RETINAL STRUCTURE IN *LATIMERIA CHALUMNAE*

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[Plates 41 to 50]

CONTENTS

The retina of *Latimeria chalumnae* contains four types of visual cells; most are rods, and there are three types of cones. Rod outer segments are cylindrical and appearances at their bases suggest that they may be renewed discontinuously from the inner segment. The rods have simple synaptic spherules, each bearing a single basal filament ending in a club-shaped expansion.

Type 1 cones contain an oil droplet, and have a complex synaptic pedicle bearing about 12 basal filaments. Type 2 cones have no droplet, and a pedicle bearing about six basal filaments and of complexity between that of rods and type 1 cones. Type 3 cones resemble type 2, except that they have a clear vacuole, but not an oil droplet, in the inner segment.

The pigment epithelium contains abundant phagosomes, but pigment granules are absent where the epithelium overlies the choroidal tapetum lucidum. Regular arrays of tubules occur in the cytoplasm, some of which appear to be formed from three interlacing hexagonal nets.

Two types of bipolar cell are present. Most are displaced bipolars, with nuclei in the outer nuclear layer. The rest are large, with nuclei in the horizontal cell layer. Both types bear Landolt's clubs, which penetrate the outer limiting membrane. Their endings contain a cilium complex, and a single large mitochondrion. Some contain 60 nm vesicles, which are also found near disrupted club endings.

Two types of horizontal cell are present. A few dark-staining cells with extensive web-like processes occur next to the outer plexiform layer. The expansions of rod basal filaments make contact with these cells. More voluminous pale staining cells with long cylindrical processes occur vitread to the dark cells.

Presumed amacrine cells form a layer vitread to the horizontal cells; they and the inner plexiform layer were not well fixed.

Sparse ganglion cells occur at the same level as the nerve fibre bundles.

Radial fibres penetrate the horizontal cell layer as compact columns. They do not contribute to the outer plexiform or horizontal cell layers, but elsewhere spread amongst the other retinal elements. Their expansions determine the inner contour of the retina.

Cells, probably microglial, which contain lysosomes are scattered amongst the other elements.

143000 myelinated fibres are present in the optic nerve, which also contains non-myelinated fibres. Retinal cell counts are given.

Introduction

The living coelacanth was first recognized, from a specimen caught near East London, South Africa in 1938, by J. L. B. Smith who named it *Latimeria chalumnae* and described the anatomy of the incomplete specimen (Smith 1939). Since that time approximately seventy other specimens have been taken, all at the Comoro Islands. The material has been extensively studied, notably by the French school of Millot & Anthony. Their results have been published in papers and in their monograph on the anatomy of *L. chalumnae*. (Millot & Anthony 1958, 1965.)

Despite the numbers of specimens caught there has been little work on the ultrastructure of the tissues and organs, doubtless owing to the inadequate state of fixation of the tissues (the Comoro Islands are tropical and remote, with poor communications and minimal scientific facilities). The coelacanths are caught by native fishermen operating from the small villages along an extensive and inhospitable coastline in dugout canoes and so the rarity and inaccessibility of the animal have hindered provision of material suitably fixed for detailed microscopical study.

Early in 1972 the Royal Society sent participants in an International Expedition to the Comoro Islands with a view to obtaining samples of tissues and organs from *Latimeria* for a variety of scientific investigations. The expedition was fortunate; a live specimen was obtained and sampling carried out immediately after death. Accounts of the circumstances of capture and observations on the living fish have been published elsewhere (Locket & Griffith 1972; Locket 1972).

Little work has been done previously on the eye and ocular tissues of *Latimeria*. Millot (1955), who made the first observations on the living fish, noted that it seemed strongly averse to light, hiding in the remote corners of the flooded boat used as a temporary aquarium. He stated that 'The greenish yellow luminescence of its eyes was very prominent and could be seen at quite a distance.'

Millot & Anthony (1965) consider this apparent luminescence in their discussion of the choroidal tapetum lucidum, and express the view that the tapetum may be responsible for the phosphorescent aspect of the eye in the living animal.

Locket & Griffith were able to observe the living fish both by night and after daybreak. When the fish was illuminated, by sunlight or by hand torches, the greenish yellow eyeshine was very prominent. In the dark, however, they saw no luminescence, and it is the author's opinion that the observed eyeshine is due solely to the tapetal reflexion.

Millot & Carasso (1955) gave a preliminary account of the eye structure, and a further description by Millot & Anthony followed (1965). Both accounts related to the eye, 60 mm in external diameter, of a specimen 128 cm in length. The retina was avascular, and the optic disk elongated, measuring 1 mm × 3 mm. There was no fovea, and the retinal pigment epithelium, which contained no pigment, remained attached to the choroid when the retina separated. The visual cells were described as almost entirely long straight rods, but very rare cones, containing a clear droplet, were also present. The layers of nerve cells contained remarkably few elements, and there were so few multipolar cells that it was hard to recognize them at all. The layer in which they are usually found was absent, so that the retina appeared incomplete. The ratio of visual cells to ganglion cells, and thus the convergence, was stated to be among the highest observed, though no figures were given; Müller's fibres were particularly numerous. Millot & Anthony did not find a lens muscle, and state that the choroidal fissure is quite obliterated. They

concluded that most of the features observed are related more to the environment in which the fish lives, dimly lit deep water, than to phylogenetic considerations.

Dartnall (1972) has succeeded in extracting a visual pigment of *Latimeria*, using the other eye of the specimen on which the present work was carried out. This pigment, which is based on retinal, shows maximal absorption at $\lambda = 473$ nm.

The present paper describes some aspects of the retinal structure from optical and electron microscopical evidence.

Methods

The specimen of *Latimeria chalumnae* was caught by a Comorian fisherman using a hand line at an estimated depth of 165 m. It was caught at 02h00, and was observed alive from 03h40 until 07h45, by which time it was moribund: sampling of tissues then began.

The work now described was carried out on samples of retina taken from the right eye, which was enucleated within 45 min of death. After samples of aqueous had been withdrawn the eye was bisected at its equator, and the anterior segment set aside. A portion of the posterior segment containing the retina and vitreous was fixed in glutaraldehyde for electron microscopy.

The glutaraldehyde fixative was formulated and prepared by Dr M. D. Lagios; its constitution was:

		mol/l	mosmol/l
glutaraldehyde	2% v/v	0.15	220
NaCl	150 mmol/l	0.15	300
KCl	10 mmol/l	0.01	20
urea	303 mmol/l	0.30	303
sodium cacodylate	500 mmol/l	0.50	110
glucose	2.7% w/v	0.003	3
polyvinyl pyrrolidine	4% w/v		2

The samples of retina were kept in this fixative in an air-conditioned room for the 7 days that they remained in the Comores. On arrival in London they were kept in a domestic refrigerator until postfixation and embedding could be undertaken.

Portions of retina were postfixed in buffered 1% osmium tetroxide. They were dehydrated through an ascending series of ethanol concentrations, and cut into small fragments in the 70% stage. These were transferred through epoxy propane to Araldite, in which they were embedded.

Radial and tangential sections were cut for optical and electron microscopy on a Cambridge Huxley or Reichert OM2 ultramicrotome, using glass knives. Sections at 1 μm for optical microscopy were stained with alcoholic toluidine blue: thin sections were mounted on uncoated grids, and stained with uranyl acetate and lead citrate. They were examined in a Zeiss EM9S electron microscope.

As well as random samples, serial 1μm tangential sections were cut for cell counting. The area of the tissue showing the least curvature was selected, and micrographs made of this area from alternate sections through the series, giving 2 μm steps. A selected area 250 μm square could be followed through the series by internal consistencies, and the cells within this area were traced on to acetate sheets. The cells of each population were traced on to separate sheets, which could then be placed in register to compare the numbers and distribution of the cell types, either alone or in combinations. The cell profiles within the 250 μm square extracted on to the acetate sheets were counted, and numbers per square millimetre obtained from these figures.

Accurate identification of cells is sometimes difficult by optical microscopy. In the present case it was possible to identify the visual cells by their outer and inner segments, and to follow the cones through to their nuclei. The nuclei in the outer nuclear layer which are not those of visual cells belong to displaced bipolar cells, and were readily counted separately. The horizontal cells of both types and the radial fibre nuclei were distinctive. No bipolar cells have been identified in the inner nuclear layer; the nuclei at this level probably all belong to amacrine cells, and were counted as such.

In the sampling area there was only a single type 1 cone. To obtain a more accurate count of these cells a piece of intact fixed retina was stained with Oil red O. The oil droplets of type 1 cones stained vividly, and were easily seen in low-power optical micrographs. From these the type 1 cones in an area of retina 3 mm × 1 mm were plotted (see figure 12) and the counts from the three 1 mm squares averaged.

To count the myelinated fibres in the optic nerve, transverse 1 μm sections of the orbital portion were stained with toluidine blue. Overlapping optical micrographs were prepared covering a whole section, and the myelinated fibres counted from these; the image of each fibre was marked as counting proceeded to minimize errors. Thin sections from the same block were examined for non-myelinated fibres.

To study the outer plexiform layer serial thin sections were prepared. Tangential thick sections beginning from the sclerad side were cut, and when the oil droplets of the type 1 cones could be seen on the block face, it was trimmed to include a single type 1 cone near its middle. Thick sections were cut until the synaptic layer was reached, when the knife was changed and serial thin sections cut. Ribbons of these sections were mounted on single hole grids with a pioloform F film, and stained with uranyl acetate and lead citrate. Serial micrographs were taken of selected areas, including the synapse of the previously identified type 1 cone.

Results

The retina of *Latimeria* contains the cell families normally found in vertebrate retina, but these are arranged in a somewhat atypical way. Radial sections (figure 1, plate 41) show a single layer of receptors, of which the majority are rods. Their nuclei are cylindrical and are arranged in

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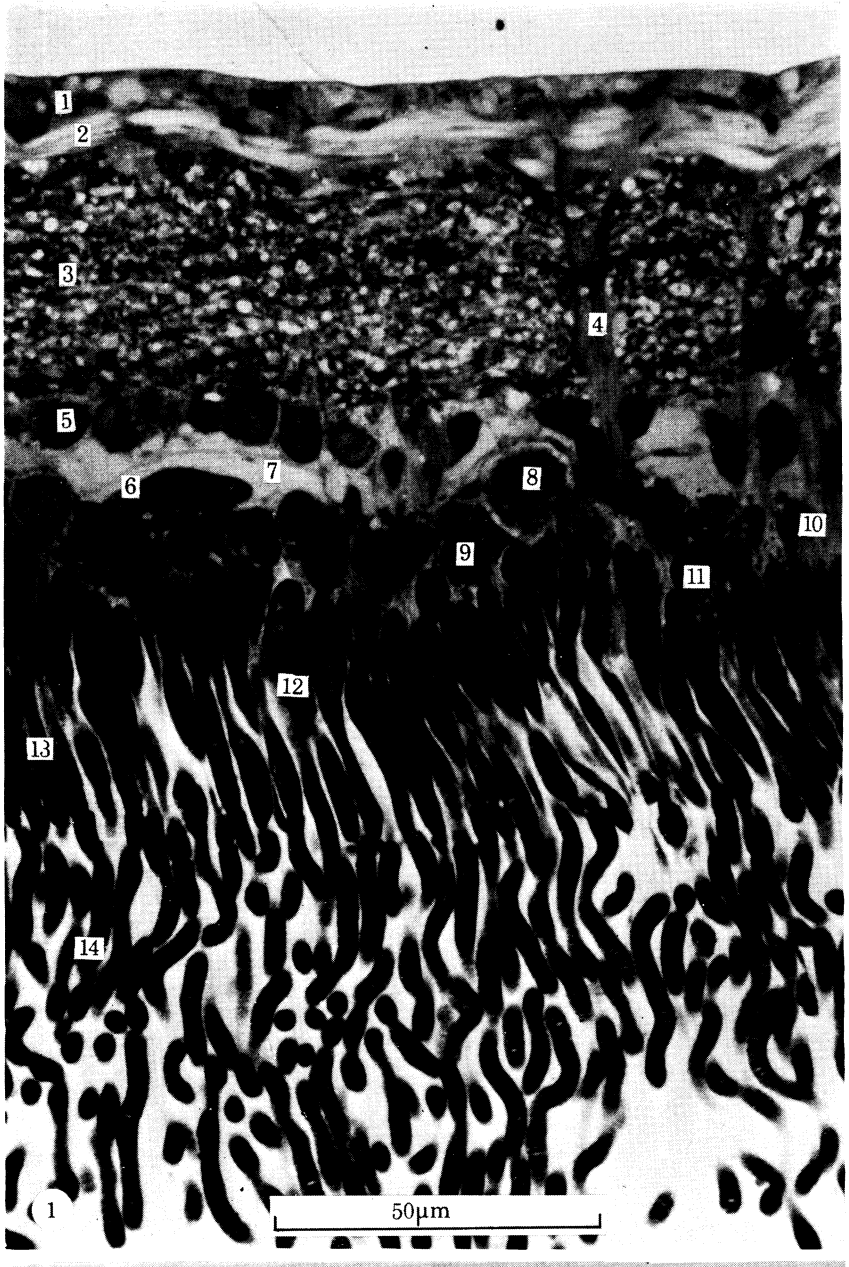
Description of plate 41

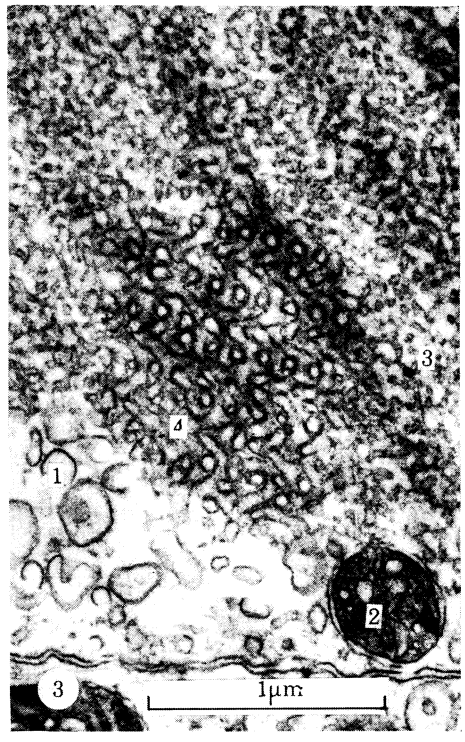
Figure 1. Radial section of retina, optical micrograph. The rod outer segments are distorted, due to the separation of the pigment epithelium during processing. (1) Radial fibre expansions beneath inner limiting membrane. (2) Nerve fibres. (3) Inner plexiform layer; the dark horizontal striation is due to bipolar cell processes. (4) Radial fibre column. (5) Amacrine cell nucleus. (6) Nucleus and perikaryon of dark horizontal cell. (7) Pale horizontal cell cytoplasm. (8) Large bipolar cell. (9) Displaced bipolar cell. (10) Rod spherule. (11) Type 2 or 3 cone nucleus. (12) Rod nuclei. (13) Rod inner segments. (14) Rod outer segments.

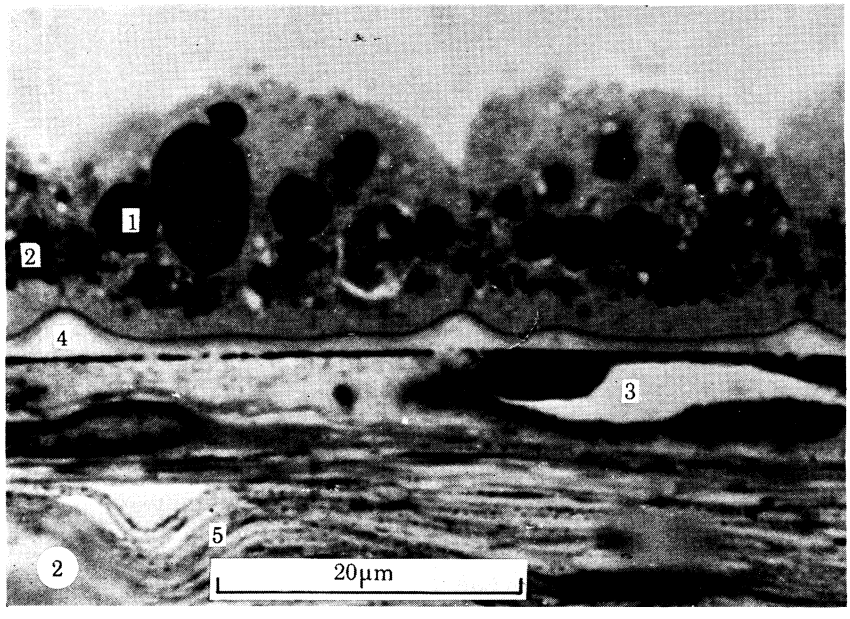
Figure 2. Pigment epithelium, optical micrograph. The dome-shaped epithelial cells contain no pigment, but large phagosomes (1) and smaller mitochondria (2) are present in the cytoplasm. The cells are separated from the choriocapillaris (3) by Bruch's membrane (4). Directly beneath the choriocapillaris is the choroidal tapetum lucidum (5).

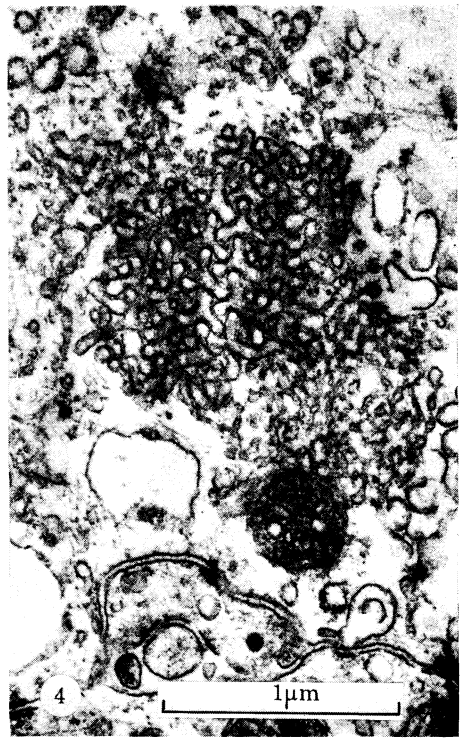
Figure 3. Tubular systems in pigment epithelium cells, electron micrograph. Vesicular profiles, many of them disrupted (1) are scattered through the cytoplasm, which also contains mitochondria (2). Patches of fine tortuous tubules (3) give way to areas showing regular patterns (4).

Figure 4. Another example of regularly arranged tubules, in this case in a pattern of intermeshing Y-shaped figures.









FIGURES 1–4. For legends see facing page

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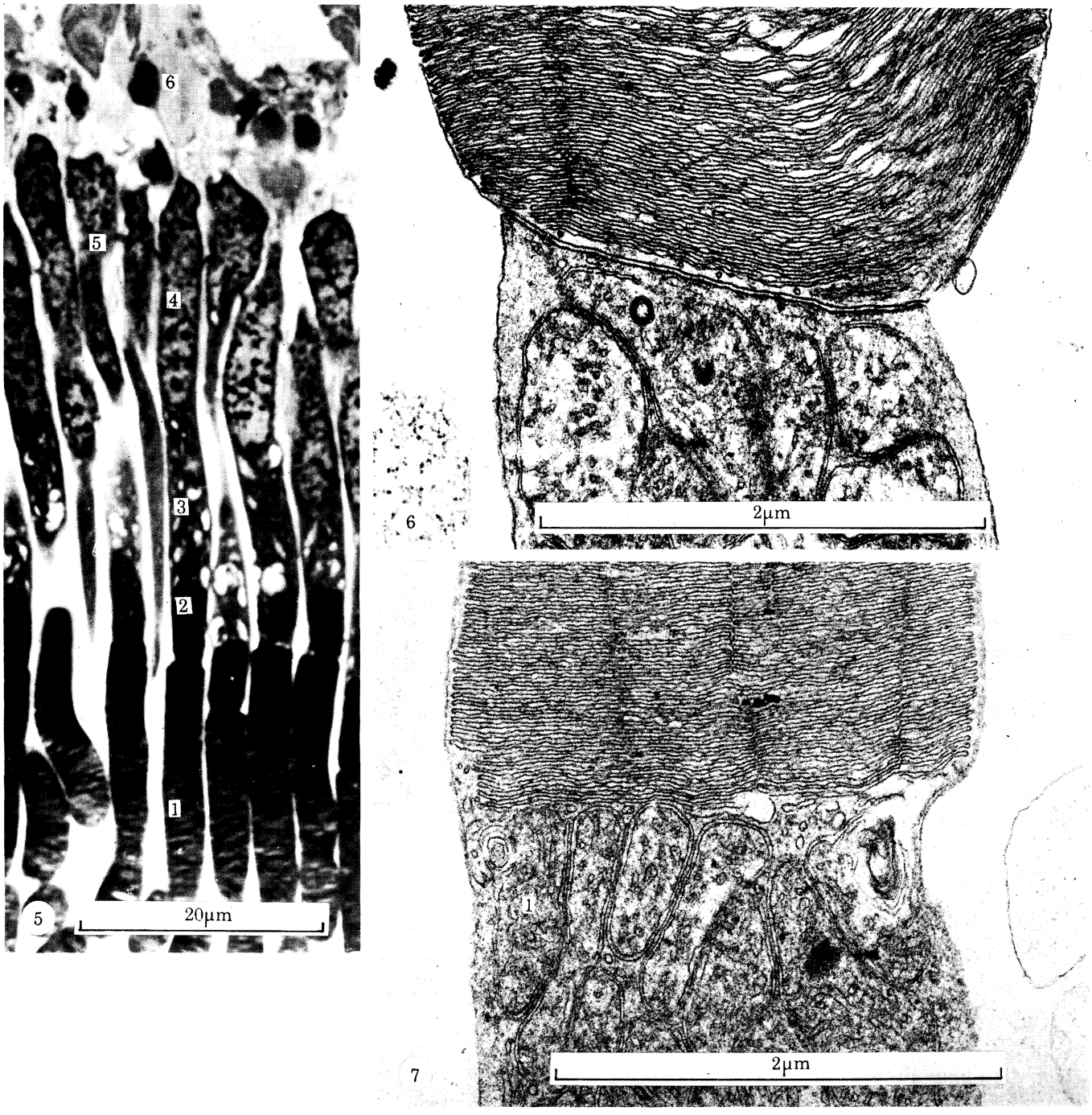
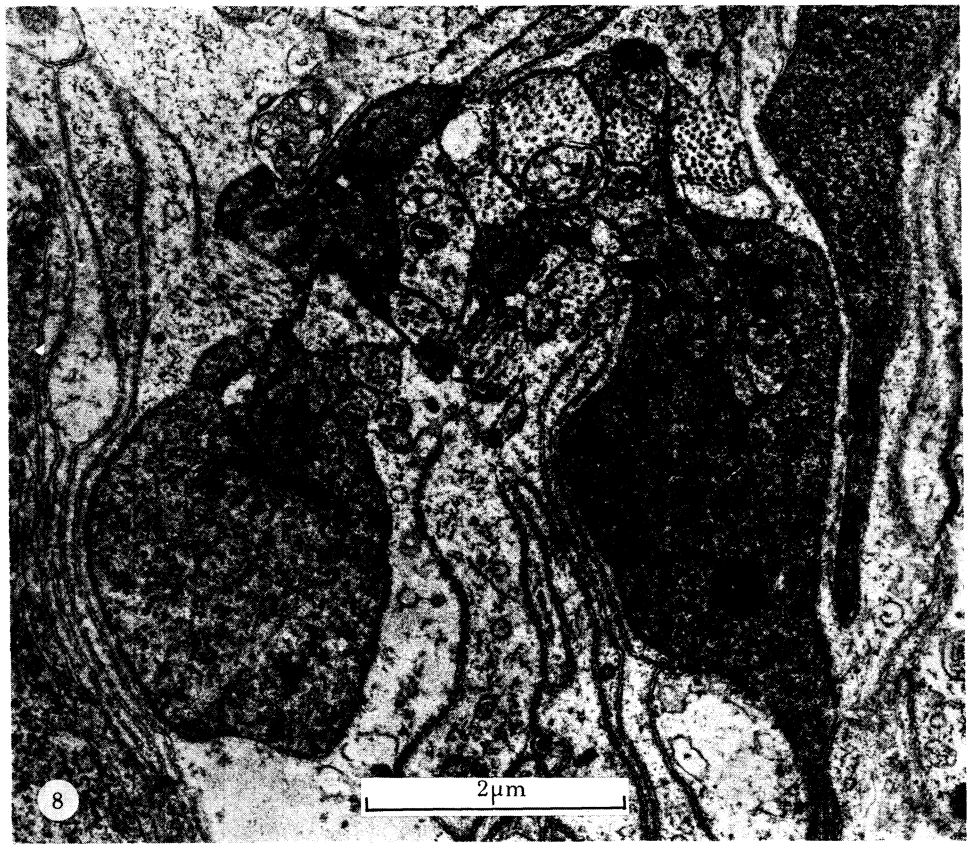


FIGURE 5. Rod, optical micrograph. The outer segment (1) is of larger diameter than the inner segment (2). The myoid (3) and the nuclear region (4) are cylindrical. The nucleus projects through the outer limiting membrane (5). The synaptic spherule (6) is borne on a fine conducting fibre.

FIGURE 6. Junction of rod inner and outer segments, electron micrograph. In this example the inner segment appears entirely separated from the outer segment by a pair of parallel membranes. The outer segment lamellae are enclosed by the plasma membrane. The inner and outer mitochondrial membranes have a similar spacing to the lamellar membranes. The mitochondrial cristae are tubular.

FIGURE 7. In this example there is no sign of the parallel membranes, and the inner and outer segments are in full cytoplasmic continuity across the width of the rod. Some mitochondria (1) appear disrupted, and apparently incomplete lamellae occur at the junctional region.



