for 2–3 h at 4 °C. The tissue then was washed in 0.28 M NaH maleate buffer and stained *en bloc* in 2 % uranyl acetate in NaH maleate buffer, pH 5.6, for 4 h at 4 °C. Tissues were dehydrated in graded 70–100 % acetone and embedded flat in Araldite 502. Sections of 1 μm thickness were obtained for purposes of orientation, stained with basic fuchsin and methylene blue, and examined in a Leitz photomicroscope. Ultra thin sections, obtained for electron microscopy, were mounted on single-slot grids coated with parlodion and stained with 2 % uranyl acetate followed by lead citrate. They were examined with a Siemens Elmiskop 1A electron microscope.

The pigment epithelial apical surface from a fourth human retina, age 38, diagnosed as a malignant melanoma of the choroid, was studied by scanning electron microscopy. The specimens of pigment epithelial–choroid–sclera were pinned flat on pieces of parawax and fixed in 3 % glutaraldehyde 0.1 M cacodylate buffer at pH 7·4, 300 mosmol/l for a minimum of

FIGURE 1. Electron micrograph montage of a longitudinal section through a cone outer and inner segment and the pigment epithelial apical processes. The cone is surrounded by rods on both sides. Several inclusions appear within the processes in the supracone space. One of these (arrow) is probably a phagosome, and it also appears in an enlargement in figure 26, plate 7. 60 year old retina. (Magn. × 3800.)

FIGURE 2. Electron micrograph montage of a longitudinal section through another cone from the retina of the 60 year old. The pigment epithelial apical processes in the supracone space appear especially thick in this section and have many dense granules protruding into them for no farther than one-half of the length of the processes. An enlargement of the apical processes appears in figure 4, plate 2. (Magn. × 3720.)

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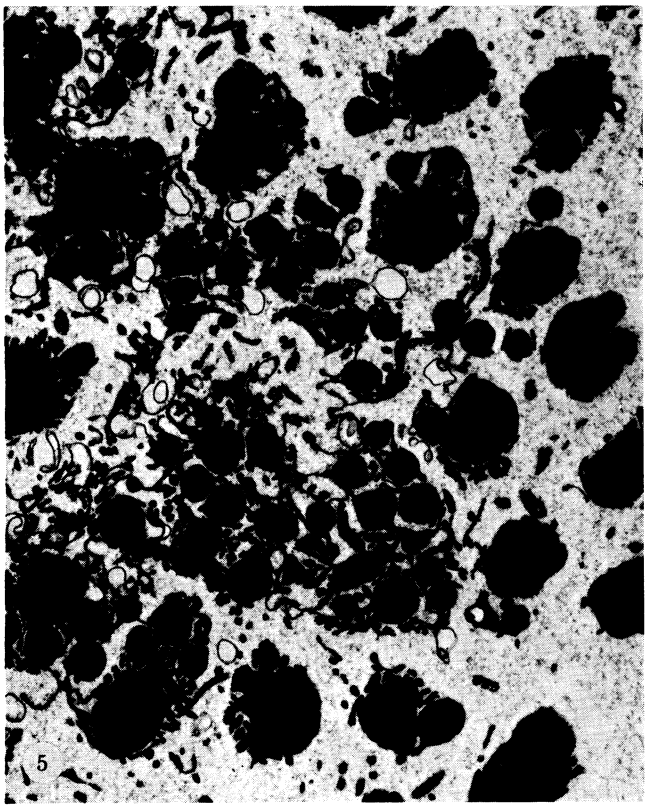
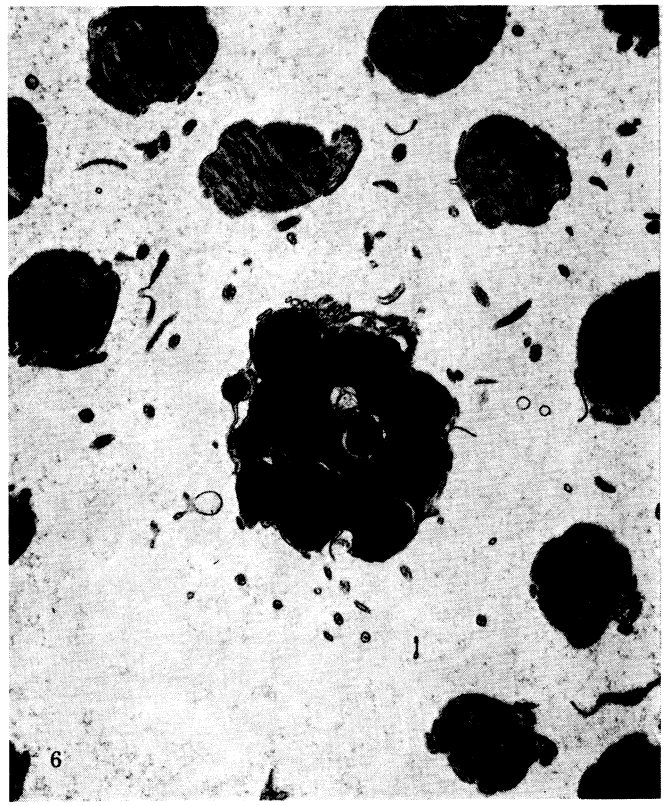
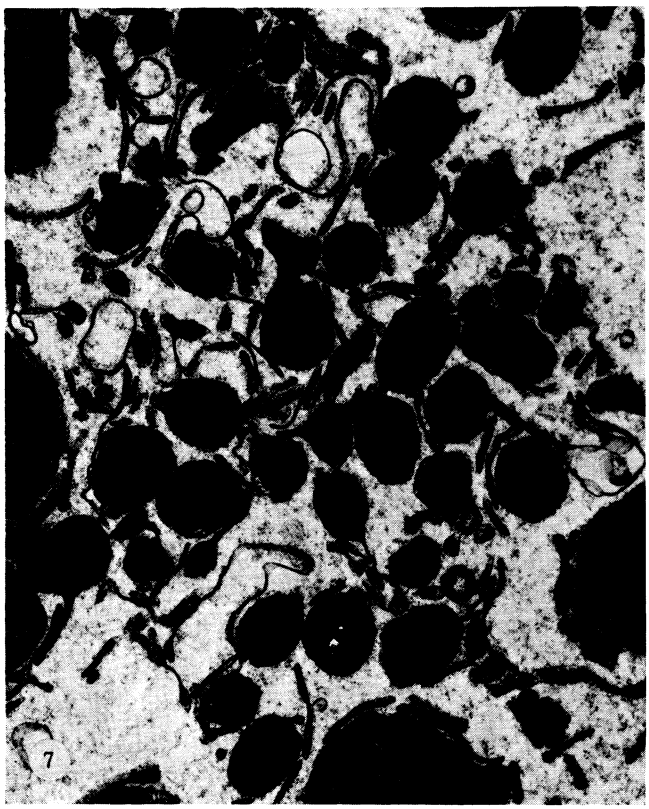
FIGURE 3. Electron micrograph of longitudinal section through pigment epithelial processes at their apical origin. A mitochondrion appears below one of the pigment granules and there are several dense bodies further vitread in the processes. The apical one-half of the pigment epithelial soma appears at the bottom of the micrograph and two rod phagosomes, having a lamellar structure, appear in the cell above a rod. 5 year old retina. (Magn. × 8800.)

FIGURE 4. Enlargement of the pigment epithelial apical processes from the cone of figure 2, plate 1. (Magn. × 8800.)







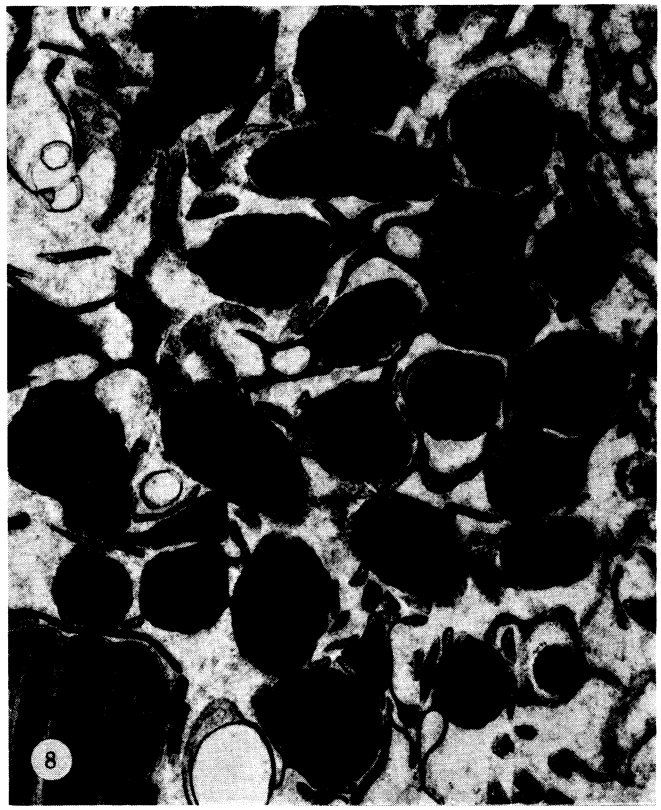


FIGURE 5. Electron micrograph of a transverse section through a supracone space. The space is surrounded by rod outer segments. It contains apical processes that assume a variety of thinly shaped profiles and many of them contain pigment granules. 5 year old retina. (Magn. × 4200.)

FIGURE 6. Electron micrograph of a transverse section through a supracone space. There is only one large process in the space and it contains pigment granules and a less dense inclusion that is probably a phagosome. 5 year old retina. (Magn. × 6400.)

FIGURE 7. Electron micrograph of a transverse section through a supracone space; similar to figure 5 except at a higher magnification. 5 year old retina. (Magn. × 10590.)

FIGURE 8. Electron micrograph of a transverse section through a supracone space. Similar to figure 7. Two processes, each containing two pigment granules, appear on the right. 5 year old retina. (Magn. × 12800.)

FIGURE 9. Electron micrograph montage of a longitudinal section through a cone outer segment and a portion of inner segment, and the pigment epithelial apical processes. One of the processes is thick and contains a variety of intracellular organelles, including a mitochondrion. A thick process extends along the left side of the outer segment. 5 year old retina. (Magn. × 3650.)

FIGURE 10. The tip of the cone outer segment of figure 9, at a higher magnification. (Magn. × 16200.)

FIGURE 11. The portion of apical process containing the mitochondrion in figure 9, at a higher magnification. (Magn. × 22500.)

FIGURE 12. Electron micrograph of a longitudinal section through a cone outer segment at its tip. Three apical processes on the left and one on the right extend alongside the outer segment. 5 year old retina. (Magn. × 35000.)

24 h at 4 °C. After fixation the tissues were washed in 20 % ethyl alcohol for 24 h to clean the surfaces. They were dehydrated in 50–100 % ethyl alcohol and brought up to room temperature in the third change of 100 % ethanol. After replacing the ethanol with amyl acetate, a polar solvent miscible with CO_2, specimens were critical point dried with CO_2. The dried specimens were cracked by applying slight pressure with a forceps as the specimens were mounted on the specimen stub. They were plated with 15 nm of gold in a vacuum of 2.7 mPa and viewed in a Kent-Cambridge model S4 scanning electron microscope at magnifications of 20000–25000 (20 kV). Photomicrographs were taken on positive–negative Polaroid Land film type 55.

Results

Identification of extrafoveal cones

Cones were readily distinguished from rods in longitudinal sections by their wider inner segments and shorter outer segments (figures 1 and 2, plate 1). Cone outer segments did not reach the apical surface of the pigment epithelium, and had an average length in the three retinas of 19 μm (Rg. 16–23). This left a space between the pigment epithelial surface and the cone outer-segment tips, the *supracone space*, which was surrounded by rod outer segments. This space had an average length of 19 μm (Rg. 15–23) and an average diameter of 11 μm (Rg. 8–13). A cone was to be expected whenever a space of this type appeared in the photoreceptor mosaic. Pigment epithelial apical processes protruded into this space, pointing at the cone (figures 1 and 2, plate 1; figures 3 and 4, plate 2).

Association of cone outer segments with pigment epithelial processes

The appearance of the pigment epithelial processes in the supracone space varied considerably from cone to cone and between different thin sections through the same cone. The ordered and symmetrical arrangement of the processes, which gave them a uniform appearance in the cat (Steinberg & Wood 1974), was not present in the human retinas. In longitudinal sections the processes most often appeared as thin, villous-like extensions of the pigment epithelium. Usually, only from one to six such processes could be identified in any one section but occasionally a larger number were arranged side by side in the supracone space near the apical surface (figure 3, plate 2).

Pigment granules were the most frequent inclusions found in the processes, and individual processes could contain more than one granule. They were usually located close to the apical surface and rarely extended into the processes farther than one-third the length of the supracone space. We did not see pigment granules in the portions of the processes that ensheathed the cone outer-segment (see below).

An accurate estimate of the numbers of pigment granules protruding into any one supracone space was difficult to obtain from individual sections. They usually showed six granules or less but occasionally the section passed through a wide region of processes and showed a much larger number of granules. Transverse sections through the supracone space (figures 5–8, plate 3) and scanning electron micrographs of the pigment epithelial surface (figures 20, 21, plate 6) indicated that as many as 30 or 40 pigment granules could occupy the processes at the apical end of the supracone space.

In these transverse sections a thin rim of process cytoplasm surrounded each pigment granule or the two pigment granules that occasionally occupied a single process. Processes that

did not contain granules were intermingled and appeared as thin cytoplasmic sheets or small circular profiles (figures 5, 7, 8, plate 3). Farther towards the cone the number of processes and the number of pigment granules decreased. Figure 6, plate 3 shows a single large process, farther in the space towards the cone, containing at least 12 pigment granules and an unidentified phagosome-like inclusion.

The processes usually appeared villous-like and relatively free of intracellular organelles. Sometimes, however, processes were enlarged into wide cytoplasmic sheets that contained a rich variety of organelles (figures 9–11, plate 4). We could identify smooth endoplasmic reticulum, ribosomes, and a variety of vesicles, which varied from clear to dense and from small to large. The appearance of some small dense membrane-bound vesicles was consistent with previous descriptions of primary lysosomes (Novikoff 1973). There also were coated vesicles and coated pits in the plasma membrane that opened to the extracellular space. Mitochondria also occurred in the processes (figure 3, plate 2; figure 11, plate 4; figure 30, plate 9) but we never saw them lower than one-half of the length of the space. Other organelles, particularly vesicles, occupied the processes all the way to the outer segment (figure 10, plate 4).

Pigment epithelial apical processes reached the cone outer segment and extended alongside of it. As many as three processes could be observed along one side of the outer segment in longitudinal sections (figure 12, plate 4). We did not see processes extend farther along the outer segment than about one-half of its length. In transverse sections, these processes appeared as cytoplasmic sheets that wrapped around each other. They could be identified both above the tip of the outer segment, in the supracone space, figure 14, plate 5, and surrounding the outer segment itself, figures 15–19, plate 5. When *several* processes surrounded the cone, as in figure 16, plate 5, it was still not clear to us if any one of them encircled it completely. *Single* processes could, however, completely ensheath the outer segment (figures 17–19, plate 5).

Surface views of the pigment epithelial processes by scanning electron microscopy are shown in figures 20 and 21, plate 6. As in cat, apical processes that associate with cones form large, distinct protruberances. Shapes of pigment granules can be seen in the processes near the

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Description of plate 5

Figure 13. Electron micrograph of a longitudinal section through a cone outer segment at its tip; from the cone of figure 38, plate 11, at a higher magnification. Two pigment epithelial apical processes on the left and one on the right extend alongside the outer segment. The inner process on the left bifurcates at the outer-segment tip and one portion of it extends along the top surface of the outer segment. 5 year old retina. (Magn. × 28600.)

Figure 14. Electron micrograph of a transverse section through a supracone space. The plane of the section is near the tip of the cone outer segment. The apical processes appear at the lower left. Three are sheet-like and concentrically arranged. A number of circular or elliptical villous-like profiles also appear. 5 year old retina. (Magn. × 23400.)

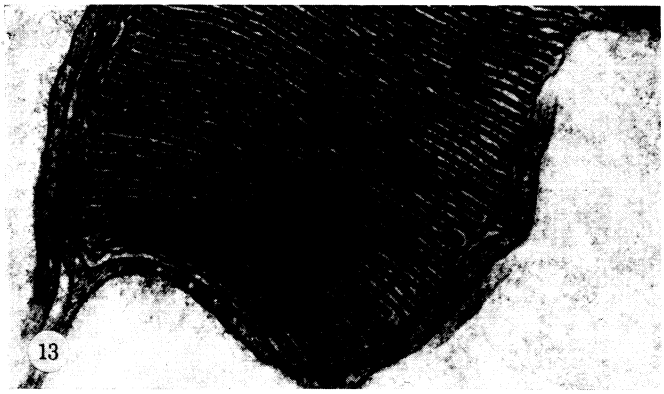
Figure 15. Electron micrograph of a transverse section through a cone outer segment somewhere within its outer one-third. The outer segment is ensheathed by multiple pigment epithelial apical processes. 5 year old retina. (Magn. × 9600.)

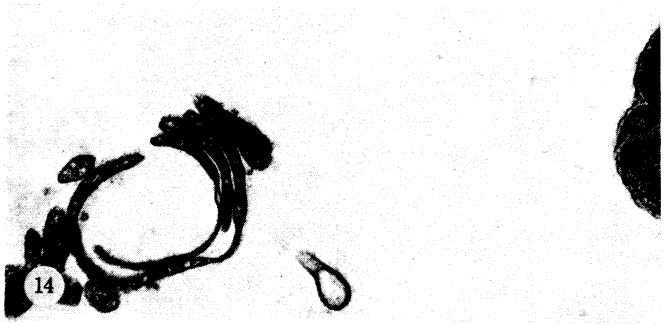
Figure 16. The outer segment of figure 15, at a higher magnification. (Magn. × 24000.)

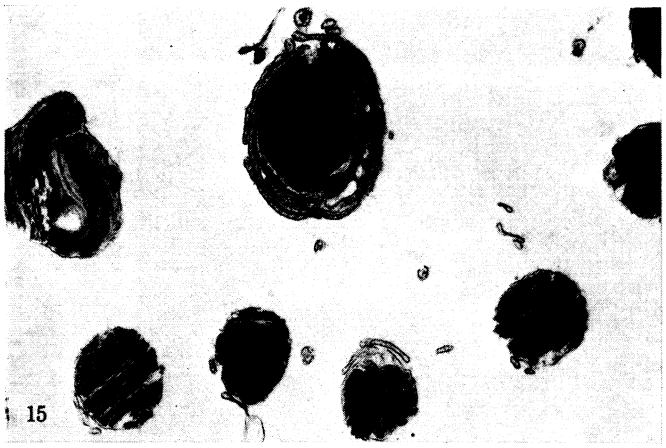
Figure 17. Electron micrograph of a transverse section through a cone outer segment somewhere along its outer one-third. Pigment epithelial apical processes surround the outer segment and one process seems to ensheath it completely. 5 year old retina. (Magn. × 36000.)

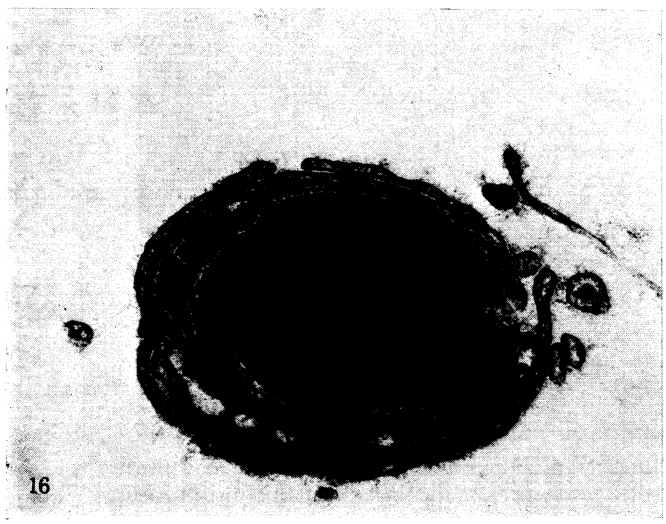
Figure 18. Electron micrograph of a transverse section through a cone outer segment somewhere along its outer one-third. One pigment epithelial apical process completely ensheaths the outer segment. There also is a circular profile of a process that contains a dense body. 5 year old retina. (Magn. × 10400.)

Figure 19. The outer segment of figure 18, at a higher magnification. (Magn. × 33600.)









FIGURES 13–19. For description see opposite.

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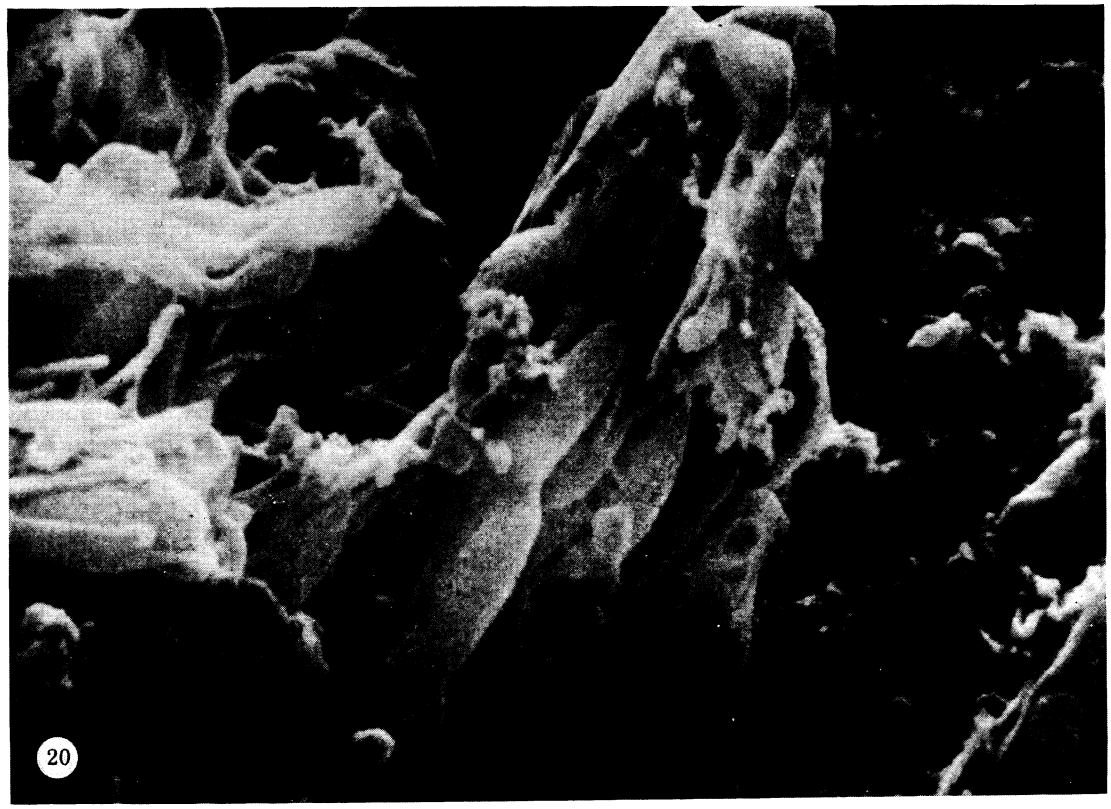


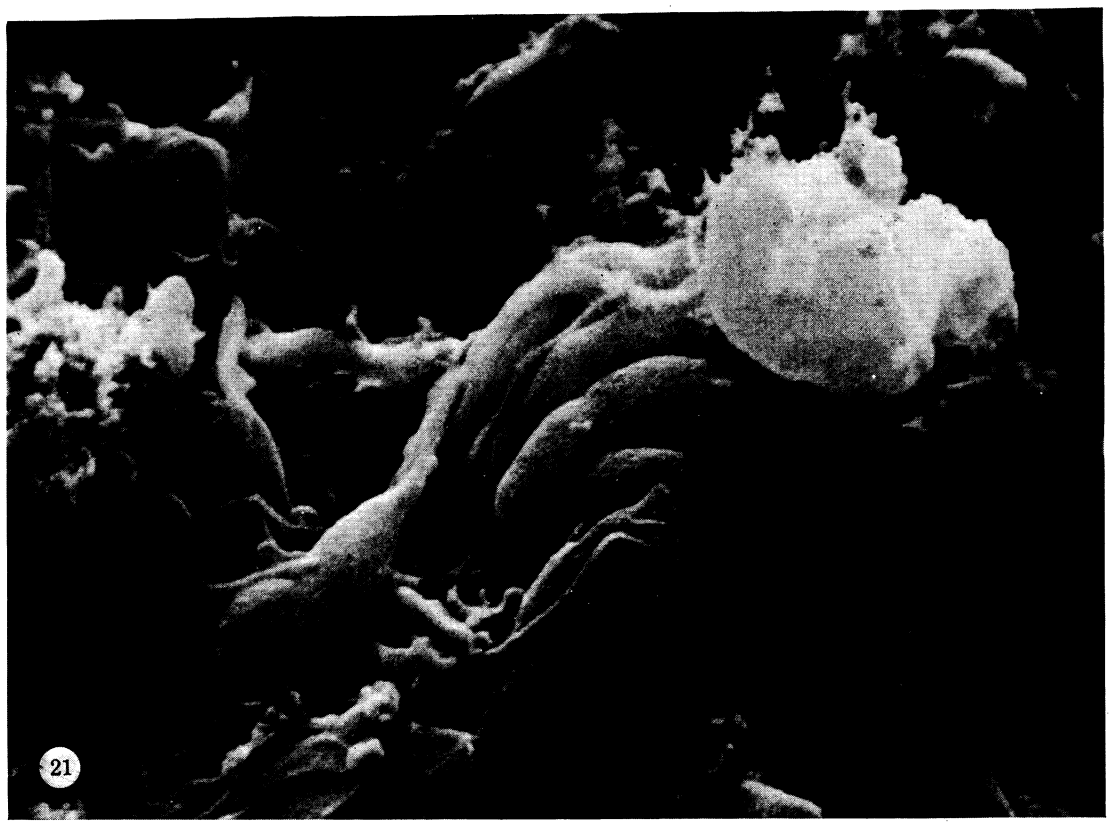


FIGURE 20. Scanning electron micrograph of pigment epithelial apical processes that associates with a cone. Profiles of pigment granules appear in the processes as they protrude from the apical surface of the pigment epithelium. Two of the processes become thin or villous-like above the pigment granules. Farther out the processes are sheet-like and, perhaps, have a tubular form. They have fallen over to the right. 38 year old retina. (Magn. × 14400.)

FIGURE 21. Scanning electron micrograph of another group of apical processes that associate with a cone. The form of the processes is similar to that of figure 20. 38 year old retina. (Magn. × 13035.)

pigment epithelial surface, and more than one pigment granule can be identified in single processes. Farther away from the surface, the processes narrow into villous-like profiles, which then become lost in a mass of membrane that forms the remainder of the protruberance. In figure 20, this appears to consist of one or perhaps two tubular projections that have fallen over.