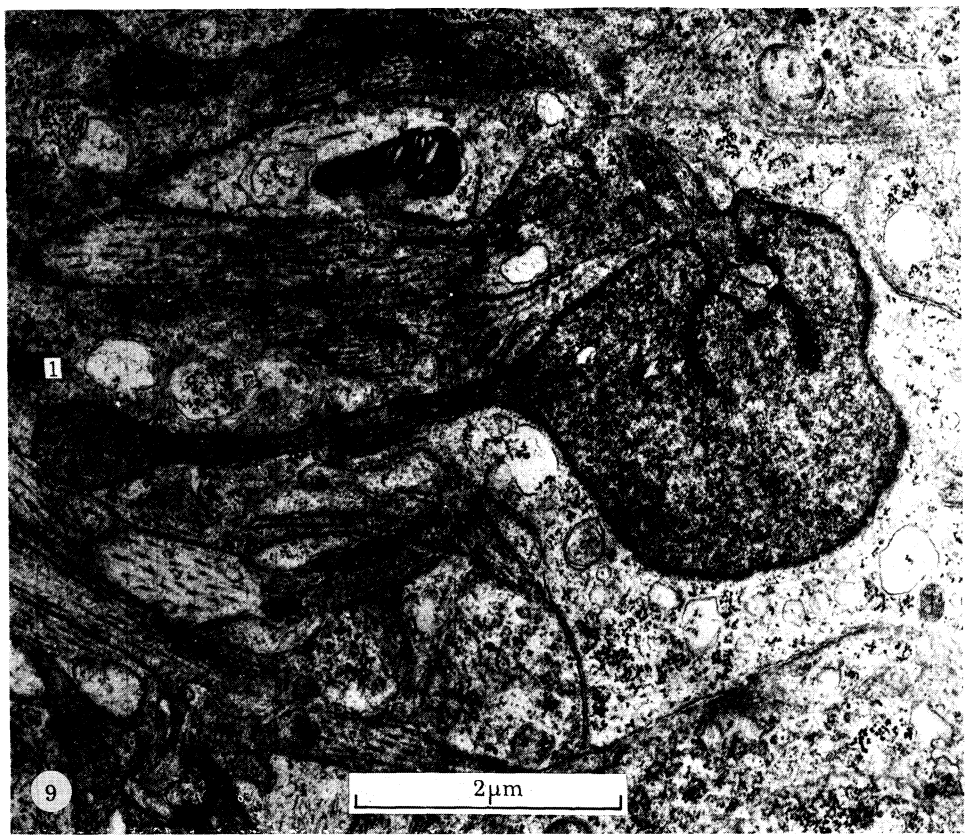
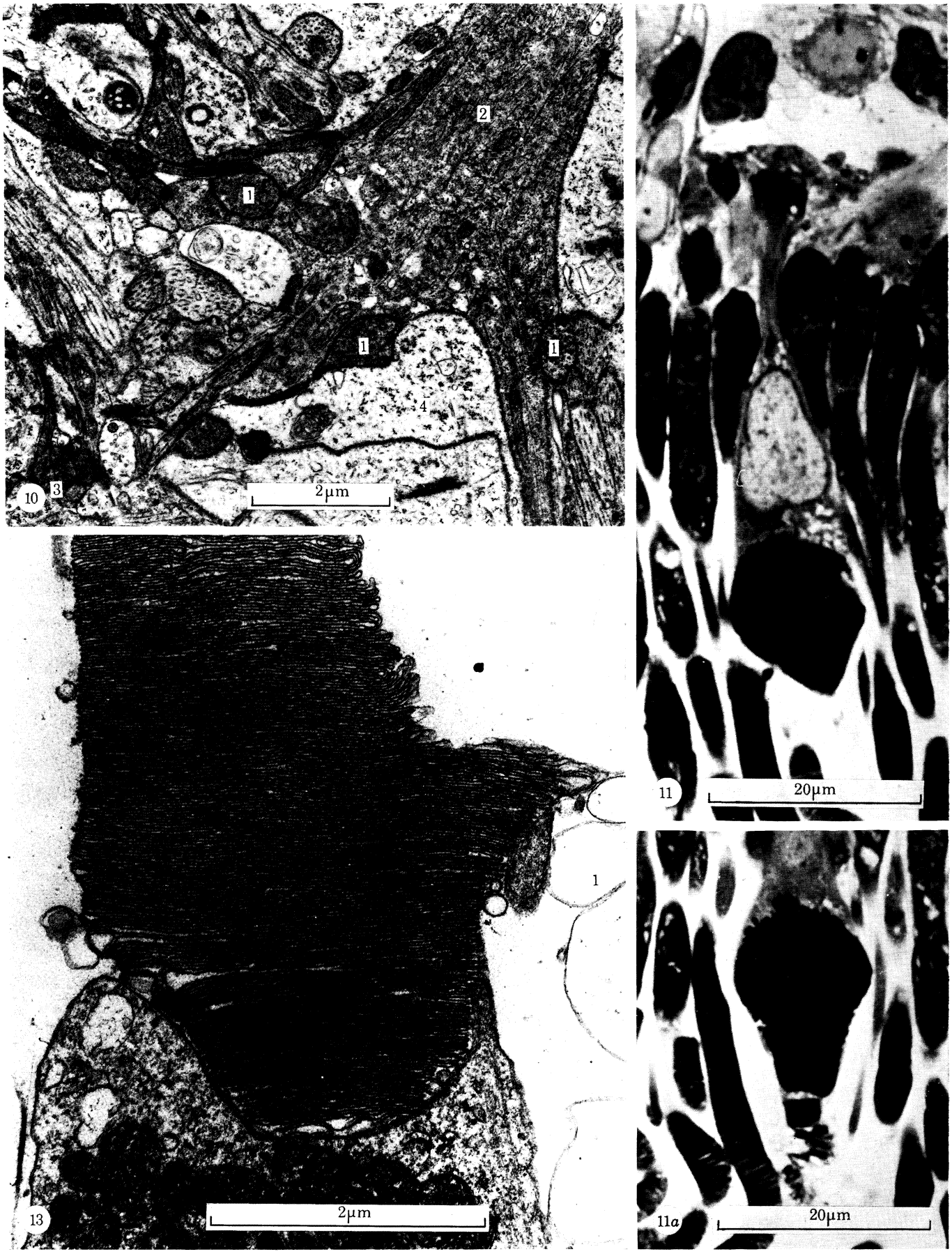


FIGURE 8. Two rod synaptic spherules. The rod synapses are of the oligosynaptic type, with few processes making contact. Fingers of rod cytoplasm project into the terminal cavity amongst the processes; three were present in a rod spherule examined in serial sections. As usual in random sections no sign of the basal filament is seen.

FIGURE 9. Rod basal filament, tangential section. This micrograph, one of a series, shows a basal filament throughout its length from the rod spherule to the expanded club. The spherule contains synaptic vesicles, but the club has a granular contents. The end club is in close contact with dark horizontal cell cytoplasm (1) but there is no sign of synaptic specialization at this site.



FIGURES 10, 11 AND 13. For legends see facing page

palisade formation; in addition to them the outer nuclear layer contains two more vitread populations of nuclei, one belonging to cones and the other to displaced bipolar cells. A further small number of cone nuclei are located entirely sclerad to the outer limiting membrane.

Immediately vitread to the outer nuclear layer are the synaptic terminals of the visual cells, marking the sclerad boundary of the outer plexiform layer. This layer is thin, and bounded vitreally by a layer of horizontal cells which are of two types, here described as pale and dark from their appearances in stained sections.

Vitread to the horizontal cells is another layer of cells which are considered to be amacrine cells; few, if any, bipolar cells are found at this level.

Situated at the level of the horizontal cells are groups of radial fibre nuclei. The fibres themselves form bundles which penetrate the inner nuclear and inner plexiform layers.

The inner plexiform layer is well developed, and shows signs of horizontal layering. Ganglion cells are not numerous, and occur in a single layer at the vitread border of the inner plexiform layer. Most of them lie in the same plane as the nerve fibres, which are gathered into bundles. Vitread to the ganglion cells and nerve fibres is a layer of radial fibre material, which determines the inner contour of the retina. The retinal constituents are now described in more detail.

Pigment epithelium

To designate the retinal epithelium as pigment epithelium in *Latimeria* is perhaps a misnomer, since it contains no pigment granules where the retina is backed by the tapetum. The term is, however, the one usually applied to the layer in question, and will be used here.

During the preparation of the tissues it was noted that the retina separated very easily from the underlying tissues, and no samples were obtained in which this had not occurred. Examination of the pigment epithelium, which remained attached to the choroid, shows features which suggest a possible reason. The vitread surfaces of the cells are smooth and domed, and there is no sign of the processes which are so prominent in many fishes whose epithelium contains pigment.

The cells (figure 2, plate 41) are somewhat lower than they are broad, and are irregularly polygonal in tangential sections. Most contain a single rounded nucleus, but some contain two, of similar shape and size. The nuclei are situated approximately in the centre of the cells, and show a well-defined chromatin pattern with a nucleolus.

The base of the cell rests smoothly on Bruch's membrane and there are few infoldings of the cell membrane in this region. At the junctions between cells there are slight irregularities near

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Description of plate 44

Figure 10. Dark horizontal cell process, tangential section. Several rod basal filament clubs (1) lie in contact with an expanded web of dark horizontal cell (2). Processes of the outer plexiform layer (3) and pale horizontal cell cytoplasm (4) are also present.

Figure 11. Type 1 cone, optical micrograph. The broad myoid and sclerally located pale nucleus are clearly seen, but the oil droplet cannot be distinguished from the mitochondria of the ellipsoid in this example. The thick conducting fibre extends between the rod nuclei to end in a large synaptic pedicle.

Figure 11*a*. This inset shows the oil droplet, inner segment mitochondria and somewhat disrupted outer segment, not visible in figure 11.

Figure 13. Cone outer segment. The lamellae of the cone outer segments are open at some point to the ventricular space, unlike those of the rods (figures 6, 7). This example is unusually well preserved; many show more swollen lamellar fragments (1). This outer segment is from a type 2 cone.

the basement membrane, but the membranes are otherwise straight. Junctional complexes are located approximately half way between the vitread and sclerad extremities of the cell borders. Situated near the cell base, and particularly near the corners of the cell, are rounded mitochondria with tubular cristae.

The cytoplasm is imperfectly fixed, but contains numerous vesicles which appear empty and sometimes disrupted. They are irregular in size, and do not appear to be in continuity with endoplasmic reticulum.

The cytoplasm also contains prominent inclusions, which range from bodies resembling outer segment material in their lamellation, found near the free border of the cells, to dense and amorphous bodies resembling lysosomes situated near the cell bases. These inclusions are regarded as phagosomes, i.e. portions of rod outer segment material phagocytosed by the pigment epithelium.

Tonofilaments are present in the cytoplasm, randomly arranged in most regions, and orientated round the nucleus; other orientated filaments are associated with the junctional complexes at the lateral borders of the cells.

Between the nucleus and the lateral cell walls the cytoplasm contains aggregates of tubules, in which two elements can be distinguished (see figures 3, 4, plate 41). The more common are patches of vesicular and tubular profiles, approximately 30 nm in diameter and random in arrangement. Associated with these patches, and commonly surrounded by them, are patches in which the tubules show striking and regular patterns.

These regular tubules are of larger diameter, approximately 60 nm, and more even than the irregular tubules. The patterns formed appear different in different sections, but certain characteristics are found to be common. Arrays of circular profiles, as of tubules cut transversely, occur at repeated intervals. Associated with these are arrays of short angled profiles; sometimes these show varieties of herring-bone patterns (figure 3) and in certain examples a pattern of interlaced Y-shaped figures is seen (figure 4).

It has not been possible to establish the continuities of the tubular systems with certainty, since the tubule dimensions are less than the section thickness. It appears, though, that one element of the regular tubules is continuous with the narrow irregular tubules surrounding the regular patches, and that another element is continuous with the background cytoplasm.

Visual cells

Rods

Cell counts (table 1) show that the rods are much the most numerous type of receptor; clearly seen in radial sections (figures 1, 5, plates 41, 42), they are present as a uniform population. The outer segments are cylindrical, and measure 80 μm in length by 5 μm diameter; their basal (most vitread) ends are surrounded by short calyx processes which lie opposite shallow incisures.

The junctional region between inner and outer segments shows two configurations. In about half the rods the inner and outer segments are clearly separated by a pair of smooth parallel membranes. If the section does not include the cilium the membranes extend the whole width of the rod so that there is apparently complete separation of the inner and outer segments (figure 6, plate 42). Other sections show that cytoplasmic continuity is present through the cilium, the membranes being continuous at the ciliary shaft.

In the second configuration (figure 7, plate 42) the parallel membranes are absent and there is

cytoplasmic continuity between the inner and outer segments across the whole width of the rod. Where the cilium is in the plane of section there is a small lacuna beside its shaft, but otherwise no trace of a gap between the two segments.

In the first configuration the most basal lamellae are mostly well formed and the full width of the outer segment, but in the second, short lamellae and vesicles are commonly seen. The mitochondria of the inner segment approach the outer segment base closely, so that their external membranes may appear to be in series with those of the lamellae. In a few examples mitochondrial membranes are apparently continuous with a basal lamella, but unequivocal evidence of this relation has not been found.

The basal lamellae are all enclosed by the outer segment membrane, and no instances have been seen in which they project free from the cilium.

The cilium is, as usual in vertebrate retinae, of the 9 + 0 type; the filamentous elements, which are double tubules, extend a few micrometres along the outer segment. The basal body of the cilium lies in an eccentrically set hilum, formed by the orientated mitochondria of the ellipsoid at the sclerad end of the inner segment. Adjacent to the basal body is a second centriole; no ciliary rootlets have been seen in connexion with either.

The ellipsoid itself is cylindrical, but usually rather narrower than the outer segment. The closely packed mitochondria, which have tubular cristae, give way abruptly to the granular cytoplasm of the myoid region. This region contains microtubules and filaments peripherally and smooth endoplasmic reticulum in a central core. Lacunae seen in places are probably artifactual dilatations of the endoplasmic reticulum. There is no sign of an aggregation of glycogen forming a paraboloid.

The nuclear region of the rods is cylindrical, and directly continuous with the myoid. The nucleus itself is cylindrical with rounded ends, usually smooth surfaced without indentations, and with a rather dense chromatin pattern. Surrounding the nucleus is a thin shell of cytoplasm containing microtubules and filaments. At approximately the half way point of the nucleus the junctions of the outer limiting membrane are situated, so that part of the nucleus projects sclerad to them.

From the vitread end of the nucleus the cytoplasm narrows sharply to a conducting fibre 0.5 μm in diameter. This fibre, which extends for approximately 6 μm, contains a few microtubules in a matrix of granular cytoplasm. At its vitread end, where it widens into the synaptic spherule, the fibre may contain some synaptic vesicles. Occasionally the synaptic apparatus is directly applied to the nucleus without the intervention of a conducting fibre, forming a sessile synapse.

The typical synaptic spherule (figure 8, plate 43), is approximately 3 μm in diameter. Its cytoplasm, which appears dense both in optical and electron micrographs due to its content of synaptic vesicles, also contains one or two synaptic ribbons. These are related to the processes making contact, which occupy a roughly spherical cavity about 1 μm in diameter. Serial sections show fingers of rod cytoplasm projecting into this cavity amongst the processes.

Study of serial sections has also shown that each rod spherule gives rise to a single basal filament (figure 9, plate 43). In some cases this filament takes origin from the vitread extremity of the spherule, when the concavity for the contacting processes opens at the side, and in some the filament arises from the side and the processes enter the apex. The filament, 3–6 μm long by 0.2 μm diameter, follows a rather straight course in the tangential plane within the outer plexiform layer, and ends as a club-shaped expansion 1 μm long by 0.7 μm diameter. This club

appears to be of similar density to the spherule, but its contents do not consist of synaptic vesicles, having a granular appearance with only occasional vesicular profiles. A few microtubules are present in the filament, but not in the spherule nor the club.

In the majority of cases in which the filament could be traced, the club has been found to lie in contact with the sclerad surface of the perikaryon or a process of a dark horizontal cell (figure 10, plate 44).

TABLE 1

cell type	number counted	number per mm²
rods	3320	53120
type 1 cones	—	22
type 2 cones	43	688
type 3 cones	6	96
bipolar cells	402	6432
dark horizontal cells	8	128
pale horizontal cells	14	224
amacrine cells	239	3824
ganglion cells	11	176
radial fibres	540	8640
'glial' cells	21	336

Myelinated fibres, optic nerve: 143104.

Cones

Three types of cone are present in the retina of *Latimeria*, which have been given numbers for ease of description.

Type 1. Type 1 cones (see figure 11, plate 44) are sparsely distributed through the retina, only some 22 occurring per mm² (figure 12). They are recognized by the possession of a large oil droplet (figure 11*a*); this may have been coloured during life, is colourless in glutaraldehyde-fixed and preserved retina and stains densely with osmium.

The outer segment of the cones of all types is much smaller than that of the rods. It measures 5–10 μm in length by approximately 3 μm diameter at its origin, tapering to 1 μm at the tip. The outer segment lamellae are less regular than those of the rods, and many appear as if they have swollen, burst and reformed into vesicular forms; in those cases where the outer segment has remained intact the packing of the lamellae closely resembles that of the rods (figure 13, plate 44). The continuous membrane surrounding the rod lamellae is absent in the cones, so that their lamellae appear open to the ventricular space.

The ellipsoid lies at the same level as the ellipsoids of the rods, and like them consists of an aggregation of mitochondria. The type 1 cone mitochondria are darkly stained, and have pale cristae, whereas those of the rods, and of the type 2 and 3 cones, are paler with dark cristae. The mitochondria are arranged in a conical form, and are most densely packed at the apex from which the outer segment arises. Surrounding the mitochondria is a thin layer of cytoplasm containing microtubules and some granules. This cytoplasm is drawn out into a shallow calyx which surrounds the base of the outer segment and ends in calyx processes.

The connecting cilium of type 1 cones has not been observed, probably due to their rarity and accidents of the plane of section.

The oil droplet is spherical, approximately 15 μm in diameter, and is difficult to preserve in thin sections. It is surrounded by a thin layer of cytoplasm containing small mitochondria and some microtubules.

The nucleus is situated immediately vitread to the oil droplet; it is paler than the nuclei of the rods or of the other cone types and shows an even chromatin pattern with a nucleolus. Lying opposite the scleral half of the rod nuclei, and thus mainly or entirely sclerad to the outer limiting membrane, the sometimes lobulated nucleus is usually widest sclerally where it is related to the oil droplet.

From the nuclear region the cone extends as a stout conducting fibre, up to 5 μm in diameter, and consisting of granular cytoplasm with microtubules, between the rod nuclei towards the outer plexiform layer.

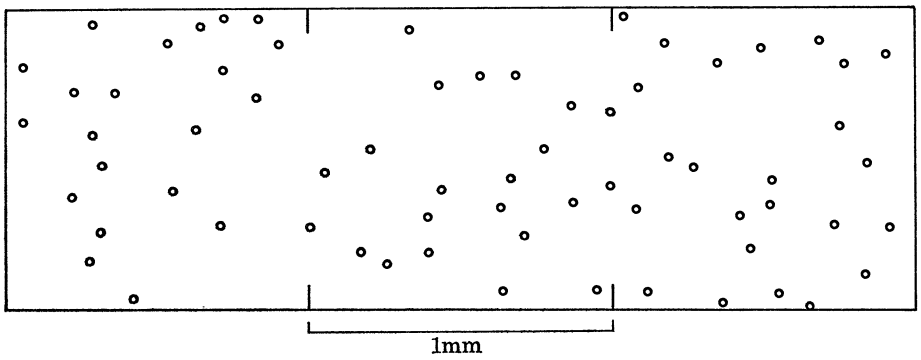

FIGURE 12. Diagram of distribution of type 1 cones. The oil droplets of type 1 cones in a piece of intact, fixed retina were stained with Oil red O, and micrographs taken by green light. This diagram was plotted from the micrographs, and shows the sparse distribution of the type 1 cones.

The synaptic pedicle is of the polysynaptic (Underwood 1968) type. It measures 6–9 μm in diameter and contains synaptic vesicles and up to 12 synaptic ribbons. The ribbons are related to the contacting processes in varieties of the dyad pattern; some dyads appear simple, but in other examples a ribbon may be related to more than one dyad, or complex assemblies of processes and ribbons may be present (figure 14, plate 45).

A serially sectioned pedicle gave rise to 13 basal filaments; most of these could not be followed to their terminations since they extended beyond the confines of the micrographs, but one was seen to end on the outside of a rod spherule, and another to form a possible synapse with a positively identified bipolar cell process (figure 15, plate 45).

Type 2. The cones of type 2 are distinguished by the absence of a droplet from the inner segment (figure 16, plate 46). They are clearly distinct from the type 1 cones and although they have strong affinities with type 3, they can usually be separated with confidence.

The outer segment resembles that of the type 1 cones very closely; the lamellae are of the open type, and in many cases appear disrupted with the formation of swollen vesicles. The outer segment is set in a cup-shaped calyx at the apex of the inner segment, the calyx processes being almost as long as the outer segment itself. A typical 9 + 0 cilium is present, from which the lamellae appear to take origin, and close to its basal body a second centriole is located.

The ellipsoid is approximately cylindrical in form; its constituent mitochondria are less dense than those of the type 1 cones and are not so closely packed. They are surrounded by granular cytoplasm in which microtubules are embedded. Typically the ellipsoid lies at the level of the rod myoids, so that the outer segment lies opposite the rod ellipsoids and outer segments.

The myoid region typically contains no droplet, and certainly no sign of an oil droplet. In some examples there are one or more rounded bodies, up to 3 μm in diameter, which have a

lightly granular appearance in electron micrographs (see figure 17, plate 46). Occasionally some clear vesicles, up to 1 μm diameter, are present which resemble the larger clear vesicles found in type 3 cones. Other examples contain neither granular bodies nor vesicles.

The myoid extends as a cylindrical process 1 to 3 μm in diameter, and dilates to contain the nucleus which is located just vitread to the rod nuclei. The more vitread portion of the myoid contains granules, some resembling glycogen and others the larger, more sclerad granular bodies. Tubular and vesicular profiles are also seen, of which some are associated with the Golgi complex. These organelles are accumulated in the centre of the myoid, the periphery containing numerous microtubules (figure 17, plate 46).

Type 2 cone nuclei are oval, and some show an indentation at their sclerad end. They resemble the nuclei of the displaced bipolar cells, among which they are situated, in size and shape, but are easily distinguished by their more distinct chromatin pattern (figure 16). Like the nuclei of the other cone types and the displaced bipolars, they contain a nucleolus.

Vitread to the nucleus there is no narrow conducting fibre like those of the rods, but a short neck which dilates at the same level as the rod spherules to form the synaptic pedicle. The pedicles appear intermediate in complexity between the oligosynaptic rod spherules and the large and complex pedicles of the type 1 cones. The pedicles of type 2 cones, and of type 3, from which they are indistinguishable morphologically, appear polysynaptic, but in an example studied from serial sections, many of the apparently separate profiles could be traced back to only two processes. The pedicle itself is considerably larger than rod spherules, and the expansions of processes larger and more widespread than in rods. About five synaptic ribbons are present (figure 18, plate 46).

Several basal filaments arise from type 2 cone pedicles. These extend for distances of several micrometers in the outer plexiform layer, and it has not been possible to trace them to their terminations.

Type 3. The cones of type 3 closely resemble those of type 2, and the description of that type applies in many respects to type 3 (figure 19, plate 47). The outer segments and the way in which they arise appear the same, and the ellipsoids are very similar. The type 3 ellipsoid is usually more conical in shape, being wider at its vitread end where it is in close relation with a vacuole.