Phagocytosis of packets of terminal disks

Phagocytosis of groups of cone disks by pigment epithelial apical processes was observed in each of the three retinas. The initial observations were made in the 5 year old retina (Hogan, Wood & Steinberg 1974), which also was the most thoroughly studied retina, providing the largest number of examples (plates 7–11, 13 and 14). Additional evidence, obtained in the 45 year old and 60 year old retinas (figure 25, plate 7; figures 40, 41, plate 12), corroborated the major findings on the 5 year old.

The terminal disks of cones, while still in their usual position at the outer-segment tips, did not appear differently from the other disks of the cone. As with monkey and squirrel rods, there was no change in membrane structure, disk spacing or density, which would indicate that a certain group of these disks might be soon shed and phagocytosed (Young 1971*b*; Anderson & Fisher 1975, 1976).

There were two different events that could be identified as the earliest stages of this process. In one, a group of terminal disks curled at their edges and began to separate from the outer segment before there was any sign that they might be phagocytosed by the apical processes. In the other, apical processes were seen phagocytosing a packet of terminal disks, which showed no evidence of having changed morphologically.

In figures 22, 23, plate 7, for example, the terminal disks have curled at their edges to form a whorl that bulges from the cone outer segment tip into the space above it. There are no degenerative changes, such as an increase in membrane density, of the disks in this packet. The packet is still enclosed by the plasma membrane of the outer segment, but this membrane pinches in on one side (arrow). The apical processes extend along both sides of the outer segment and give no indication that they may soon phagocytose the terminal packet.

Figures 27–29, plate 8 and figures 30 and 31, plate 9 represent a different view of the earliest events. In figures 27 and 28, plate 8, the terminal forty disks are almost completely surrounded by an apical process. These disks have not undergone any observable change in their position with respect to the outer segment, nor do they show any degenerative changes. The phagocytosing apical process has spread over the tip and down the outer segment past the terminal forty disks. Just below disk forty it sends in a pseudopod that cleaves the terminal packet away from the remainder of the outer segment. (It is possible, however, that the plasma membrane of the cone may have pinched in below the fortieth disk as an even earlier event.)

A packet of forty disks appears above the tip of the cone in figures 30 and 31, plate 9. The participation of apical processes in phagocytosing this group of disks is less clear than in plate 8. There is an apical process between the packet and the outer-segment tip, but we cannot determine if the packet actually has been incorporated into any one of the processes. In this example the disks in the terminal packet appear slightly more electron dense than their counterparts in the outer segment.

In the next stage, the detached packet of disks appears above the tip of the outer segment in the form of a phagosome: surrounded by pigment epithelial plasma membrane and located within an apical process. The phagocytosed disks always are more electron dense than those ot

the outer segment. They may have curled at their edges as in figures 35 and 36, plate 10, or remained relatively flat, as in figure 33, plate 9, and figure 39, plate 11. The packet of disks assumes different orientations with respect to the outer segment, sometimes remaining in its usual orientation (figures 35, 36, plate 10; figure 39, plate 11), and at other times rotating so that disk edges face into the process and toward the pigment epithelium (figure 33, plate 9; figure 39, plate 11; figure 40, plate 12).

An apical process that has recently phagocytosed a packet of disks appears to remain closely associated with the cone outer segment. It descends toward the cone from the region of the phagosome, extends down along both sides of the outer segment, and encloses the extracellular space that lies between the phagosome and the outer segment tip (plates 10–12). The top surface of the outer segment may be covered by apical processes that have grown over it, as in figure 13, plate 5, or it may be free of apical processes, as in figures 34 and 35, plate 10.

Phagosomes in apical processes also were identified in transverse sections through the supracone space. They appeared inside single processes, enclosed in pigment epithelial plasma membrane, and surrounded by a rim of process cytoplasm (figures 42–45, plate 13). As in the longitudinal sections, the disks may have changed their orientation so that disk edges face the epithelium (figures 42–44, plate 13).

Multiple phagosomes above the tips of the outer segments were commonly found in the 5 year old retina, and were only occasionally seen in the 45 and 60 year old retinas. The four phagosomes above the cone in figure 27, plate 8 was the largest number ever observed. Figure 34, plate 10, shows three phagosomes, while there are two above the cones in figure 32, plate 9, and figure 38, plate 11. The disks of the oldest phagosomes, those closest to the epithelial surface, frequently have turned 90° away from their usual orientation in the outer segment (figure 33, plate 9; figure 39, plate 11).

Successive phagosomes above cones may be contained within a single apical process or in separate processes. The two phagosomes of figure 39, plate 11, for example, are inside one apical process. Notice that they are separated by an elliptically shaped portion of extracellular space whose boundaries are formed by apical process plasma membrane, and the situation is similar for the two phagosomes of figure 33, plate 9. The successive phagosomes of figure 49, plate 14, however, are contained in separate processes.

Description of plate 7

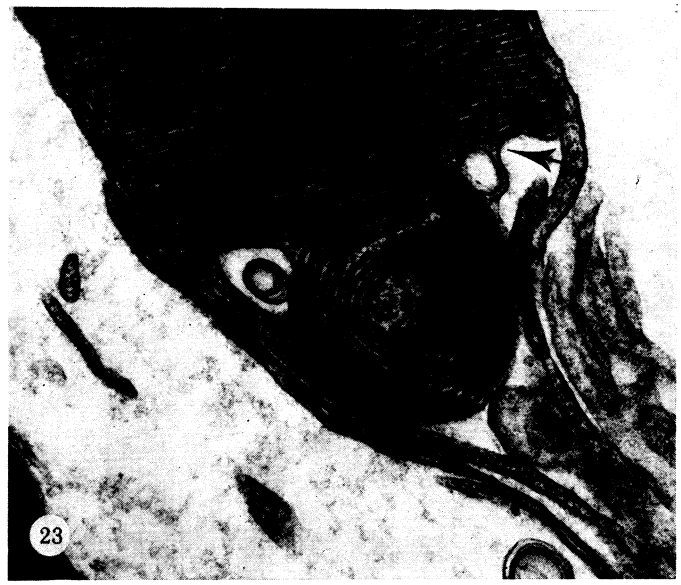
Figure 22. Electron micrograph montage of a longitudinal section through a cone outer segment, portion of inner segment, and pigment epithelial apical processes. A process, swollen with intracellular organelles, appears immediately below the apical surface. At the outer-segment tip there is a whorled packet of disks. 5 year old. (Magn. × 4250.)

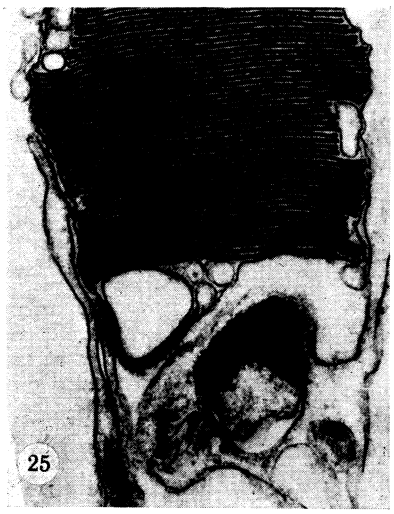
Figure 23. The tip of the cone outer segment of figure 22, at a higher magnification. Pigment epithelial apical processes extend along both sides. The terminal nine disks have curled into a circular packet that is still enclosed by outer-segment membrane. The outer segment membrane appears to be pinching inward on the right (arrow) and, therefore, separates the packet from the remainder of the outer segment. (Magn. × 23 600.)

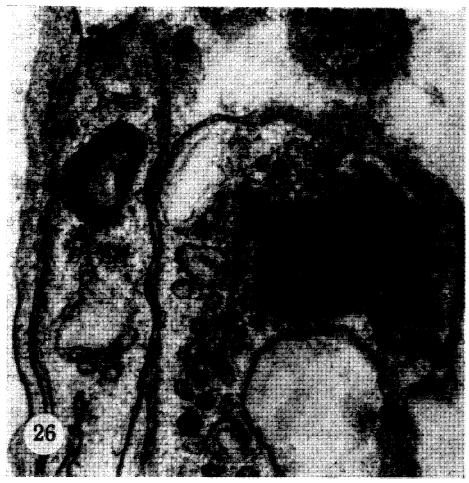
Figure 24. The apical processes near the pigment epithelial surface, from figure 22 at a higher magnification. (Magn. × 7800.)

Figure 25. A longitudinal section through the tip of a cone outer segment and its pigment epithelial apical processes. A small phagosome, with unclear lamellar structure, appears in a process immediately above the outer-segment tip. 60 year old retina. (Magn. × 21 000.)

Figure 26. A small phagosome from the cone of figure 1, plate 1, at a higher magnification. The lamellar structure is not clear. (Magn. × 28 800.)







FIGURES 22–26. For description see opposite.

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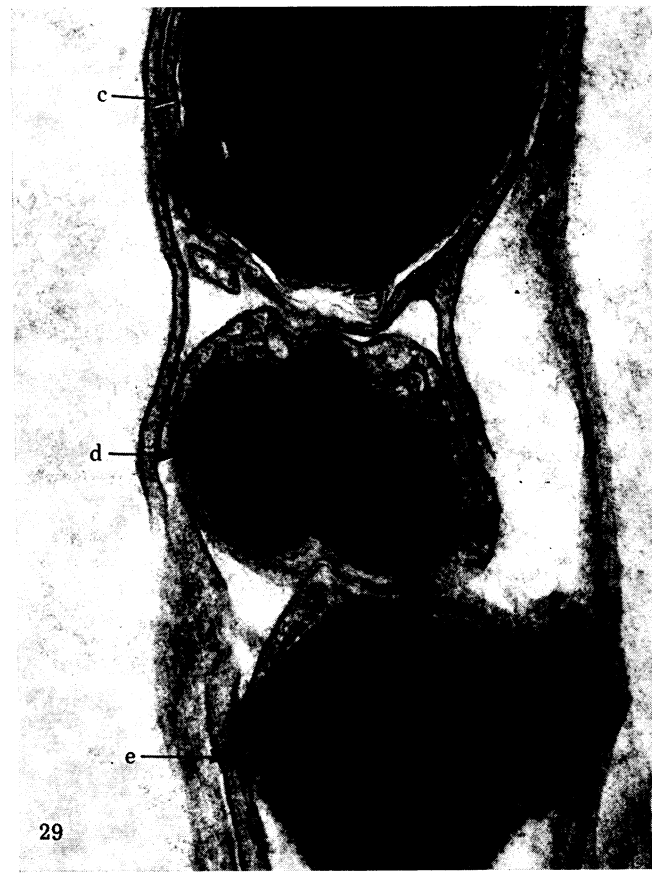

FIGURE 27. Electron micrograph montage of a longitudinal section through a cone outer segment, portion of inner segment and pigment epithelial apical processes. Four phagosomes (*b–e*) appear lined up within the processes above the outer-segment tip and a terminal packet of disks (*a*) is in the process of being phagocytosed by a single pigment epithelial apical process. 5 year old retina. (Magn. × 3700.)

FIGURE 28. The outer-segment tip and terminal packet of disks from the cone of figure 27, at a higher magnification. An apical process bifurcates at the tip of the outer segment (lower left) and sends a branch across the tip and down along the right side. At the right side the process then sends a pseudopod across the outer segment so that a terminal packet of 40 disks is completely encircled. (Magn. × 43400.)

FIGURE 29. Phagosomes *c, d, e* in the apical processes from the cone of figure 27, at a higher magnification. (Magn. × 44800.)

Once having identified disk shedding and phagocytosis at the terminal portions of cones and having seen phagosomes in apical processes above the cone tips, we became certain that similar inclusions found in processes near the apical space of the pigment epithelium, also were cone phagosomes. These phagosomes tended to be more electron dense than the more vitreal ones and it was often difficult to distinguish individual disks (figures 46–48, plate 14). Other inclusions also were observed at various levels in the processes that were difficult to identify as phagosomes because of their high density and an absence of disk clarity (figures 25, 26, plate 8).

Diagrammatic representation of phagocytosis

A diagrammatic representation of phagocytosis by the pigment epithelial apical processes is shown in figure 50, plate 15. The villous-like processes expand at the outer segment tip into cytoplasmic sheets and, thereby, ensheath a portion of the outer-segment tip. There may be more than one ensheathing process. To phagocytose the terminal disks a single process grows across the top surface of the cone and extends pseudopodia across the outer segment immediately below the last disk of the shed packet. The phagosome then ascends in the process toward the pigment epithelium, and a space appears between the phagosome and the cone tip. We assume that this is a portion of extracellular space that is enclosed within the ensheathing process. To phagocytose the next group of disks these events are repeated. Sometimes the next phagosome appears in a different process, indicating that the process that phagocytosed the first packet must have come off the cone or have been actively displaced by the new process (not shown).

Discussion

Form of the sheath

Multiple villous-like pigment epithelial apical processes protrude into the spaces above extrafoveal cones. They diminish in number as they descend and a few of the processes become cytoplasmic sheets that surround the cone outer segment for no more than one-half of its length. The upper one-third of the outer segment may be ensheathed by a single process or by several overlapping processes. Pigment granules occur in the villous-like processes but do not appear in the ensheathing region. Sometimes individual processes are sheet-like in the supracone space and contain a variety of cell organelles; some of these organelles can be found in the process cytoplasm along side of the outer segment. The sheet-like processes usually are seen above cones that do not have phagosomes in *any* of their processes. When there are phagosomes, there is a scarcity of other intracellular organelles in the process *below* the phagosome. It is as if the phagosome, as it ascends towards the apical surface, pushes the cytoplasm ahead of it into the soma and also blocks cytoplasmic flow into the process below it. When the phagosome enters the soma there then may be a rush of cytoplasm into the process. Since processes that contain phagosomes have become sheaths, the expanded internal area could then fill with cytoplasm.

Comparison with cat cones

The human cone sheath differs considerably from the cat's (and from the rabbit's as well; Scullica & Tangucci 1968; Sjöstrand & Nilsson 1964; Steinberg & Wood 1974). The cat's sheath is a more regularly ordered structure. Its walls are thicker and it forms a tunnel that encloses the supracone space. Each process begins at the apical surface as a leaf-like cytoplasmic

sheet that narrows in width as it descends toward the cone. The enclosure of the supracone space is formed by concentric whorling of the processes in the space. The outer segment always is ensheathed with multiple laminae and at least one extends the full length of the outer segment (Steinberg & Wood 1974).

These differences are not based on the length of the supracone space, which was 14 μm in cat and 19 μm in human; the rabbit has a sheath similar to the cat's while its cones abut against the apical surface. One obviously significant factor, however, is the much greater photoreceptor packing density in cat and rabbit compared to human, due to a much greater rod density. At 10° in the temporal periphery, for example, the cone density of both cat and human is about 5000/mm^2, but rod densities are 450000/mm^2 and 125000/mm^2, respectively (Steinberg, Reid & Lacy 1973). Compared with cat, human rods are considerably farther away from the cones, and from each other, as they surround a cone. Extrafoveal human cones, therefore, are surrounded by much more space than cat cones, and the greater thickness and regularity of the cat's sheath could mean that isolation of cones from rods is a more important function of the sheath in cat than it is in human (Steinberg & Wood 1974).

In human, an abundance of intracellular organelles in the processes suggests that there is active metabolic exchange across the extracellular space to the cone. In cat, the processes were empty except for regularly spaced microfilaments and occasional ribosomes, suggesting that they may not be as metabolically active with the cone. The enclosed supracone space in cat may be the more important route for transport to and from the pigment epithelial cell (Steinberg & Wood 1974).

Comparison with human rods

The ensheathment of human extrafoveal cones is similar to that of human rods. Spitznas & Hogan (1970) described the rod as ensheathed by a 'single solid cytoplasmic sheet', and examination of their figures 2 and 3 suggests that the sheath also may have multiple laminae. There are more processes protruding into the space above a cone than the space surrounding the rod and ensheathment with more than one lamina may be more typical for cones. The major anatomical differences are the much greater length of the processes in the supracone space, the variations of form that they assume, and the intracellular organelles that they may

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Description of plate 9

Figure 30. Electron micrograph montage of a longitudinal section through a cone outer segment, portion of inner segment, and pigment epithelial apical processes. A mitochondrion and a cluster of pigment granules appear in the processes near the apical surface. A detached packet of disks is positioned above the outer-segment tip. 5 year old retina. (Magn. × 3800.)

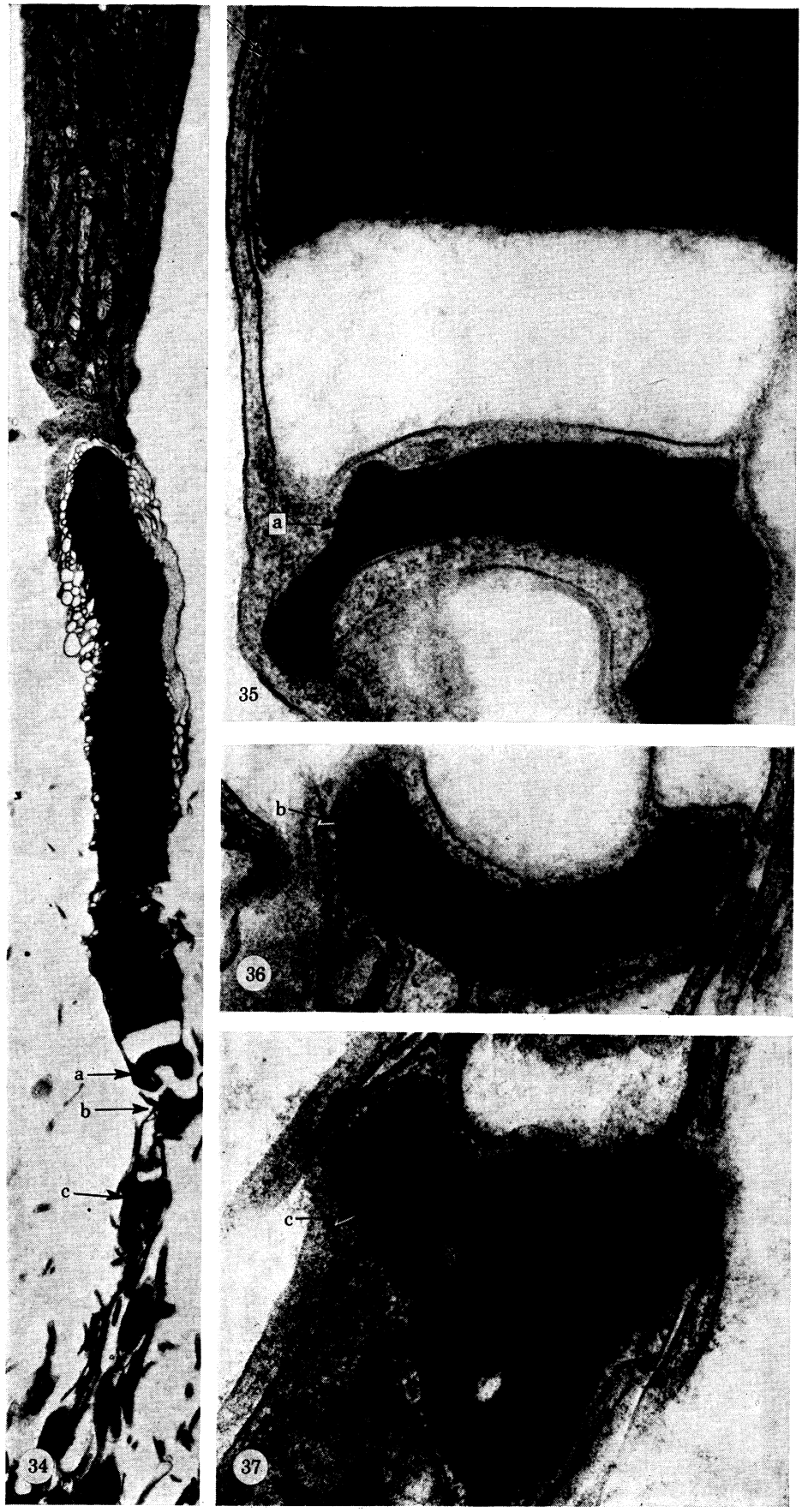
Figure 31. The outer portion of the cone outer segment of figure 30 at a higher magnification. Pigment epithelial processes extend along both sides of the outer segment. A packet of forty disks appears surrounded by apical processes immediately above the outer-segment tip. It is not certain if the disks are contained, as a phagosome, within any one process. (Magn. × 16800.)

Figure 32. Electron micrograph montage of a longitudinal section through a cone outer segment, portion of inner segment, and pigment epithelial apical processes. The apical processes contain two phagosomes (*a*, *b*). 5 year old retina. (Magn. × 3800.)

Figure 33. The two phagosomes of figure 32, at a higher magnification. Phagosome *a* is a packet of about 45 disks that has rotated about 45° so that the disk edges are beginning to face the pigment epithelial cell. Phagosome *b*, a packet of about 25 disks, has rotated a full 90°. Observe that both phagosomes are contained within a single apical process. (Magn. × 24800.)

FIGURES 30–33. For description see opposite.

(*Facing p.* 466)



FIGURES 34–37. For description see opposite.

contain. Since it is the ensheathing processes of rods that phagocytose the shed disks (Spitznas & Hogan 1970), the similarity in form extends to a similarity in function.

Phagocytosis

In previous studies, the number of rods and cones observed, at any one time, to be shedding disks and undergoing phagocytosis was considered to be 'a minority' (Young 1971 *b*; Anderson & Fisher 1976). It is especially difficult to make this estimate for human extrafoveal cones because of the smaller size of the sample. Cones have a much lower density, extrafoveally, than rods, and must be well aligned both at their outer segments and along the supracone space. We probably have not examined more than 100 completely aligned cone outer segments and apical processes, although a great many more partial structures have been studied. That cone phagosomes actually were observed in all three retinas suggests that it is not a rare event. In the 5 year old retina, about one-third of the cones showed it, suggesting that the 'minority' may be a larger one than for rods. Contributing to the large 'catch' of cone phagosomes, also, may be the long distance that phagosomes must travel to reach the soma and a relatively slow rate of movement (see below).

Our observations indicate that extrafoveal human cones, like human rods and the rods of other vertebrates, shed their terminal disks in packets. The packets are then phagocytosed and digested by the pigment epithelium. Is this usual for cones or is something wrong with these three retinas? If disk shedding and phagocytosis were abnormal, then we might expect to find other defects in these retinas, but this was not the case. The rod and cone cells were intact and the pigment epithelium was not unusual in any respect. There were no isolated macrophages clearing up debris as would be expected if the photoreceptor layer were degenerating. Furthermore, the number of disks in human cone phagosomes did not seem to be any larger than the number of disks observed in rod phagosomes of other vertebrate retinas; cone phagosomes ranged in size from 9 to 62 disks, the average was 29 (n = 12). Cones, therefore, were not sloughing large portions of their outer segments. Equally important, the process of *rod* disk shedding and phagocytosis was observed to be no different from previous descriptions in the literature and the numbers of rod phagosomes did not appear excessive (Spitznas & Hogan 1970; Young 1971 *b*; Anderson & Fisher 1975, 1976). Yet, we wonder whether the intense exposure to light, which these retinas may have received during ophthalmoscopic examination in the hours and days prior to surgery, could have a causal rôle in these findings. There now is

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Description of plate 10

Figure 34. Electron micrograph montage of a longitudinal section through a cone outer segment, portion of inner segment, and pigment epithelial apical processes. The apical processes contain three phagosomes (*a–c*). The disk edges of phagosomes *a* and *b* both have curled and the convexities of the packets face in opposite directions. 5 year old retina. (Magn. × 5560.)

Figure 35. Phagosome *a* and the outer-segment tip of figure 34 at a higher magnification. Phagosome *a* is a packet of about 15 disks. The disk edges have curled so that the convexity of the packet faces the outer segment. The process that encloses the phagosome continues down both sides of the outer segment, presumably, ensheathing it. Observe that the 18th disk from the top of the outer segment is open to the extracellular space on the left (arrow). (Magn. × 47600.)

Figure 36. Phagosome *b* of figure 34, at a higher magnification. This phagosome is a packet of 11 disks that have slightly curled so the convexity faces the epithelium. (Magn. × 54400.)

Figure 37. Phagosome *c* of figure 34, at a higher magnification. The lamellar structure of this phagosome is no longer clear and the edges of the phagosome have become irregular. (Magn. × 52500.)

evidence from studies in albino rats and *Rana pipiens* tadpoles that the number of *rod* phagosomes in pigment epithelial cells can be *increased* by a recent history of light exposure (M. M. LaVail, personal communication; J. G. Hollyfield, personal communication). In dark-adapted albino rats, for example, sudden exposure to light, moderate in intensity, is followed by a burst of disk shedding that peaks after about 30 min of light, although this appears to follow a circadian rhythm (M. M. LaVail, personal communication). We would hope that a light effect of this type, if it occurred in the human retina, only accelerated a normal process of cone disk turnover and thereby increased the number of instances available for observation (see Anderson & Fisher (1976) for a recent discussion of the question of disk renewal and phagocytosis in cones.)

Disk shedding and phagocytosis

The earliest change observed by Young (1971 *b*) in monkey rods was curling of disk edges in the terminal group. This was followed by folding or pinching in of the outer membrane and finally shedding of the packet into the extracellular space. The pigment epithelium then engulfed the packet. That the pigment epithelium can participate more actively in the detachment of the packet was shown by Spitznas & Hogan (1970). Pseudopodia, from the ensheathing process may extend into the outer segment to separate the packet from it. Recently, in squirrel rods and cones, Anderson & Fisher (1976) found instances of both types of early event – invagination of the outer membrane without epithelial participation; and inward penetration of pigment epithelial processes that pinched off the group of terminal disks. In the present study, there was also evidence of both types of event.

The apparent controversy about the relative rôles of disk shedding and phagocytosis might be resolved if we accept Young's disk shedding sequence but assume that the *timing* of the epithelial response can vary and that there exists a not yet visualized signal from the terminal disks that precedes disk curling. According to this hypothesis, pinching of a disk packet by epithelial pseudopodia is a fast response to this signal; the disks have not had time to curl or detach. This would explain why the disks that are being engulfed by pseudopodia in figure 28, plate 8 are not curled and, also, the presence in the processes of other packets in which the disks are not curled. In a slower response the disks curl and the packet may detach before phagocytosis (Young 1971 *b*; Anderson & Fisher 1976) as in figure 23, plate 7; and some phagosomes in the processes also have curled disks, (figures 35, 36, plate 10). Finally, an example of a still slower response would be where detached disks are still present within squirrel cones, but show further degenerative changes – increases in electron density and membrane disintegration (Anderson & Fisher 1976). The epithelium had not yet responded.