It is the possession of this vacuole which distinguishes type 3 cones from type 2. Typically it is between 4 and 7 μm in diameter, and thus considerably smaller than the droplets of type 1 cones, from which it is clearly distinguished by its staining characteristics. The vacuole appears quite clear in toluidine blue stained sections, and does not stain with Oil red O in intact retina. In electron micrographs (figure 20, plate 47) the vacuole has a pale amorphous appearance,

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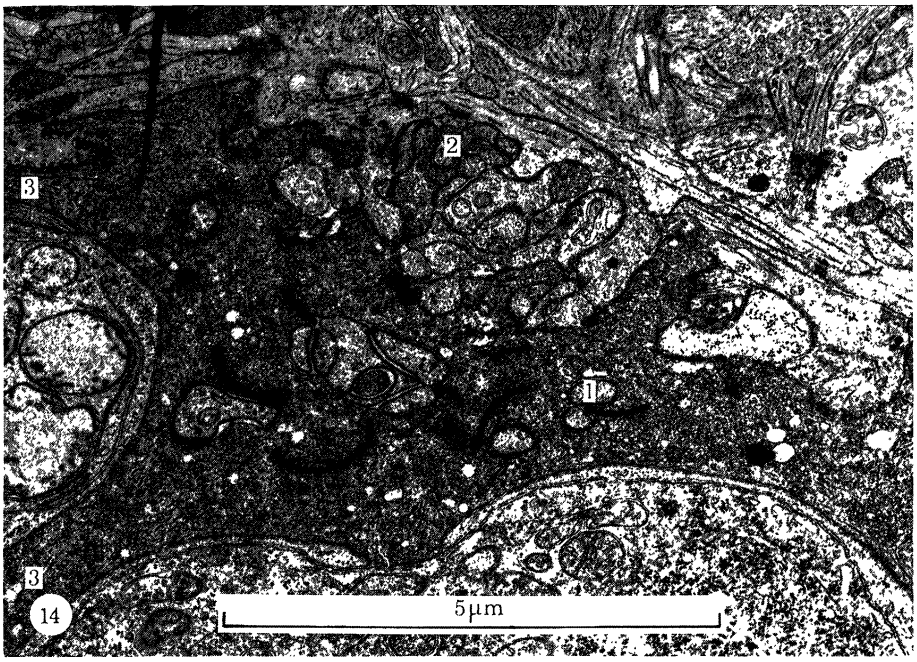
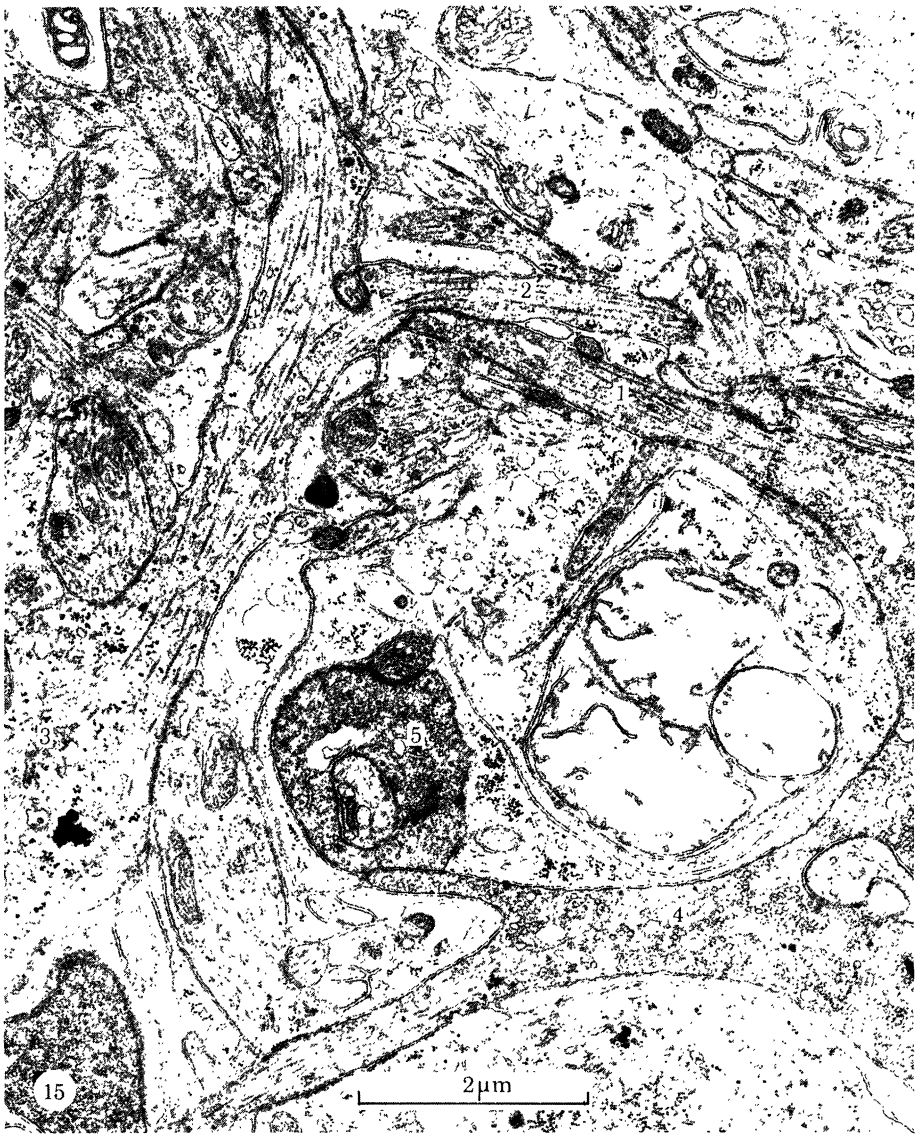
Description of plate 45

Figure 14. Type 1 cone synapse. This micrograph is one of a tangential series, and suggests the complexity of type 1 cone connections. Synaptic ribbons are related to deeply penetrating processes (1) mostly in variants of a dyad pattern. Other processes make more superficial contacts (2). Basal filaments, of which this pedicle had 13, are seen taking origin (3).

Figure 15. Type 1 cone basal filaments, tangential section. The type 1 cone pedicle seen in figure 14 here gives two basal filaments. One of these (1) contain sysnaptic vesicles where it ends against a process (2) which arises from a positively identified bipolar cell perikaryon (3). The other basal filament (4) sends a small branch to the exterior of a rod spherule (5). Note the morphological similarity of the basal filaments and bipolar processes; this makes their identification in random sections difficult.





FIGURES 14 AND 15. For legends see facing page

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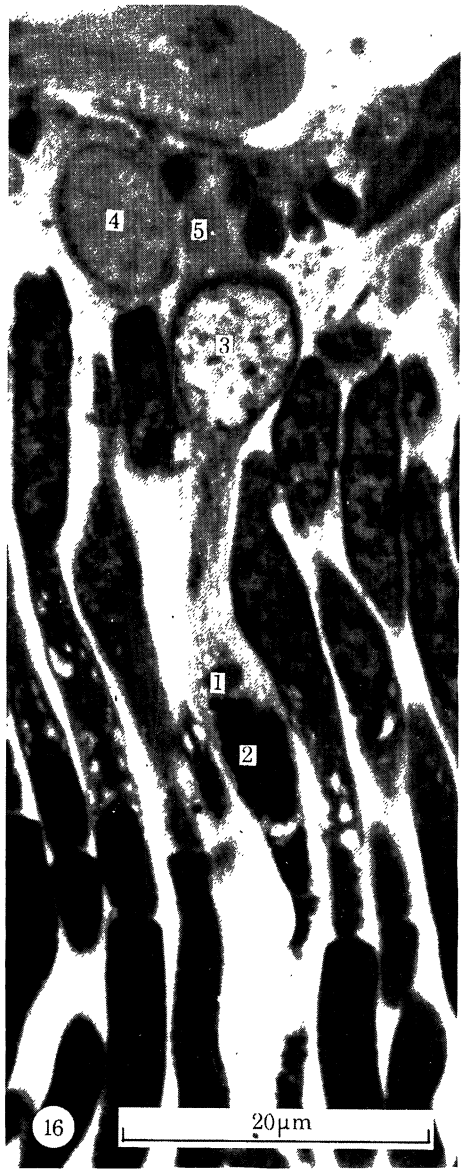

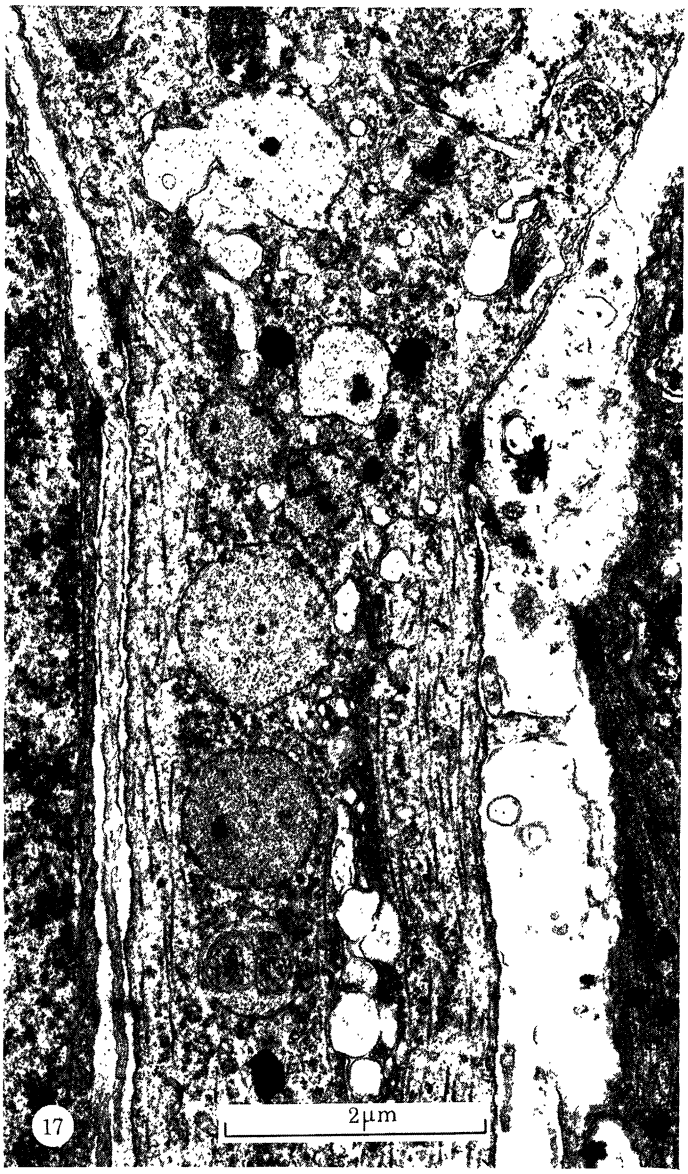

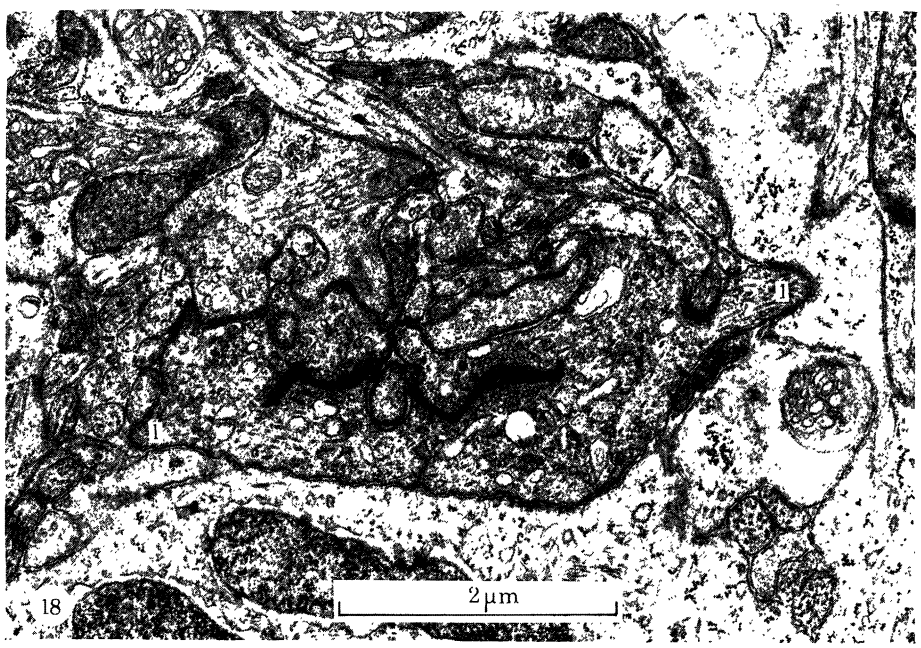

FIGURES 16–18. For legends see facing page

similar to that seen in blood vessels, and suggesting that the clear appearance is not merely due to some labile substance having leached out during processing; some of this amorphous material appears aggregated around the edge of the vacuole, which is membrane bounded. In some examples more than one vacuole are present; when this is so they are of the same appearance, though may be of different sizes.

The myoid region resembles that of type 2 cones, and like them contains a few granules, but no aggregation of glycogen forming a paraboloid.

The location of the nucleus amongst those of the displaced bipolar cells, and thus well vitread to the outer limiting membrane, is the same as for type 2 cones, and the nuclei of the two types cannot be separated morphologically.

The conducting neck and synaptic pedicles are also similar; both the pedicle and the neck contain synaptic vesicles, and about five synaptic ribbons are present. Basal filaments arise from the vitread edges of the pedicle, but these have not been traced to their terminations.

Bipolar cells

Two types of bipolar cell have been identified; the more common have their nuclei in the outer nuclear layer and so are called displaced bipolars, whilst the less common are larger and are centred in the horizontal cell layer.

The nuclei of the displaced bipolar cells are located at the same level of the outer nuclear layer as the nuclei of the types 2 and 3 cones. They are of similar size and shape, but are easily distinguished by their pale, even staining (figure 16, plate 46). The cell bodies are pear-shaped, broad vitreally, where they contribute lateral processes to the outer plexiform layer, and narrow sclerally, where the cell body merges into a Landolt's club. The perikaryon contains numerous mitochondria, mainly alined with the Landolt's club, in a lightly granular cytoplasm. This also contains similarly alined microtubules and filaments.

A Golgi apparatus and clusters of vesicles are located close to the nucleus, commonly at the vitread end of the cell. More vesicles, resembling synaptic vesicles but not associated with membrane specializations, occur away from the Golgi apparatus in the vitread region of the perikaryon, and at the roots of the Landolt's clubs.

In addition to the processes extending laterally into the outer plexiform layer, the vitread aspect of the perikaryon gives rise to a vitread process which reaches the inner plexiform layer. Both vitread and lateral processes contain orientated microtubules in a matrix of granular cytoplasm, and, near their origins, alined mitochondria. To reach the inner plexiform layer the

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DESCRIPTION OF PLATE 46

FIGURE 16. Type 2 cone, optical micrograph. This cone has no droplet, but there is a small granule, (1) just vitread to the mitochondria of the ellipsoid (2). The nucleus (3) unlike those of type 1 cones, is located amongst the displaced bipolar nuclei (4) from which it is distinguished by its darker chromatin pattern. The conducting fibre (5) is short and stout, and the synaptic pedicle intermediate between those of rods and type 1 cones.

FIGURE 17. Myoid region of a type 2 cone. This region contains microtubules, scattered vesicles and a few granules which resemble glycogen. Some larger membrane bounded granules are present, but there is no sign of an oil droplet.

FIGURE 18. Type 2 cone synapse, tangential section. This pedicle, which was serially sectioned, is intermediate in complexity between type 1 cone pedicles and rod spherules. The projections (1) are the roots of basal filaments, of which five were present.

vitread process traverses the horizontal cell layer, and this it does among the penetrating radial fibre trunks (figure 21, and figure 22, plate 47).

From the perikaryon sclerad to the nucleus a stout tapering process extends to the region of the outer limiting membrane. This process, the Landolt's club, contains mitochondria, mainly radially alined, in lightly granular cytoplasm which also contains radially directed microtubules. The club is approximately circular in section and is surrounded mainly, if not exclusively, by radial fibre material.

At the outer limiting membrane the club is constricted, and shows the junctions with adjacent cells which together make up the 'membrane'. The club has a characteristic ending sclerad to the outer limiting membrane, and thus free among the visual cells and radial fibre microvilli. Beyond the constriction the club expands to a diameter of approximately 2 μm; within the expanded portion is located a single large mitochondrion, up to three times the size of those elsewhere in the cell, together with two centrioles (figure 23, plate 47). One of these forms the basal body of a short cilium, and gives rise to striated ciliary rootlets which extend vitreally within the club stalk. The cilium has the 9 + 0 filament pattern, and arises at one side of the expansion, to extend in an obliquely radial direction for up to 2 μm. It is associated with one or more calyx processes which arise from the expansion just proximal to the cilium.

In addition to the large mitochondrion the expansion contains several dark bodies, and commonly, but not always, a number of vesicles. These measure 50 to 70 nm in diameter, and thus are larger than the 40 nm synaptic vesicles. Mostly they are clear, but some examples show a small central dense point. Where the vesicles are present they may be confined to the expanded club, but in a number of cases the limiting membrane of the club is discontinuous, perhaps artifactually, and vesicles lie both within the expansion and free in the ventricular space (figure 24, plate 47).

The second family of bipolar cells are larger than the displaced bipolars, measuring up to 20 μm across the perikaryon and with nuclei 10–12 μm in diameter (figure 25, plate 48). Their nuclei are located in the horizontal cell layer, and thus vitread to the outer plexiform layer, as in conventional bipolar cells. The perikaryon extends to the inner plexiform layer vitreally, and merges into a broad Landolt's club sclerally. Apart from their size and location, these cells are distinguished from the displaced bipolars by their pale cytoplasm, in which the mitochondria stand out clearly.

Description of plate 47

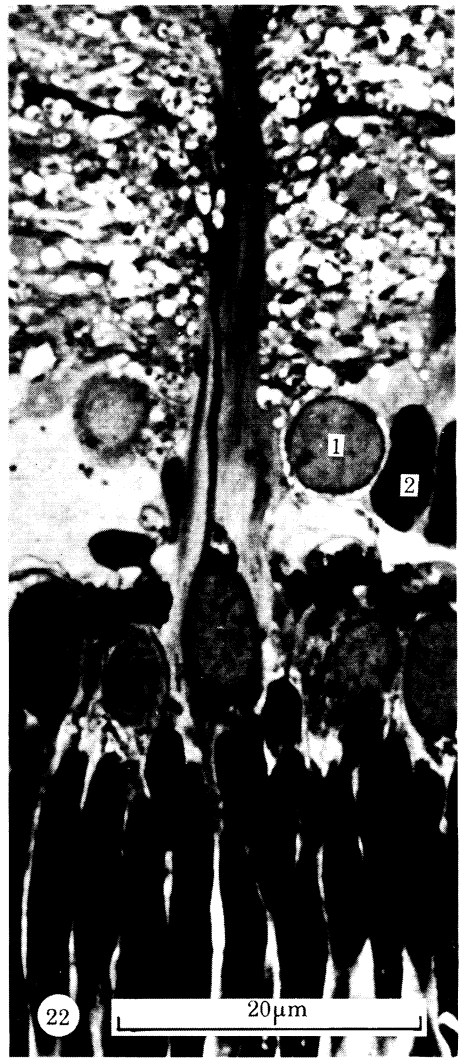
Figure 19. Type 3 cone, optical micrograph. This cone closely resembles type 2, except for the presence of a clear vacuole just vitread to the ellipsoid.

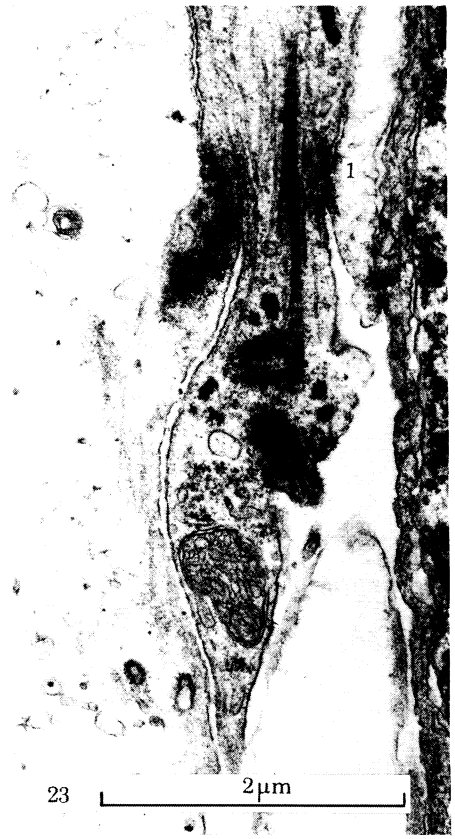
Figure 20. Type 3 cone vacuole. The vacuole is membrane bounded and contains scattered granules. Some smaller vacuoles of comparable appearance are located nearby.

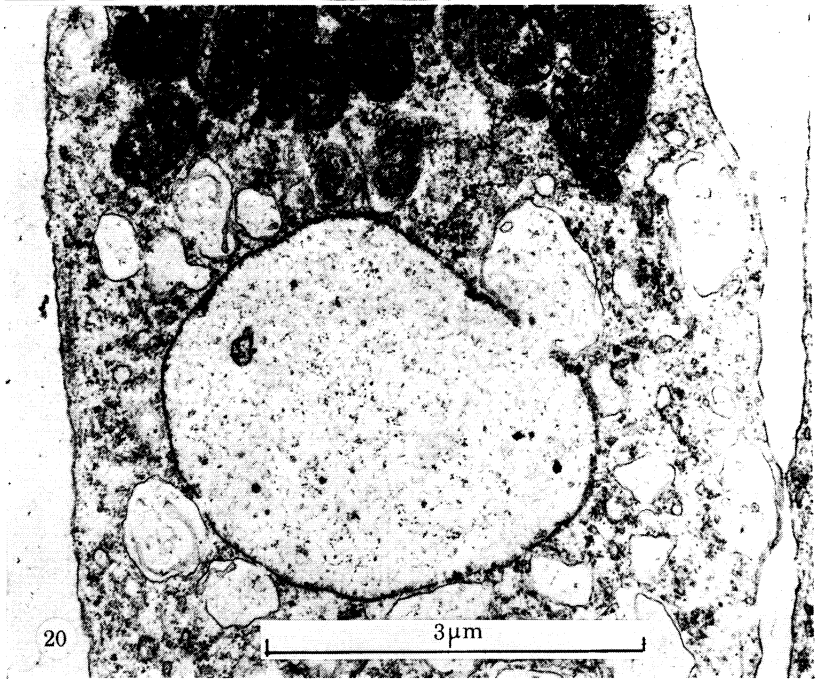
Figure 22. Displaced bipolar cell, optical micrograph. This example shows the vitread process extending to the inner plexiform layer with a radial fibre column. The Landolt's club is visible extending sclerally to the outer limiting membrane. The nuclei of amacrine cells (1) are clearly distinct from those of radial fibres (2).

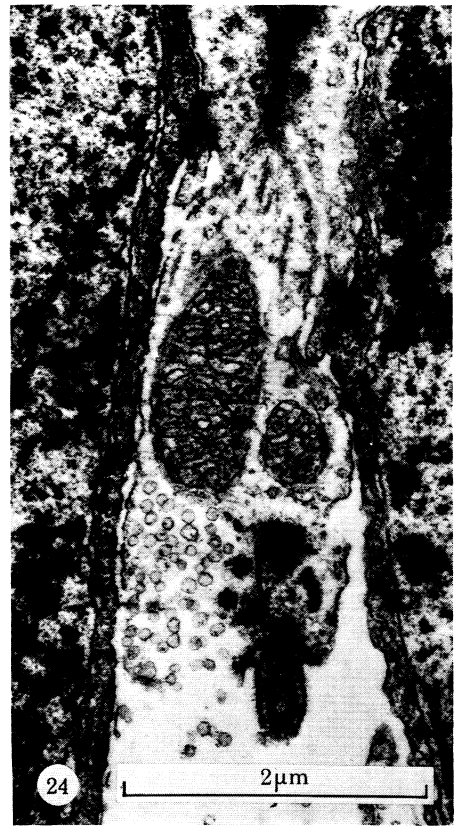
Figure 23. Ending of Landolt's club. The expanded ending extends into the ventricular space beyond the outer limiting membrane (1). The club contains two centrioles, one giving rise to a cilium, and a large mitochondrion. This example contains granules but no vesicles.

Figure 24. Another example of a Landolt's club ending. The large mitochondrion and cilium are seen, and in this example 60 nm vesicles are present within the end sac, of which the membrane is broken, and in the ventricular space nearby.









FIGURES 19, 20; 22–24. For legends see facing page

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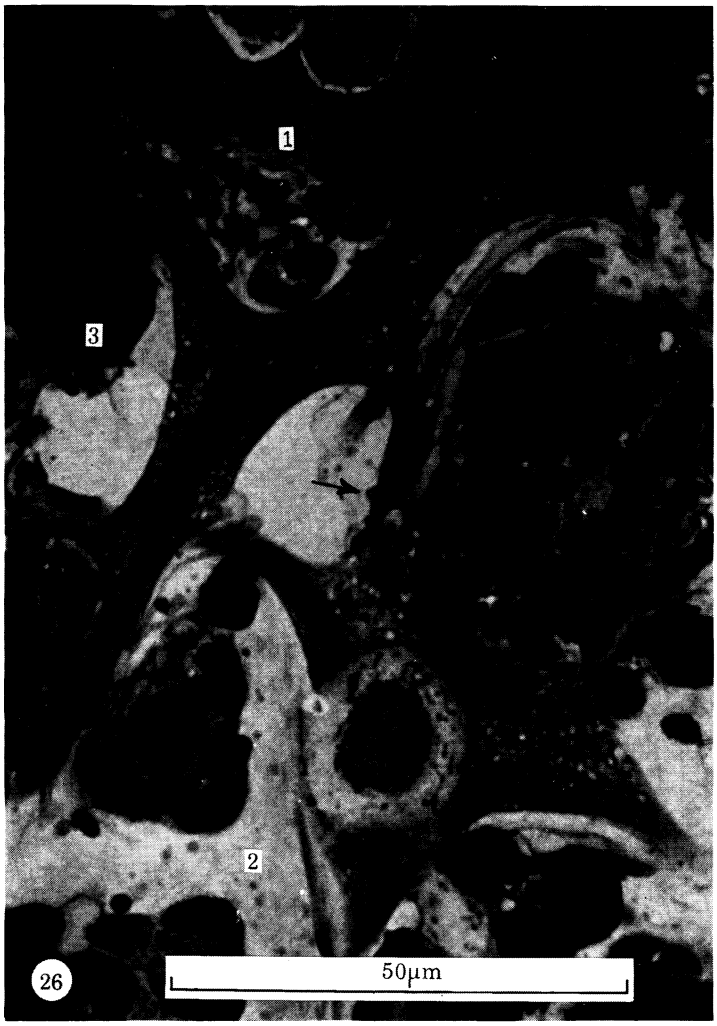