Digestion of phagosomes

Cone phagosomes in the processes have some of the same appearances, which are associated with degeneration, as rod phagosomes in the pigment epithelial somata: increase in density, blurring of disk edges, distortion and compression of disk membrane, shrinkage and irregularities of outer membranes. In the apical process, degeneration of disk membranes appears to *increase* as the phagosomes approach the pigment epithelial somata. Similarly, for rod phagosomes, there is a progression from the apical to the mid- and basal zones of the cell (Baraiti & Orzalesi 1968; Ishikawa & Yamada 1970; Spitznas & Hogan 1970; Marshall & Ansell 1971; Young 1971 *b*; Anderson & Fisher 1976). In the soma there are phagosomes that are essentially granular, but which can be identified by a few remaining lamellae. There also

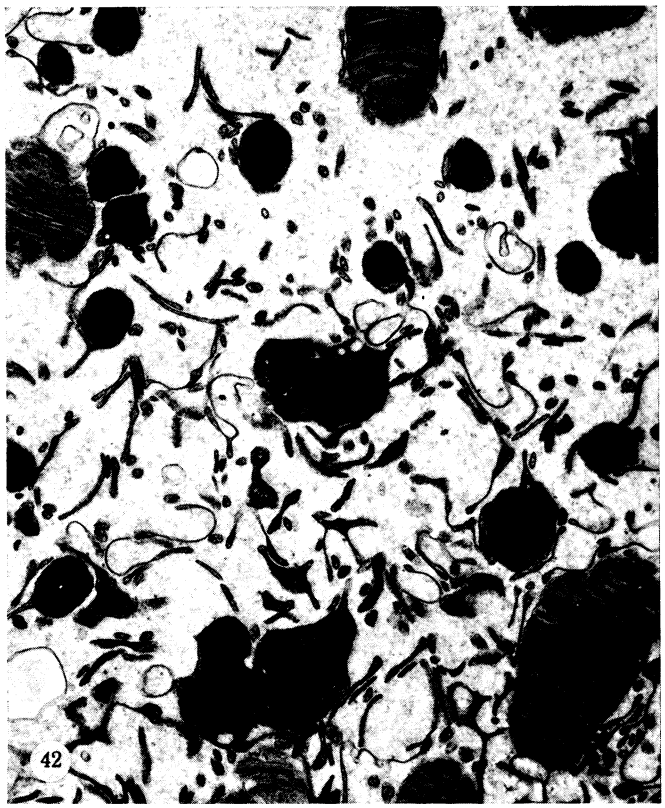
FIGURE 38. Electron micrograph montage of a longitudinal section through a cone outer segment, portion of inner segment, and pigment epithelial apical process. The apical processes contains two phagosomes (*a*, *b*). An enlargement of the tip of this outer segment was presented in figure 13, plate 5. 5 year old retina. (Magn. × 3650.)

FIGURE 39. Phagosomes *a* and *b* and the apical processes of figure 38, at a higher magnification. Observe that both phagosomes are enclosed in the same apical process, and the process continues to the outer segment where it ensheaths the tip (figure 13, plate 5). Phagosome *a* is a packet of 62 disks that have approximately maintained their usual orientation. Phagosome *b*, probably contains about 20 disks, and the packet has rotated 90° so that the disk edges face the pigment epithelium. (Magn. 20400.)

(*Facing p.* 468)

FIGURE 40. Electron micrograph montage of a longitudinal section through a portion of a cone outer segment and its pigment epithelial apical processes. The processes contain a phagosome that has rotated 90°. 60 year old retina. (Magn. × 16800.)

FIGURE 41. Electron micrograph montage through a portion of a cone outer segment and its pigment epithelial apical processes. There is a single phagosome in the apical processes; a packet of less than 20 disks that has rotated almost 90°. The apical process that enclosed the phagosome continues to the outer segment where it lies along both sides, presumably ensheathing it. 45 year old retina. (Magn. × 13980.)



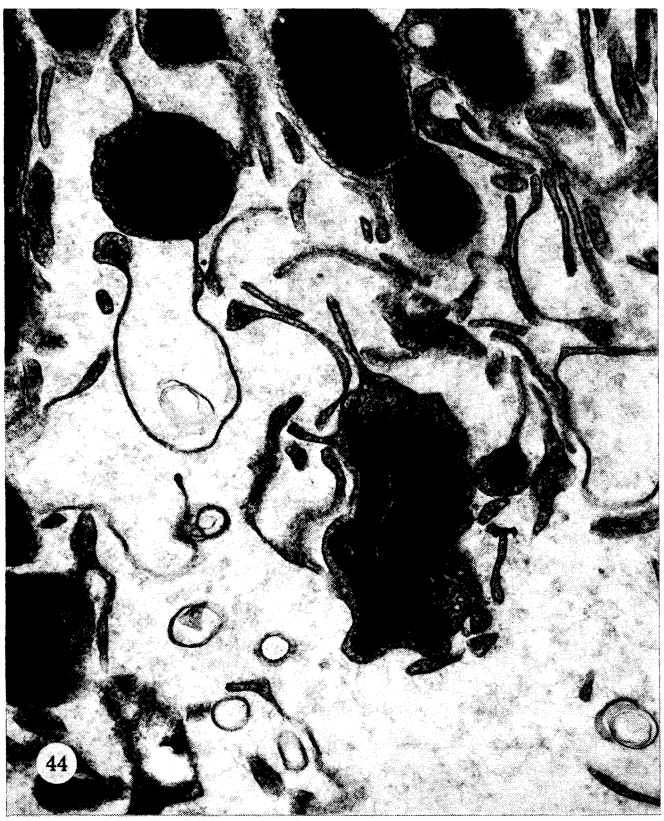


FIGURE 42. Electron micrograph of a transverse section through a supracone space. The space is surrounded by rods. An apical process that contains a phagosome occurs in the center of the space and it is surrounded by other processes that contain pigment granules. 5 year old retina. (Magn. × 7200.)

FIGURE 43. The phagosome of figure 42, at a higher magnification. (Magn. × 27 750.)

FIGURE 44. Electron micrograph of a transverse section through the supracone space of a process that contains a phagosome. 5 year old retina. (Magn. × 9600.)

FIGURE 45. Electron micrograph of a transverse section through the supracone space of a process that contains a phagosome. 5 year old retina. (Magn. × 20 400.)

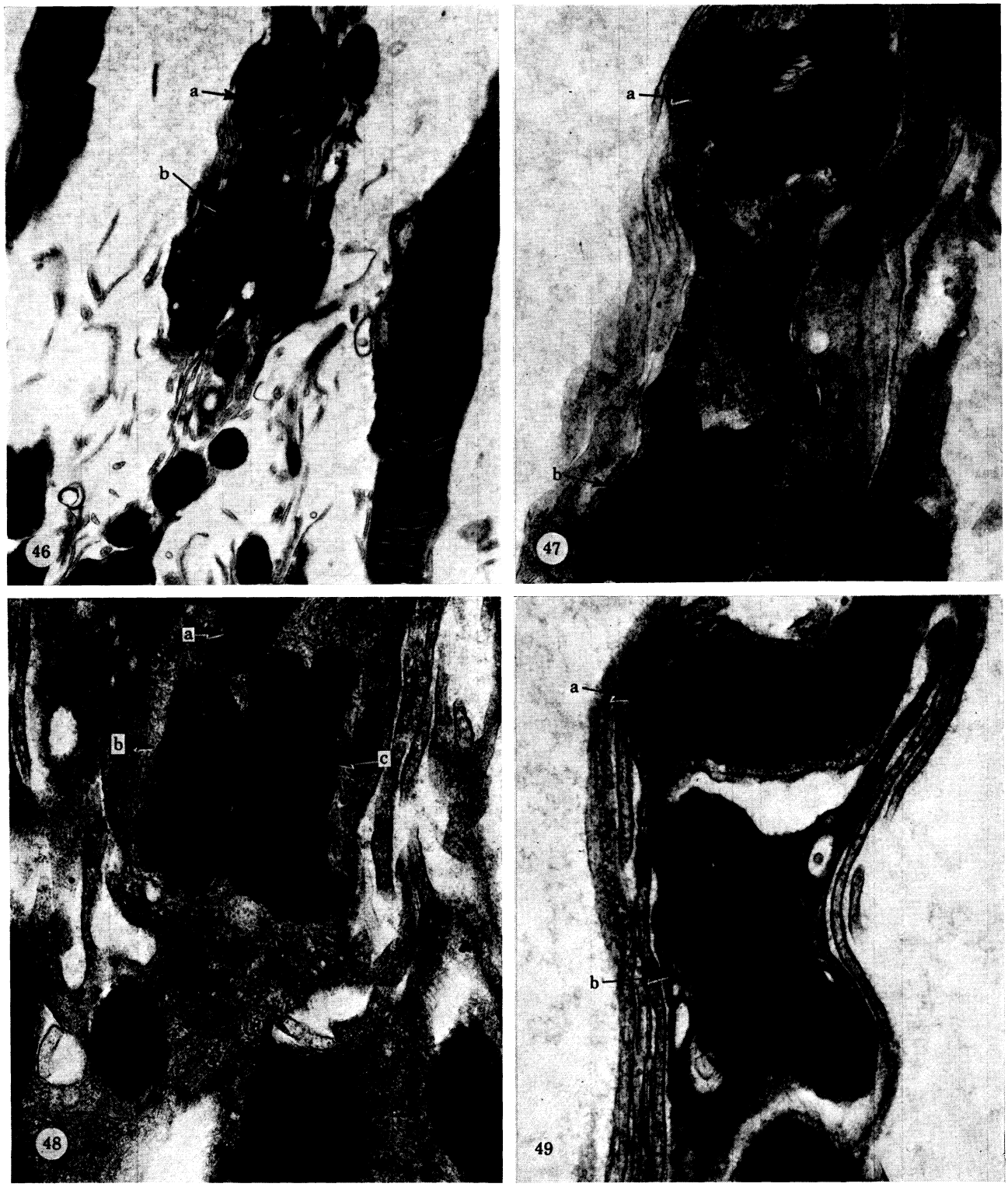


FIGURE 46. Electron micrograph of a longitudinal section through pigment epithelial apical processes near the apical surface. Two phagosomes are located in one of the processes (*a*, *b*). A second process contains two pigment granules. 5 year old retina. (Magn. × 6800.)

FIGURE 47. The phagosomes of figure 46, at a higher magnification. In both phagosomes the lamellar structure is obscured and the margins of the packets are irregular. (Magn. × 17 000.)

FIGURE 48. Electron micrograph of a longitudinal section through pigment epithelial apical processes at their origin at the apical surface. Three phagosomes (*a–c*) appear very near the apical surface of the pigment epithelial cell. The lamellar structure of these phagosomes is not clear and their margins are irregular. 5 year old retina. (Magn. × 18 800.)

FIGURE 49. Electron micrograph of a longitudinal section through pigment epithelial apical processes in a supracone space. There are two phagosomes (*a*, *b*), each in a *separate* apical process. The packet of phagosome *a* is in its usual orientation with respect to the outer segment and contains about 25 disks. Phagosome *b* has rotated almost 90° and contains about 30 disks. (Magn. × 27 000.)

are occasional inclusions in the processes that have a similar appearance, suggestive of a later stage of digestion.

For *rod* phagosomes, investigators have not yet shown which phase of degeneration is definitely associated with the onset of digestion by the pigment epithelial cell. Digestion would be expected to begin when lysosomal enzymes enter the phagosome. An increase in the osmiophilia of disk lamellae usually appears in the fresh phagosome, but it is not possible to accept this as a sign of digestion, since Anderson & Fisher (1976) have observed it in detached squirrel cone

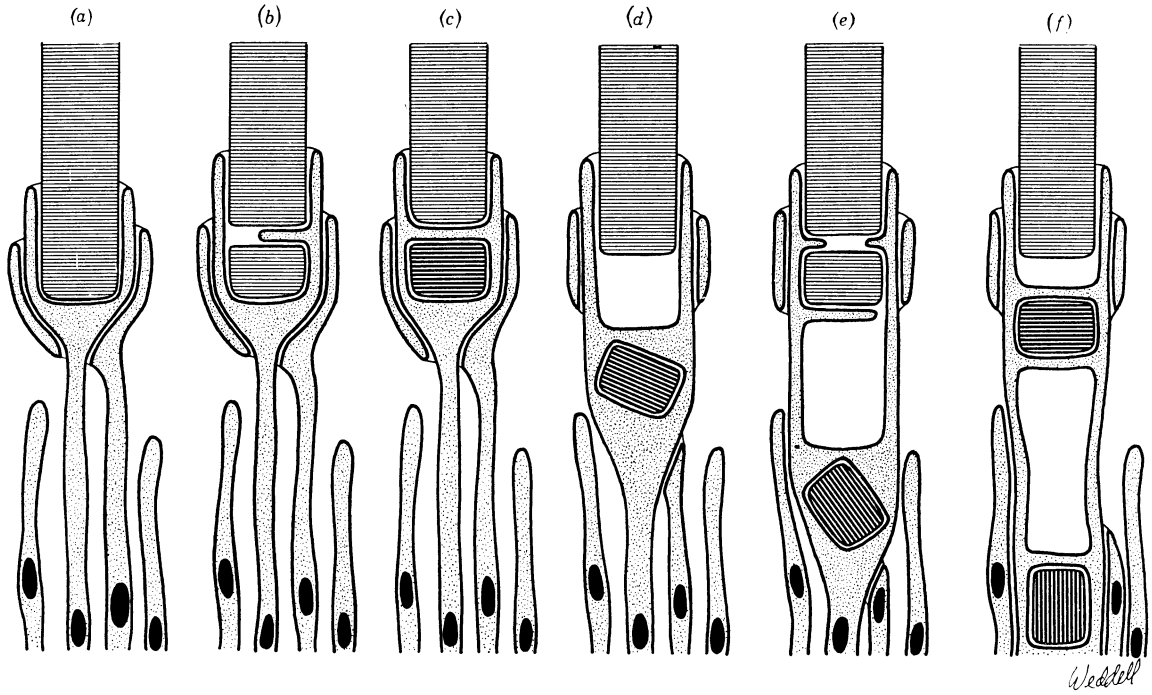


FIGURE 50. Summary diagram of phagocytosis. Long villous-like pigment epithelial apical processes reach toward the cone from the pigment epithelium. The processes that reach the cone (two are arbitrarily shown) expand to ensheath a portion of the outer segment (*a*). The sequence (*a*)–(*f*) shows the successive phagocytosis of two packets of disks by one apical process. We assumed that separation of the packet and resealing of outer-segment, plasma membrane occurred *before* phagocytosis, (*b*) and (*e*). The forms assumed by apical process pseudopodia-(*b*) and (*e*), were arbitrarily selected to illustrate several possibilities. The disks in the packets are shown becoming more osmiophilic than those in the outer segment, after they have been phagocytosed. Notice that as the first phagosome ascends in the process, (*d*) → (*f*), the sheath-like region of the process lengthens. Phagocytosis of the second packet of disks might also occur by a different process which, for some reason, displaces the first process. Diagram drawn by J. Weddell.

disks while they were still present in the outer segment. Also, studies of degenerating axonal terminals have shown that an increase in density, shrinkage, disintegration and fragmentation of intracellular organelles *all* can occur before phagocytosis by glia (Wong–Riley 1972). (The possibility cannot be excluded that enzymes, already within the neuron, or for that matter, within the cone are responsible for these changes. It is difficult to imagine, however, how inner-segment lysosomal enzymes could readily reach the outer-segment tip.)

Evidence from acid phosphatase studies on rat rod phagosomes indicates that reaction product can be found inside the phagosome at an early stage -- when disk lamellae are still distinct (Ishikawa & Yamada 1970); (although in another study on rat, frog and pigeon, fresh phagosomes of this type were described as containing very little reaction product (Marshall &

Ansell 1971).) Certainly, the lysosomal enzymes are actively working in the later stages that follow. The presence, in the processes, of cone phagosomes that no longer have distinct lamellae suggests, therefore, that *enzymatic digestion begins in the pigment epithelial apical processes*. The occurrence in the processes of small dense granules that look like primary lysosomes supports this hypothesis. This could mean a spatial separation, at the pigment epithelial cell, of the early stages of rod and cone phagosome breakdown. This could be based on a need to separate the lysosomal enzyme system, which might be distinct for rods and cones; or, perhaps, a need to isolate early digestive products from each other. Also, some product(s) might be returned to the cone at an early phase.

Movement of phagosomes

Whether digestion begins in the processes or in the somata also must depend on the length of time it takes phagosomes to reach the somata. That we have been able to observe phagosomes in the processes at all, and that they are often multiple, suggests that movement towards the apical surface is relatively slow, perhaps in the range of hours or even days. Note that we have not proven that phagosomes finally leave the processes to enter the soma, although their position just below the apical surface in the processes strongly suggests this (figure 46–48, plate 14).

To reach the soma from the outer segment tip, a phagosome must travel at least 19 μm. It does not appear that the processes themselves are withdrawn back to the soma, rather that the phagosome moves within the process while the process remains associated with the outer-segment tip. We have not found any fibrillar or tubular attachments to the phagosomes that suggest they are pulled up. Some pushing from below could occur when there is a succession of phagosomes in one process, but some processes only have one phagosome. Several movement mechanisms can be hypothesized, but there is one that is implied in the form taken by an ensheathing process after it has phagocytosed a packet of disks. This mechanism is sketched in figure 50. We suggest that the phagosome ascends away from the outer segment tip because of growth of the apical process below it. If the process is anchored at the outer segment tip or grips it in some way, addition of new membrane to the process will lengthen the invagination above the outer segment tip; in effect, there is a lengthening of the portion of the process that is sheath-like in form. The new growth of plasma membrane might be stimulated by loss of the membrane that was used to surround the packet.

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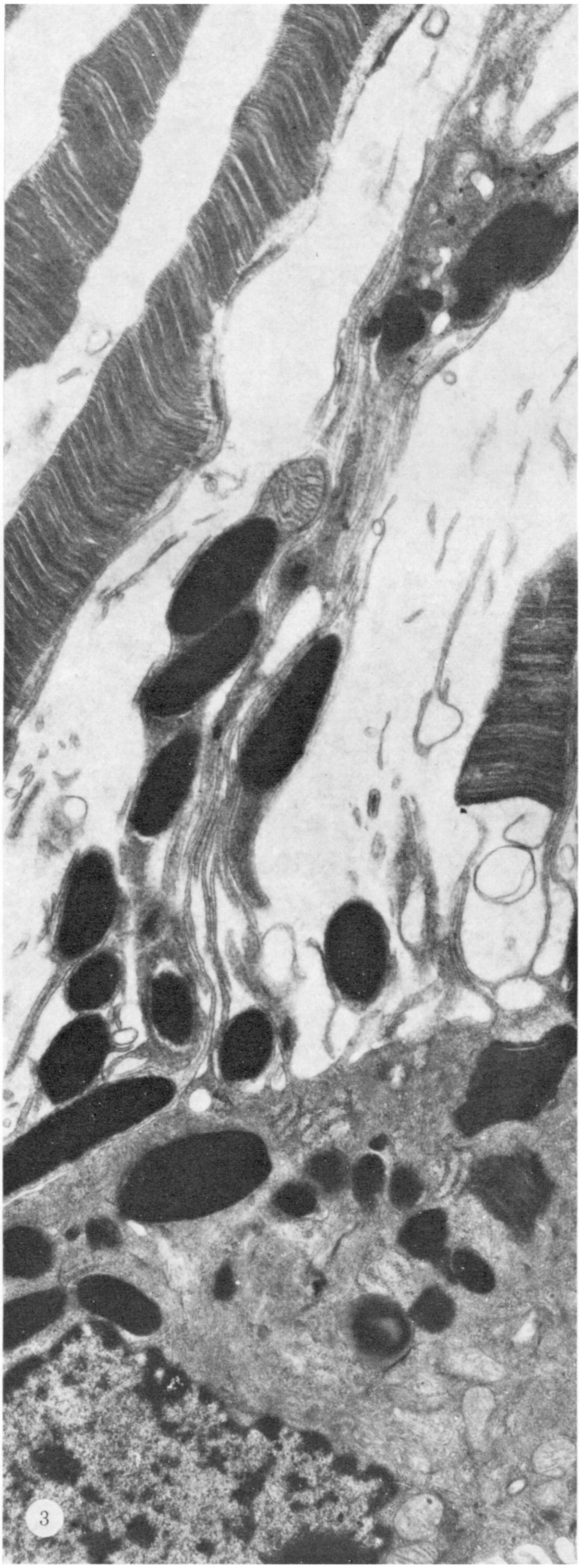
FIGURE 1. Electron micrograph montage of a longitudinal section through a cone outer and inner segment and the pigment epithelial apical processes. The cone is surrounded by rods on both sides. Several inclusions appear within the processes in the supracone space. One of these (arrow) is probably a phagosome, and it also appears in an enlargement in figure 26, plate 7. 60 year old retina. (Magn. × 3800.)

FIGURE 2. Electron micrograph montage of a longitudinal section through another cone from the retina of the 60 year old. The pigment epithelial apical processes in the supracone space appear especially thick in this section and have many dense granules protruding into them for no farther than one-half of the length of the processes. An enlargement of the apical processes appears in figure 4, plate 2. (Magn. × 3720.)



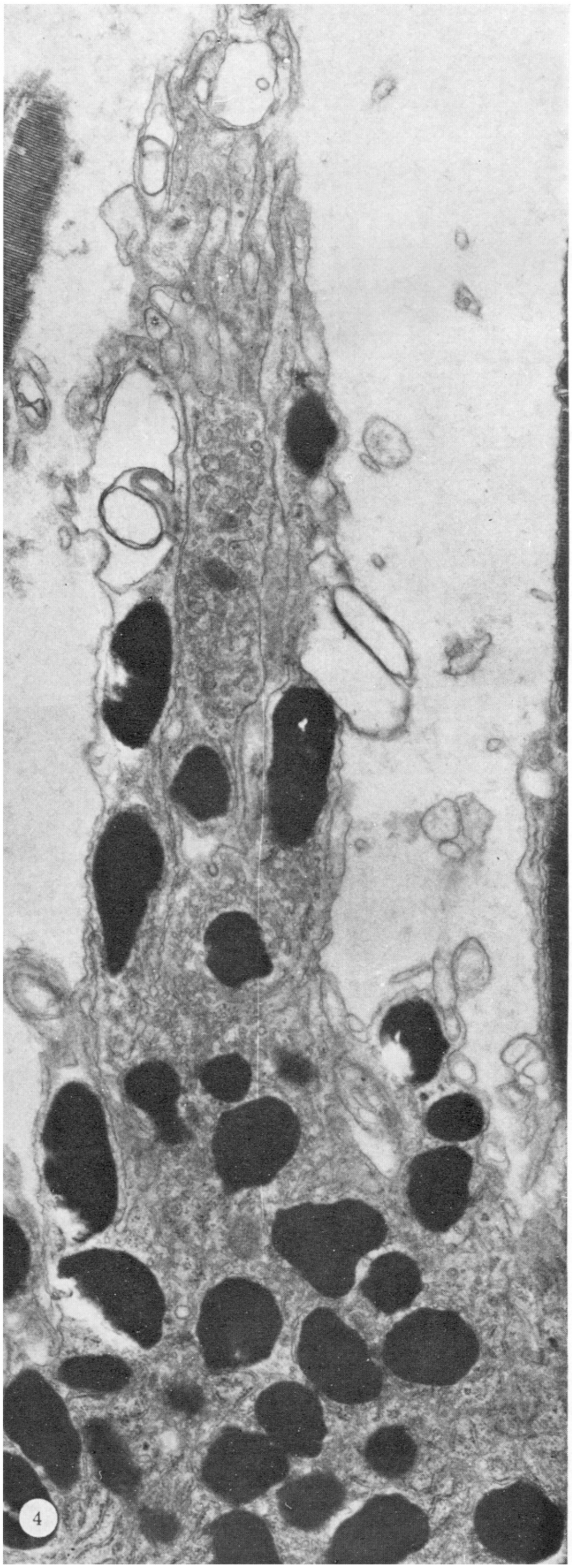
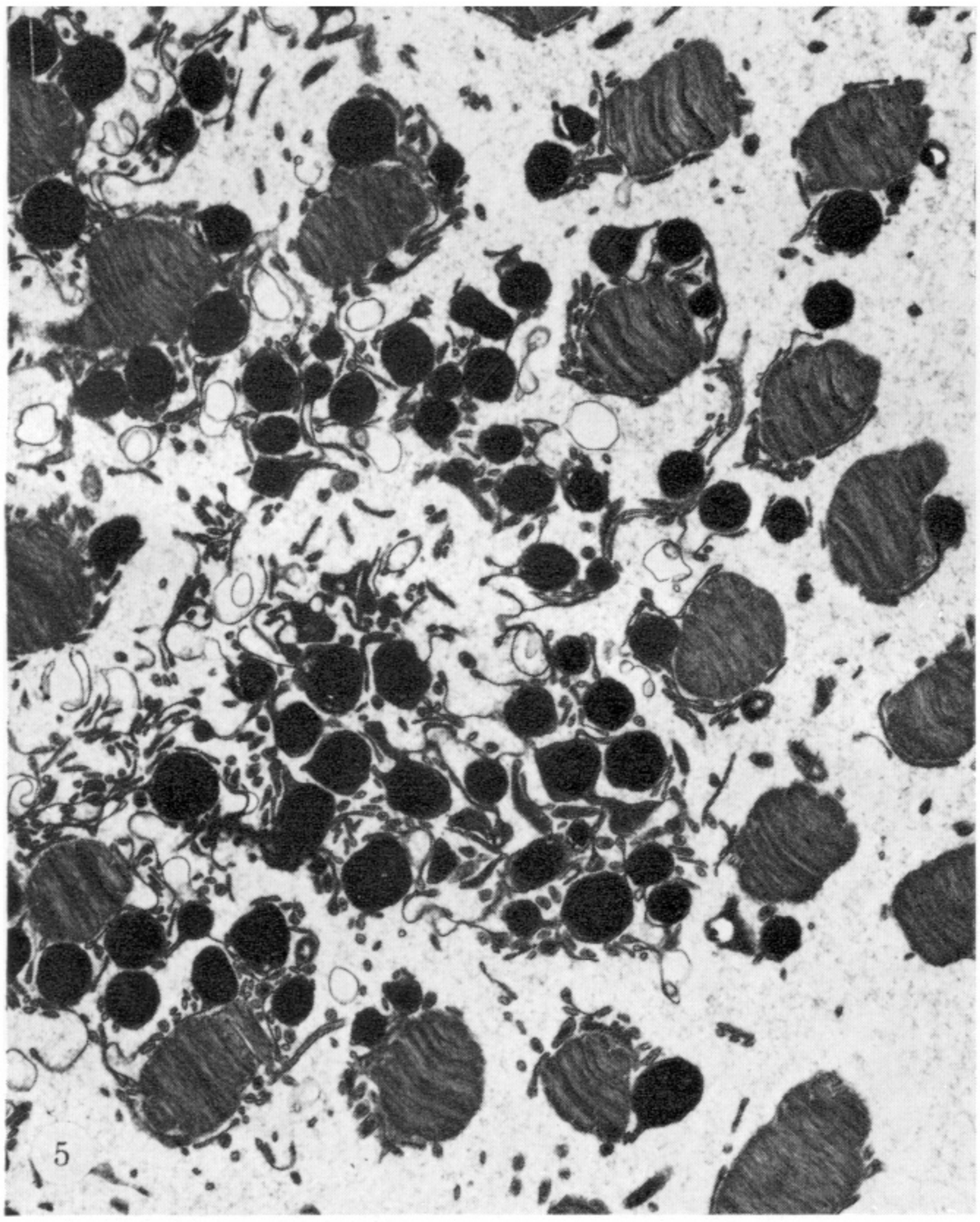


FIGURE 3. Electron micrograph of longitudinal section through pigment epithelial processes at their apical origin. A mitochondrion appears below one of the pigment granules and there are several dense bodies further vitread in the processes. The apical one-half of the pigment epithelial soma appears at the bottom of the micrograph and two rod phagosomes, having a lamellar structure, appear in the cell above a rod. 5 year old retina. (Magn. × 8800.)

FIGURE 4. Enlargement of the pigment epithelial apical processes from the cone of figure 2, plate 1. (Magn. × 8800.)



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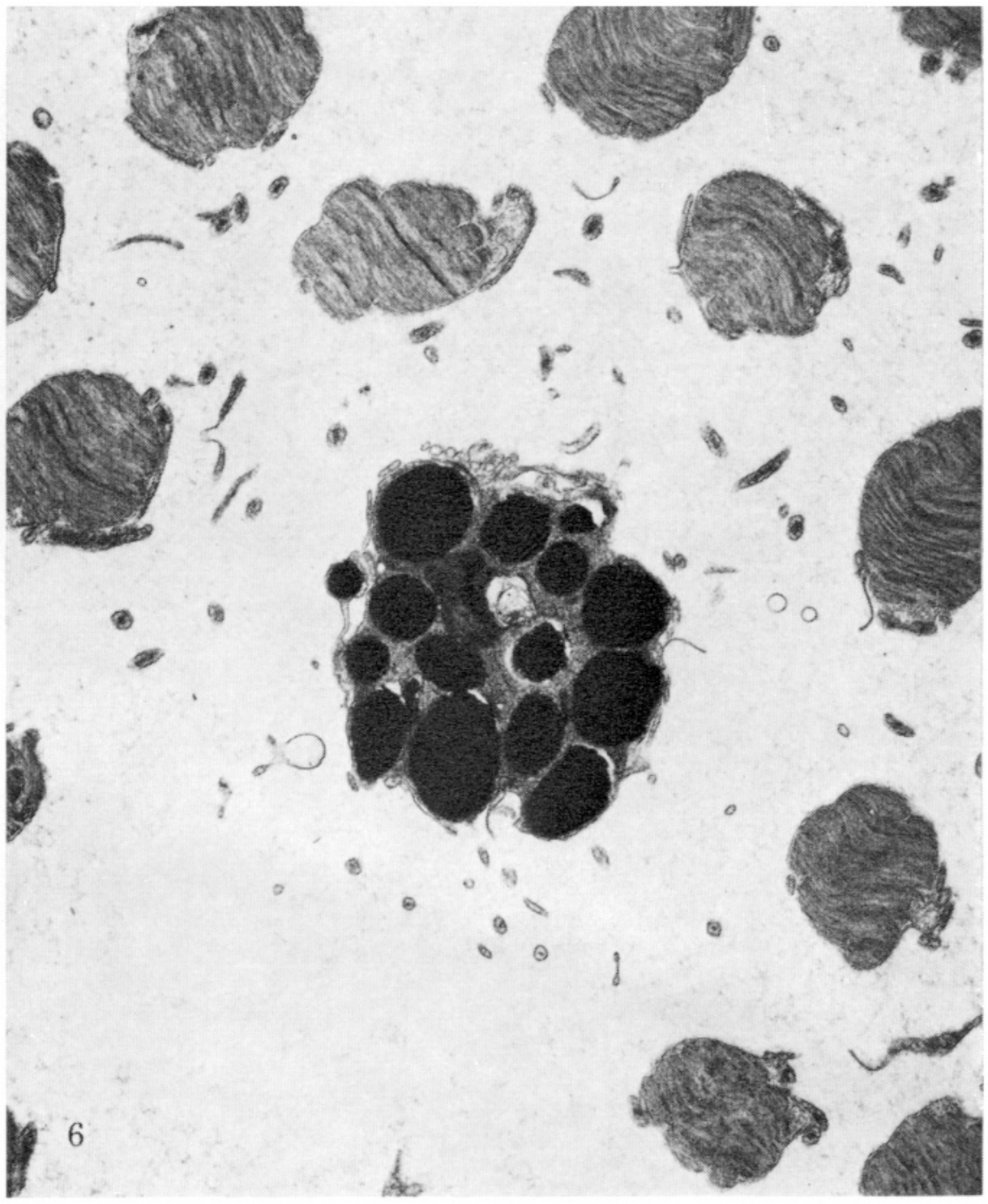

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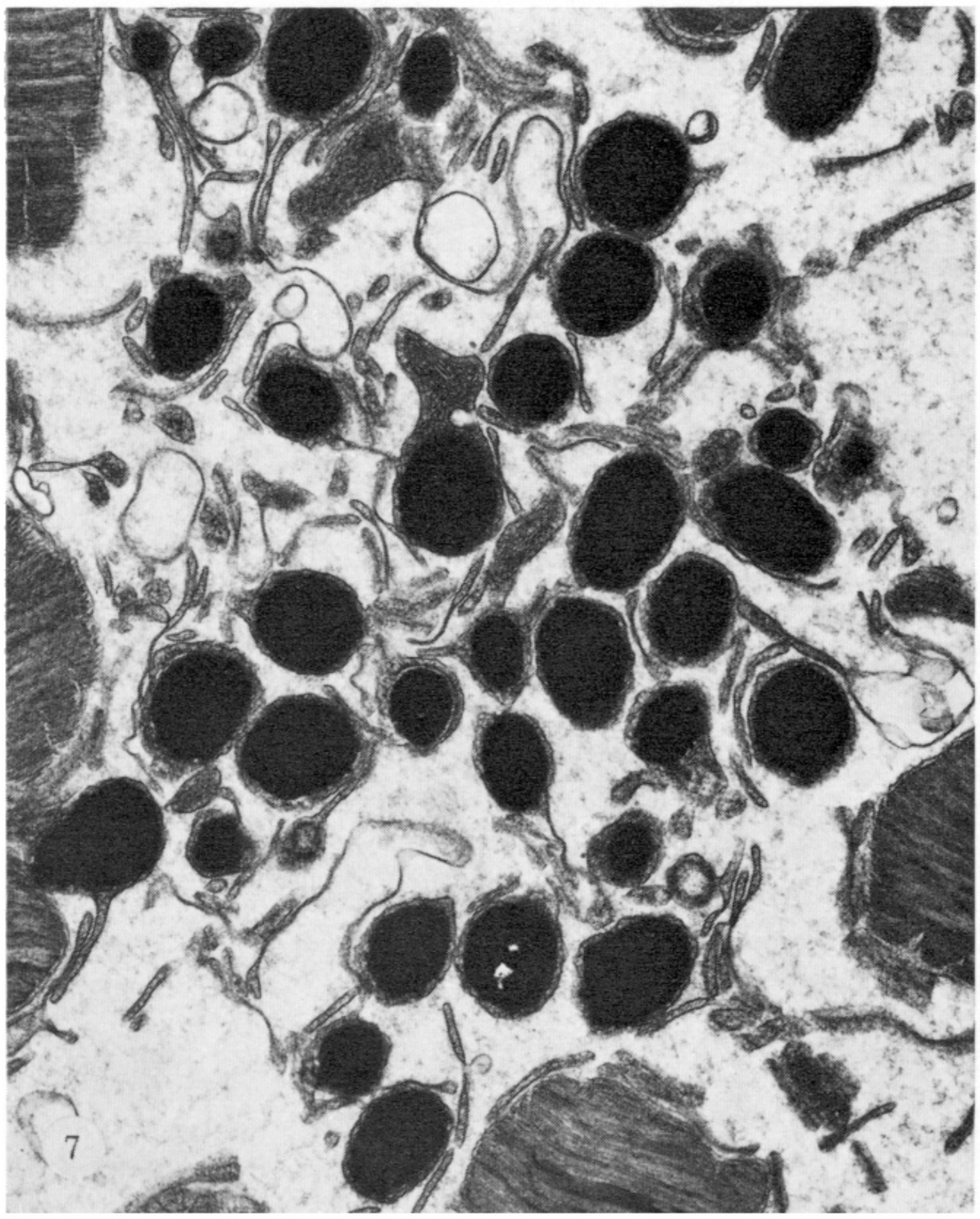

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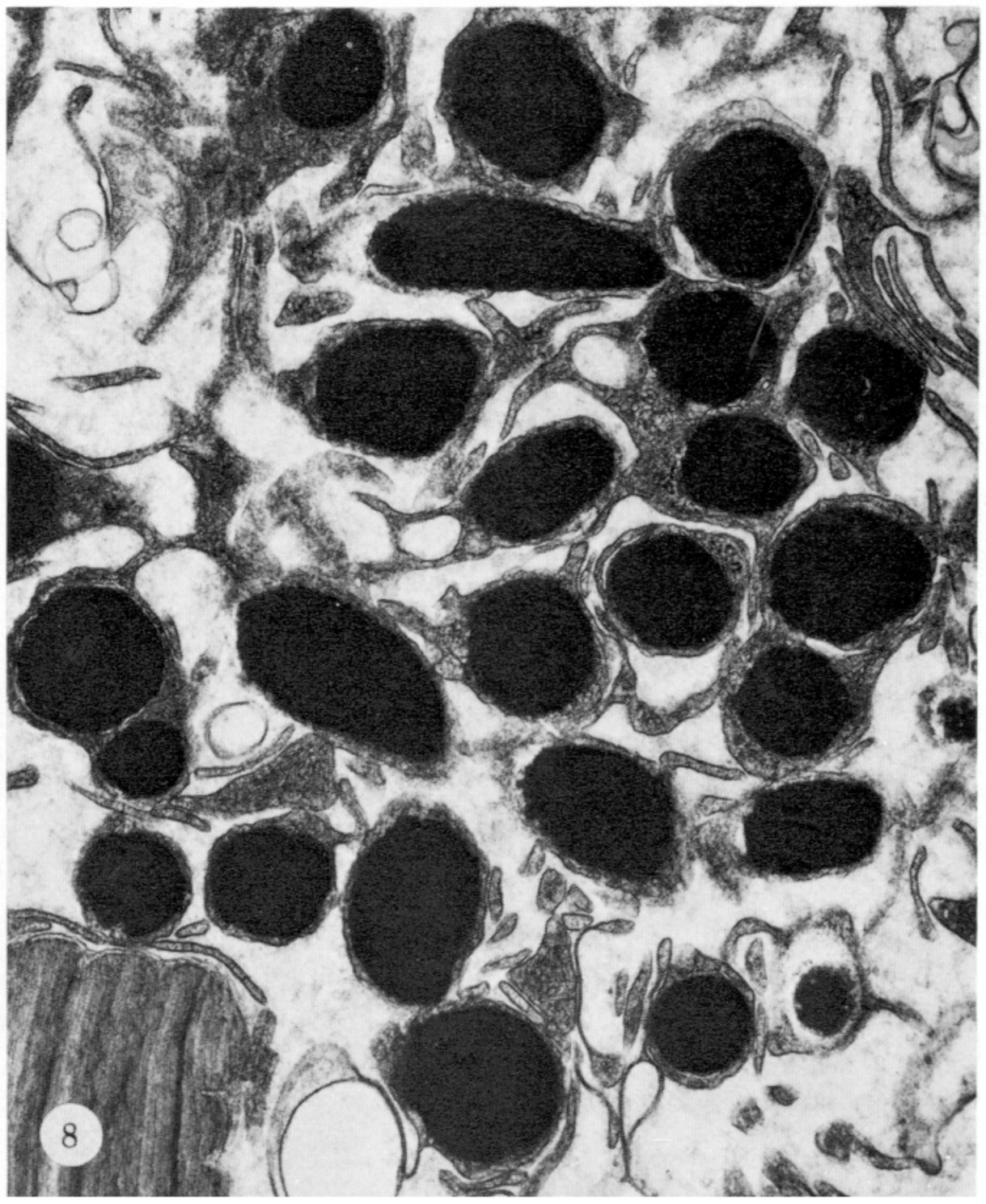

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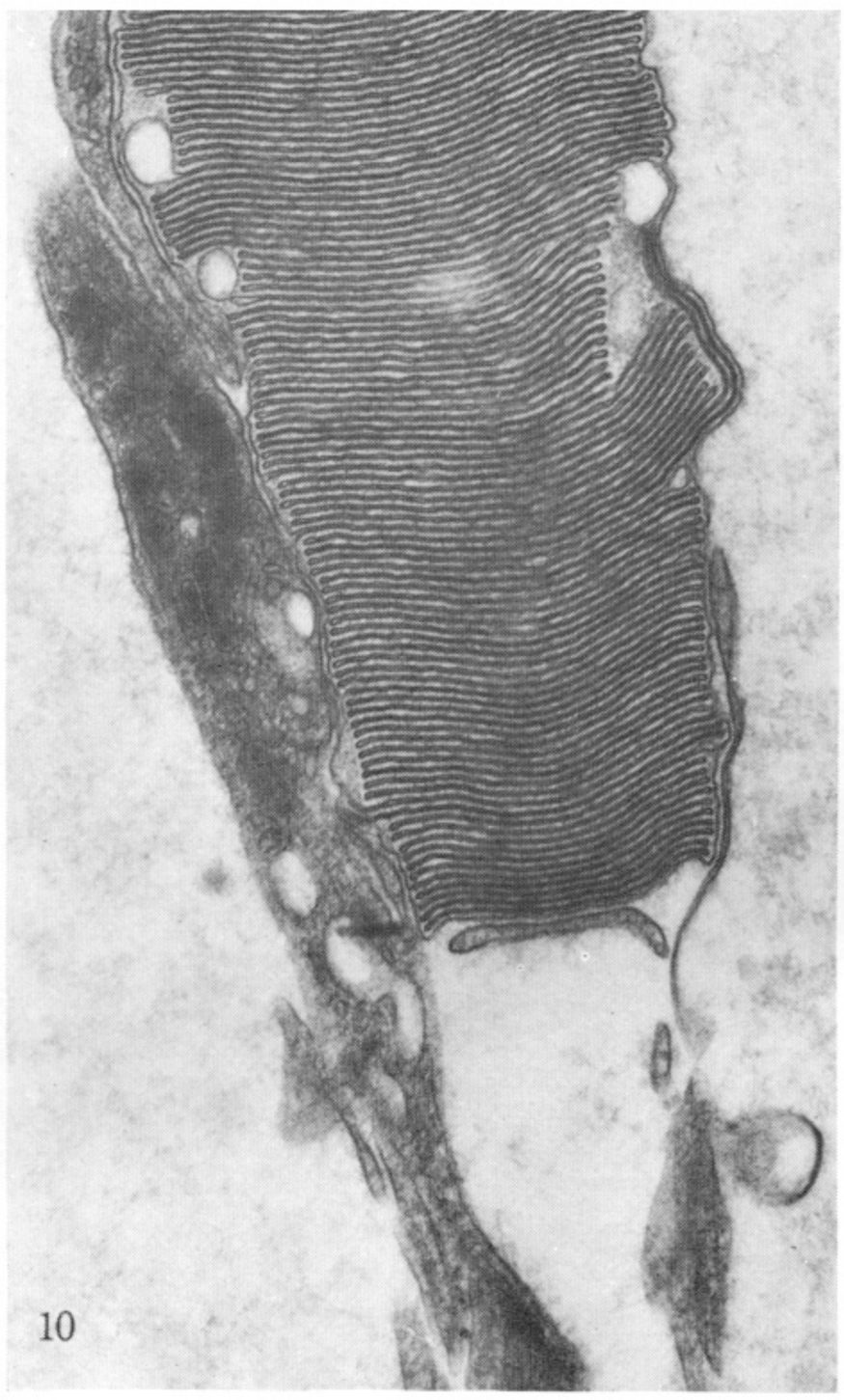
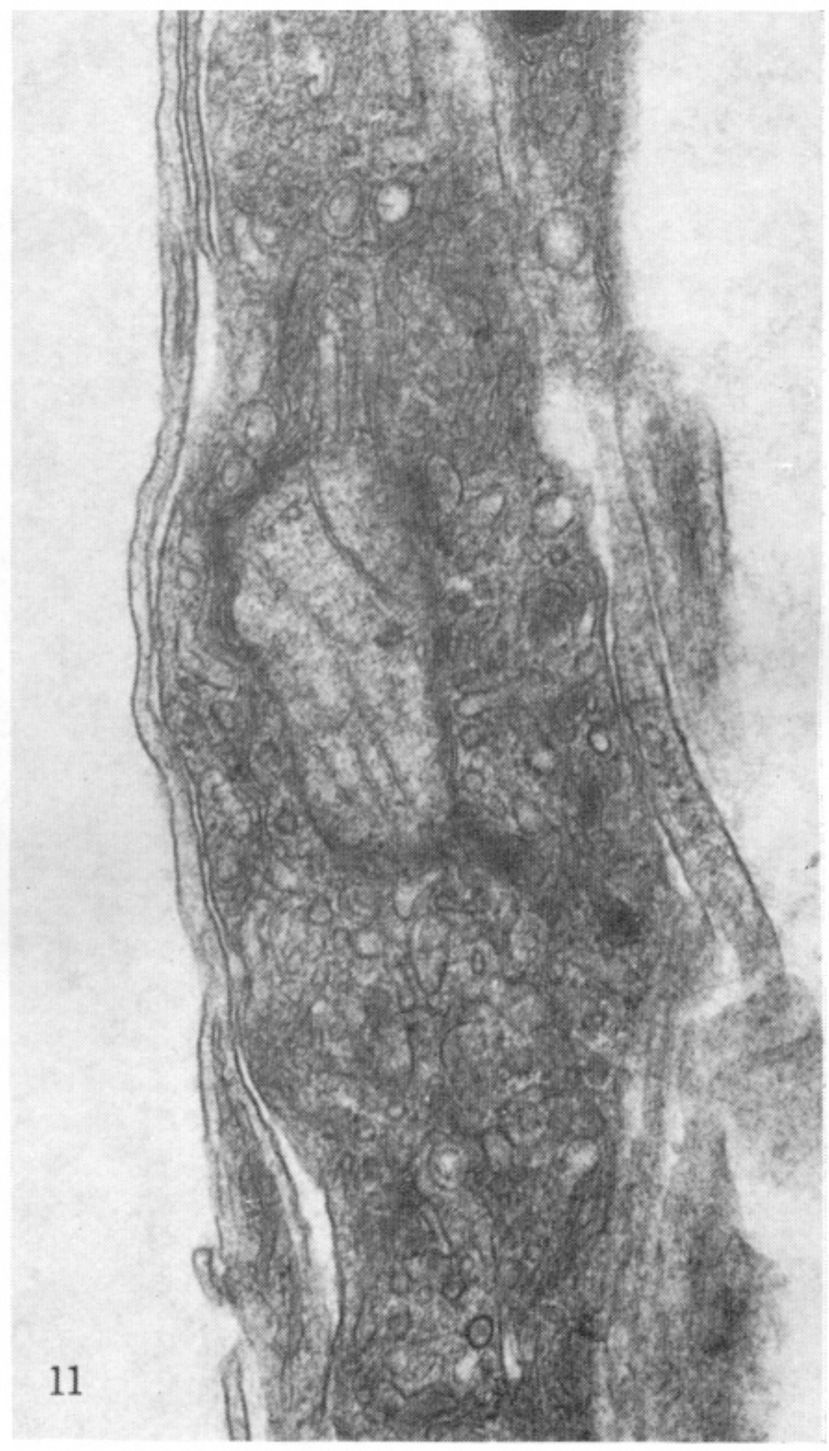
FIGURE 5. Electron micrograph of a transverse section through a supracone space. The space is surrounded by rod outer segments. It contains apical processes that assume a variety of thinly shaped profiles and many of them contain pigment granules. 5 year old retina. (Magn. $\times 4200$.)

FIGURE 6. Electron micrograph of a transverse section through a supracone space. There is only one large process in the space and it contains pigment granules and a less dense inclusion that is probably a phagosome. 5 year old retina. (Magn. $\times 6400$.)

FIGURE 7. Electron micrograph of a transverse section through a supracone space; similar to figure 5 except at a higher magnification. 5 year old retina. (Magn. $\times 10590$.)

FIGURE 8. Electron micrograph of a transverse section through a supracone space. Similar to figure 7. Two processes, each containing two pigment granules, appear on the right. 5 year old retina. (Magn. $\times 12800$.)





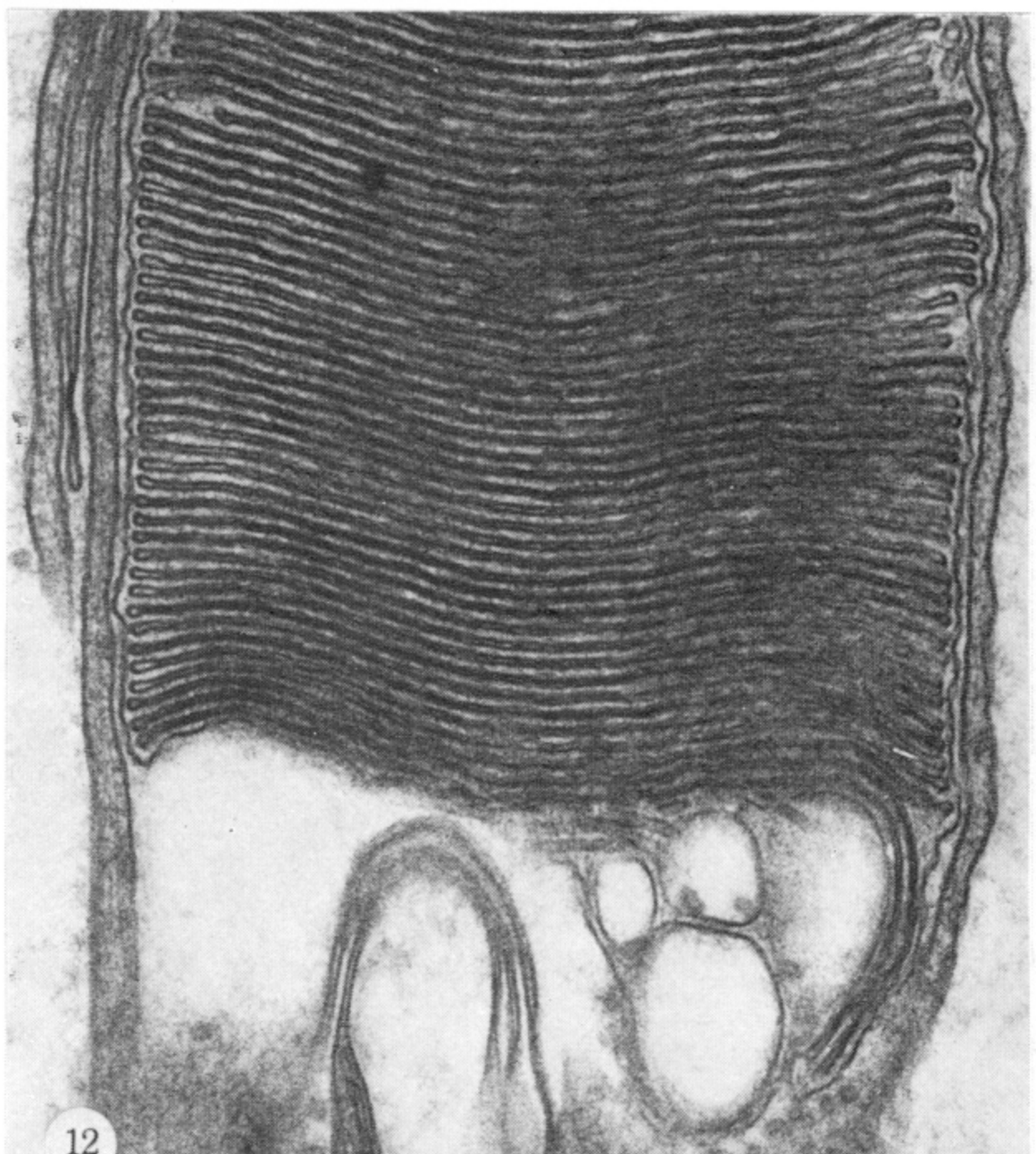
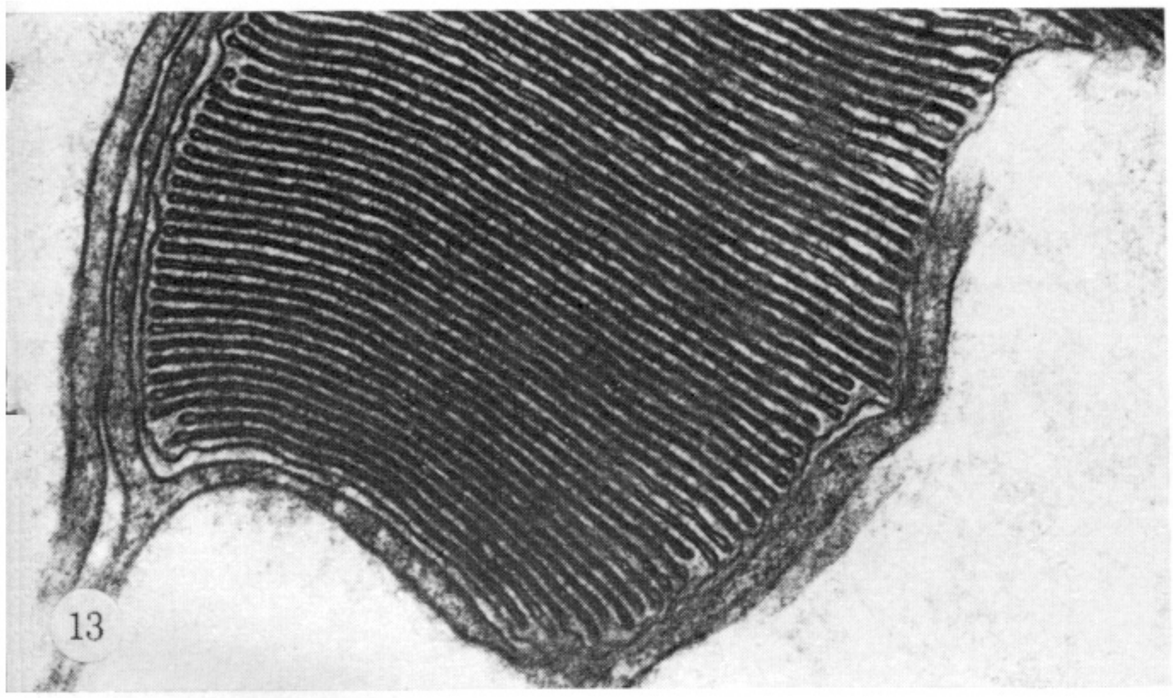


FIGURE 9. Electron micrograph montage of a longitudinal section through a cone outer segment and a portion of inner segment, and the pigment epithelial apical processes. One of the processes is thick and contains a variety of intracellular organelles, including a mitochondrion. A thick process extends along the left side of the outer segment. 5 year old retina. (Magn. × 3650.)

FIGURE 10. The tip of the cone outer segment of figure 9, at a higher magnification. (Magn. × 16 200.)

FIGURE 11. The portion of apical process containing the mitochondrion in figure 9, at a higher magnification. (Magn. × 22 500.)