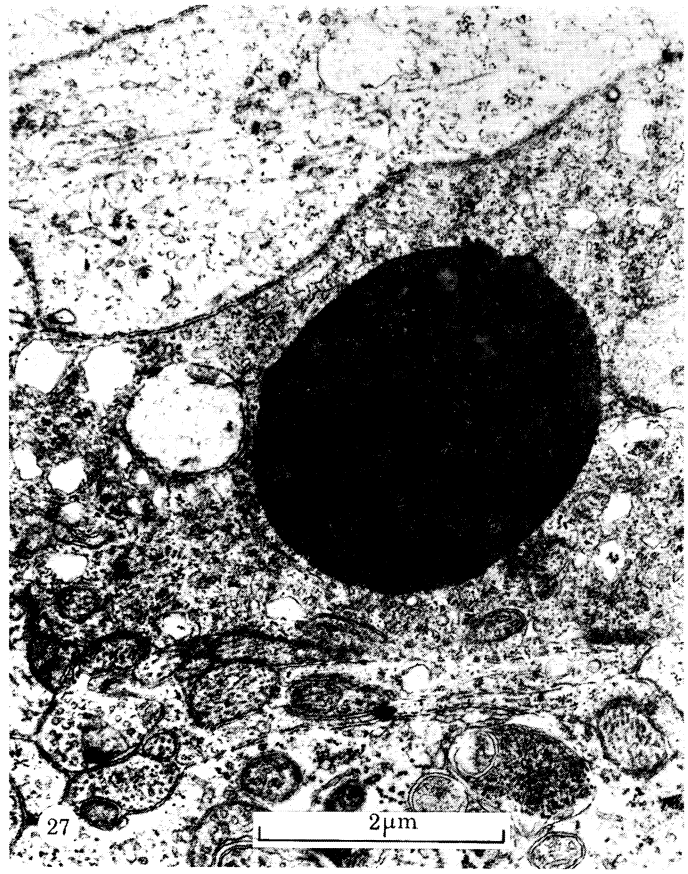

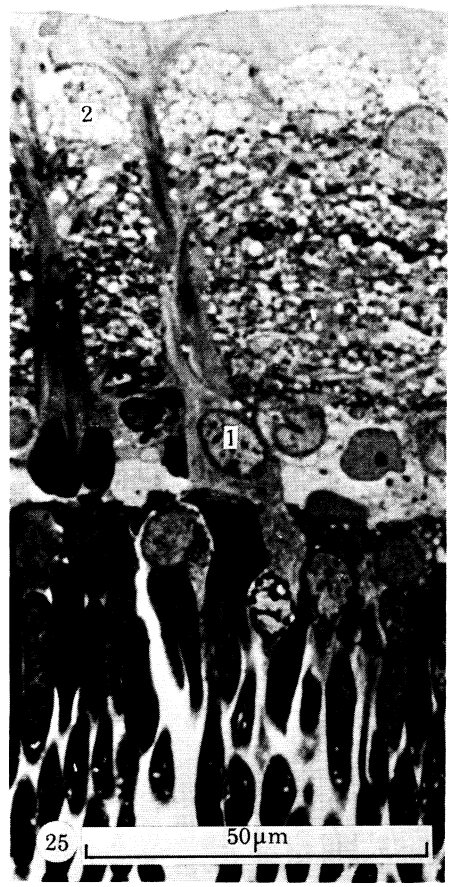

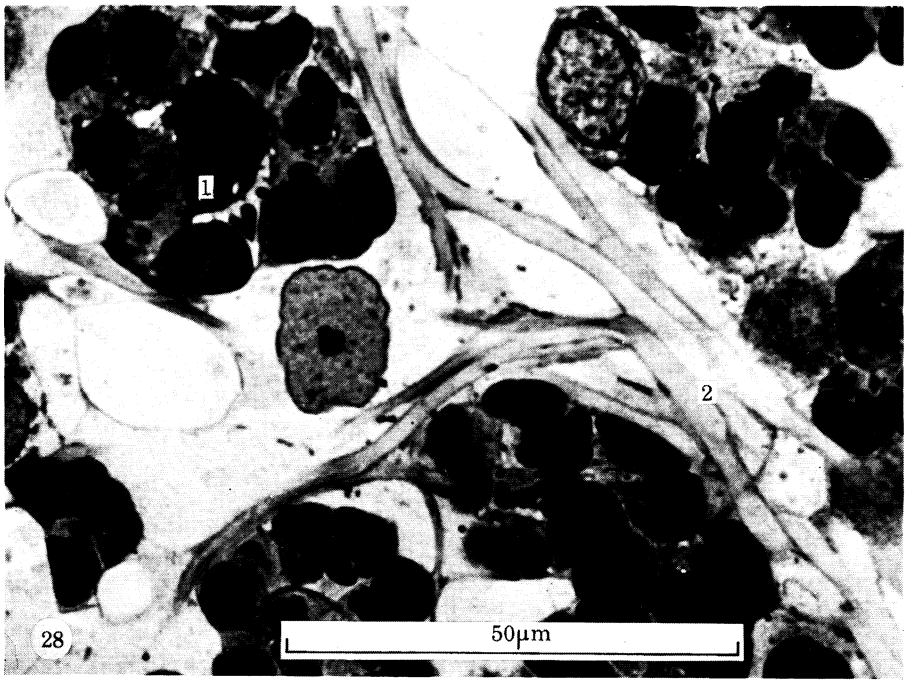

FIGURES 25–28. For legends see facing page

Their lateral processes arise from the junction of the perikaryon and the Landolt's club, and thus sclerad to the nucleus, unlike those of the displaced bipolar cells. These stout lateral processes have been traced for distances of up to 40 μm, but their terminations have not been positively identified.

Outer plexiform layer

The outer plexiform layer is approximately 3 μm in thickness; it consists of cell processes, most of which appear in sections as circular or oval profiles, or as short segments of apparently straight processes. On the sclerad side the layer is bounded by the rod and cone synapses, by the vitread extremities of the bipolar cells, and by the radial fibre cytoplasm which lies between these elements. The vitread limit of the layer is formed by the pale horizontal cells, or by the perikarya and processes of the dark horizontal cells where these are present. The outer plexiform layer is discontinuous where it is penetrated by the radial fibre trunks and by the large bipolar cells.

It has not been possible to characterize the component processes of the layer with certainty. Displaced bipolar cells undoubtedly contribute lateral processes to the layer, and these are lightly granular and contain microtubules. They have irregular contours near their origins but become cylindrical. These cylindrical bipolar processes are indistinguishable morphologically from the basal filaments of cones; both classes of process extend for distances exceeding 10 μm, and it has not been possible to follow them from origin to ending, even in serial sections (figure 15, plate 45).

In addition to the granular processes there are numerous cylindrical processes ranging in diameter from 0.2 to 1.3 μm, which contain prominent microtubules in a pale matrix (see figure 8, plate 43); these processes resemble those which arise from the large bipolar cells and from the pale horizontal cells. Certainly the large bipolar cells do contribute lateral processes to the layer; the pale horizontal cell processes which can be positively identified are found in the vitread portion of the horizontal cell layer, where they are smooth, cylindrical and seldom branched. Due to their size, up to 3 μm diameter, and the thickness of pale cells between the processes and the outer plexiform layer, up to 8 μm, it has not been possible to trace them in serial sections.

A family of processes which can be identified more confidently is that of the rod basal filaments (figure 9, plate 43). Serial sections have shown that these are of rather constant diameter,

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DESCRIPTION OF PLATE 48

FIGURE 25. Large bipolar cell, optical micrograph. The nucleus of this cell (1) is located at the horizontal cell level. A broad Landolt's club extends through the outer nuclear layer to the outer limiting membrane, and a vitread process extends into the inner plexiform layer. In this section the nerve fibre bundles (2) are cut transversely.

FIGURE 26. Dark horizontal cells, tangential section, optical micrograph. Portions of two dark horizontal cells are present, their broad processes spreading from the web-like perikaryon. Rod spherules are seen (1) and rod basal filament clubs can be identified against the horizontal cell processes; one is arrowed. Clear cytoplasm of pale horizontal cells (2) and dark radial fibre nuclei within columns (3) can also be recognized.

FIGURE 27. Dark horizontal cell lysosome. Dark bodies of this kind, containing linear and vesicular profiles, are found in the perikaryon and processes of the dark horizontal cells. Similar bodies are particularly abundant in the cells described as glial cells.

FIGURE 28. Pale horizontal cells, tangential section, optical micrograph. The space between radial fibre columns (1) is occupied by pale horizontal cells, of which a nucleus with a prominent nucleolus is seen. The pale cells give rise to long cylindrical processes (2).

0.2–0.3 μm, and that they contain a few tubules in a darkly granular matrix. Their terminal expansions are also characteristic, and once identified from serial sections have been readily recognizable (figure 10, plate 44).

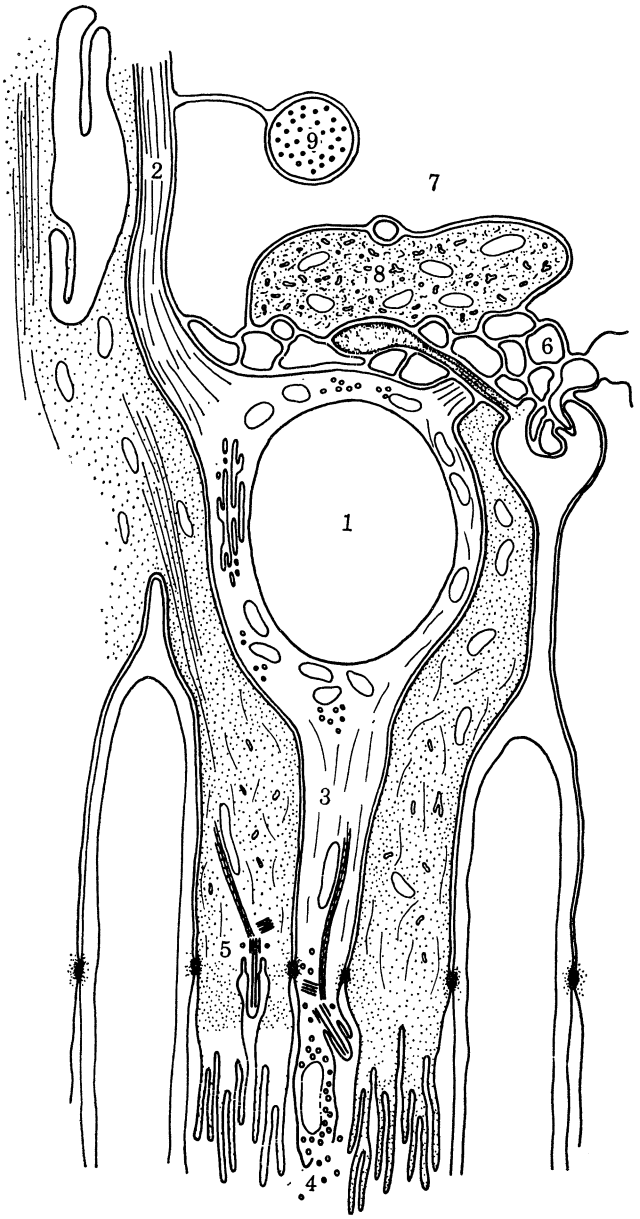

FIGURE 21. Diagram of displaced bipolar cell. A displaced bipolar cell (1) gives rise to a vitread process (2) which reaches the inner plexiform layer along a radial fibre column. The sclerad process of the bipolar cell is the Landolt's club (3) which extends beyond the outer limiting membrane. The sclerad extremity of the club contains two centrioles, one being the basal body of a short cilium, and giving rise to striated rootlets. Beyond the cilium is an expanded sac containing a large mitochondrion. In some examples the sac contains vesicles 60 nm in diameter; in others there are no vesicles, and in a proportion the membrane is broken and vesicles are present within and without the sac, as shown here (4). The radial fibres also have a pair of centrioles and a cilium at their sclerad extremity, but the cilium is recessed vitread to the outer limiting membrane (5). The radial fibres penetrate the outer plexiform layer (6) only as columns. The outer plexiform layer is overlain vitreally by pale horizontal cells (7) and, sandwiched between the two, dark horizontal cells, of which a sectioned process is shown (8). A rod basal filament is shown traversing the outer plexiform layer to end as a club in contact with the dark horizontal cell. Cylindrical processes (9) probably from pale horizontal cells, are invaginated into the bodies of those cells.

The dark horizontal cell contribution to the outer plexiform layer presents difficulties. In radial sections both perikaryon and processes are clearly identifiable, and appear as thick discrete masses sandwiched between the outer plexiform layer and the pale horizontal cells (figure 1, plate 41). Serial tangential sections through a dark horizontal cell process confirmed the impression gained from random sections, but additionally the special relation of the rod basal filament clubs to dark horizontal cells was observed. These serial sections did not show any rounded processes arising from the horizontal cell to enter the outer plexiform layer.

Serial sections of the type 1 cone pedicle shown in figure 14, plate 45, showed certain processes which could be traced from the interior of the pedicle to broad processes, which closely resemble dark horizontal cell material. Due to their size these processes could not be traced back to their cells of origin, so positive identification is lacking.

Within the outer plexiform layer itself the processes lie in contact with each other with no intervention of radial fibre material. Though the pale horizontal cells appear to limit the layer abruptly on the vitread side, a few protoplasmic processes from these cells have been identified which penetrate the outer plexiform layer to make invaginating contact with rod synapses.

Horizontal cells

The region of retina between the outer plexiform and amacrine cell layers is occupied by horizontal cells and large bipolars, except where radial fibre trunks, and the bipolar cell processes which accompany them, penetrate. The horizontal cells are of two kinds, a sclerally situated type which stains darkly, and a vitreally located pale type.

The dark horizontal cells are flattened, stellate cells which measure up to 120 μm across their processes. They occur in a single layer closely applied to the outer plexiform layer, and because their branches are few and large, are hard to visualize from radial sections. In suitable tangential sections (figure 26, plate 48) the nucleus, approximately 12 μm in diameter, is seen to lie centrally in the perikaryon, which gives rise to the broad processes. These narrow at first, but expand to a flat web from which further processes may arise. The major processes extend for distances of at least 30 μm, and some curve round the radial fibre bundles before terminating.

The cells stain darkly with toluidine blue (figure 26, plate 48) and in the electron microscope their cytoplasm is also dense (figure 10, plate 44). The density is due to an overall granularity of the cytoplasmic matrix, but other components are present. Tubular and vesicular profiles, some resembling smooth endoplasmic reticulum, are present in the perikaryon, and microtubules which appear in random arrangements in the perikaryon, are alined in the processes. Though mitochondria are scarce in the perikaryon, they occur in the processes, particularly near their terminations. Near the flattened edges of the perikaryon, and in the webs, clear lacunae are seen. Some of these may be artifactual, but others are membrane bounded and contain granules. A large proportion of the dark horizontal cells contain densely staining round bodies, with granular, vesicular and linear profiles in them (figure 27, plate 48). These bodies, which range from 0.5 to 4 μm in diameter, are also found in the glial type of cells described below, and are described as lysosomes.

The vitread surface of the dark horizontal cells is smoothly convex, and is overlaid by the pale horizontal cells. The sclerad aspect is flat where it abuts against the outer plexiform layer, but is indented by the processes which comprise that layer.

The modes of termination of the dark horizontal cells have proved difficult to interpret: some radial sections show large, discrete portions of horizontal cell apparently ending abruptly

between the pale horizontal cells and outer plexiform layer. Tangential sections suggest that these may be obliquely cut processes, and that the endings are of the flat web type. Such a region was examined in serial sections; it gave no straight processes, but some tortuous cytoplasmic processes do appear to arise. Serial sections from cone pedicles show cytoplasmic contacts from processes with the characteristics of dark horizontal cell, but which can not be positively identified (figure 14, plate 45).

The dark horizontal cells have no processes which run vitreally, nor do they give rise to a recognizable axon. Though their processes extend towards those of other dark horizontal cells no areas of contact have been seen.

The pale cells which occupy the vitread portion of the horizontal cell layer are very different in structure. They fill all the space at this level which is not occupied by the cells of the other families, in the way that the radial fibres do elsewhere in the retina. They are in such close association, and their cell boundaries are so faint to optical microscopy, that it is not possible to make out their individual shapes (figures 1, 28).

Their nuclei are approximately 15 μm in diameter, slightly flattened and without indentations; they stain lightly and have a prominent nucleolus. The cytoplasm is pale and amorphous to optical microscopy; electron micrographs show scattered granules, tubular and vesicular profiles of smooth endoplasmic reticulum and scanty microtubules. A few mitochondria and a small Golgi apparatus are present.

The vitread portions of the cells give origin to cylindrical processes, which contain abundant orientated microtubules and few filaments, in a pale matrix which resembles that of the perikaryon. These processes, which also contain a few mitochondria, form bundles which course between the radial fibre trunks (figure 28, plate 48). Occasionally they can be seen to branch, but most appear as smooth cylinders. Certain of these processes slope gradually towards the outer plexiform layer, and these are invaginated into the bodies of the pale horizontal cells which they encounter. Thus sections show a process buried within the pale cytoplasm, with the surface membrane of the cell reflected inwards and round the process in the manner of a Schwann cell mesaxon (figure 29, plate 49).

The pale cells meet each other over extensive smooth areas, but there are no membrane specializations at these sites and in particular no areas of close contact such as occur between

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Description of plate 49

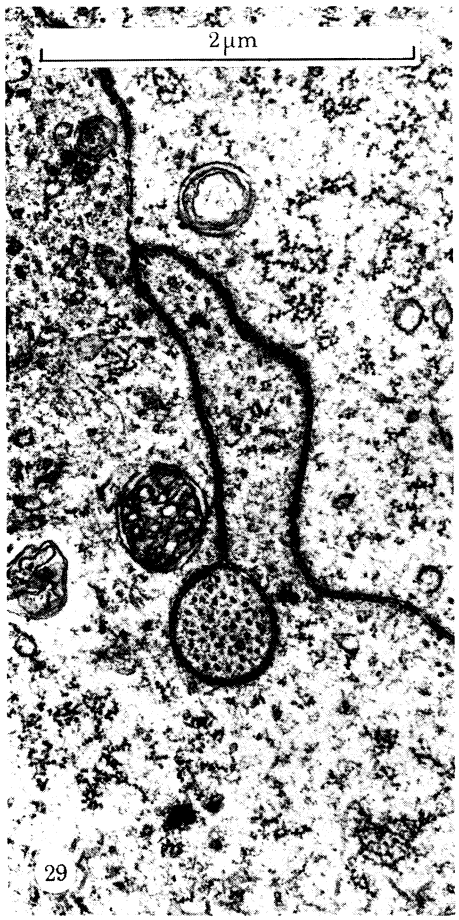
Figure 29. Pale horizontal cell, radial section. Portions of two pale horizontal cells are seen. A cylindrical process, probably of pale horizontal cell origin, is enfolded by pale cell cytoplasm. The relations recall those of unmyelinated nerve fibres to Schwann cell cytoplasm.

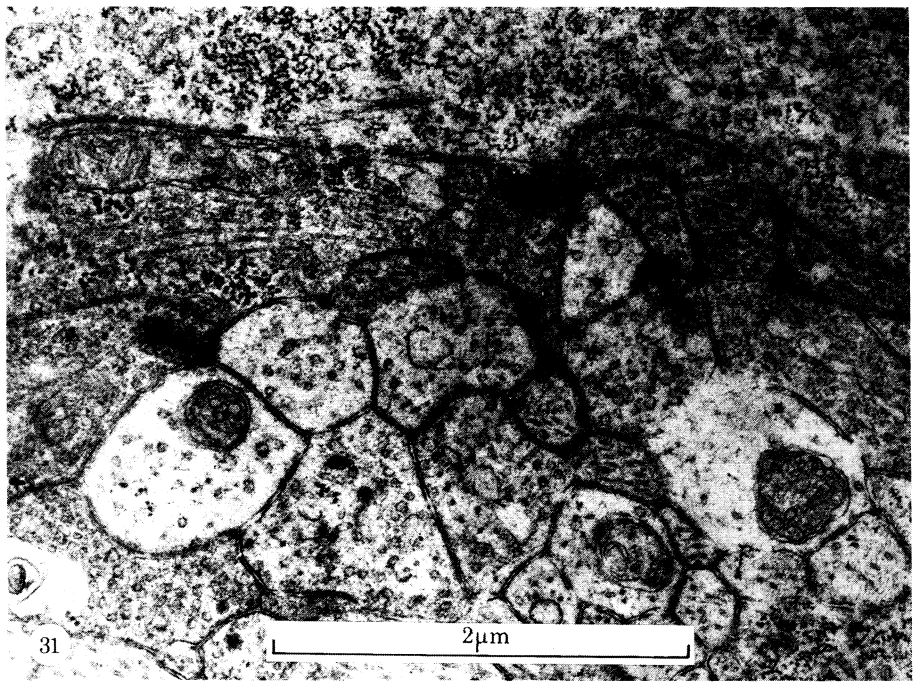
Figure 30. Gap junction, pale horizontal cell. A process, probably of pale horizontal cell origin, is seen surrounded by pale horizontal cell cytoplasm. The membranes are separated by the 10–20 nm gap found elsewhere, except for a region where they are only 2–3 nm apart; this separation is characteristic of gap junctions associated with electrotonic synapses.

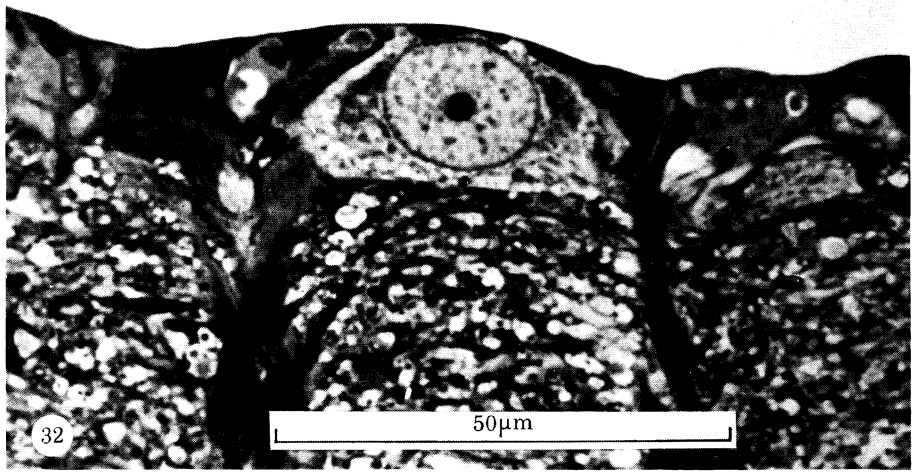
Figure 31. Inner plexiform layer. Processes containing synaptic ribbons and vesicles are believed to originate from bipolar cells. Fixation at this level is unsatisfactory.

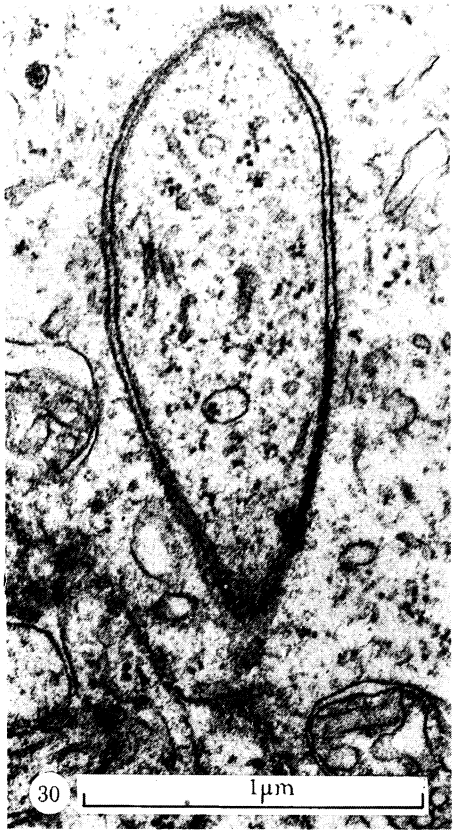
Figure 32. Ganglion cell, optical micrograph. A large ganglion cell is situated unusually close to the inner limiting membrane. Radial fibre columns contribute processes to the inner plexiform layer, and lamellae beneath the inner limiting membrane, the contours of which they determine.

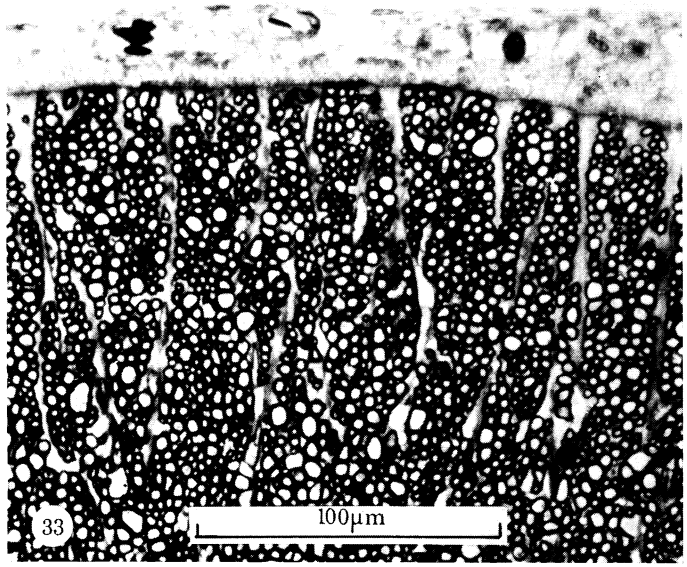
Figure 33. Optic nerve, optical micrograph. The myelinated fibres of the optic nerve are clearly distinguished. A montage of the whole nerve section was made from such micrographs and the myelinated fibres counted. Electron microscopy shows a significant number of non-myelinated fibres in addition, which cannot be distinguished in optical micrographs.