FIGURE 12. Electron micrograph of a longitudinal section through a cone outer segment at its tip. Three apical processes on the left and one on the right extend alongside the outer segment. 5 year old retina. (Magn. × 35 000.)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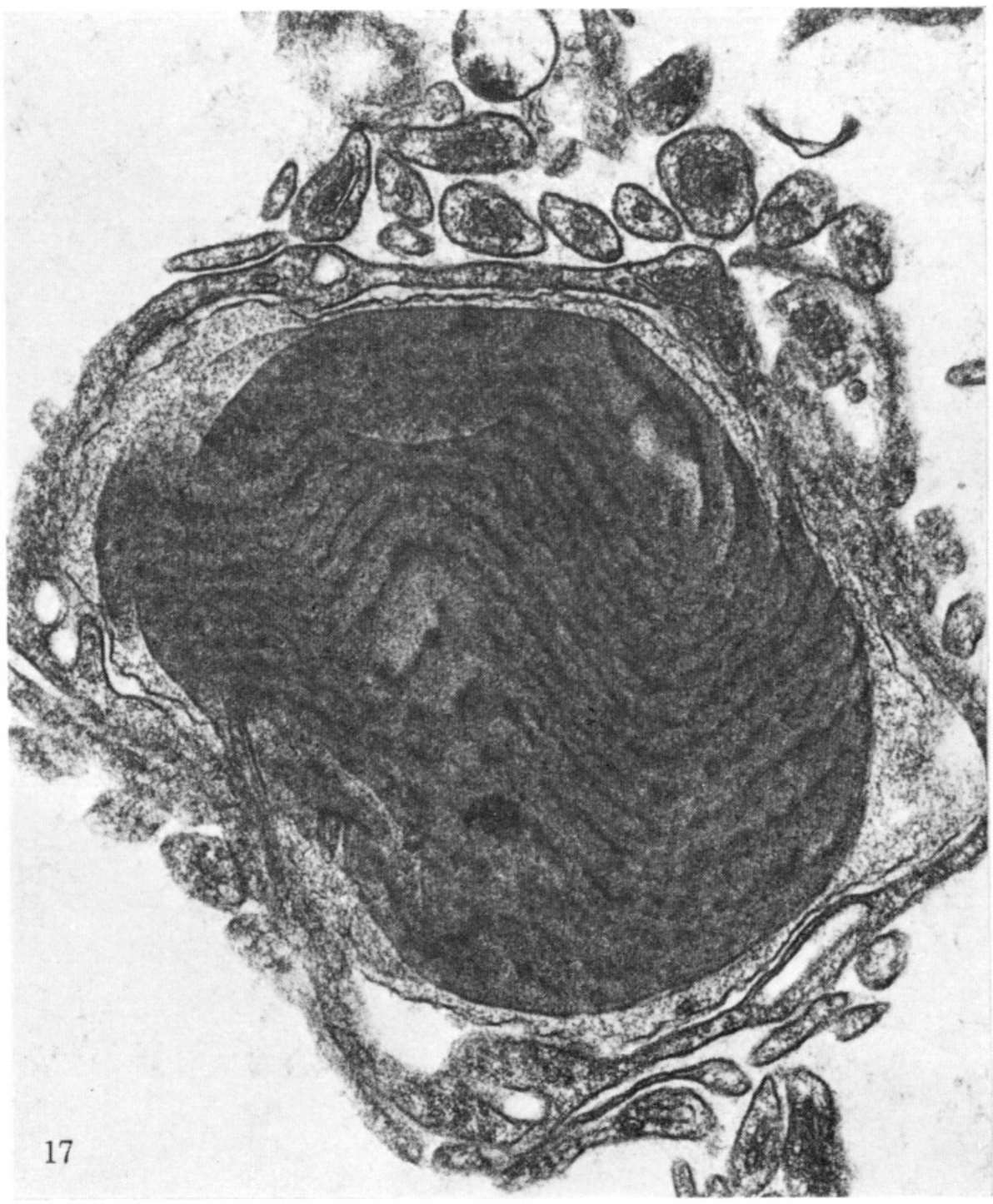
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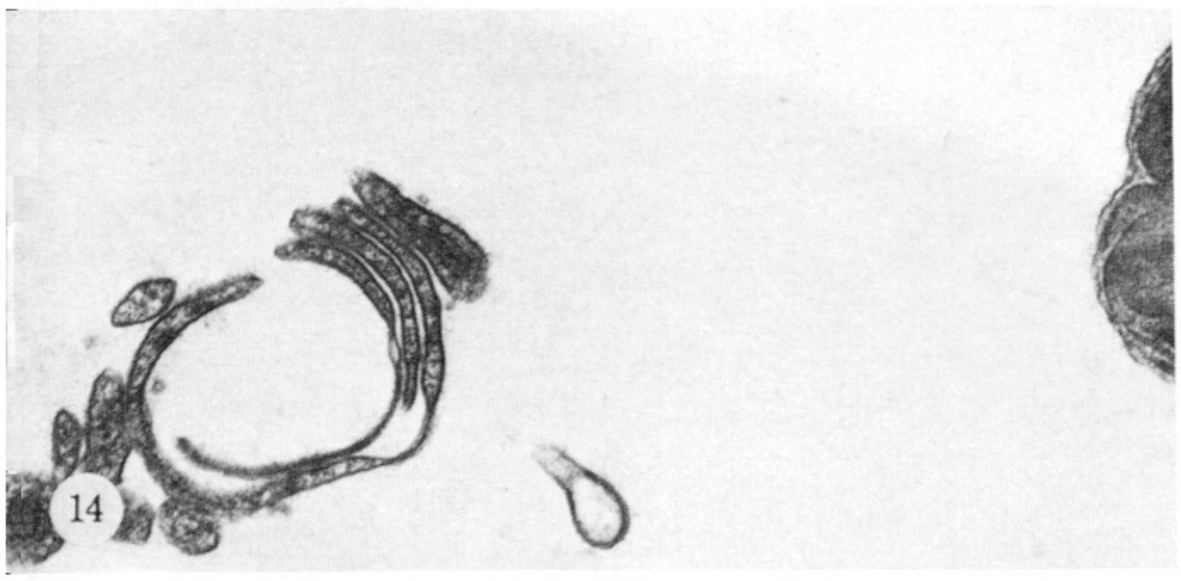
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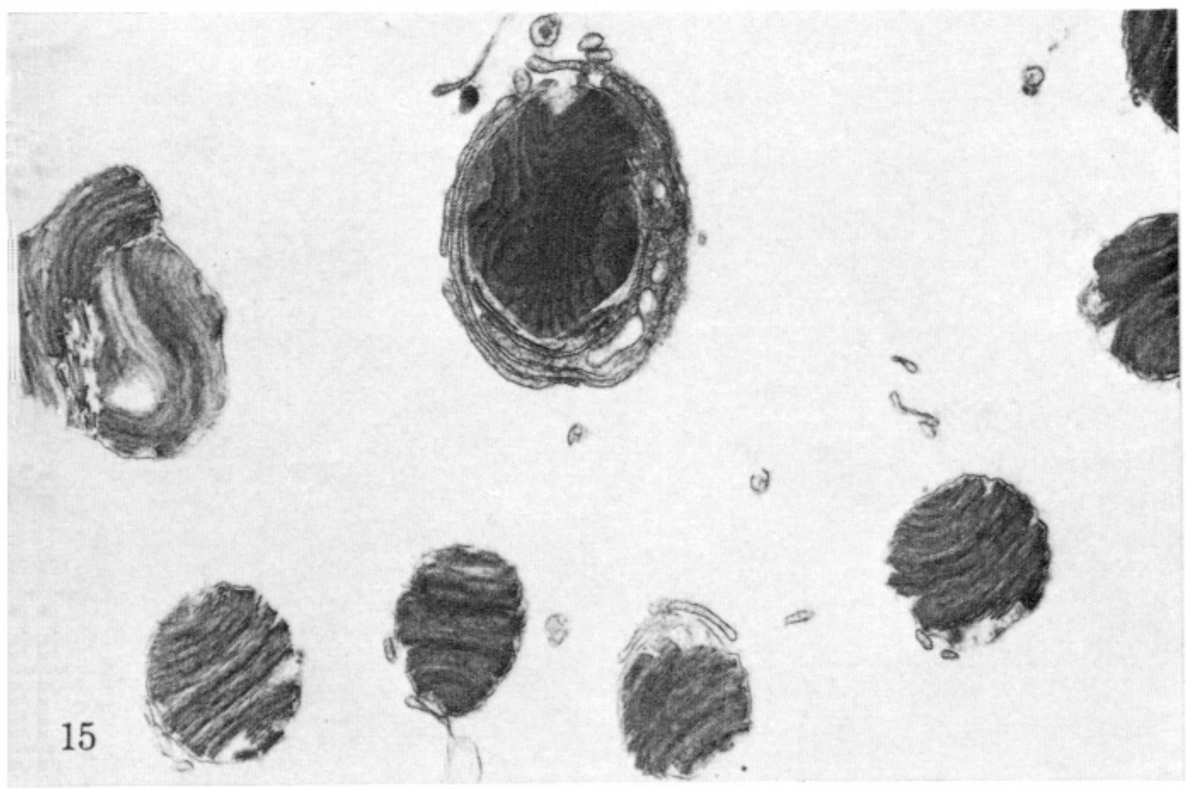
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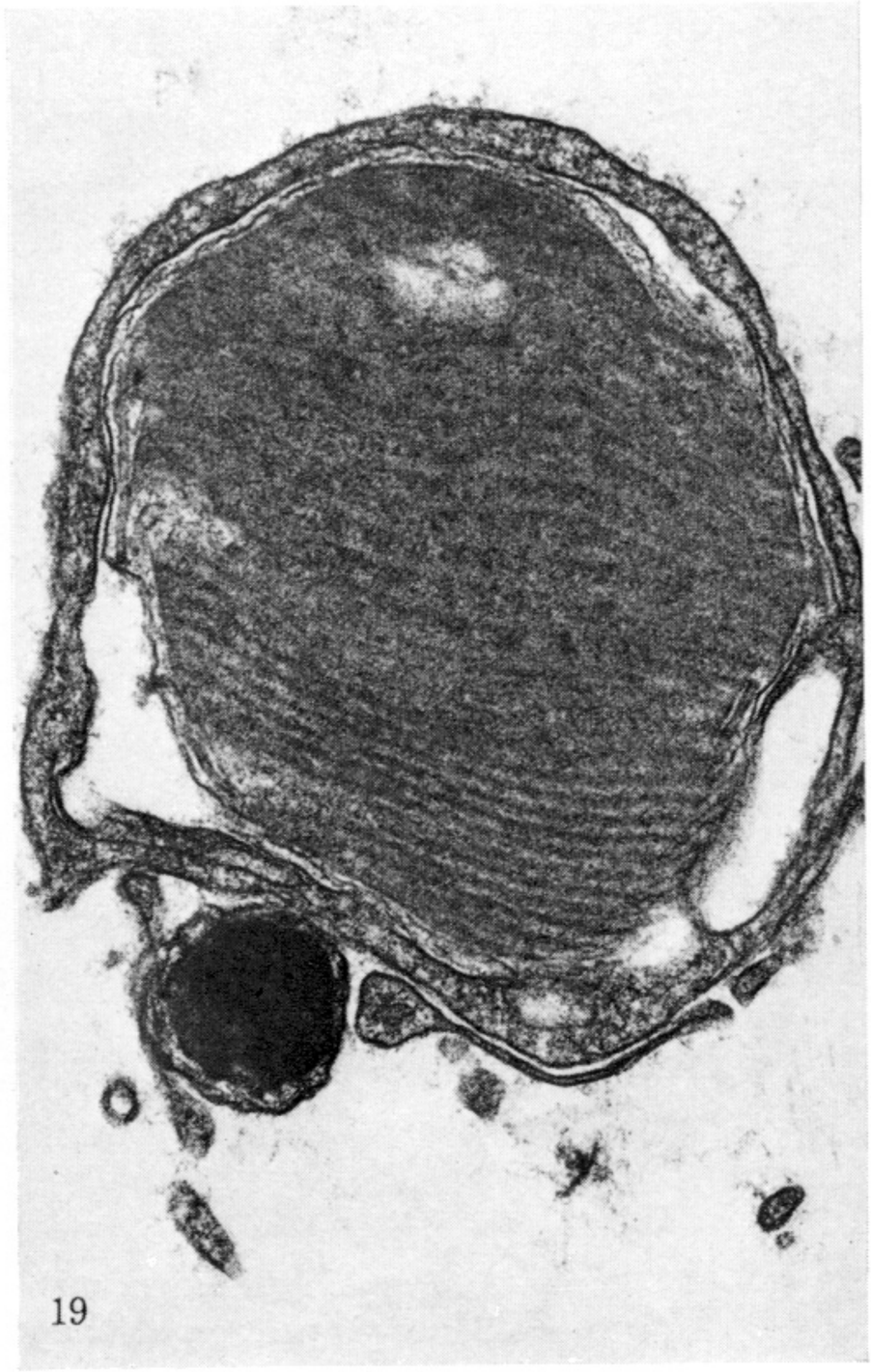
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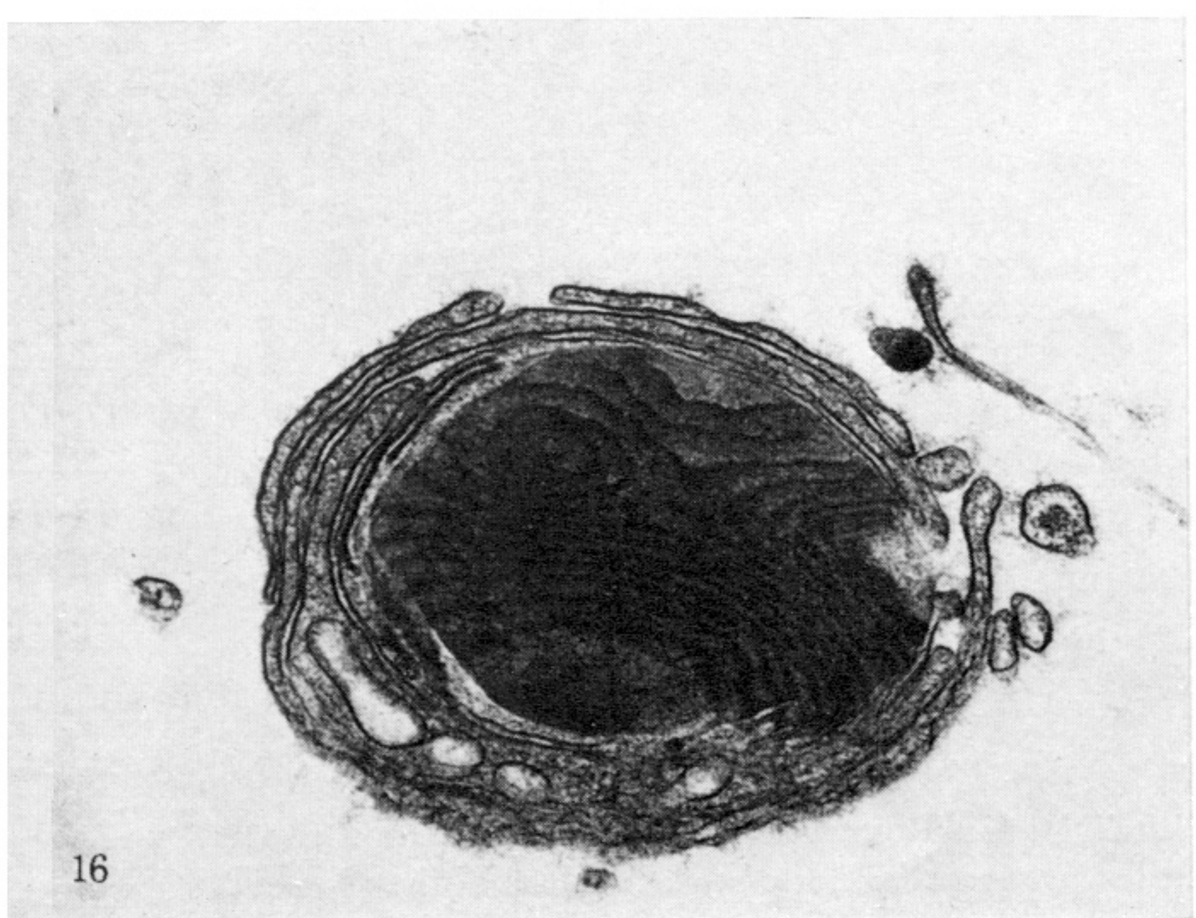
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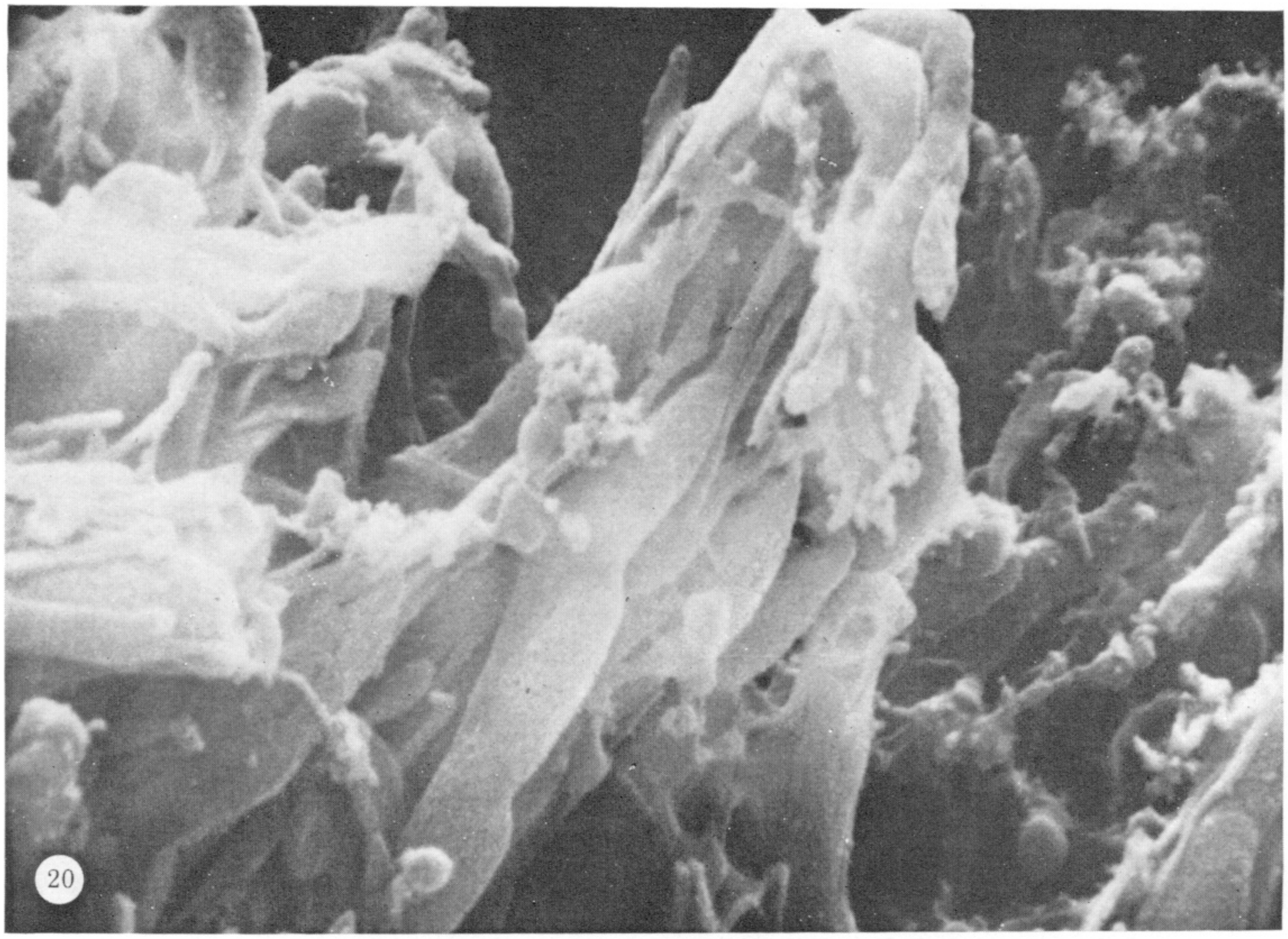
FIGURES 13–19. For description see opposite.



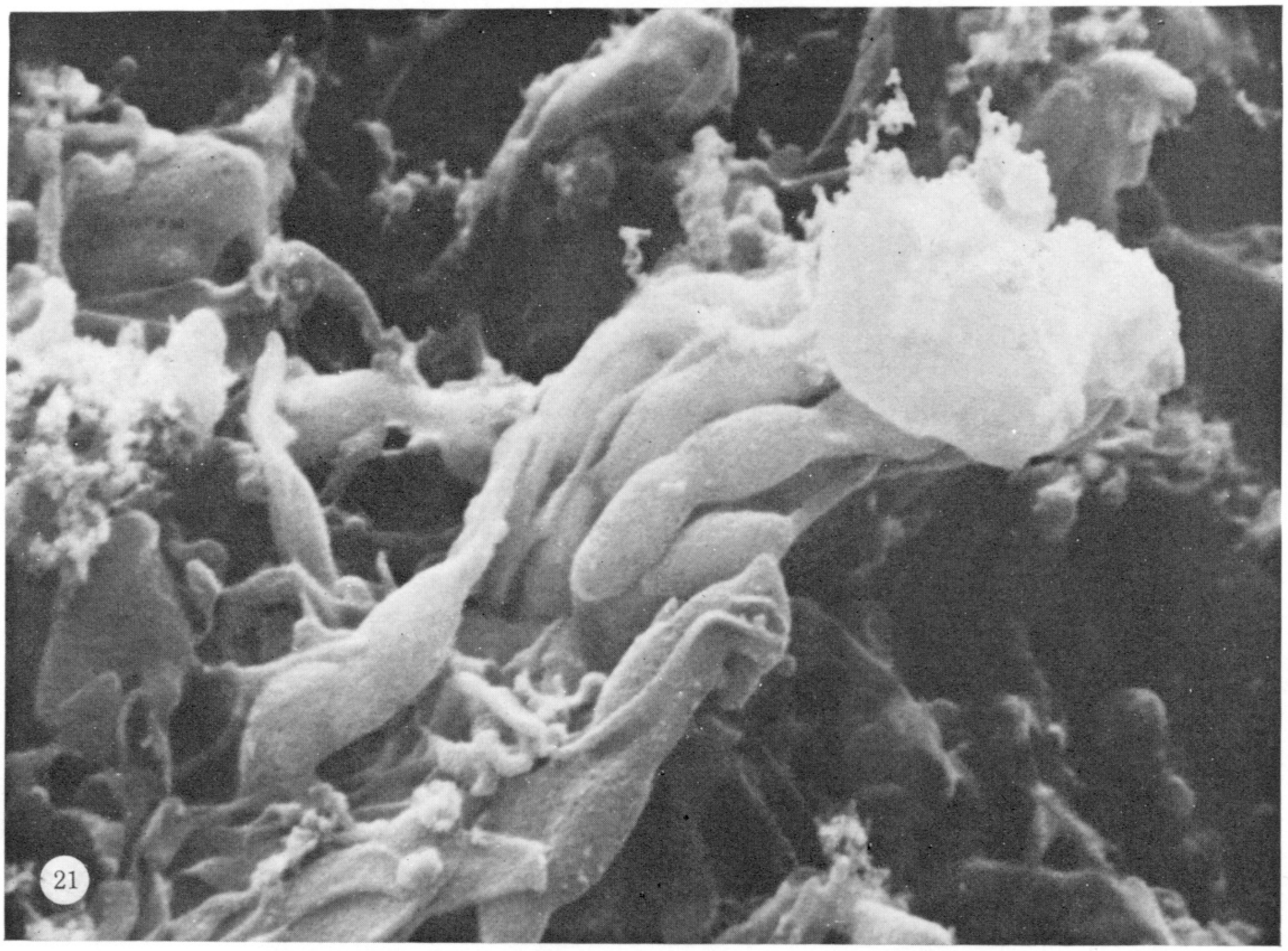
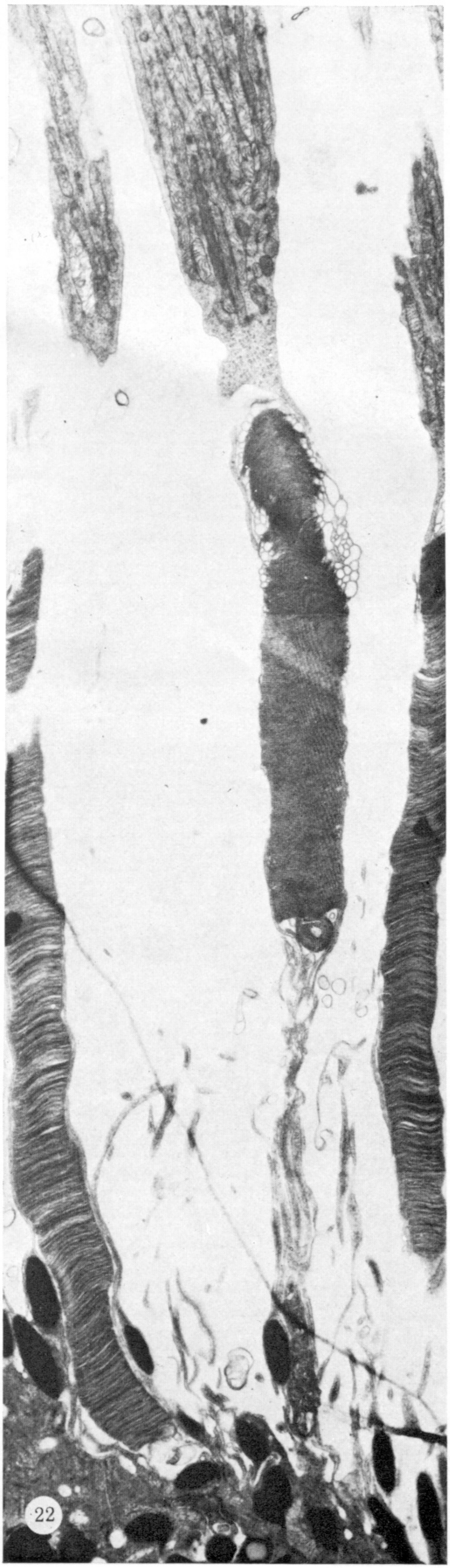


FIGURE 20. Scanning electron micrograph of pigment epithelial apical processes that associates with a cone. Profiles of pigment granules appear in the processes as they protrude from the apical surface of the pigment epithelium. Two of the processes become thin or villous-like above the pigment granules. Farther out the processes are sheet-like and, perhaps, have a tubular form. They have fallen over to the right. 38 year old retina. (Magn. × 14400.)

FIGURE 21. Scanning electron micrograph of another group of apical processes that associate with a cone. The form of the processes is similar to that of figure 20. 38 year old retina. (Magn. × 13035.)