FIGURES 29–33. For legends see facing page

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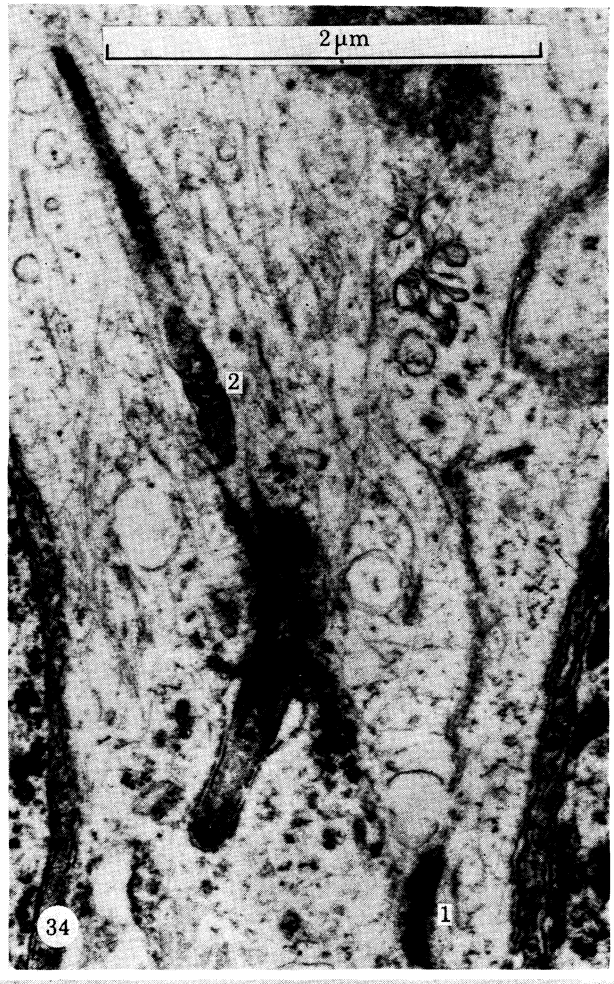

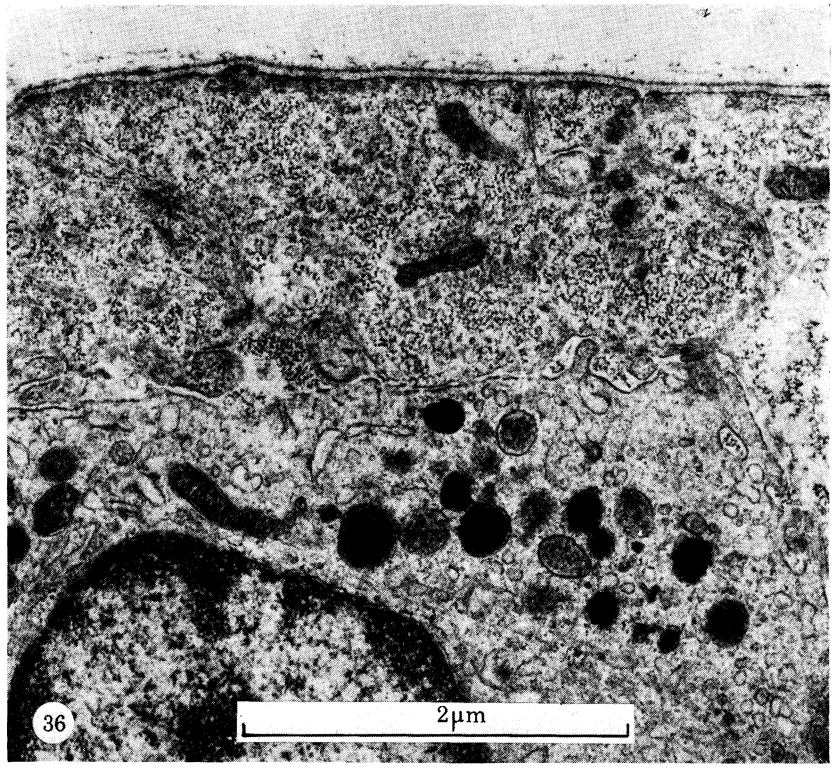

FIGURE 34. Radial fibre cilium. Radial fibre cytoplasm just vitread to the outer limiting membrane (1) contains a cilium complex. There are two centrioles, of which one is the basal body of the cilium. This centriole gives rise to striated rootlets, beside which alined mitochondria are located (2). The cilium appears to be buried in the cytoplasm.

FIGURE 36. Inner limiting membrane. The inner boundary of the retina is formed by radial fibre expansions with the closely adherent vitreous condensation. The cell beneath the radial fibre is tentatively identified as a microglial cell.

horizontal cells in certain other fishes. Occasional examples of such close junctions have however been seen between pale horizontal cell bodies and unidentified processes just vitread to the outer plexiform layer (figure 30, plate 49).

Because the processes of these cells are so long it has not been possible to trace them to their terminations; there are processes in the outer plexiform layer which resemble them (figure 8, plate 43) but the large bipolar cells give similar processes and those observed cannot be rigorously assigned to either type.

Where the pale cell bodies lie against the outer plexiform layer they, like the dark horizontal cells, are indented by the processes. They give rise to cytoplasmic processes which extend into the outer plexiform layer, and some of these have been seen to make contact with rod spherules.

Amacrine cells

Situated between the vitread aspect of the pale horizontal cells and the inner plexiform layer is an apparently uniform population of cells, which are identified as amacrine cells. Their nuclei are approximately spherical, commonly with an incisure, and most appear pale and evenly stained; a nucleolus is present (figure 1).

The cytoplasm is granular; it contains a moderate number of mitochondria, randomly arranged microtubules and areas of rough endoplasmic reticulum or Nissl substance. Clear

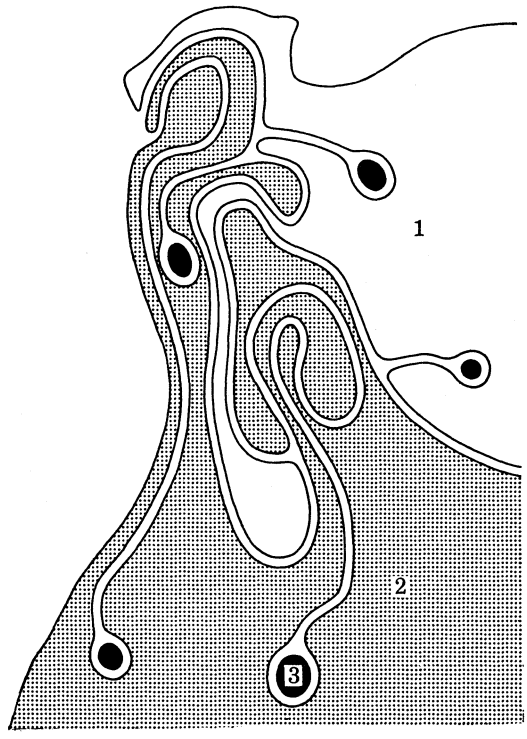

FIGURE 35. Diagram to show relations of radial fibre cytoplasm and rod conducting fibres. The outlines were traced from a micrograph of a tangential section at the level of the rod conducting fibres. Portions of radial fibres (1) and (2) interdigitate with each other. The radial fibre surfaces are also invaginated by the conducting fibres (3) which at this level are surrounded by radial fibre cytoplasm.

vesicles are associated with the Golgi apparatus and larger (100 nm) dense cored vesicles are also present. In some examples a short cilium has been seen projecting from the cell surface to lie free in the intercellular space; associated with the basal body are striated ciliary rootlets and a second centriole. Processes, which contain alined microtubules and a few mitochondria near their origins, arise from the vitread aspects of the perikaryon to enter the inner plexiform layer.

Inner plexiform layer

The fixation of the inner plexiform layer is not sufficiently good for valid conclusions to be drawn about its organization. Processes from bipolar cells, amacrines and ganglion cells are certainly present, and synaptic areas between processes can be recognized. Certain dark staining processes which occur at definite horizons (figure 1) are of bipolar origin. These processes make ribbon synapses, forming dyads (figure 31, plate 49). Besides the neural processes the inner plexiform layer contains irregular processes extending into it from radial fibre trunks.

Ganglion cells

The ganglion cells (figure 32, plate 49) are present in small numbers, counts showed 176/mm^2, and do not form a separate layer. They occur at the same level as the nerve fibres, and tend to be arranged in loose rows between the fibre bundles. Their sclerad aspect reaches the inner plexiform layer, to which their processes contribute. The ganglion cell nuclei are round and pale staining, up to 15 μm in diameter and containing a prominent nucleolus. The lightly granular cytoplasm contains numerous small mitochondria, a Golgi apparatus with associated vesicles, and scattered microtubules. Though not prominent, areas of Nissl substance are present. Dark granules, apparently with an amorphous content and membrane bounded, may be lysosomes. It has not been possible to follow the ganglion cell dendrites in the inner plexiform layer, nor to define the origin of their axons.

Nerve fibres and optic nerve

The nerve fibres within the retina occur at the same level as the ganglion cells, and are mainly grouped in bundles. These are separated from each other and from the ganglion cells by radial fibre material, but the individual fibres within a bundle are not so separated.

All the nerve fibres in the retina itself are non-myelinated, but the optic nerve contains myelinated and non-myelinated fibres. The myelinated fibres are easily recognized by optical microscopy, and could be counted in matched overlapping micrographs of a transverse section through the whole nerve; figure 33, plate 49, is an example of the micrographs used for counting. The number of myelinated fibres counted in the whole nerve was 143104.

Electron microscopy shows that the nerve also contains non-myelinated fibres; though exact counts have not been made, it is estimated that the proportion of myelinated to non-myelinated fibres is approximately 3 to 1.

Radial fibres

Typically radial fibres extend from the outer to the inner limiting membrane and appear as filling-in material between the bodies and processes of the other retinal cell families; their distribution in *Latimeria* is in some ways atypical.

Projecting sclerally from the region of the outer limiting membrane are flattened radial fibre processes, which subdivide into microvilli about 10 μm long by 0.3 μm diameter. The radial

fibres from which these processes arise fill the spaces between the other retinal elements in the outer nuclear layer, and so have complex forms with concave outlines in tangential sections.

Just vitread to the outer limiting membrane each radial fibre shows a pair of centrioles. One of these forms the basal body, orientated approximately radially, of a short 9 + 0 cilium (figure 34, plate 50). The cilium arises vitread to the outer limiting membrane, unlike those of the Landolt's clubs, and is ensheathed by radial fibre cytoplasm throughout its length. Some examples appear to be buried in the cytoplasm, and cannot be seen to emerge beyond the outer limiting membrane. Ciliary rootlets extend vitreally from the basal body.

At the level of the rod nuclei the radial fibre cytoplasm is pale staining, containing a few granules, some randomly arranged filaments, and few microtubules. Scattered vesicles of unequal sizes are also present and elongated mitochondria are found near the ciliary rootlets.

At the level of the rod conducting fibres the radial fibres show a different arrangement; they fill in between the other elements, but the rod fibres are enclosed in complex and extensive folds (figure 35). At this level the radial fibres contain numerous mitochondria, and, as further sclerally, they isolate the other cells from each other.

Vitread to the bipolar cell and type 2 and 3 cone nuclei are the synaptic pedicles and cell processes of the outer plexiform layer; in many animals there is a radial fibre contribution to this layer, but in *Latimeria* this is not the case. Radial fibre elements extend to the sclerad surface of the outer plexiform layer, but they penetrate it only at well-defined sites where columns of radial fibre pass through to the inner plexiform layer (figures 1, 26, 28). The radial fibre nuclei, dark staining and elongated in a radial direction, are located in these columns. In tangential sections the nuclei appear much folded.

Vitread to the horizontal cells, the radial fibres give lateral extensions which ramify amongst the amacrine cells. The trunks continue in a radial direction, giving further lateral expansions to the inner plexiform layer, and end beneath the inner limiting membrane. In the trunks, particularly from the horizontal cell layer to the inner limiting zone, the radial fibres display bundles of fine parallel fibrils which stain densely. From the inner plexiform layer to the inner limiting zone the cytoplasm itself also stains darkly (figure 36, plate 50).

Beneath the inner limiting membrane the radial fibre cytoplasm extends as tangentially orientated lamellae, which overlap each other and the other retinal elements, here largely nerve fibres. At the inner limiting membrane these lamellae determine the inner contour of the retina, their vitread face and the adjacent condensation of the vitreous forming the 'membrane' itself.

Glial cells

Tangential sections of the nerve fibre layer show a number of cells which appear different from the typical ganglion cells. These cells, which are believed to be microglia, have a dark staining nucleus about 4 μm diameter, with a prominent chromatin pattern and a nucleolus. The nucleus may be round, but in some instances is elongated or indented. Though the outline of the cell is usually approximately round, it may bear short cytoplasmic processes which are unlike the neural processes of the inner plexiform layer. The cytoplasm contains cisternae of smooth endoplasmic reticulum, but no Nissl substance; mitochondria are present in small numbers, one or more centrioles may be present and a Golgi apparatus is located near the nucleus.

The most conspicuous feature of these cells is the presence of lysosomes, which may be small,

or may almost fill the cytoplasm. These range from 0.1 to 4.5 μm in diameter, stain darkly and are membrane bounded. The smaller ones appear amorphous, but the larger examples are closely packed with granular, vesicular and fibrillar elements.

The microglial cells are not confined to the ganglion cell and nerve fibre layer, though they are most numerous there. Examples have also been seen in the inner plexiform layer, and adjacent to radial fibre trunks at the horizontal cell level.

Discussion

In addition to occupying a unique position as the surviving crossopterygian, *Latimeria* is an inhabitant of deep water, and apparent specializations in the retinal structure may relate to habitat as well as phylogeny.

The depths at which coelacanths have been caught are probably between 100 and 400 m (Millot, Anthony & Robineau 1972). Exact depths of capture are difficult to ascertain; the fishermen do not usually measure it, and if they do their estimates are subject to considerable errors. Attempts were made to measure the depth from which the present specimen was taken by using a sounding line at the position in which the fisherman thought he had been at the time of capture; the depth recorded was 165 m.

The shallow limit of Millot, Anthony & Robineau's figures is probably a true one, since fishing does take place in shallower water. Their deep limit, however, may be set by the length of the lines used, rather than the true vertical distribution of the fish.

The low λ_{max} of the visual pigment recorded by Dartnall (1972) accords with a deep water habitat; he quotes comparable values only from deep-sea teleosts, elasmobranchs and cetaceans. Though the pigment appears to be a deep-sea one, the known depths of capture are well within the zone to which daylight penetrates. In the clear waters of the Indian Ocean this depth may lie between 500 and 1000 m. Denton & Warren (1957) give a value of 1100 m as the maximum at which deep-sea fishes could perceive light, and they considered the clearest oceanic waters: *Latimeria* inhabits coastal waters which are likely to be more turbid.

In *Latimeria* the retinal epithelium overlying the tapetum does not contain the pigment granules which are abundant in most fish retinae. This situation is found in other fishes with a choroidal tapetum, e.g. sturgeons (Nicol 1969) and some elasmobranchs (Denton & Nicol 1964).

Owing to the absence of pigment granules the other cell components stand out clearly in micrographs. These components include numerous phagosomes, some of which have well-ordered lamellae resembling those in the intact rod outer segments. Others have disordered lamellae, and in the densest, usually located most sclerally in the cell, no lamellar structure is visible.

The presence of these phagosomes suggests that the process of renewal and destruction of rod outer segments, established in frogs and mammals by Young and his colleagues (Young 1971 for references), also occurs in *Latimeria*. Due to the separation of the epithelium from the retina during processing the relation of intact rod tips to the epithelial cells could not be studied, but the epithelial cells lack the processes which extend between the outer segments in many vertebrates, and which Young showed are involved in the phagocytic process in the rhesus monkey.

As well as phagosomes and mitochondria, the cytoplasm contains abundant vesicular and tubular profiles, at least some of which represent smooth endoplasmic reticulum. In some species

the cell membrane adjacent to the choriocapillaris is extensively folded into the interior of the cell, but this is not the case in *Latimeria*; nor are the vesicular profiles seen to connect with the surface membranes facing the adjacent cells.

Areas of fine and apparently tortuous tubules are present, and related to these the ordered patterns of tubules of larger diameter seen in figures 3 and 4, plate 41. Some of these appear to consist of two tubular systems intimately related to each other (figure 3); the components in this case could be endoplasmic reticulum and the ground cytoplasm. In some cases (figure 4) the tubules are seen to form a pattern of tightly interlaced Y-shapes, and here the situation is more complicated. Some of the Y-shaped elements join with others, and appear to form portions of a hexagonal net. Attempts to reconstruct the observed pattern were made using molecular models used for demonstrating chemical structures, and it was found that three interlacing nets were required. The regular patterns without Y-shapes (figure 3) may be formed if there are layers of nets connected in stacks, the apparent irregularities being due to the plane of section. If there are indeed three networks, their components may be derived from the large diameter smooth endoplasmic reticulum, the small diameter tortuous tubules and the ground cytoplasm. The function of these systems of closely apposed tubules is obscure, but they may be compared with systems of tubules in the chloride cells of certain teleost gills (Conte 1969) and with the labyrinth cells in the olfactory mucosa of seatrout (Bertmar 1972); in both these cases, however, there is only a single net of tubules, and this is in continuity with the surface membrane. Both these cell types are concerned with osmotic regulation and ion transport, and the retinal epithelium, being a pathway between the choroidal blood supply and the neuroretina, is comparable in function.

The visual cells in *Latimeria* are mainly rods, as stated by Millot & Anthony (1965) and confirmed by the present study. Some of their features are characteristic of typical vertebrate rods, thus they have a cylindrical outer segment consisting of discrete lamellae enclosed within the cell membrane. Their inner segment consisting of ellipsoid and myoid, but not paraboloid nor oil droplet, is also typical, as is the narrow conducting fibre and oligosynaptic spherule. Millot & Anthony stated that the outer segments are, at 70–80 μm, long; though they are certainly longer than those of many shallow water teleosts, they are much shorter than the rods of certain deep-sea species. Lengths of 150 μm in *Platytroctes* (Locket 1971*a*) and 525 μm in *Diretmus* (Munk 1966) have been recorded in specimens with much smaller eyes than *Latimeria*.

The appearances described at the base of the outer segments are unusual, and raise questions about the mode of formation of the lamellae. Two configurations are found in this region; in one the outer and inner segment are separated by a distinct pair of membranes, and in the other there is cytoplasmic continuity between the two across the full width of the rod.

In certain species, including some teleosts, the basal few disks have been shown to project from the cilium into the ventricular space between the inner and outer segments (Moody & Robertson 1960; Cohen 1961, 1963, 1972; Locket 1971*a*, *b*; Young 1971), but this is not so in *Latimeria*. Investigations on the early development of rod outer segments have suggested that they are formed by a process of infolding of the plasma membrane of the outer segment precursor (Tokuyasu & Yamada 1959; Carasso 1959; De Robertis & Lasansky 1961; Olney 1968).

The work of Droz, Young and Bok in following the fate of labelled amino acids by radioautography has established the pattern of protein incorporation and turnover in frogs, rats and monkeys (Young 1971 for references). The label is first identified in the inner segment, and moves to the base of the outer segment, apparently through the cilium, as a continuous process. The labelled band so formed at the base of the outer segment migrates sclerally and is eventually

lost from the tips, when it is phagocytosed by the pigment epithelium cells. The characteristic phagosomes formed from this outer segment debris are abundant in *Latimeria*, strongly suggesting that outer segment formation and renewal takes place in this species as it has been shown to do in others.

Assuming that the rod outer segments are renewed during life, the appearances at the outer segment base can be explained in two ways. It may be that all the rods have both an area of parallel membranes and one of cytoplasmic continuity. If this is so, then examples showing both patterns should be present in a large sample of rods. Occasional examples only have been seen, and in the large majority of rods one pattern or the other is quite clear. Alternatively, some rods with one configuration and others with the second were present at the time of fixation; examination of the sections suggests that this is the true explanation.

If this is so, then either two types of rod are present, or the two configurations alternate in the rods of a uniform population. In the configuration with cytoplasmic continuity it looks as if the lamellae are being formed by, or in close association with, the inner segment mitochondria. In the other configuration the mitochondria are separated from the lamellae by the parallel membranes. The appearances, as suggested elsewhere (Locket 1973) lead to the conclusion that outer segment formation, as well as destruction, may be a discontinuous process in *Latimeria*.

The other three types of visual cell present are described as cones, and all have small conical outer segments with their lamellae open to the ventricular space. Their synaptic pedicles are all of the complex variety typical of cones; though those of type 1 cones show greater complexity than those of types 2 and 3, all are clearly distinct from the rod spherules. This distinction contrasts with the situation in dipnoans and amphibians, in which both rods and cones have polysynaptic pedicles.

Oil droplets occur in the cones of a wide variety of vertebrates. Among fishes they are present in the dipnoans *Protopterus*, *Lepidosiren* and *Neoceratodus*, the chondrosteans *Acipenser* and *Polyodon* and the brachiopterygian *Calamoichthys* (Munk 1969). They are absent in teleosts, elasmobranchs and the holosteans *Amia* and *Lepisosteus*. Oil droplets are also present in type 1 *Latimeria* cones, and their small number, 22/mm^2, accords with Millot & Carasso's finding (1955). The type 2 and 3 cones are difficult to recognize in paraffin embedded material, and were doubtless missed by the French authors for this reason. Though a vacuole is present in the inner segment of the type 3 cones, this contains some labile substance and not the oil of type 1 cones.

It may be that the labile vacuole in type 3 cones is not a sufficiently important feature to divide them from type 2, in which it is absent. These two types resemble each other very closely except for that single feature.

From the retinal cell and optic nerve fibre counts some approximate ratios may be calculated. The cell count gives 53926 visual cells, 6432 bipolar cells and 176 ganglion cells per square millimetre. From this the approximate ratio of visual cells to bipolars is 8:1; that of visual cells to ganglion cells, the convergence ratio, 306:1, and that of bipolar cells to ganglion cells 36:1.

The number of cells counted may also be compared with the retinal area. The diameter of the posterior eye cup was approximately 37 mm (Dartnall 1972). If we assume that two-thirds of the eye is lined by retina then the area of the retina will be given by $\frac{2}{3}\pi d^2$, i.e. $\frac{1}{3}(6.28 \times 1369) = 2860$ mm^2. If we assume that the distribution of ganglion cells is uniform over the whole retina, it will contain $2860 \times 176 = 503360$ cells. This figure may be compared with the number of fibres counted in the optic nerve, 143000, and suggests that there are 360360 ganglion cells

whose fibres escaped counting. A discrepancy would be expected if some cells give rise to non-myelinated fibres; although such fibres certainly occur in the optic nerve they do not appear to be numerous enough to explain the figures. It is perhaps more likely that the distribution of ganglion cells is not uniform, and that the piece of retina used for counts was from a cell-rich area. These calculations take no account of possible centrifugal fibres in the optic nerve.

In higher vertebrates the bipolar cell bodies are usually located in the inner nuclear layer; they give a sclerad process, which branches to be distributed to the visual cell synapses, and a vitread process which ramifies in the inner plexiform layer. In certain groups a proportion of the bipolar cell bodies are located sclerad to the outer plexiform layer. These cells, the displaced bipolar cells (Ramón y Cajál 1893) are well known in fishes lower than teleosts. They were described in *Acipenser* by Dogiel (1883) as subepithelial ganglion cells; Neumayer (1897) recorded them in selachians, Munk (1964) in *Calamoichthys*, and they also occur in dipnoans (personal observation) together with normally sited bipolars. Most of the bipolars in *Latimeria* are of the displaced variety, with nuclei in the outer nuclear layer. These nuclei are distinctive in appearance, enabling them to be counted separately from those of the rods and cones which occur at the same level.

The club-shaped process described by Landolt (1871) was established as arising from bipolar cells by 1885, largely as a result of the work of Ranvier (1882) and Dogiel (1883, 1885). The clubs have since been observed in all vertebrate groups except mammals, nocturnal birds and teleosts; Stell (1972), reviewing the small amount of work on the clubs, concludes that their function is still obscure.

In most cases the clubs end at, or just sclerad to, the outer limiting membrane, though in *Protopterus* they extend beyond the visual cells to make intimate contact with the pigment epithelium (Locket 1970). In the present case the clubs extend a short distance beyond the outer limiting membrane, and their endings show some unusual features.

The centrioles with $9+0$ cilium and rootlets are expected findings, but the expanded sac and its contents are less so. The large mitochondrion is a constant finding, but whether it may have some functional significance is unknown. It is related, at least in a structural sense, to the presence of the 60 nm vesicles described above. In some examples these vesicles are confined to the sac, in some they are absent and in others they occur within and around a disrupted sac. These appearances would be consistent with a process of accumulation and intermittent release of vesicles from the club, and thus the bipolar cytoplasm, into the ventricular space, though the disruption of the sac may be artifactual.

If Landolt's clubs do release vesicles, then their contents are of interest. Synaptic vesicles found within the retina, and elsewhere in the nervous system, are believed to contain transmitter substances, but the nature of these in retina is still unknown (Stell 1972). The Landolt's club vesicles are larger than the 40 nm synaptic vesicles in visual cell synapses, and there is no other structure suggestive of synaptic specialization in the clubs.

Stell observes that Landolt's clubs are not found in retinae in which the rod synapses are typical spherules, i.e. oligosynaptic. Apart from the possession of a single basal filament, the rod synapses in *Latimeria* are typically oligosynaptic, so they may be an exception to the usual association of complex synapses with the possession of Landolt's clubs.

The structure and arrangement of horizontal cells varies widely amongst fishes; even within the teleosts they range from four layers of prominent cells in, for example, Mugilidae (Parthe 1972) to no recognizable horizontal cells in some deep-sea forms (Locket 1971*a*). Among lower

fishes they are present in two layers in lampreys (W. Müller 1874), and sturgeons (Dogiel 1883); Munk (1964) describes a single layer in *Calamoichthys*, and at least one layer can be seen in his figures of *Amia* and *Lepisosteus* (Munk 1968). Of the lungfishes *Lepidosiren* has no prominent horizontal cells, but *Protopterus* has one layer of inconspicuous cells and *Neoceratodus* a layer of large flat cells and a more vitread layer of cylindrical processes (personal observation).

In many teleosts the horizontal cells are present in three or four layers, (Krause 1886; Parthe 1972) each consisting of stellate cells whose broad lateral processes meet those of the adjacent cells at areas of gap junctions (Brightman & Reese 1969). Such teleost horizontal cells give rise to processes which pass sclerally between the horizontal cells of intervening rows to make contact with the synaptic terminals of the visual cells. Typically the sclerad horizontal cells contact cones, and only the most vitread layer contacts rods (Ramón y Cajál 1893; Stell 1972).

Besides the stellate horizontal cells a layer of intertwining processes adjacent to the vitread aspect of the horizontal cells is sometimes present. These processes, described as internal horizontal cells by Ramón y Cajál and as anucleate concentric cells by Schiefferdecker (1886), have seldom been related to a cell type of origin. Kaneko (1970) has recorded S-potentials from them in the goldfish however, and Stell has identified them as an expansion of cone horizontal cell axons in the same species (personal communication). Comparable processes are present in *Latimeria*, and here examples have been seen to take origin from the pale horizontal cells.

The dark horizontal cells do not resemble the sclerad cells in teleosts; their diffuse arrangement and apparent lack of contact with each other is unlike the mutually connected layer of cells in the higher fishes. Though it has not been possible to work out their connexions exhaustively, some points have emerged. In teleosts the most sclerad horizontal cells usually make contact with cones. In the present case processes related to the ribbons in cone pedicles have been traced to areas of cytoplasm with the characteristics of dark horizontal cells. This identification has not been confirmed by tracing back to an unequivocal horizontal cell, but the cytoplasm is certainly not that of a pale horizontal cell. It is possible that the processes are from bipolar cells; the cytoplasmic characteristics of these and dark horizontal cells are similar in the layer in question. It has, however, been well established in other vertebrates that the processes related laterally to ribbons in synaptic pedicles are from horizontal cells (Stell 1972, for discussion), and it seems justified to assume that this is the case in *Latimeria*, and that hence it is the dark horizontal cells which contact the cones.