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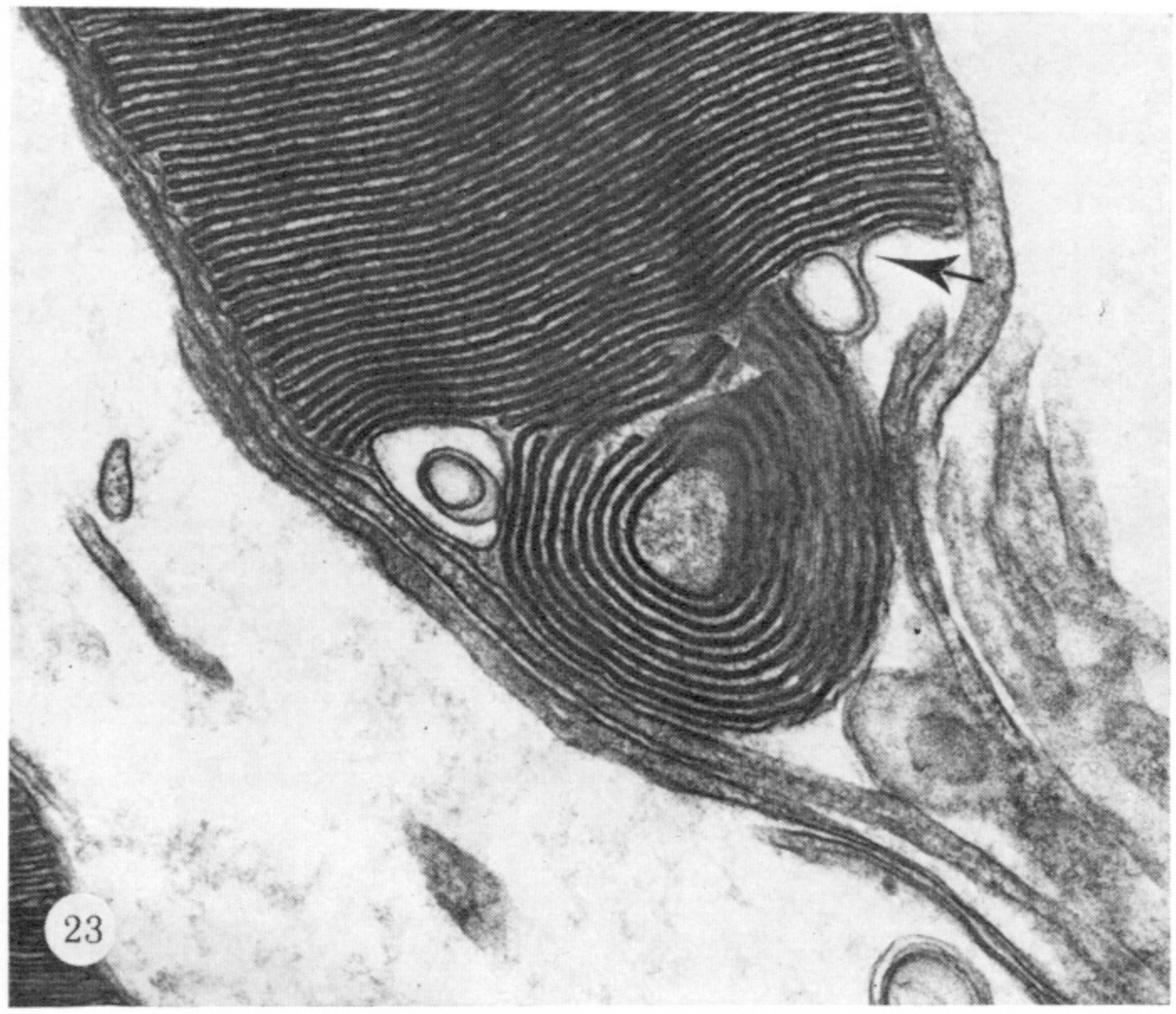

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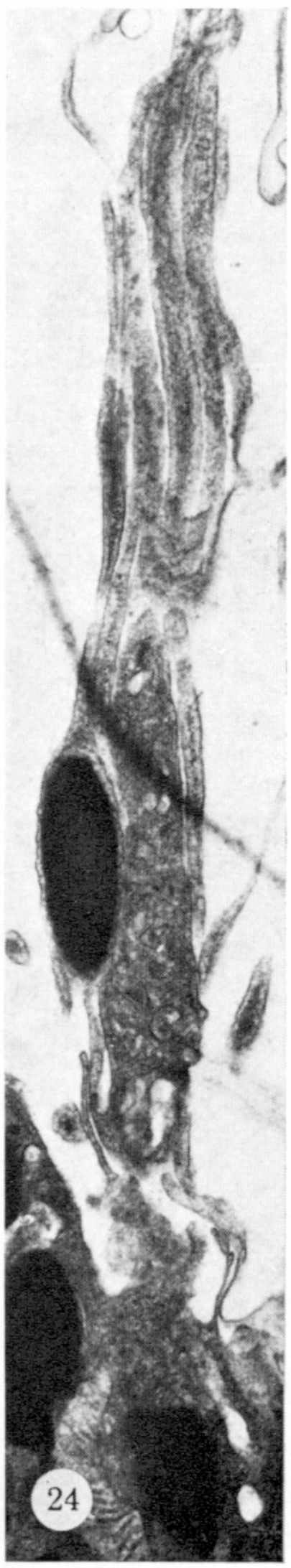

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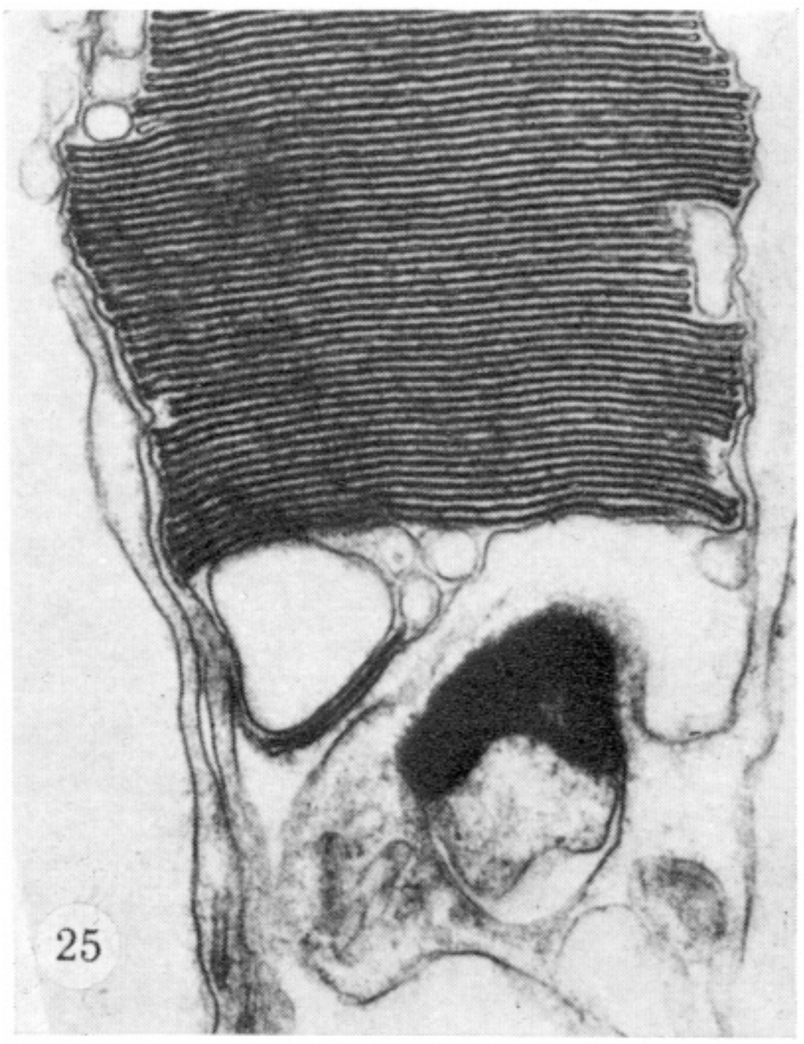

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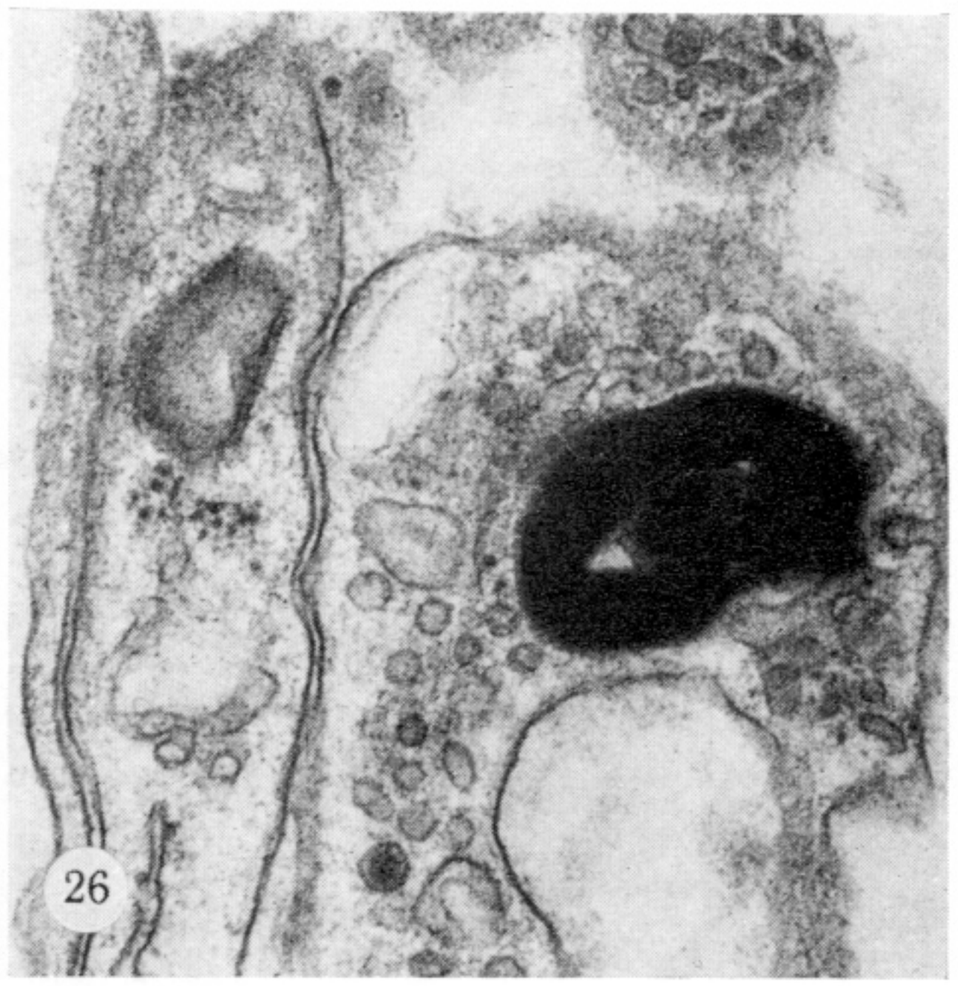

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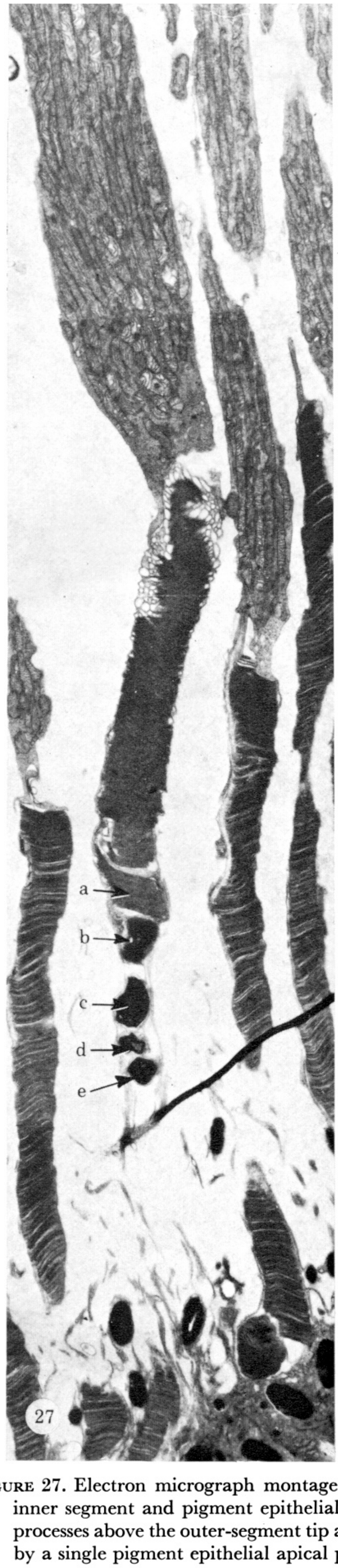
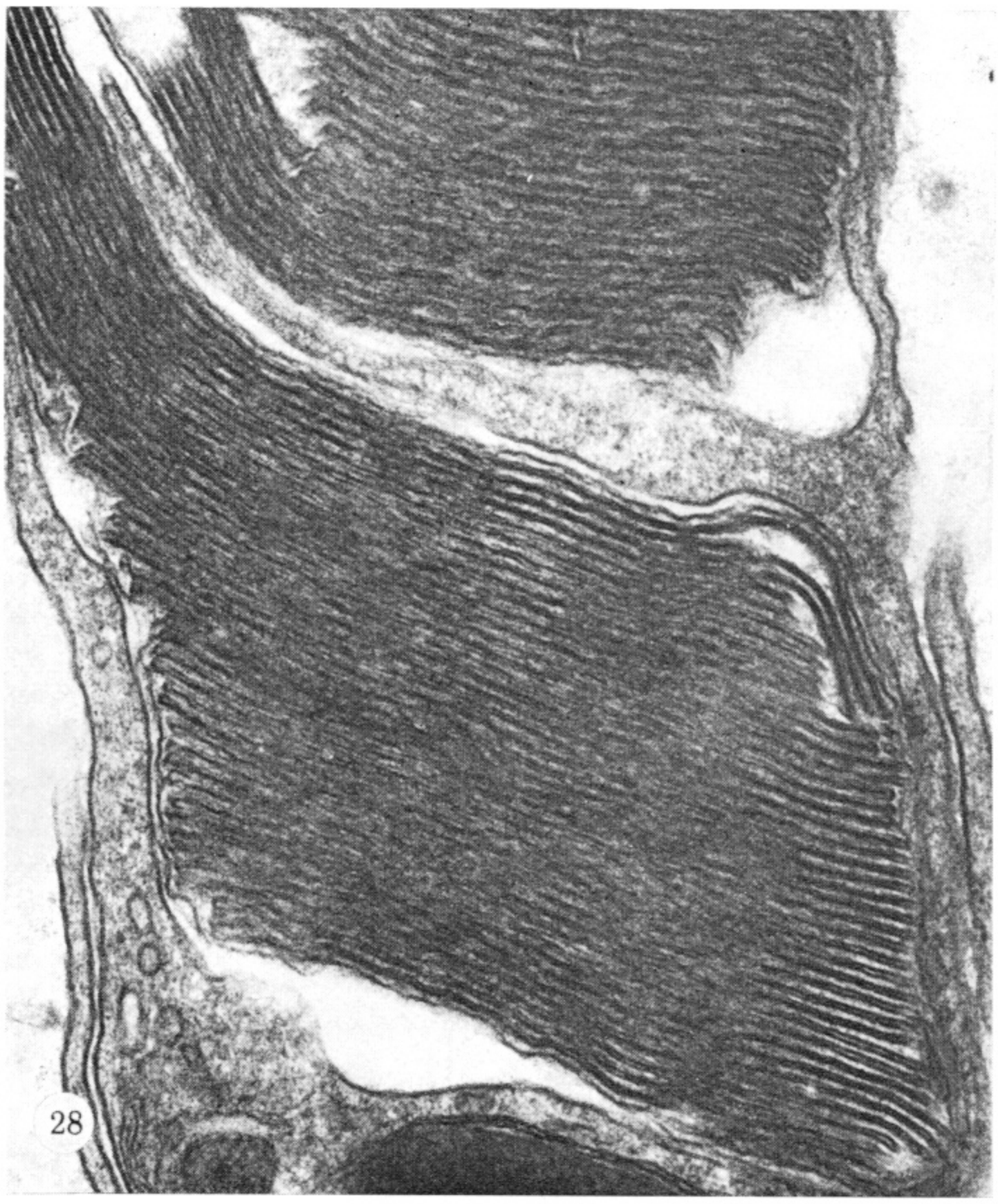
FIGURES 22–26. For description see opposite.





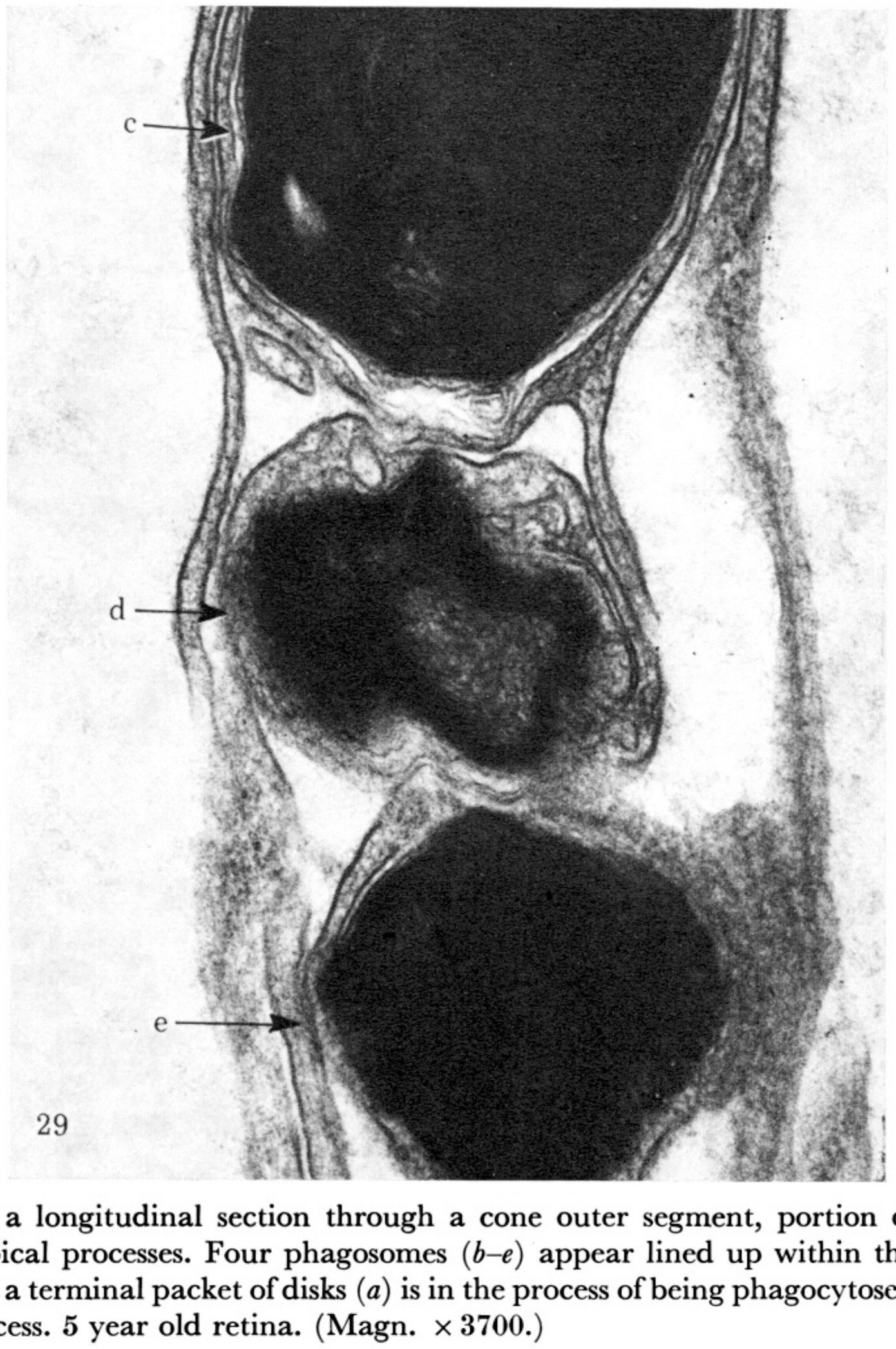
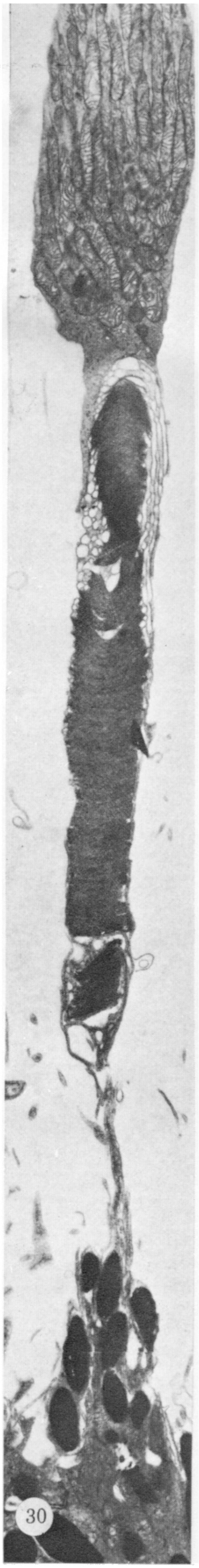
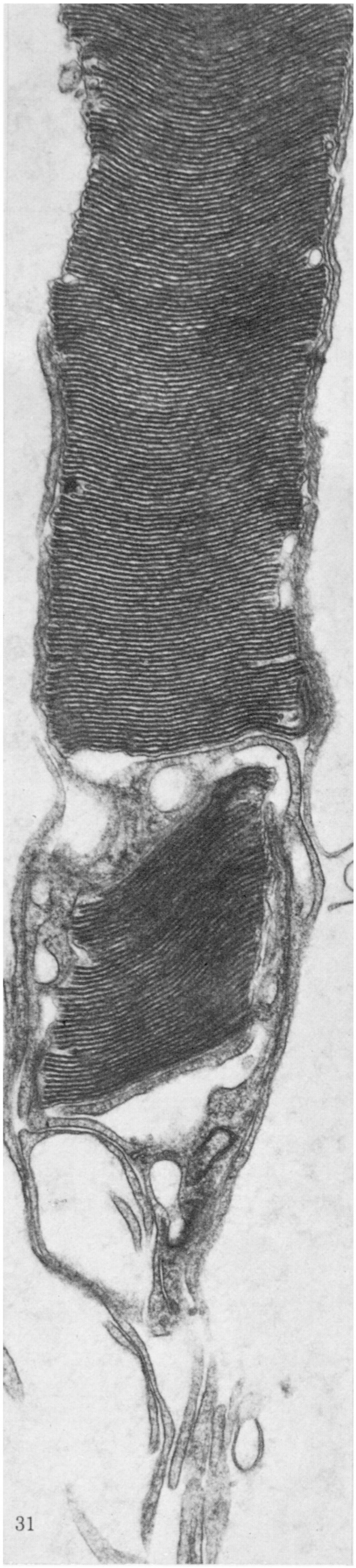


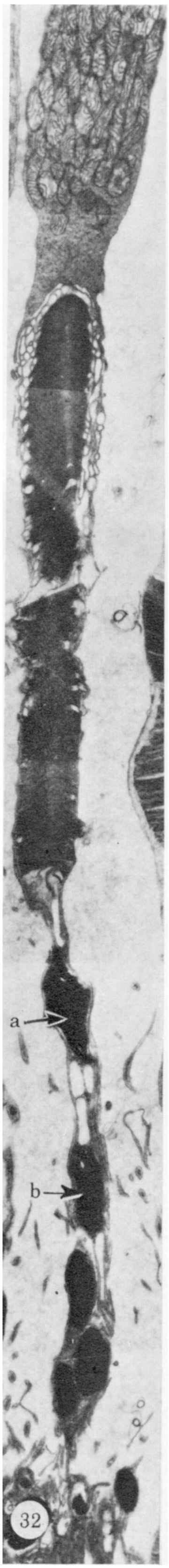
FIGURE 27. Electron micrograph montage of a longitudinal section through a cone outer segment, portion of inner segment and pigment epithelial apical processes. Four phagosomes (*b–e*) appear lined up within the processes above the outer-segment tip and a terminal packet of disks (*a*) is in the process of being phagocytosed by a single pigment epithelial apical process. 5 year old retina. (Magn. ×3700.)

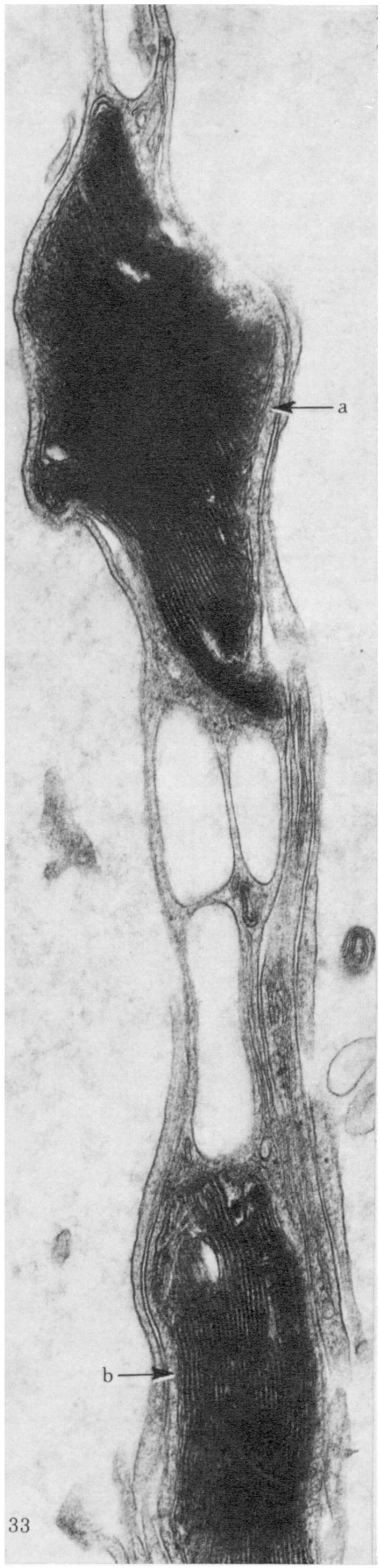
FIGURE 28. The outer-segment tip and terminal packet of disks from the cone of figure 27, at a higher magnification. An apical process bifurcates at the tip of the outer segment (lower left) and sends a branch across the tip and down along the right side. At the right side the process then sends a pseudopod across the outer segment so that a terminal packet of 40 disks is completely encircled. (Magn. ×43400.)

FIGURE 29. Phagosomes *c*, *d*, *e* in the apical processes from the cone of figure 27, at a higher magnification. (Magn. ×44800.)