In addition to these contacts, in which horizontal cell processes extend to a cone, there are numerous areas of contact where rod basal filaments extend to a horizontal cell. That the majority, if not all, of the rod basal filaments make contact with dark horizontal cells, and that their ends are expanded into clubs where they do so, suggests a special functional relation. Though the basal filaments arise from synaptic terminals, their end clubs show no typically synaptic specializations; they contain granules and not the synaptic vesicles of the parent spherule, and there are no synaptic thickenings of the membranes, either of the club or of the horizontal cell. There are no vesicles in the horizontal cell opposite the club, nor is there a gap junction suggestive of an electrotonic synapse. Thus whether these sites of contact are functional synapses, and if so in which direction transmission occurs, remains obscure.

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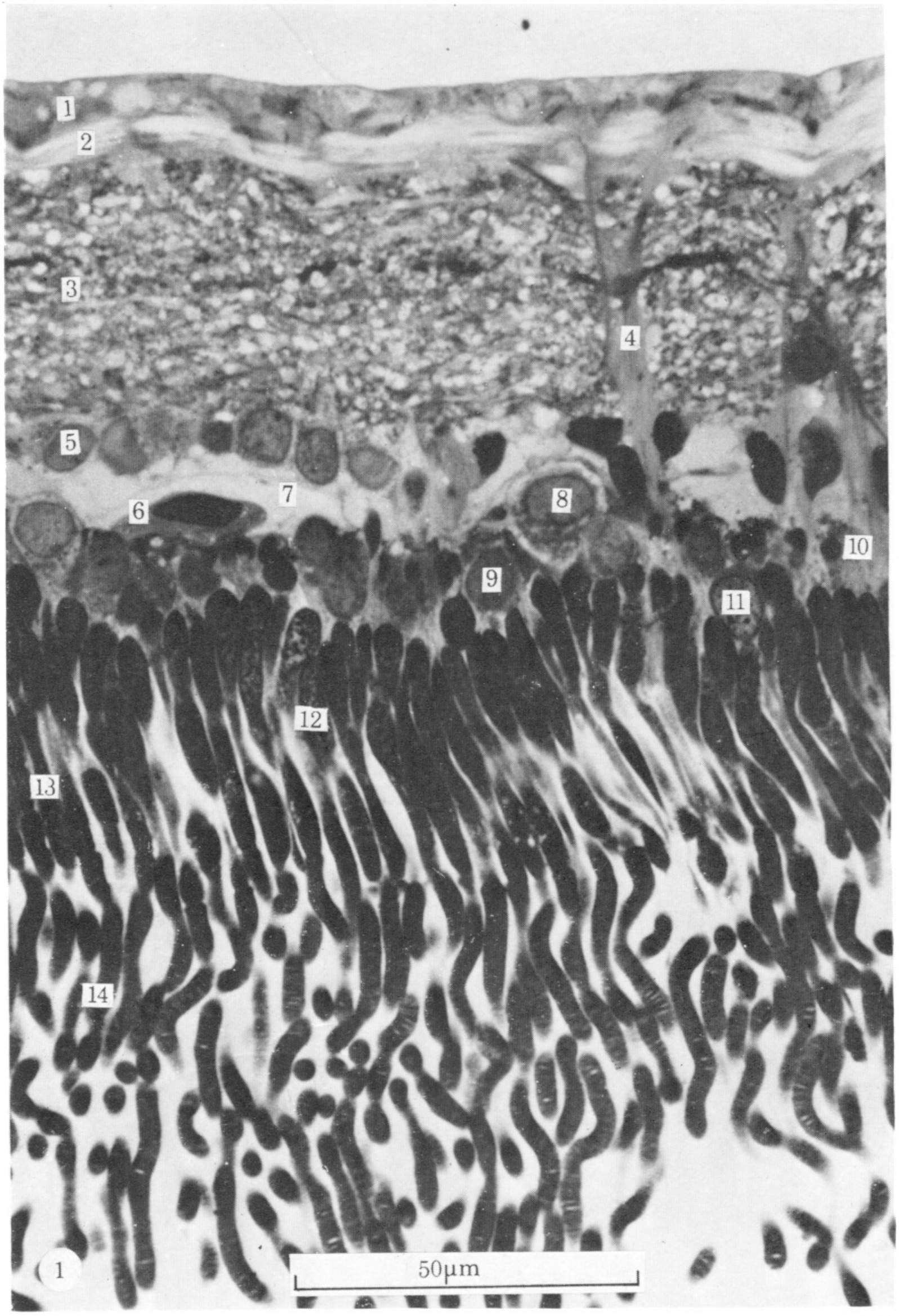

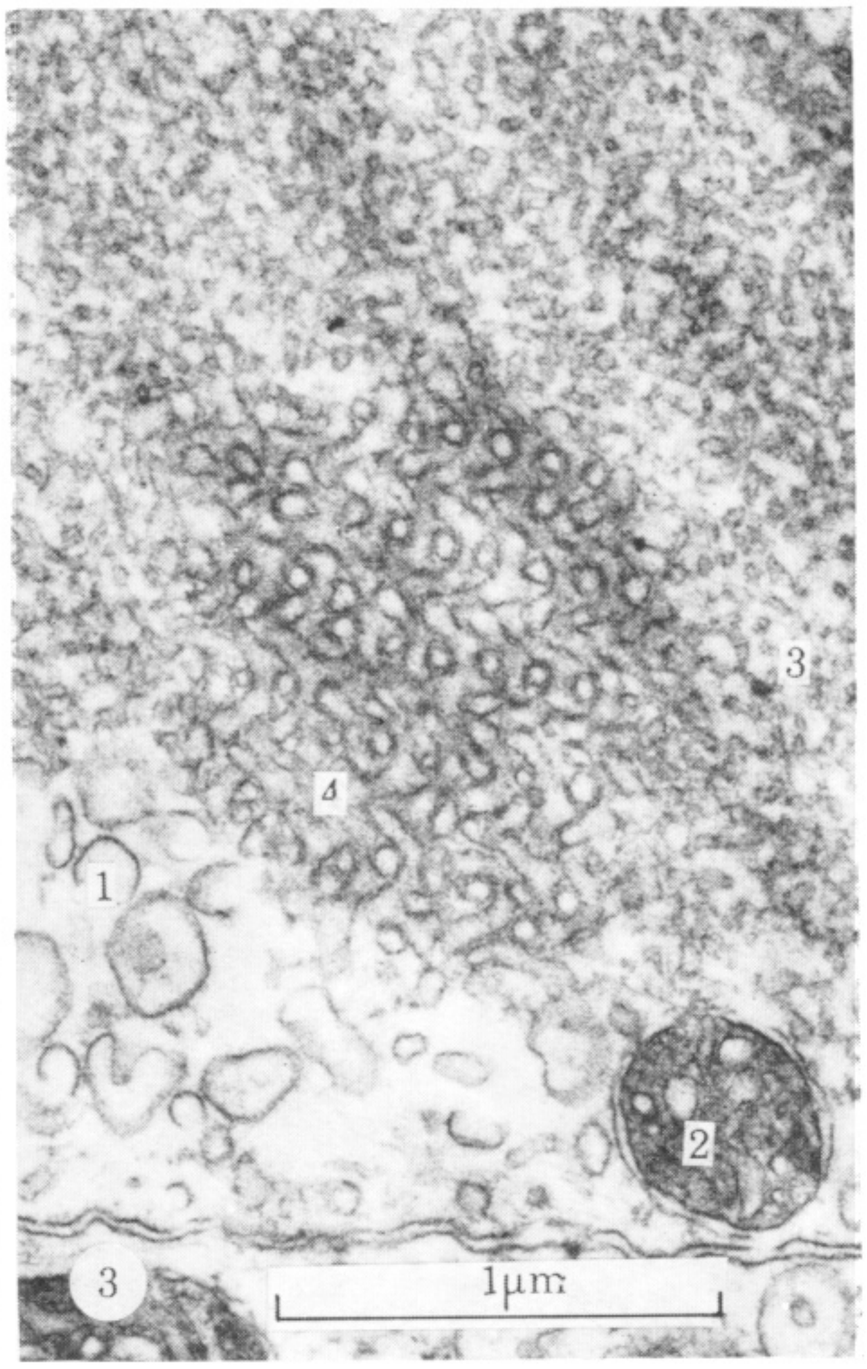

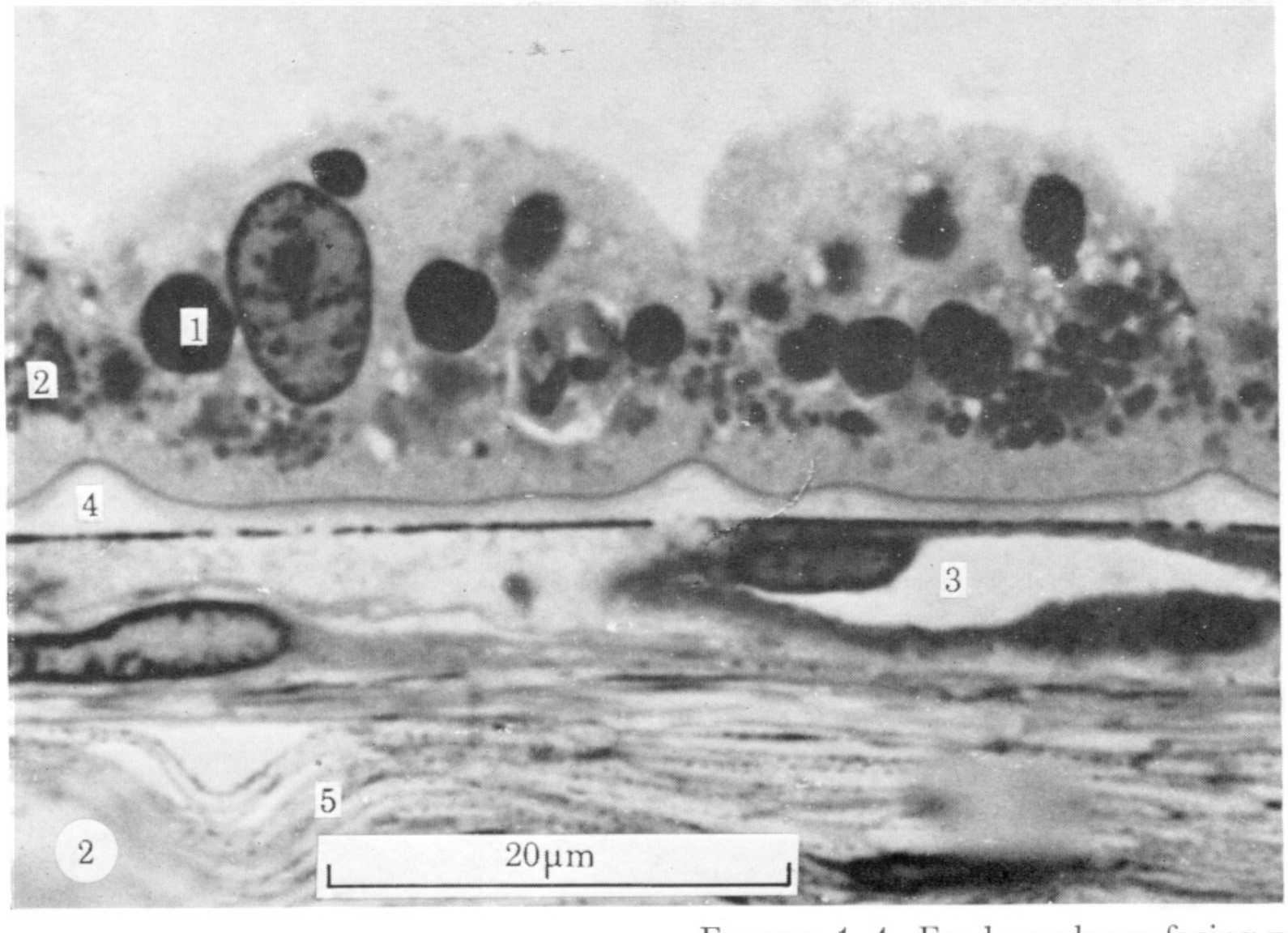

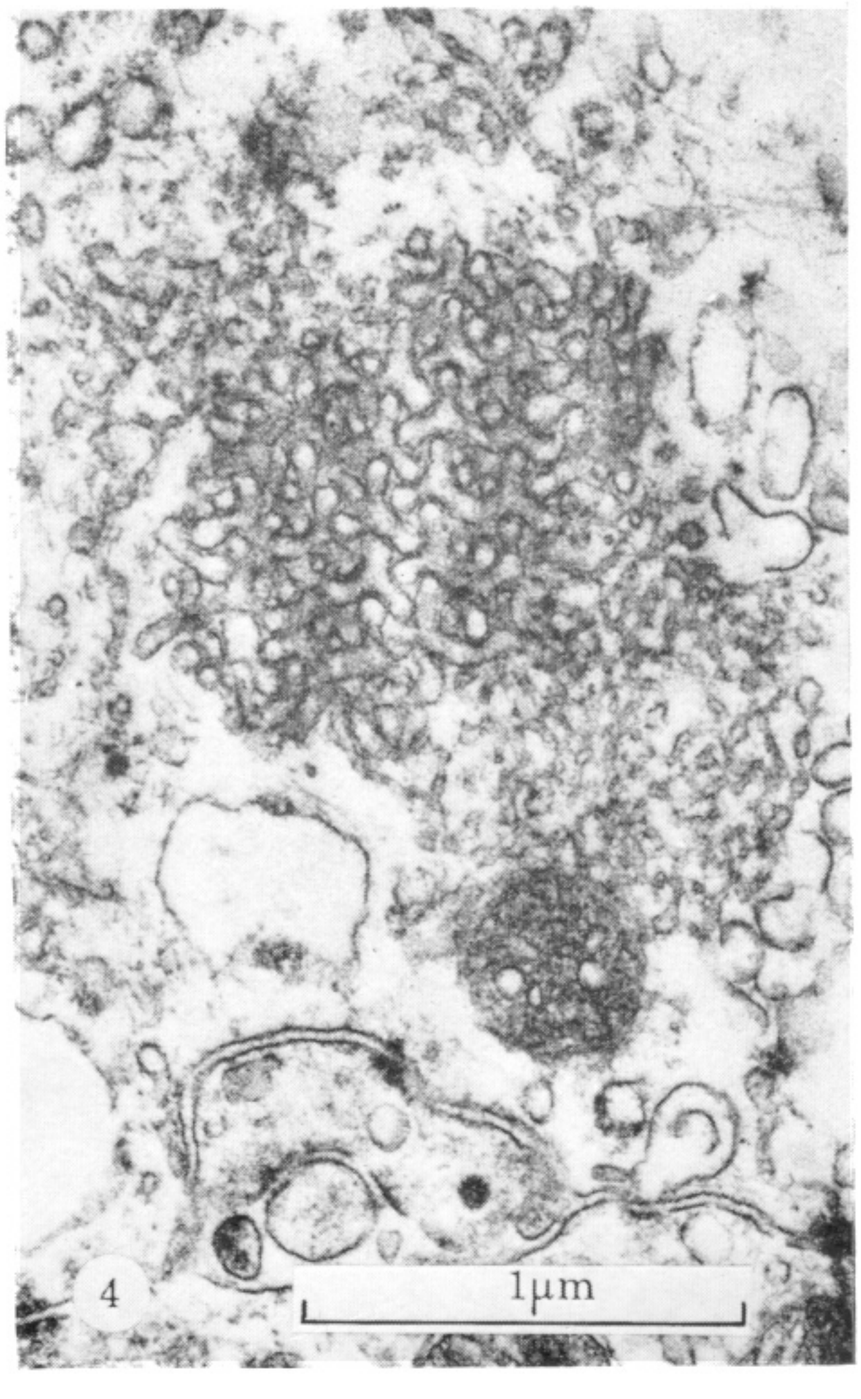

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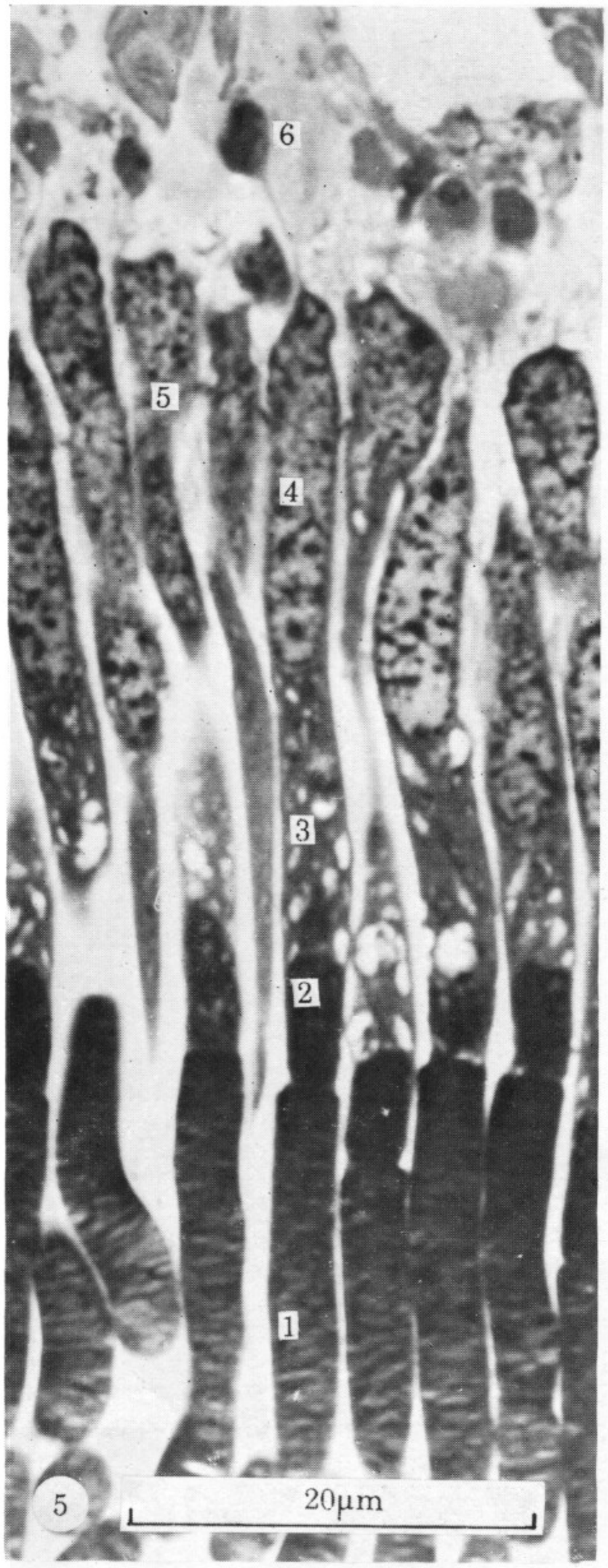
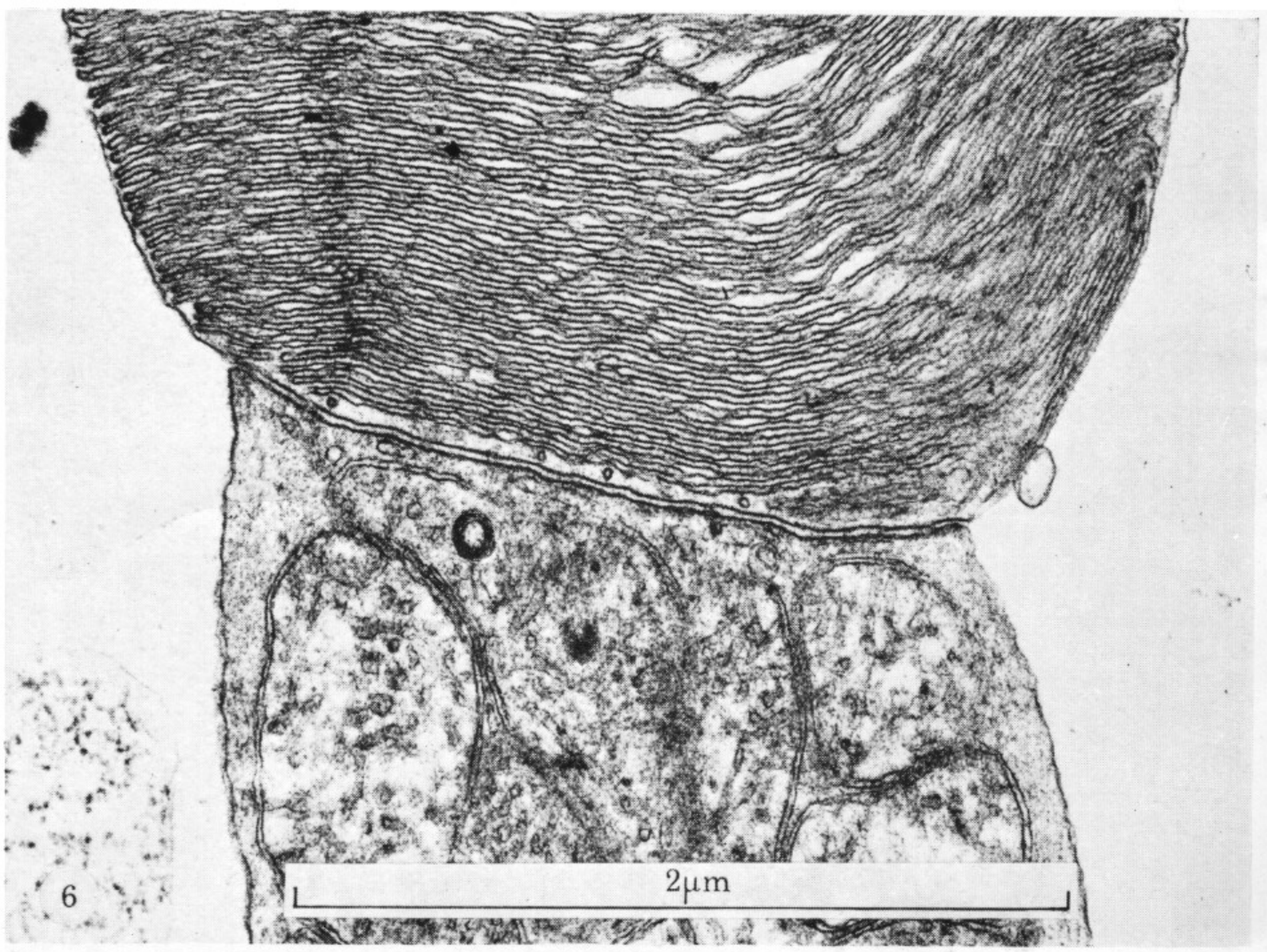




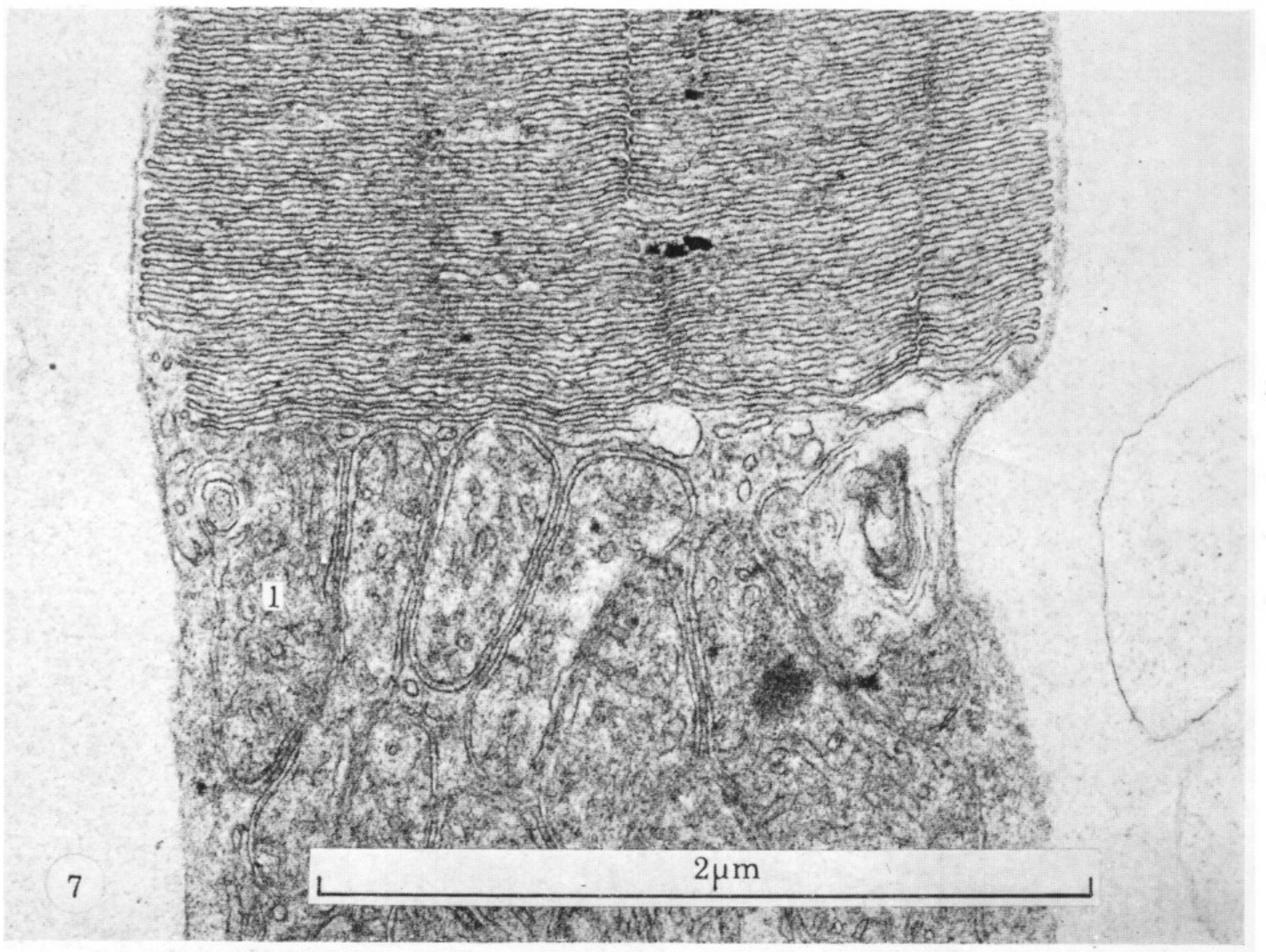
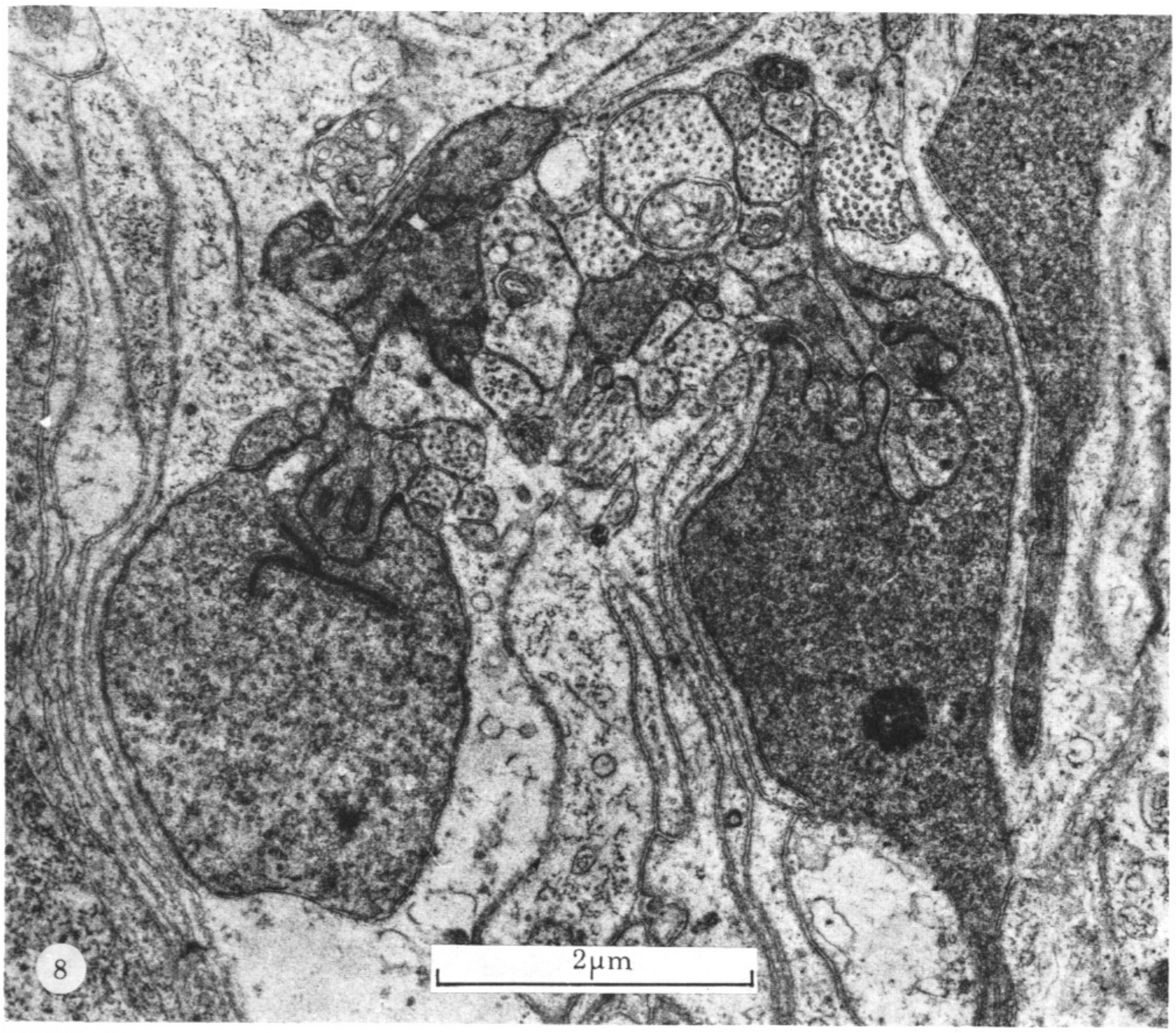


FIGURE 5. Rod, optical micrograph. The outer segment (1) is of larger diameter than the inner segment (2). The myoid (3) and the nuclear region (4) are cylindrical. The nucleus projects through the outer limiting membrane (5). The synaptic spherule (6) is borne on a fine conducting fibre.

FIGURE 6. Junction of rod inner and outer segments, electron micrograph. In this example the inner segment appears entirely separated from the outer segment by a pair of parallel membranes. The outer segment lamellae are enclosed by the plasma membrane. The inner and outer mitochondrial membranes have a similar spacing to the lamellar membranes. The mitochondrial cristae are tubular.

FIGURE 7. In this example there is no sign of the parallel membranes, and the inner and outer segments are in full cytoplasmic continuity across the width of the rod. Some mitochondria (1) appear disrupted, and apparently incomplete lamellae occur at the junctional region.



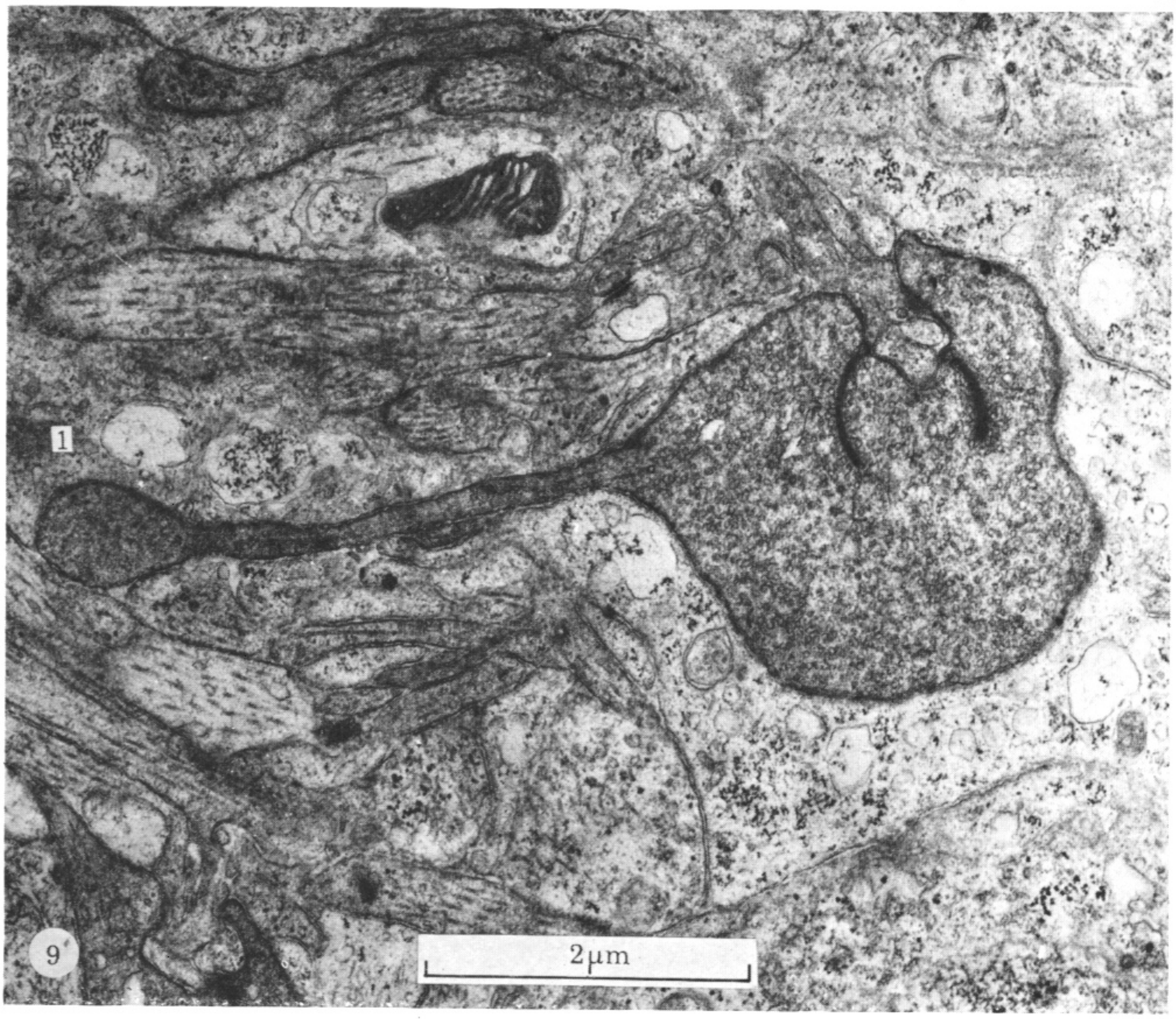
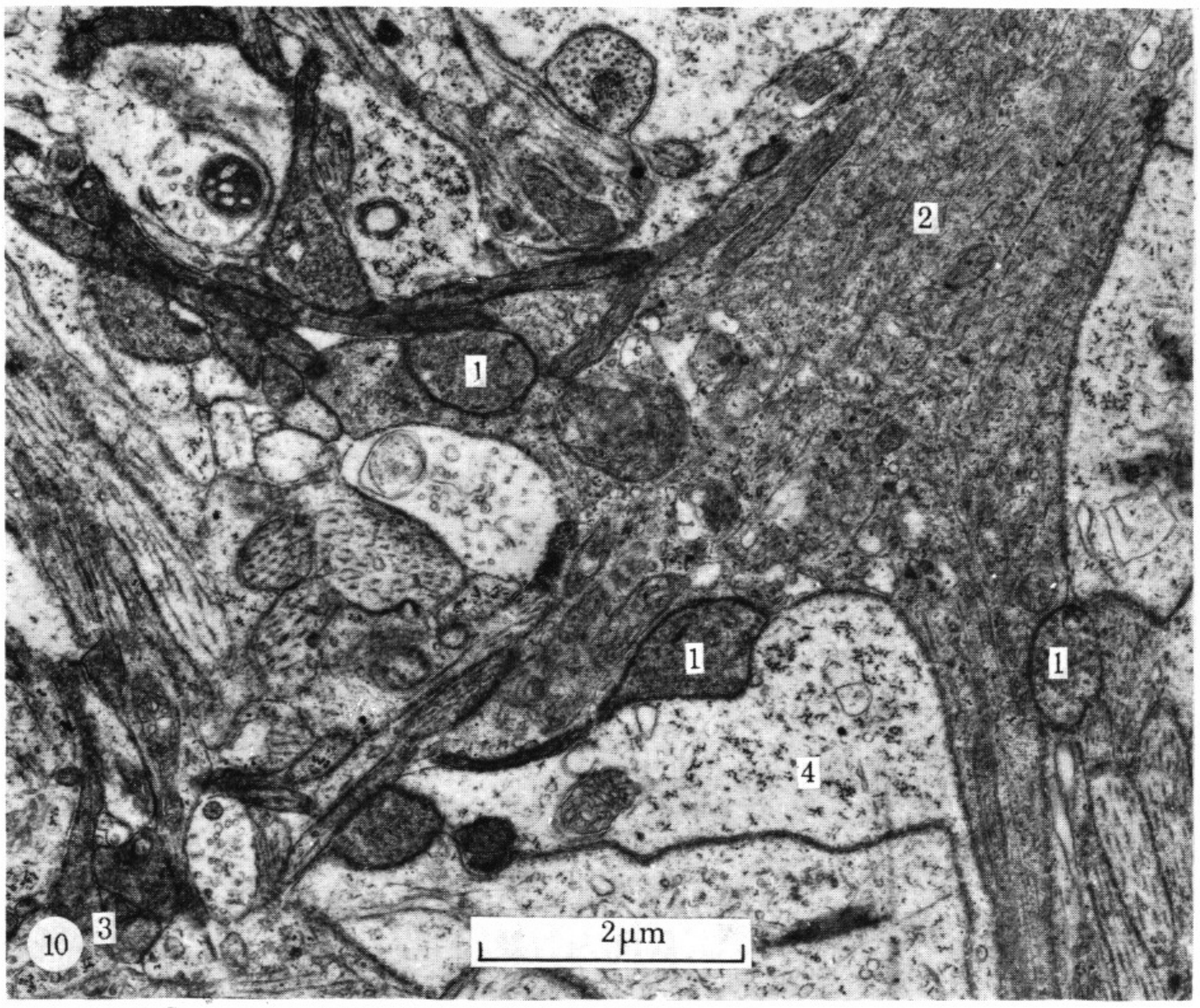


FIGURE 8. Two rod synaptic spherules. The rod synapses are of the oligosynaptic type, with few processes making contact. Fingers of rod cytoplasm project into the terminal cavity amongst the processes; three were present in a rod spherule examined in serial sections. As usual in random sections no sign of the basal filament is seen.

FIGURE 9. Rod basal filament, tangential section. This micrograph, one of a series, shows a basal filament throughout its length from the rod spherule to the expanded club. The spherule contains synaptic vesicles, but the club has a granular contents. The end club is in close contact with dark horizontal cell cytoplasm (1) but there is no sign of synaptic specialization at this site.



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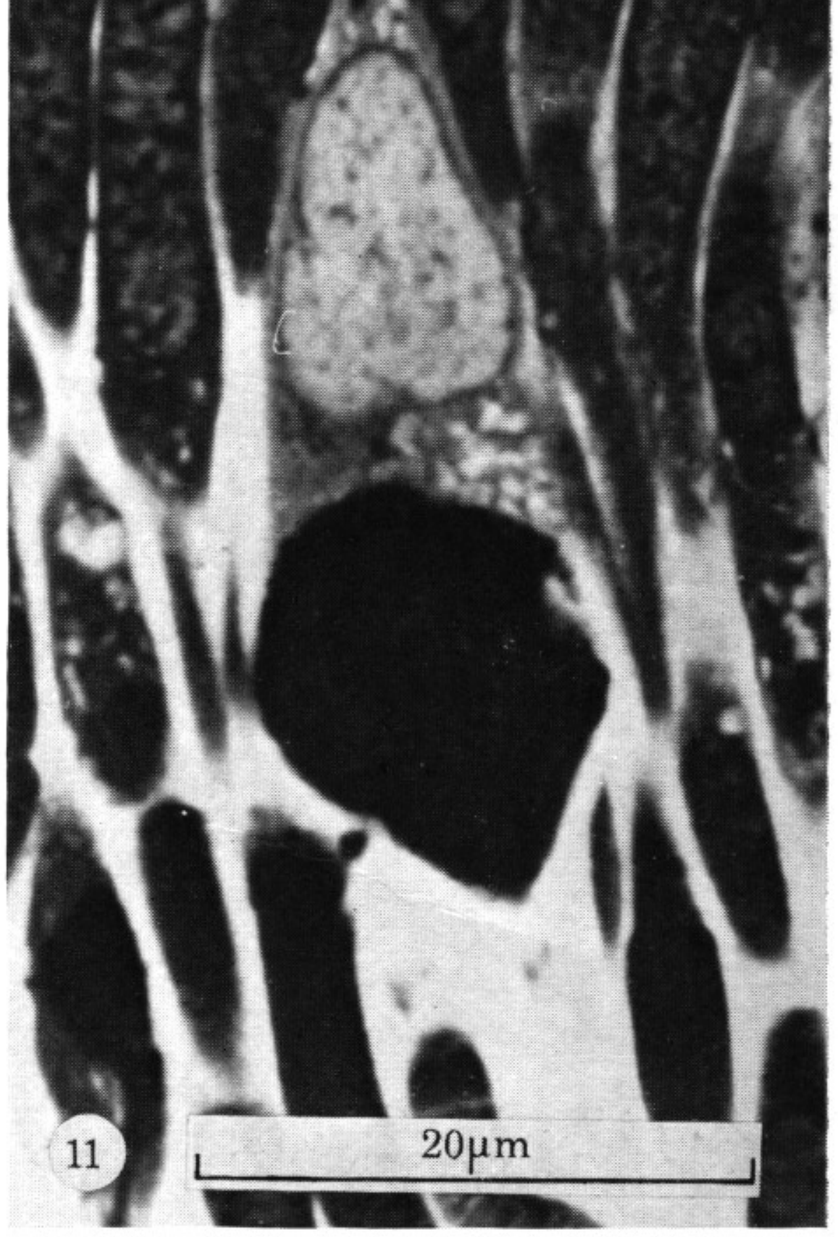

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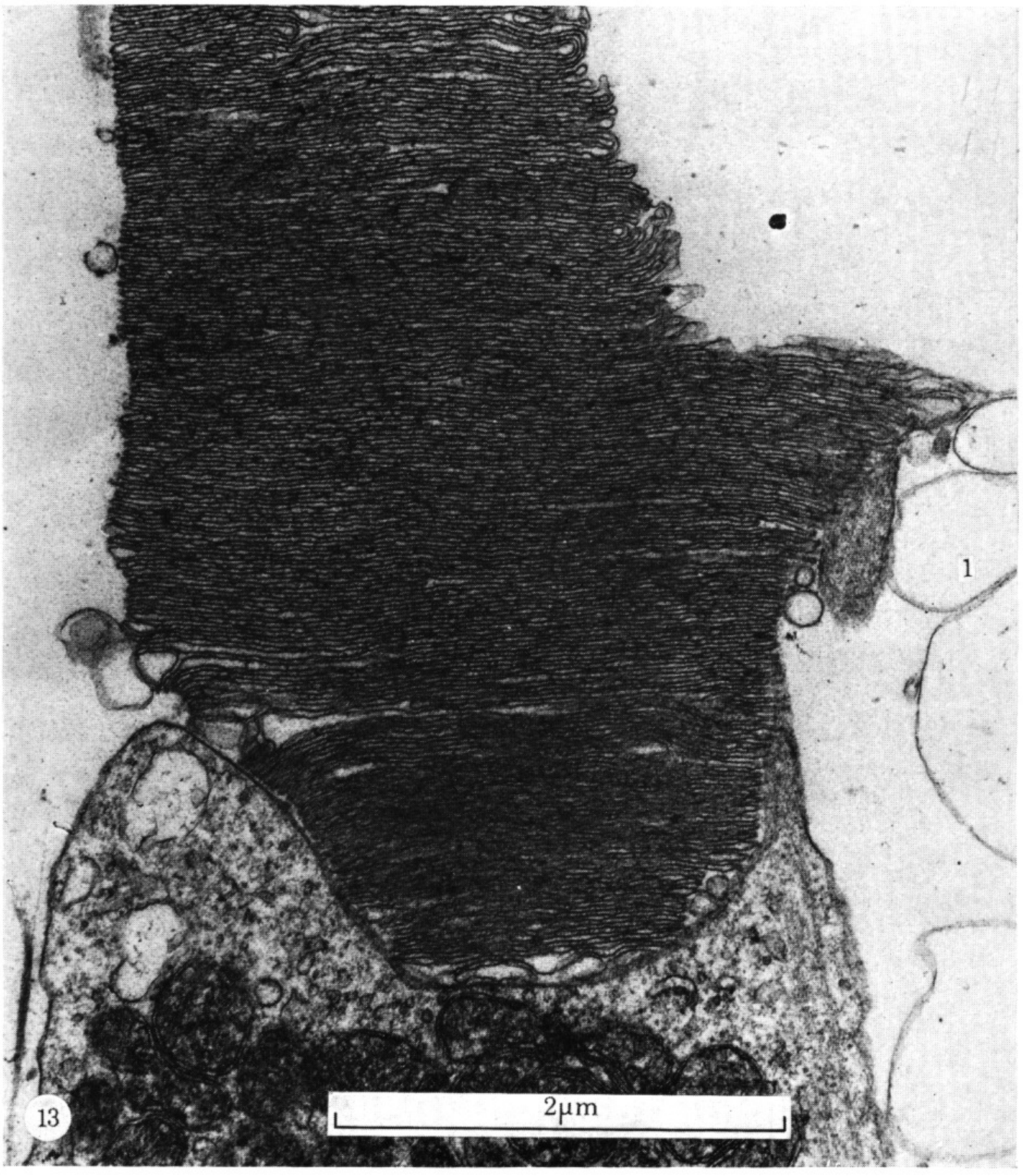

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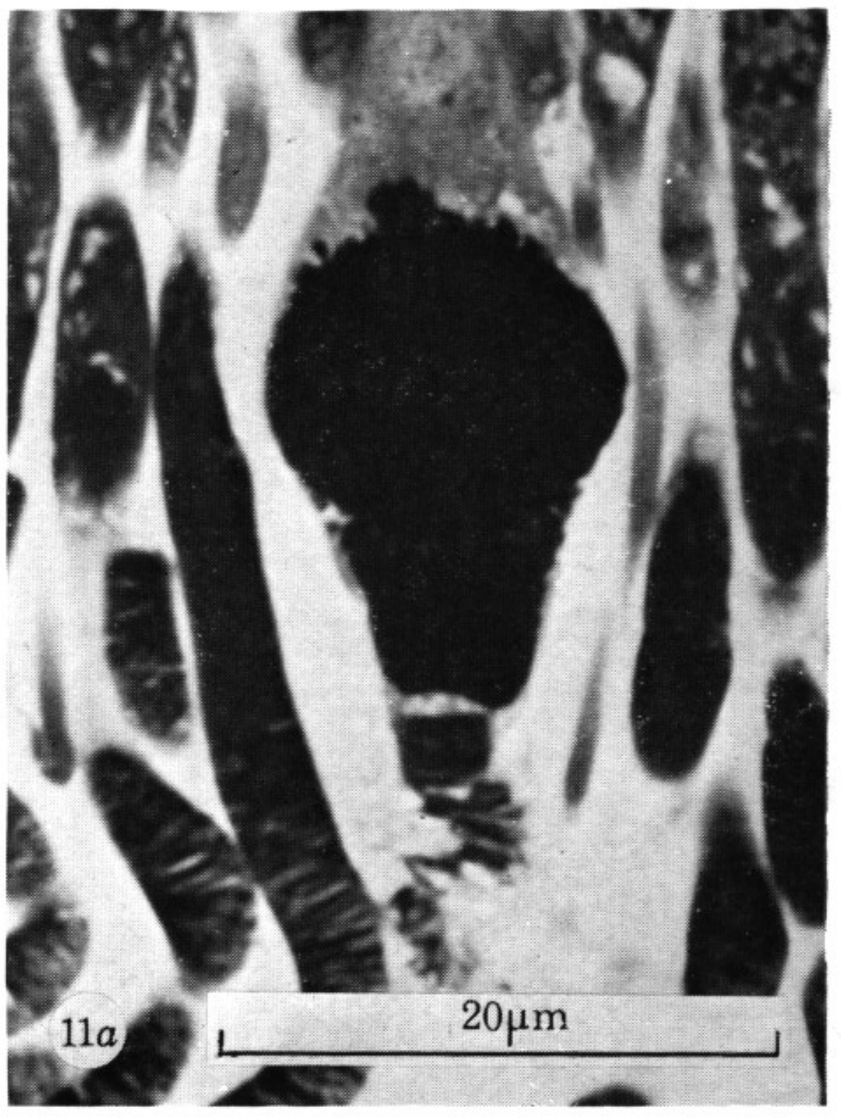

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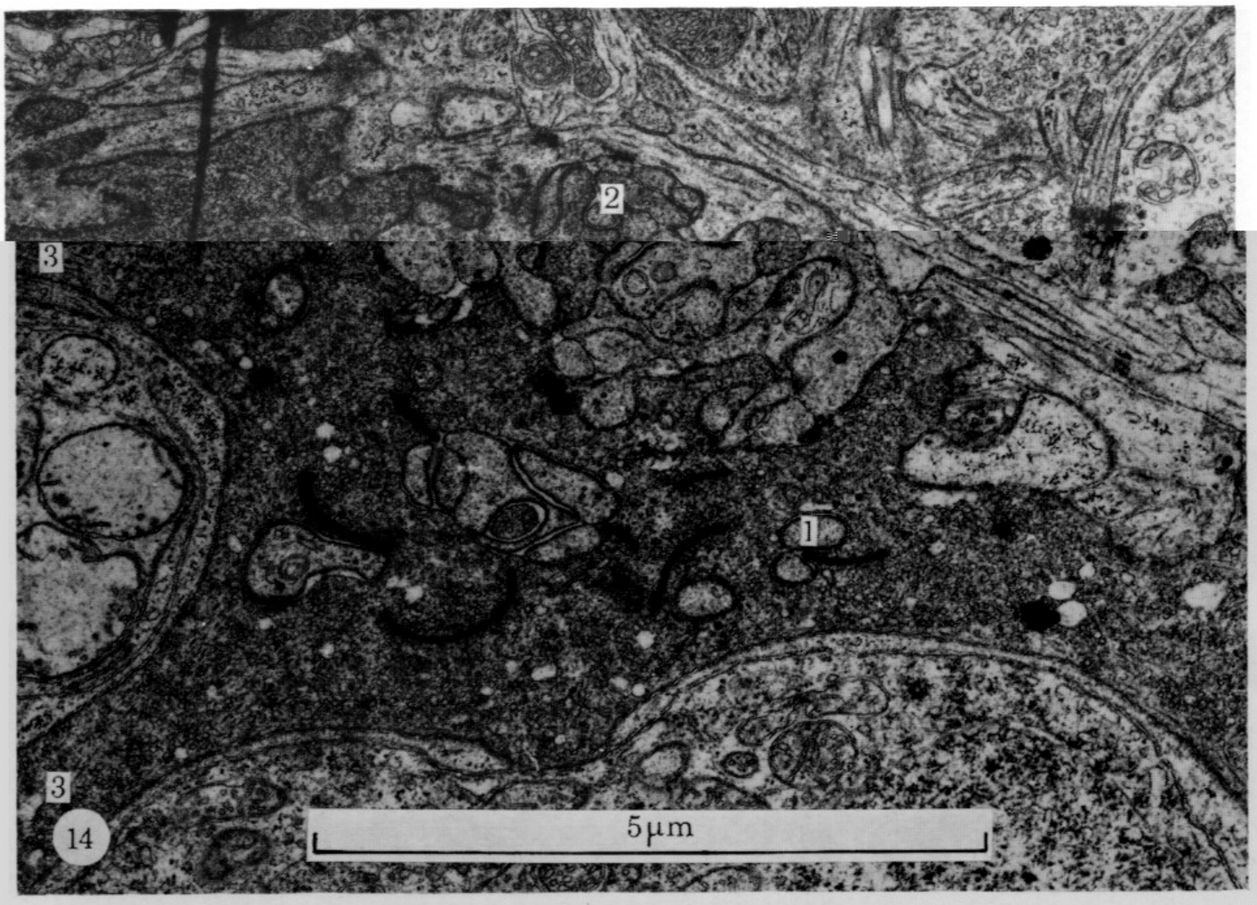