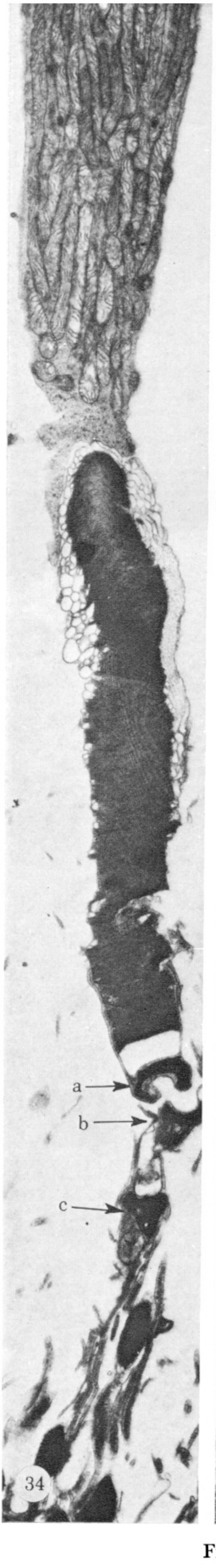








FIGURES 30–33. For description see opposite.



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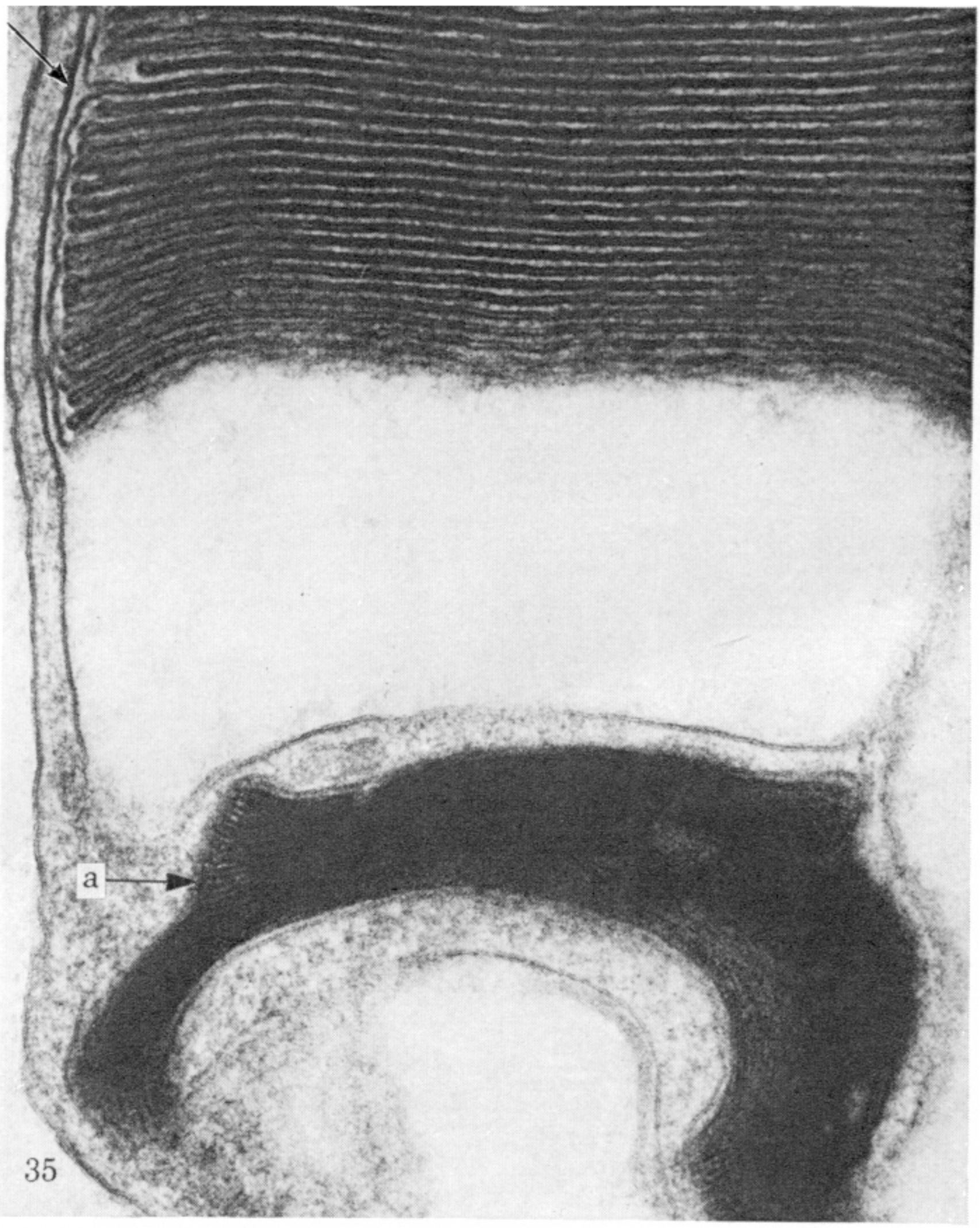

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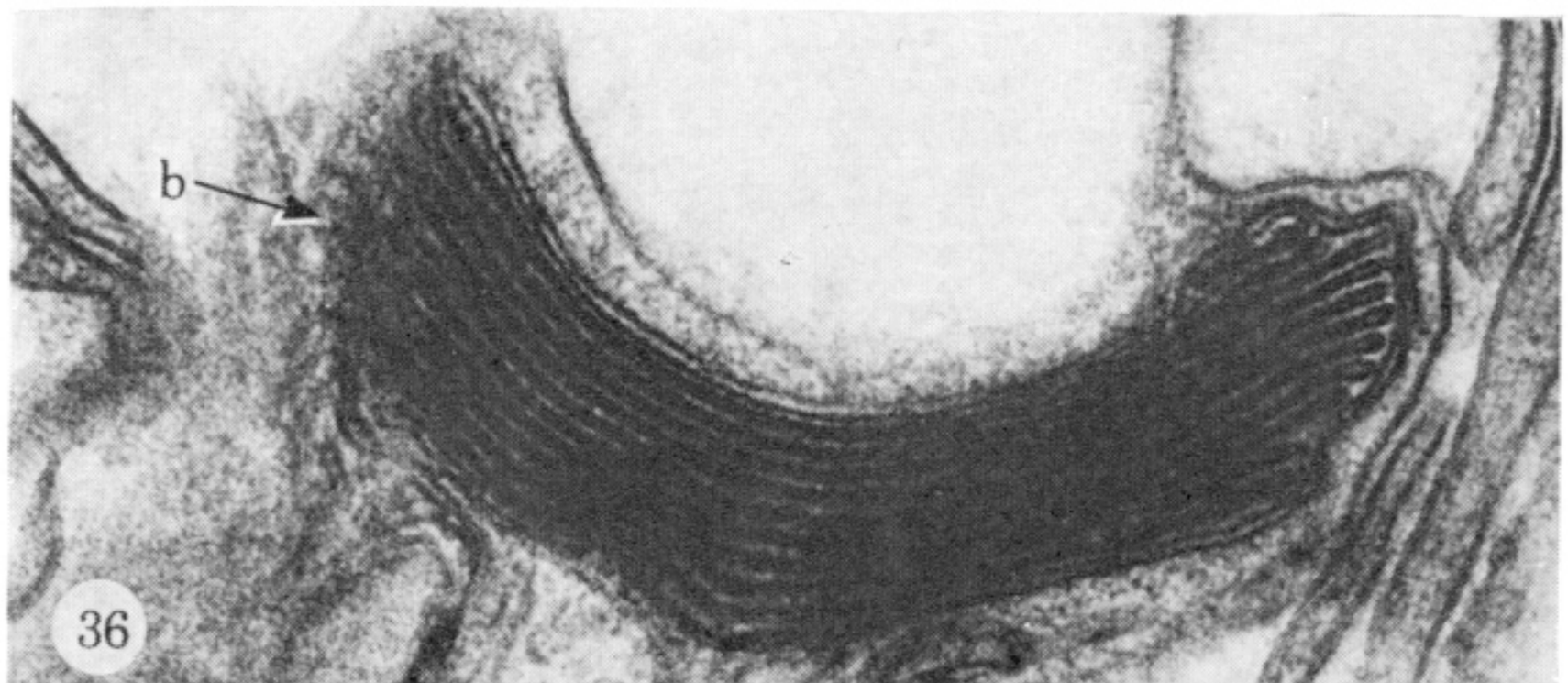

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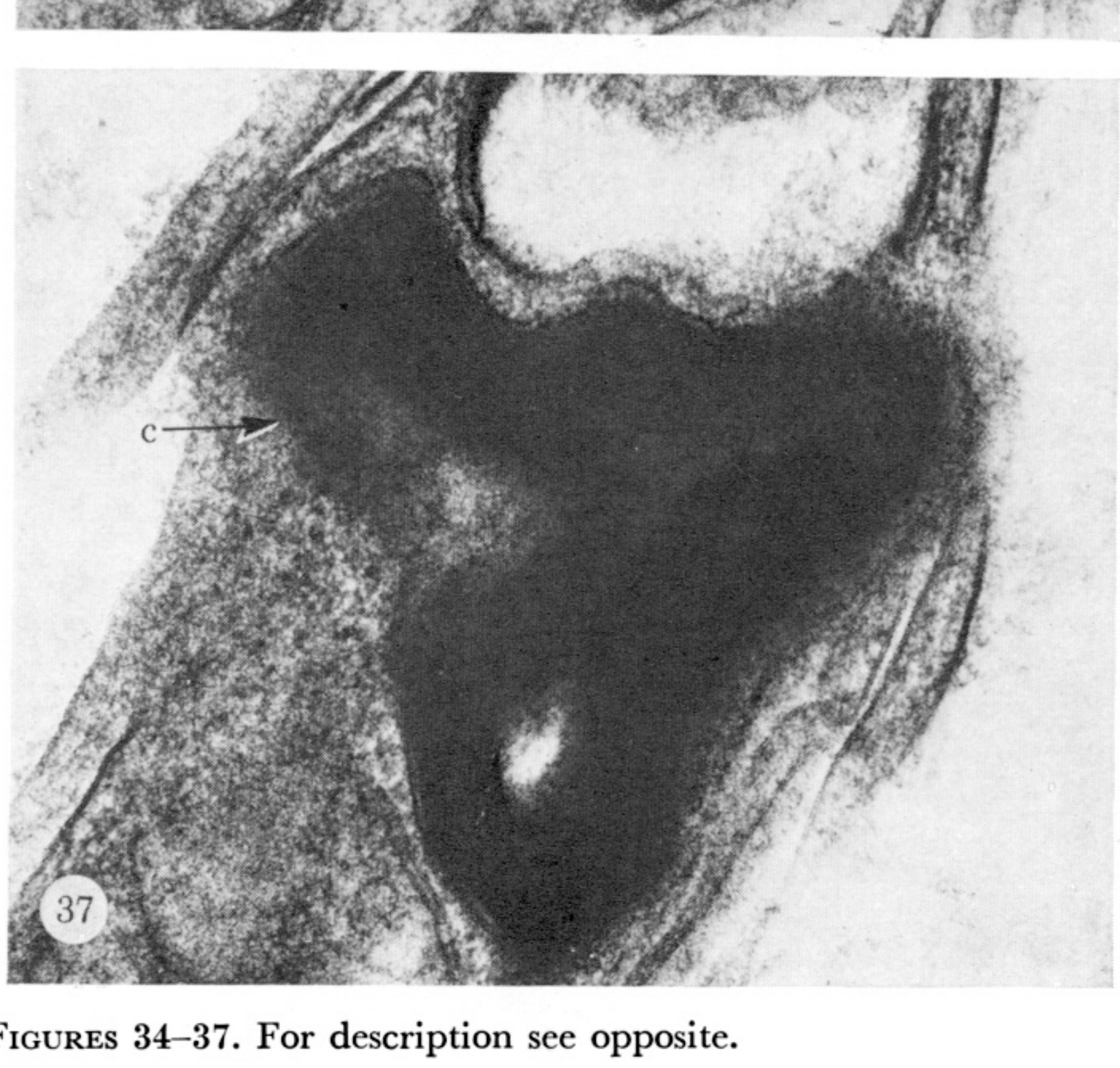

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FIGURES 34–37. For description see opposite.

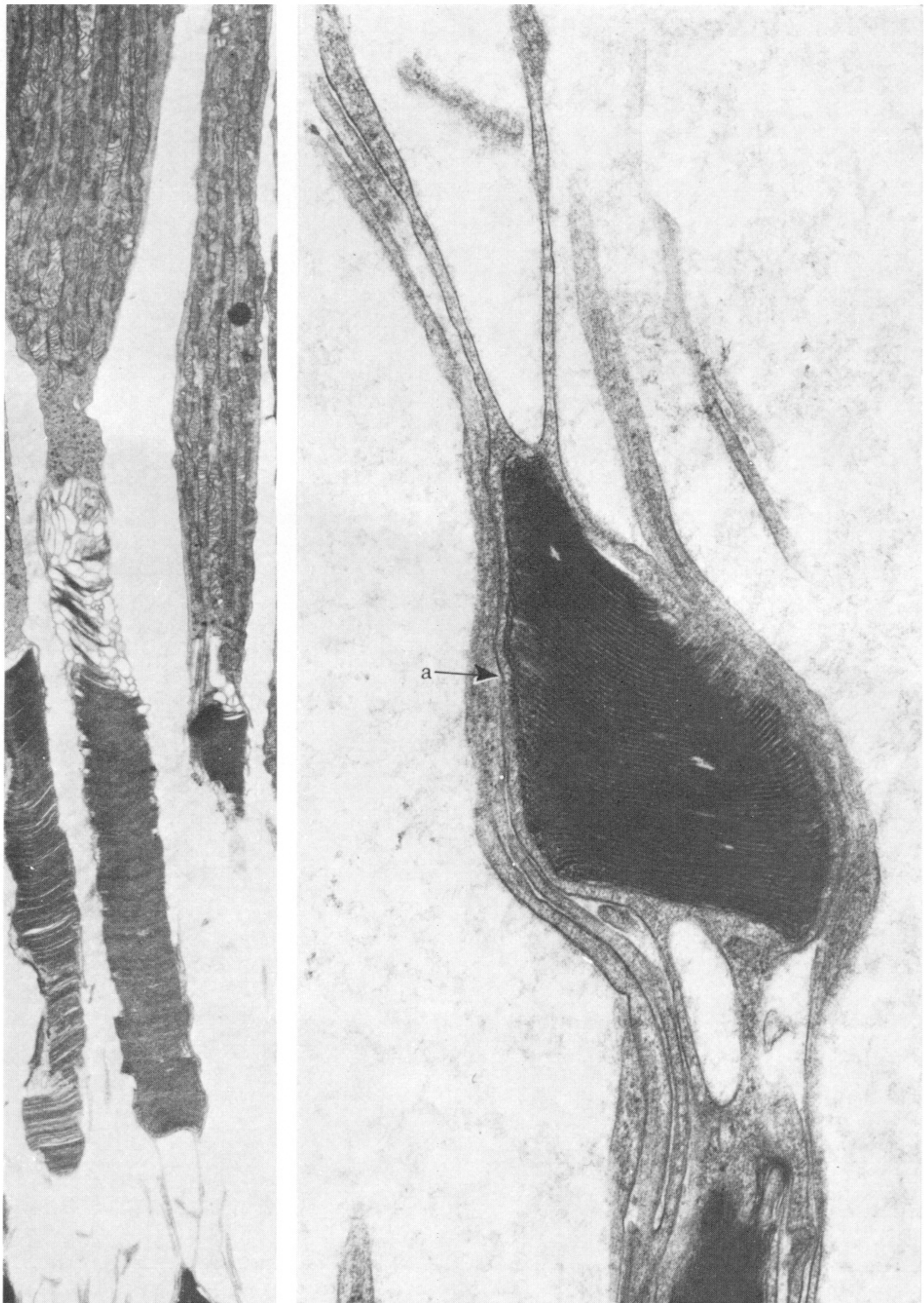
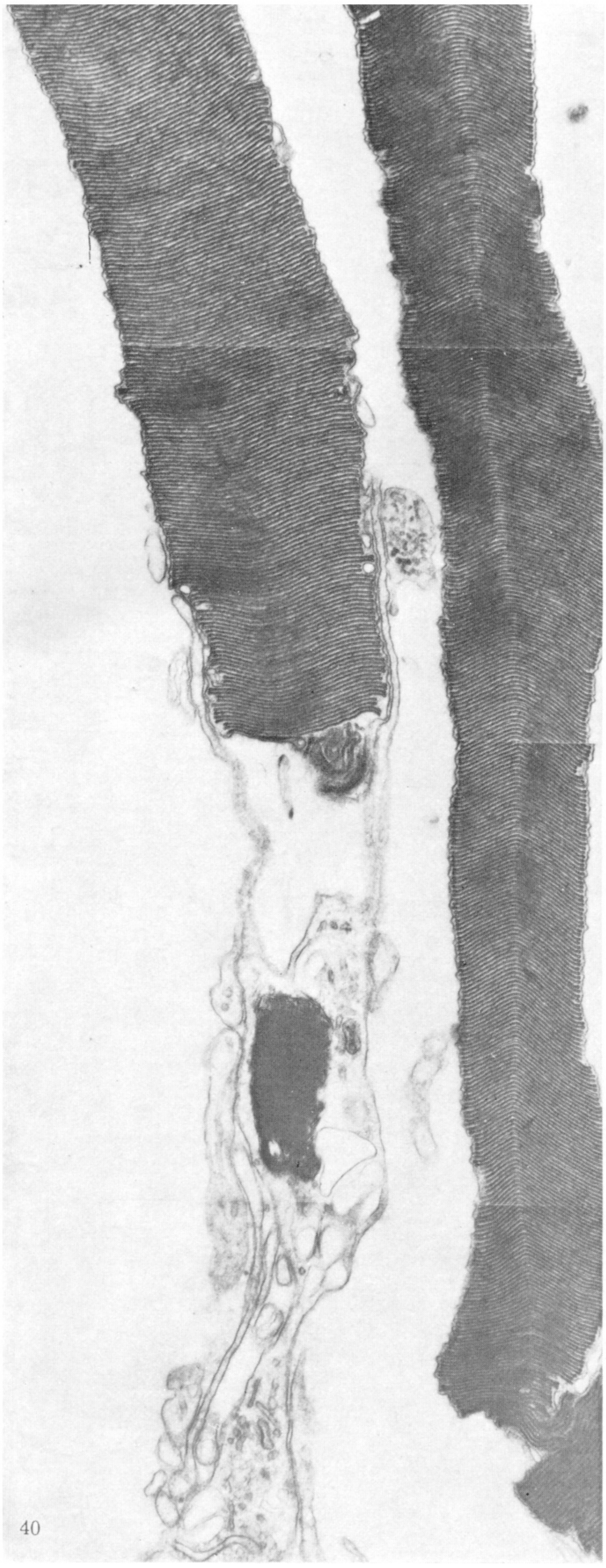


FIGURE 38. Electron micrograph montage of a longitudinal section through a cone outer segment, portion of inner segment, and pigment epithelial apical process. The apical processes contains two phagosomes (*a*, *b*). An enlargement of the tip of this outer segment was presented in figure 13, plate 5. 5 year old retina. (Magn. × 3650.)

FIGURE 39. Phagosomes *a* and *b* and the apical processes of figure 38, at a higher magnification. Observe that both phagosomes are enclosed in the same apical process, and the process continues to the outer segment where it ensheaths the tip (figure 13, plate 5). Phagosome *a* is a packet of 62 disks that have approximately maintained their usual orientation. Phagosome *b*, probably contains about 20 disks, and the packet has rotated 90° so that the disk edges face the pigment epithelium. (Magn. 20400.)



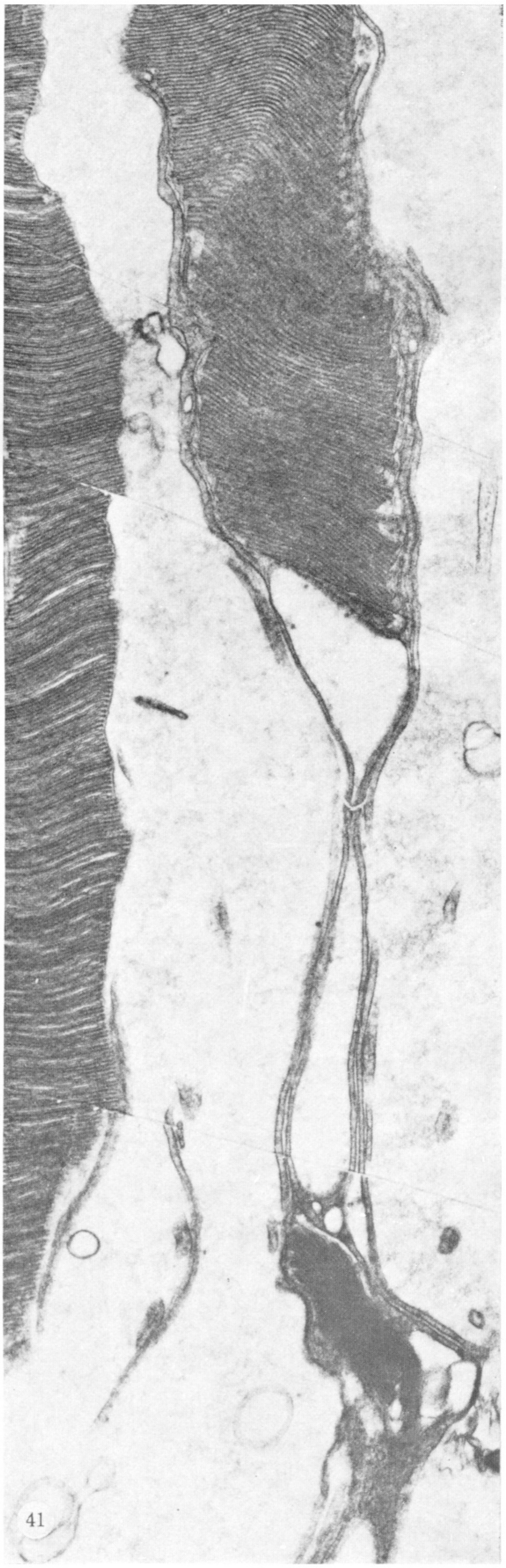
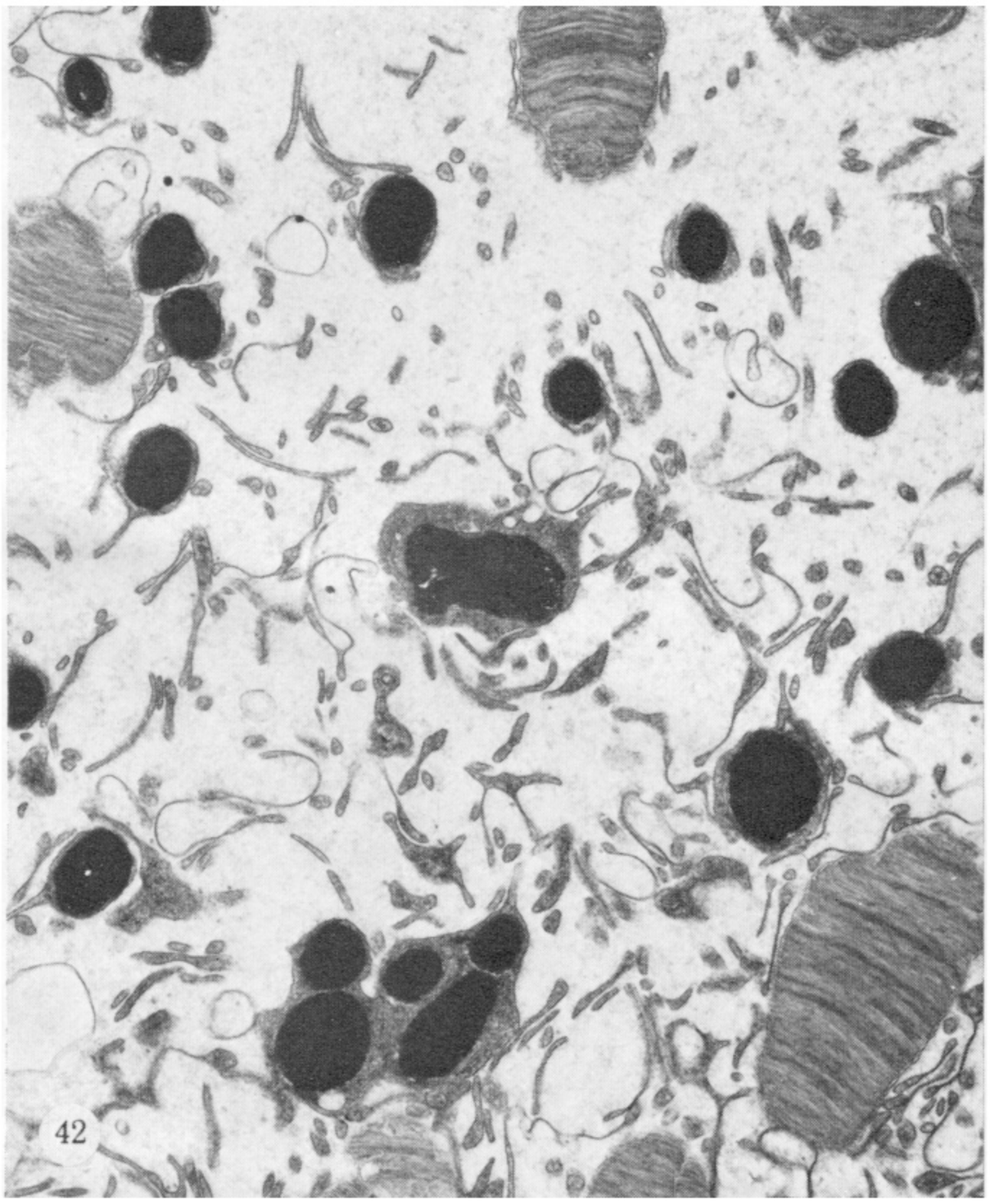
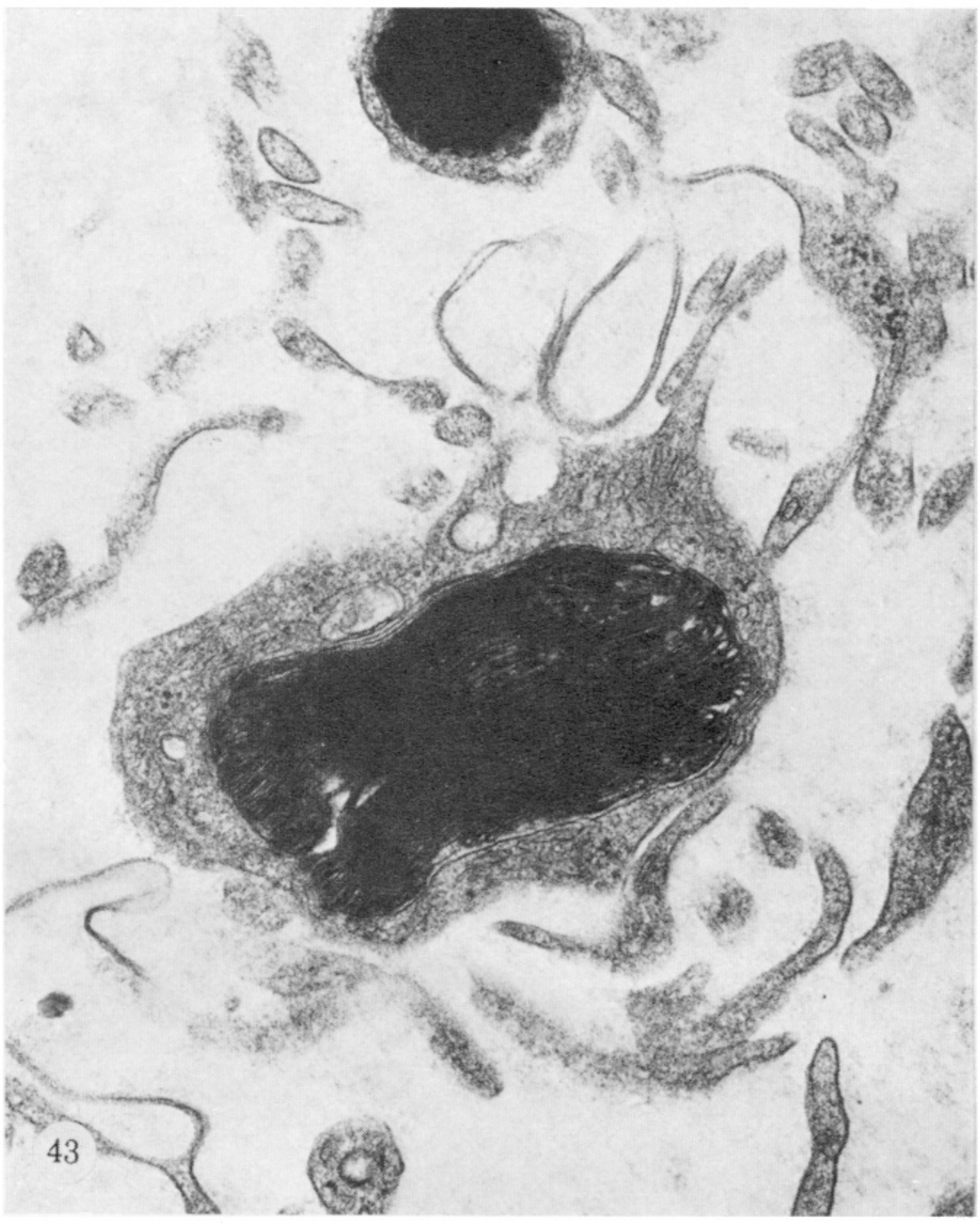
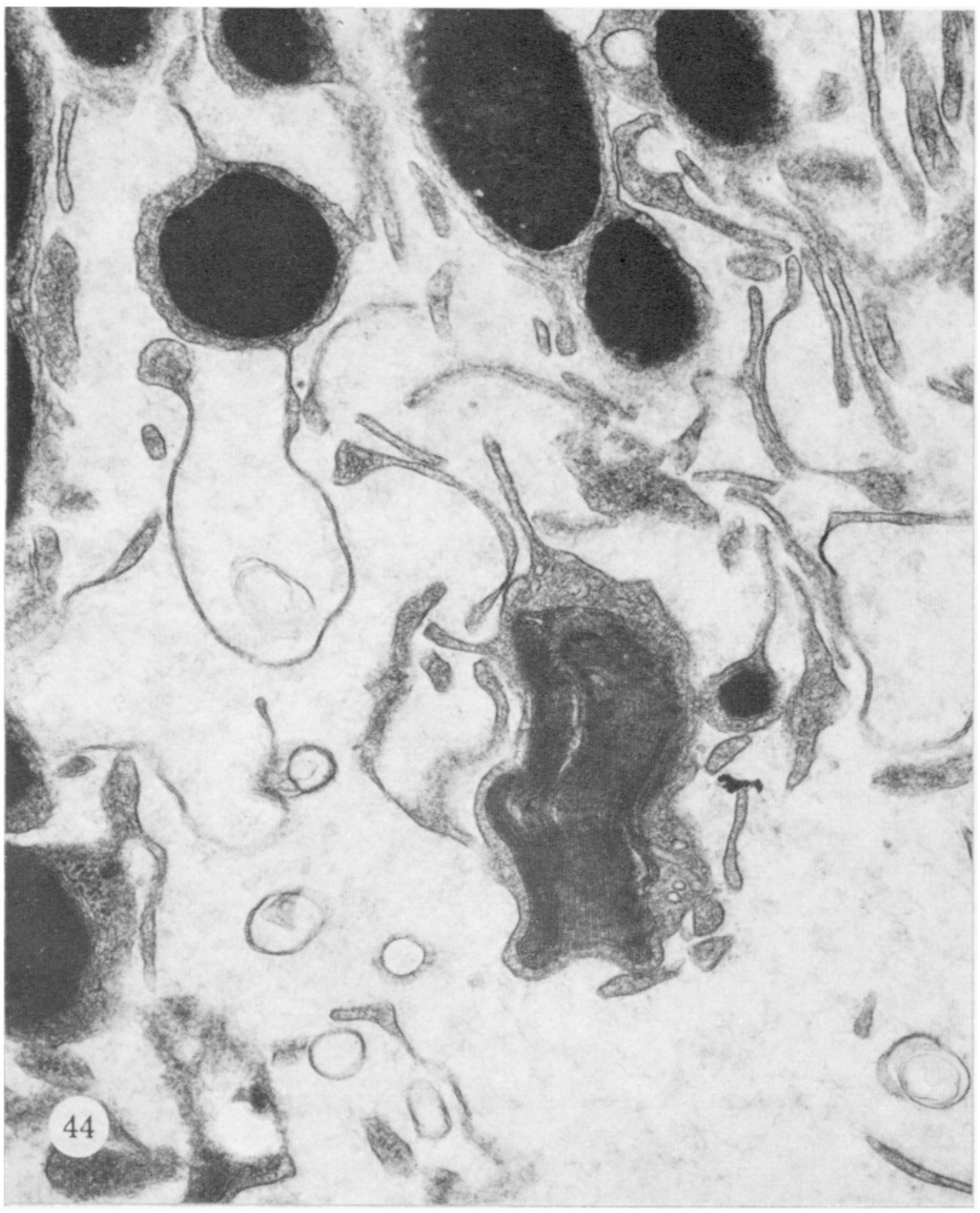


FIGURE 40. Electron micrograph montage of a longitudinal section through a portion of a cone outer segment and its pigment epithelial apical processes. The processes contain a phagosome that has rotated 90°. 60 year old retina. (Magn. × 16 800.)

FIGURE 41. Electron micrograph montage through a portion of a cone outer segment and its pigment epithelial apical processes. There is a single phagosome in the apical processes; a packet of less than 20 disks that has rotated almost 90°. The apical process that enclosed the phagosome continues to the outer segment where it lies along both sides, presumably ensheathing it. 45 year old retina. (Magn. × 13 980.)







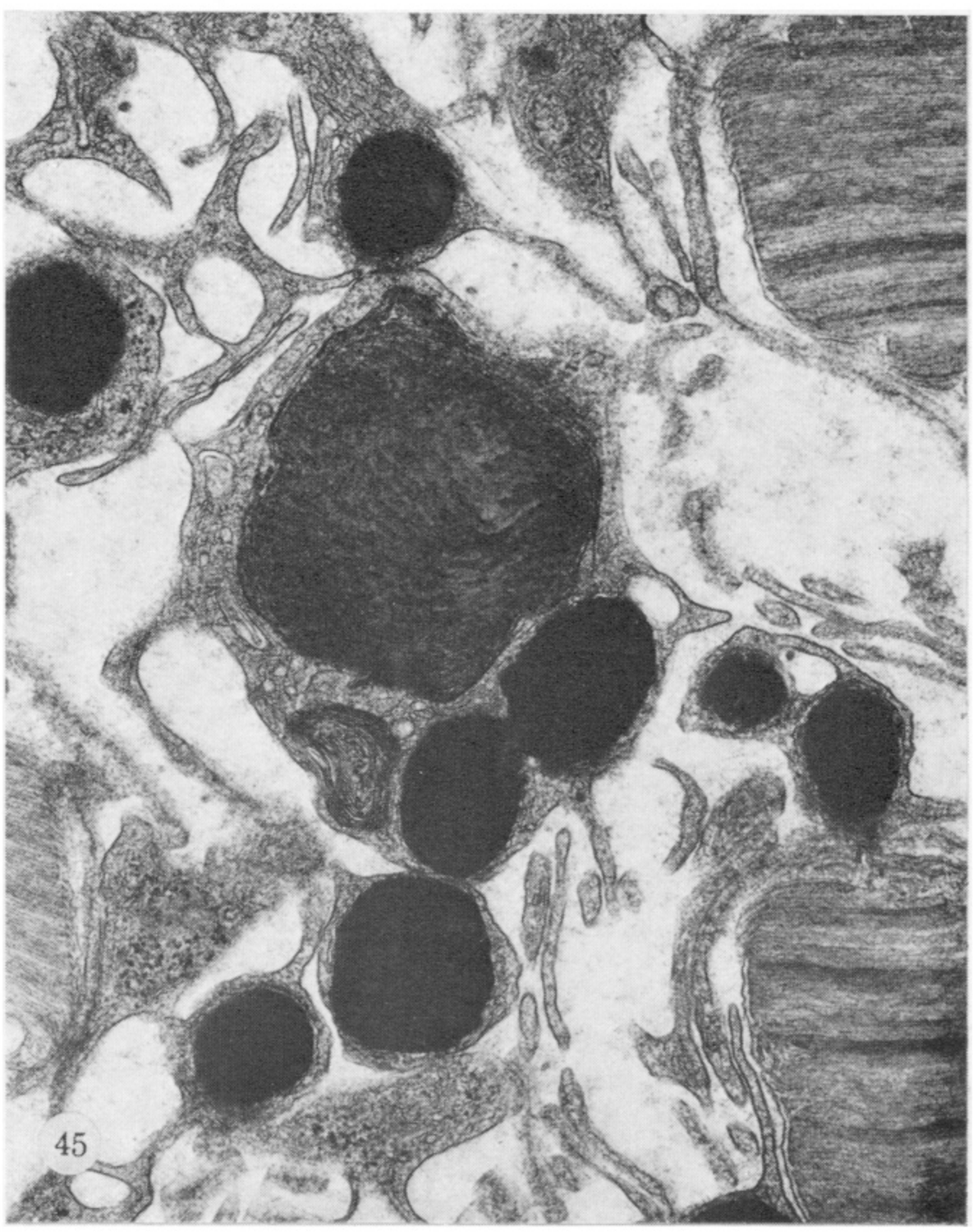
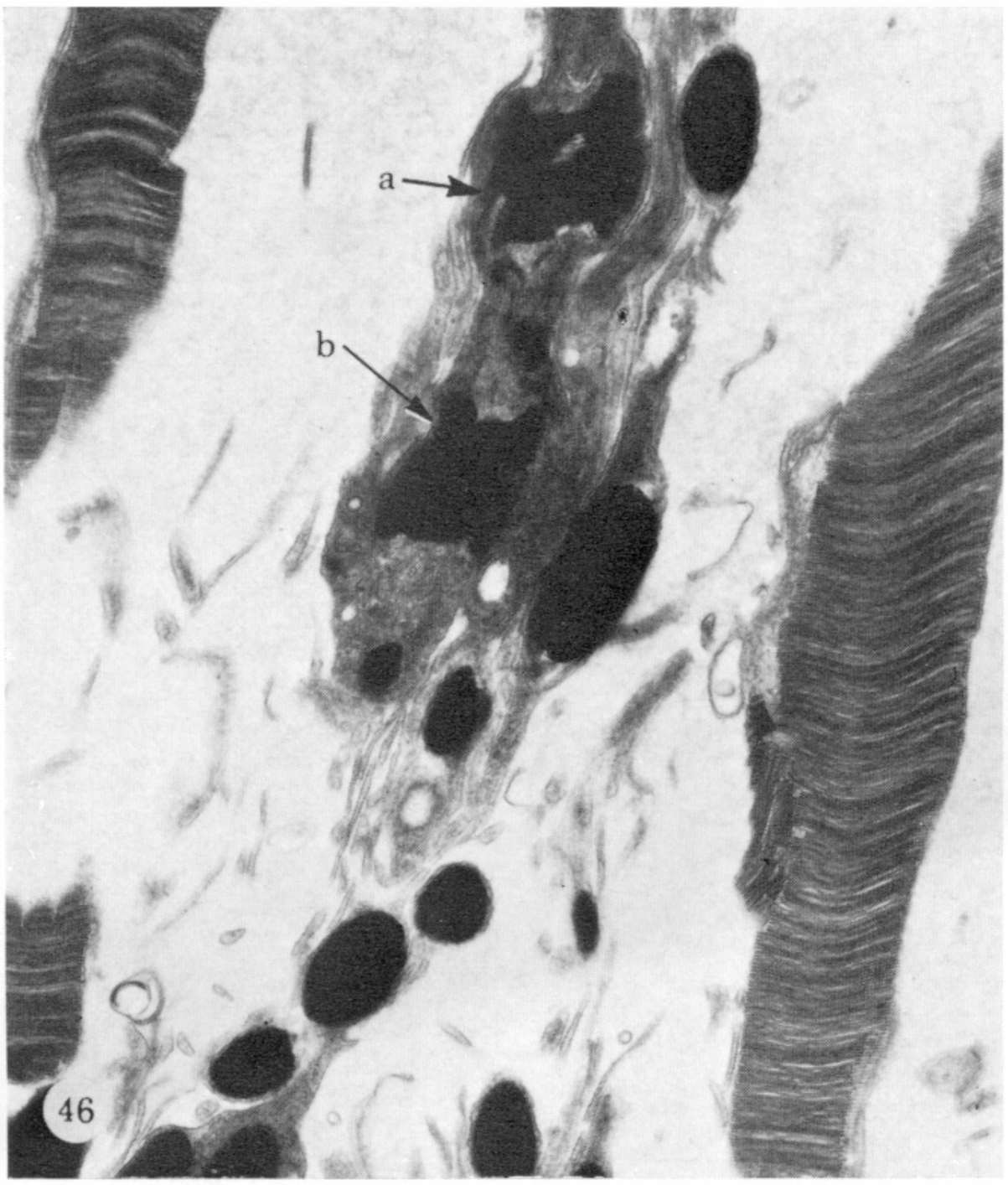


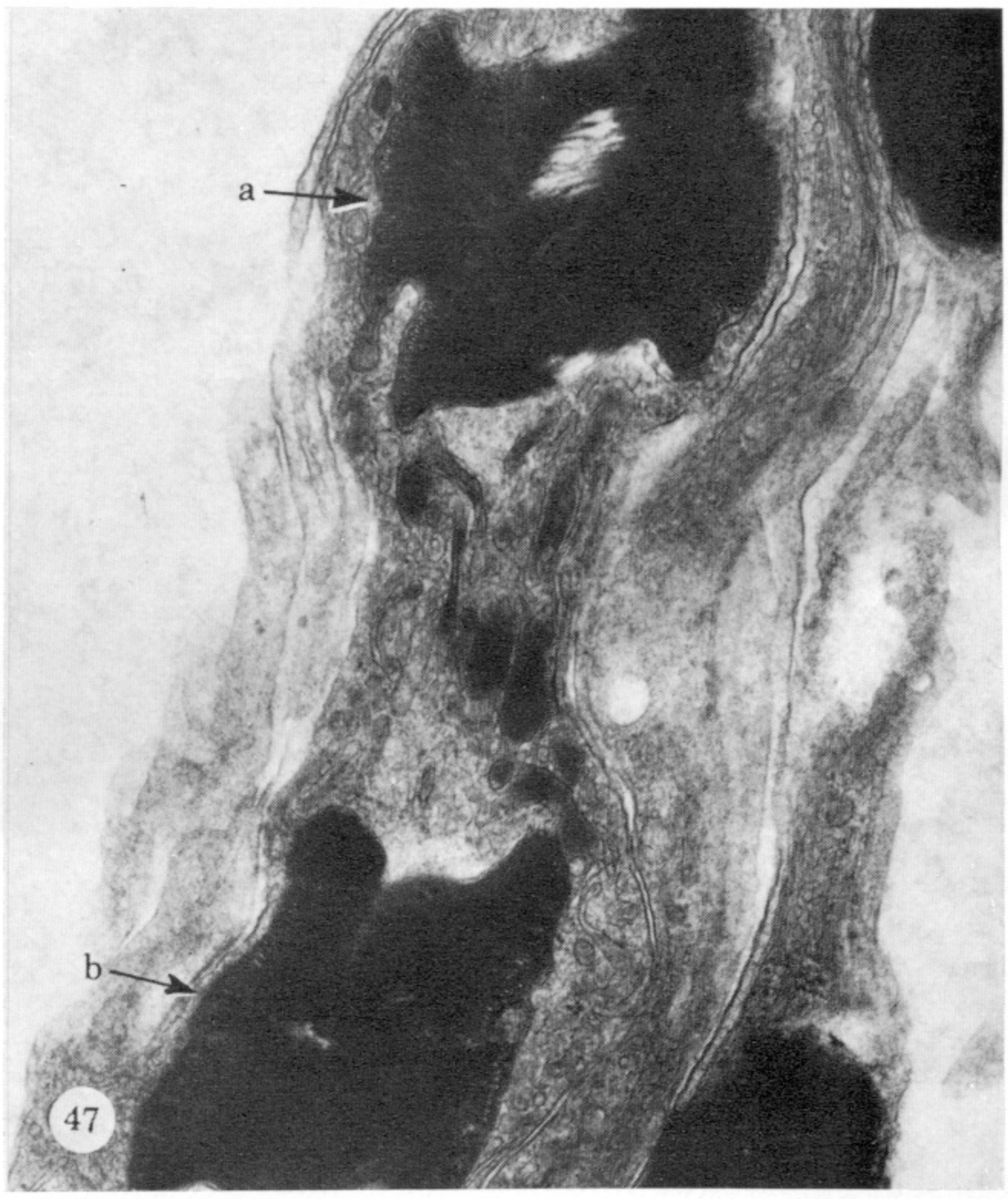
FIGURE 42. Electron micrograph of a transverse section through a supracone space. The space is surrounded by rods. An apical process that contains a phagosome occurs in the center of the space and it is surrounded by other processes that contain pigment granules. 5 year old retina. (Magn. × 7200.)

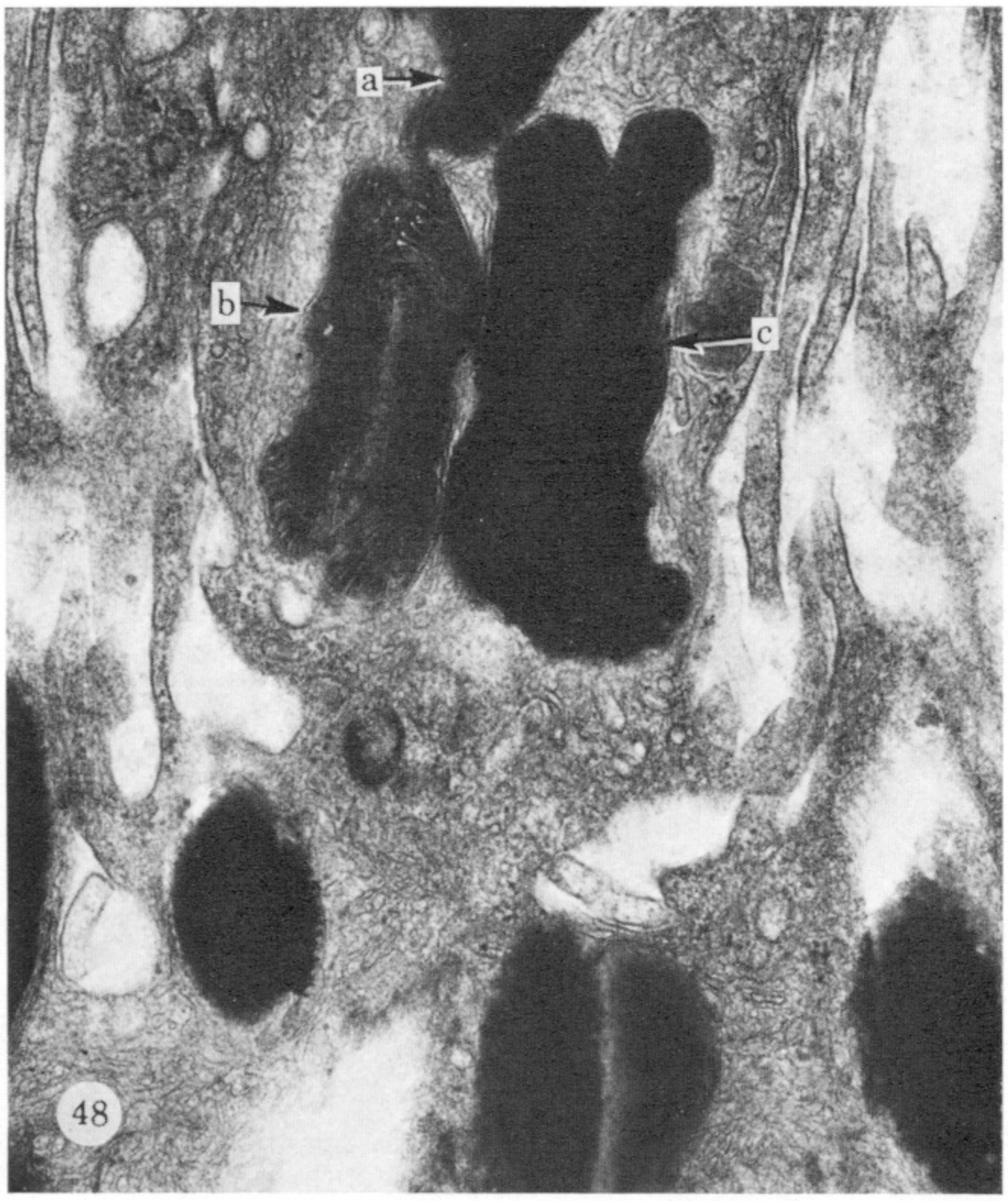
FIGURE 43. The phagosome of figure 42, at a higher magnification. (Magn. × 27 750.)

FIGURE 44. Electron micrograph of a transverse section through the supracone space of a process that contains a phagosome. 5 year old retina. (Magn. × 9600.)

FIGURE 45. Electron micrograph of a transverse section through the supracone space of a process that contains a phagosome. 5 year old retina. (Magn. × 20 400.)







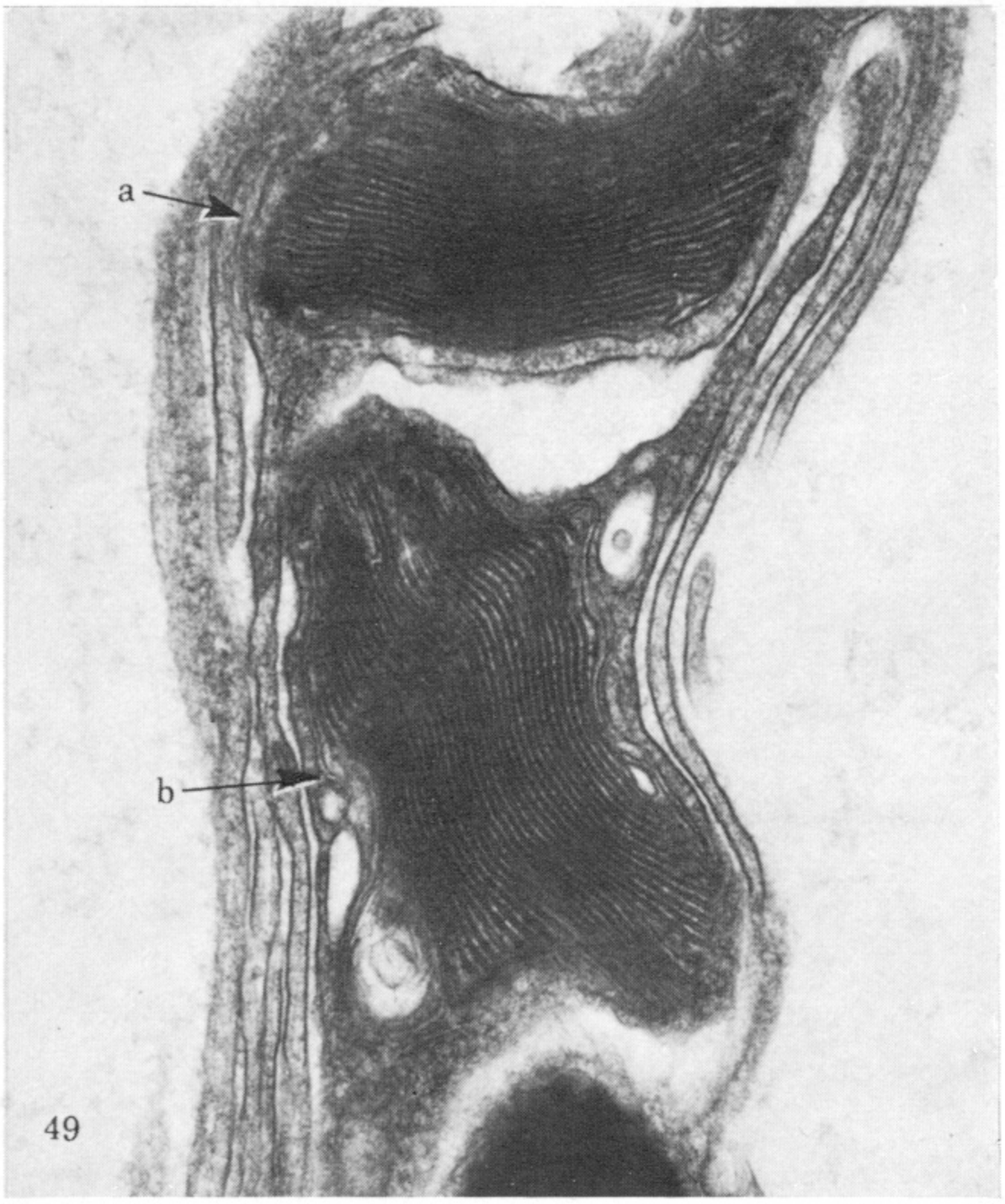


FIGURE 46. Electron micrograph of a longitudinal section through pigment epithelial apical processes near the apical surface. Two phagosomes are located in one of the processes (*a*, *b*). A second process contains two pigment granules. 5 year old retina. (Magn. × 6800.)

FIGURE 47. The phagosomes of figure 46, at a higher magnification. In both phagosomes the lamellar structure is obscured and the margins of the packets are irregular. (Magn. × 17 000.)

FIGURE 48. Electron micrograph of a longitudinal section through pigment epithelial apical processes at their origin at the apical surface. Three phagosomes (*a–c*) appear very near the apical surface of the pigment epithelial cell. The lamellar structure of these phagosomes is not clear and their margins are irregular. 5 year old retina. (Magn. × 18 800.)

FIGURE 49. Electron micrograph of a longitudinal section through pigment epithelial apical processes in a supracone space. There are two phagosomes (*a*, *b*), each in a *separate* apical process. The packet of phagosome *a* is in its usual orientation with respect to the outer segment and contains about 25 disks. Phagosome *b* has rotated almost 90° and contains about 30 disks. (Magn. × 27 000.)