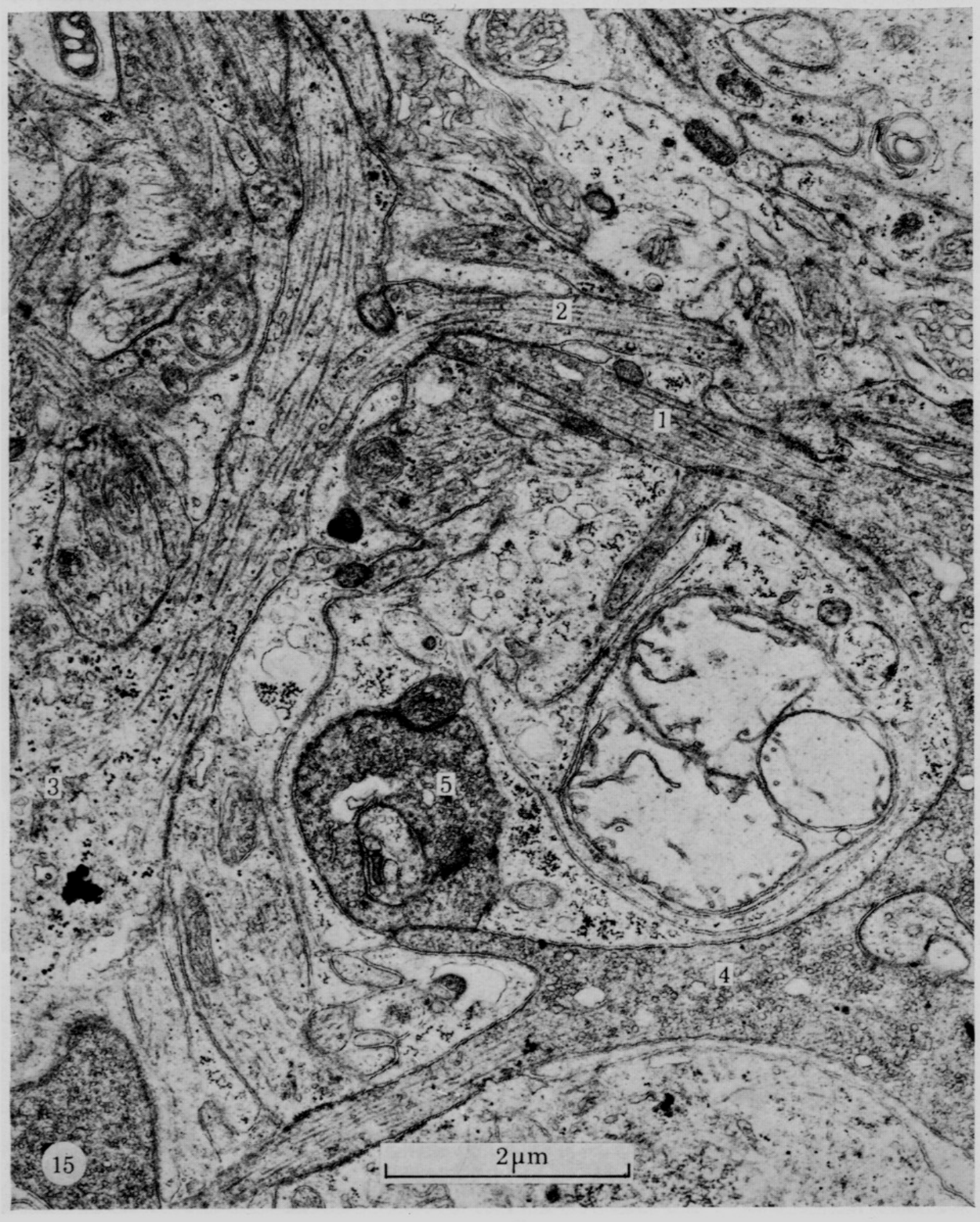

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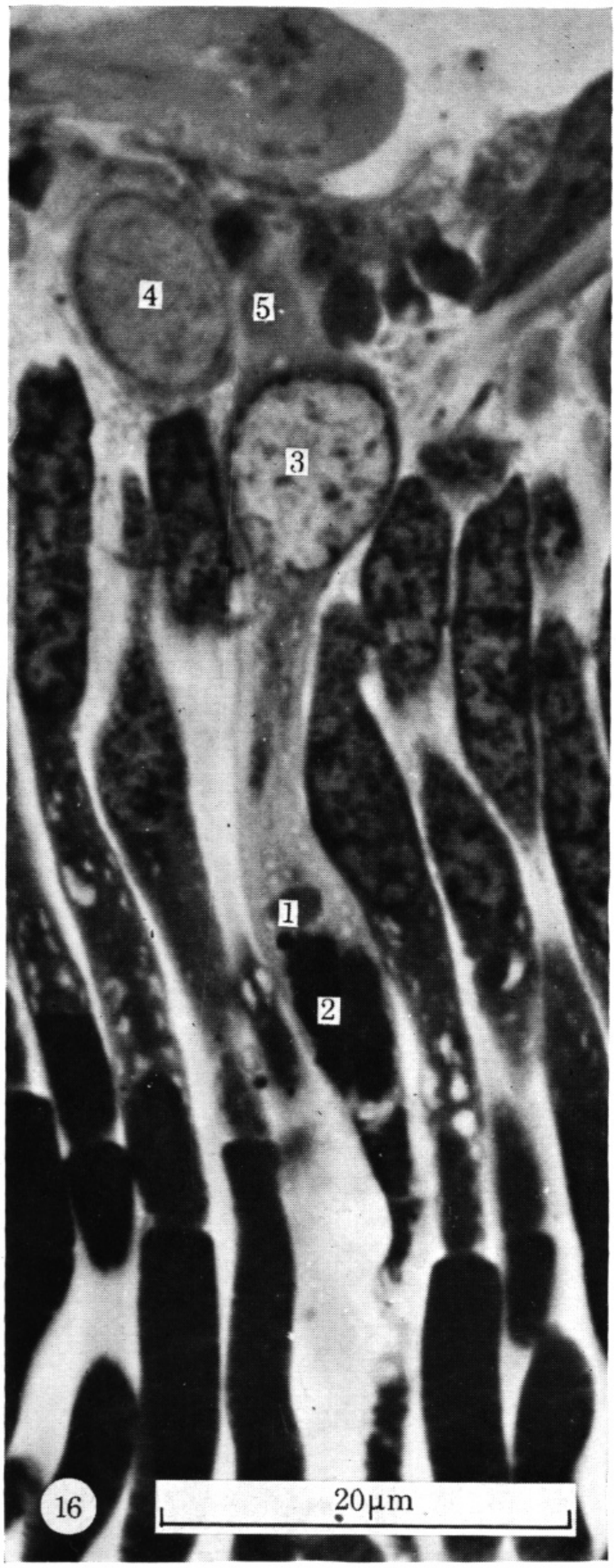

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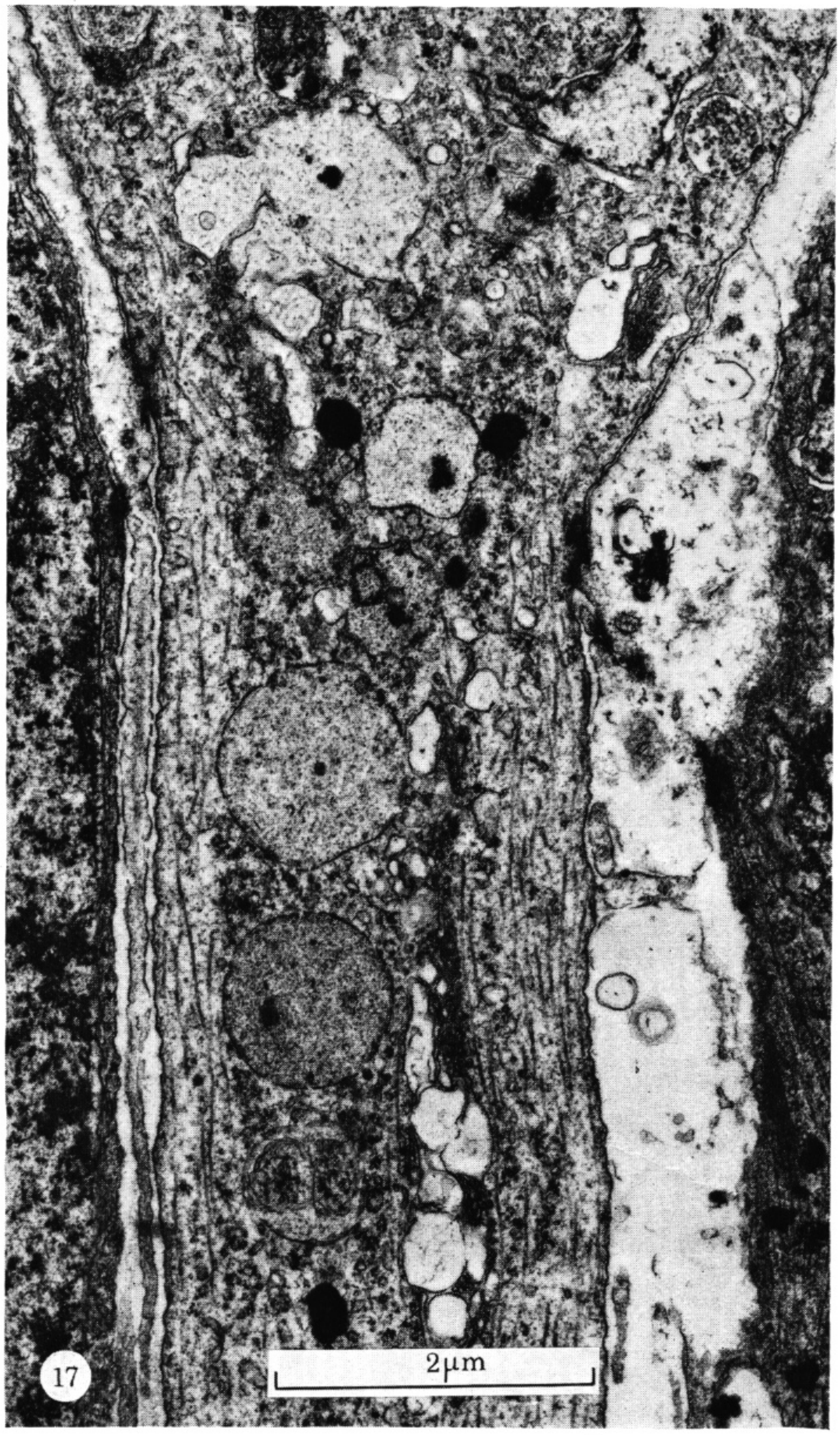

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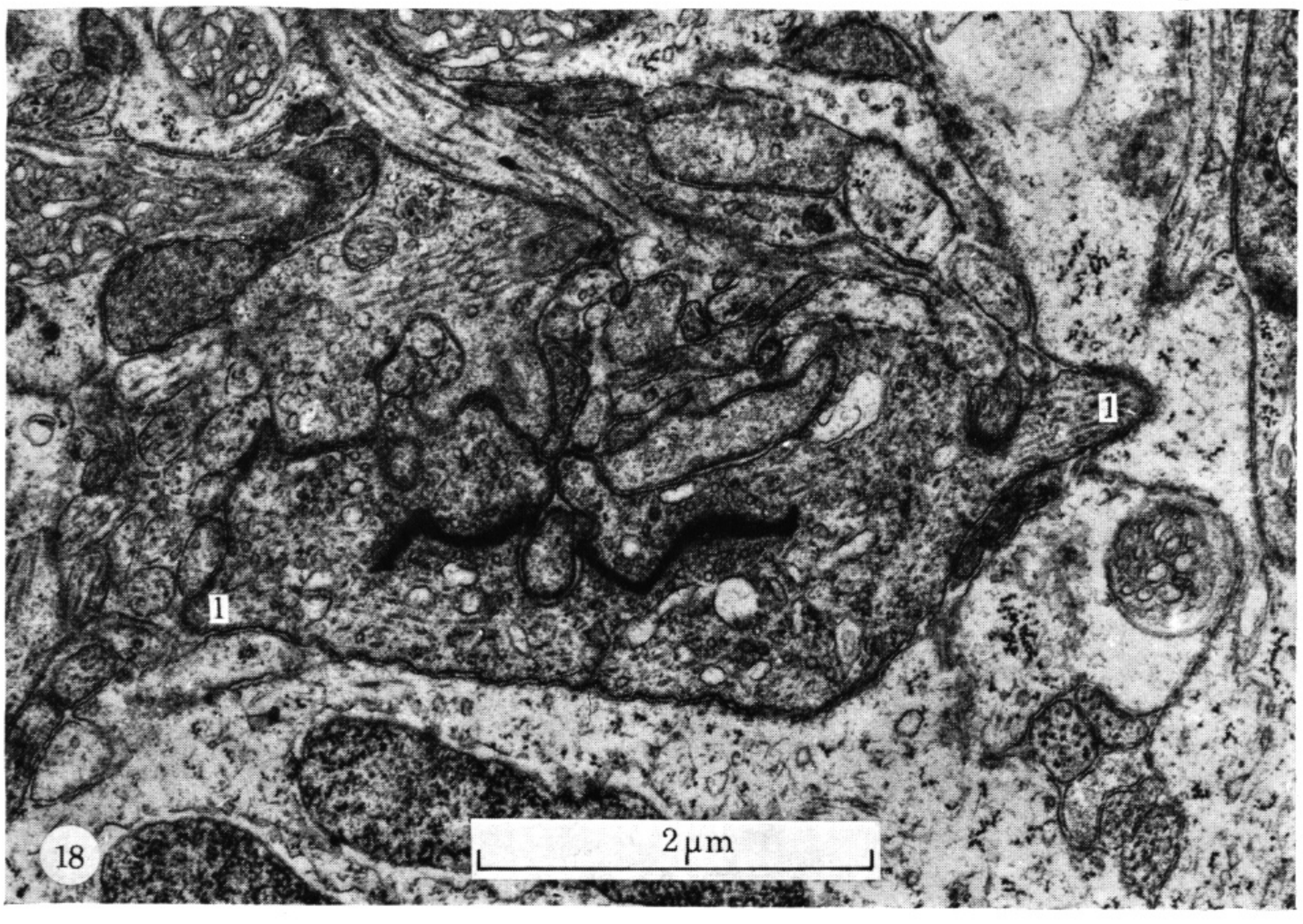

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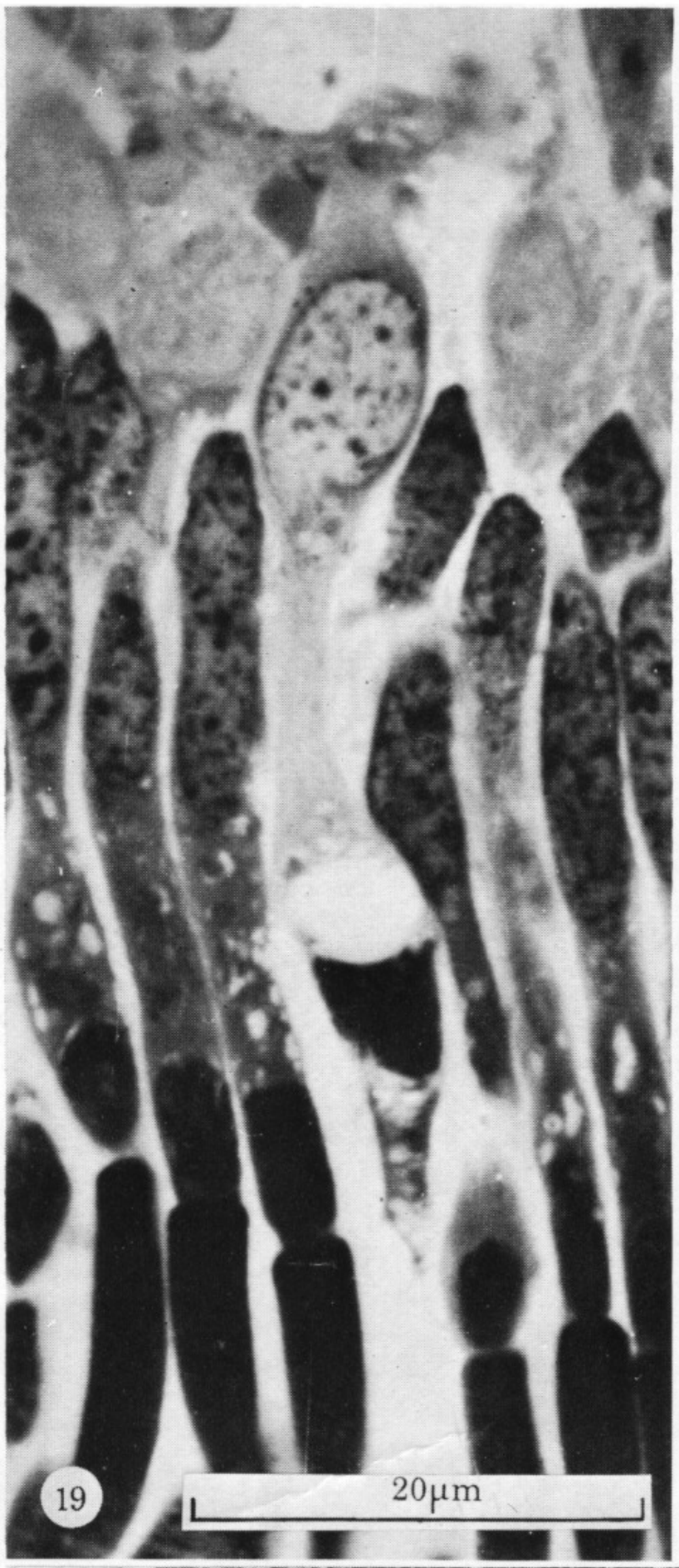
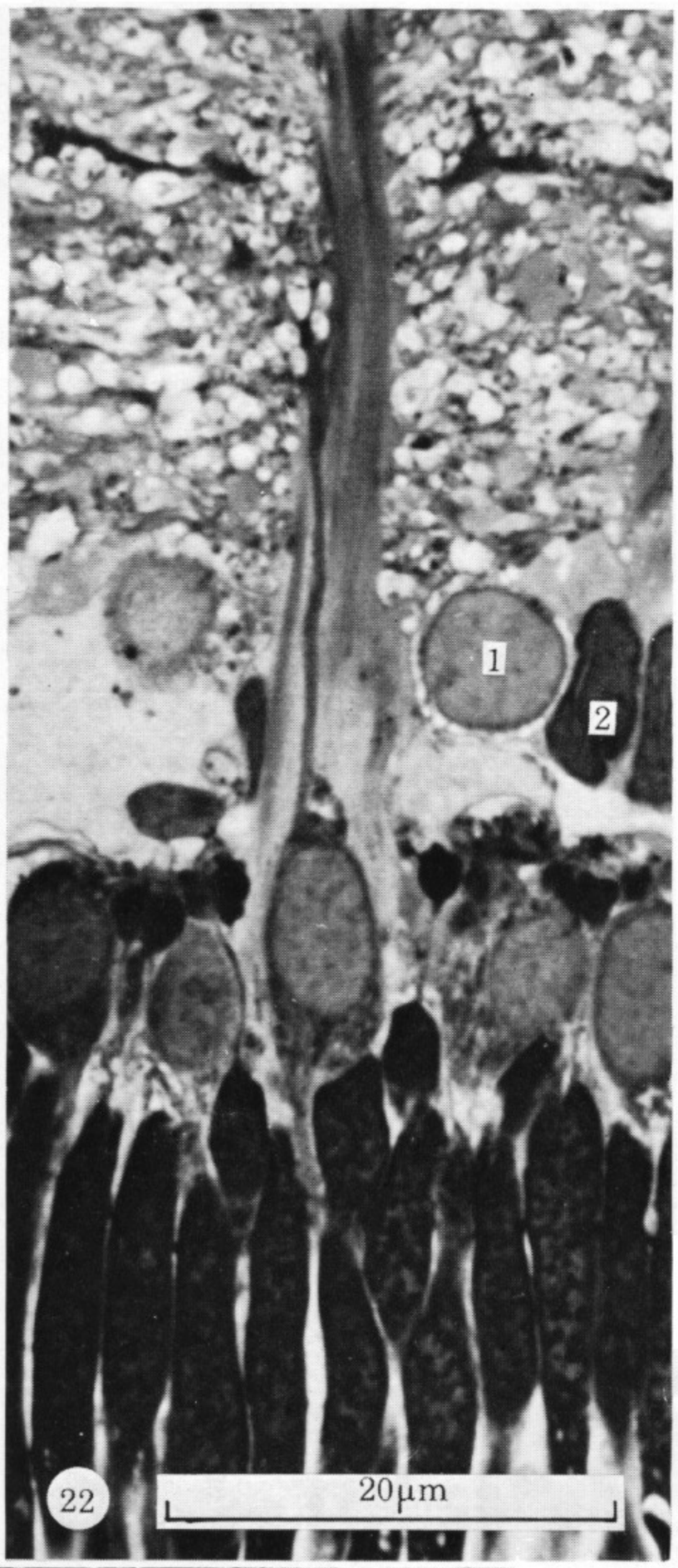
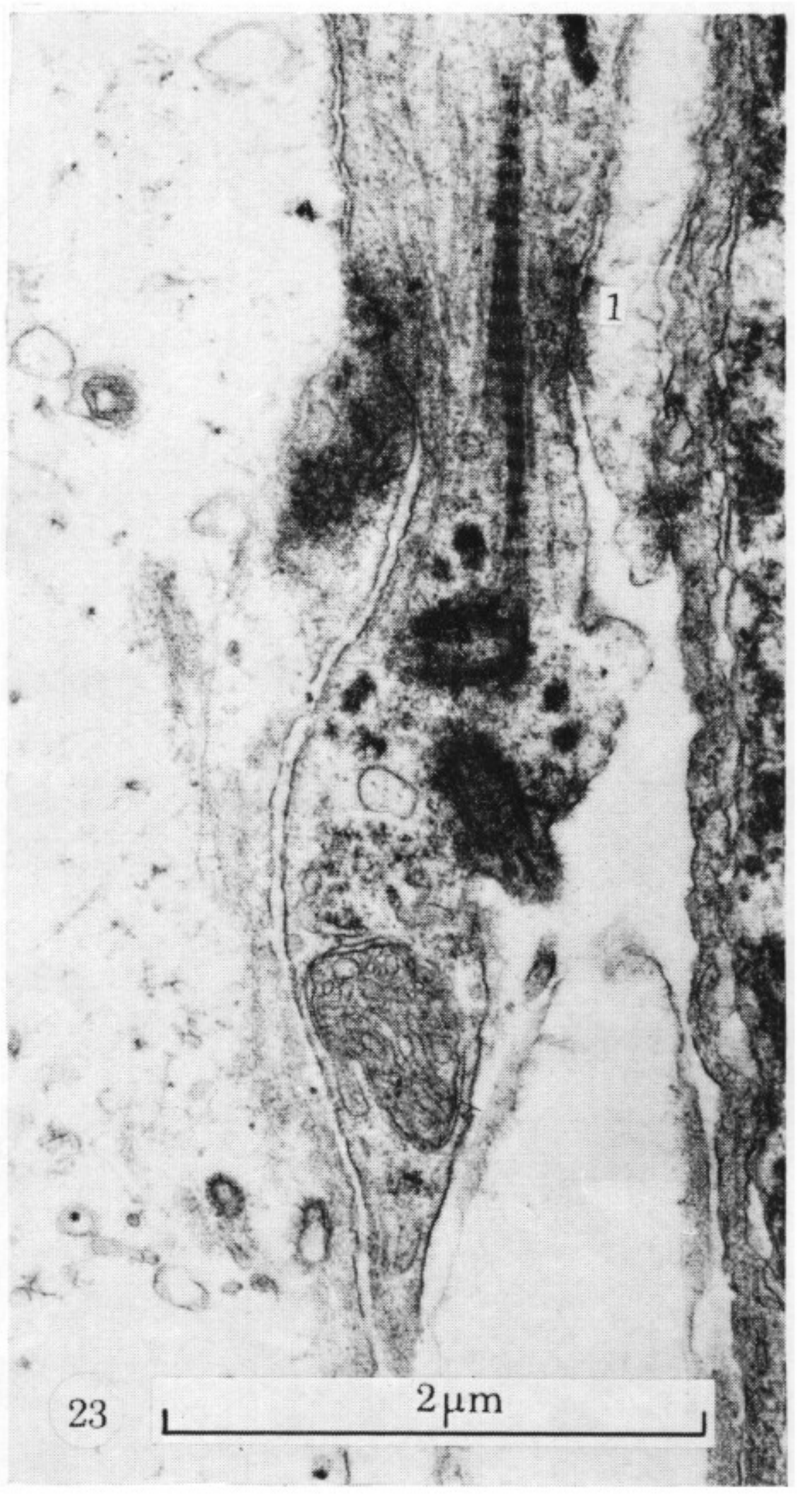
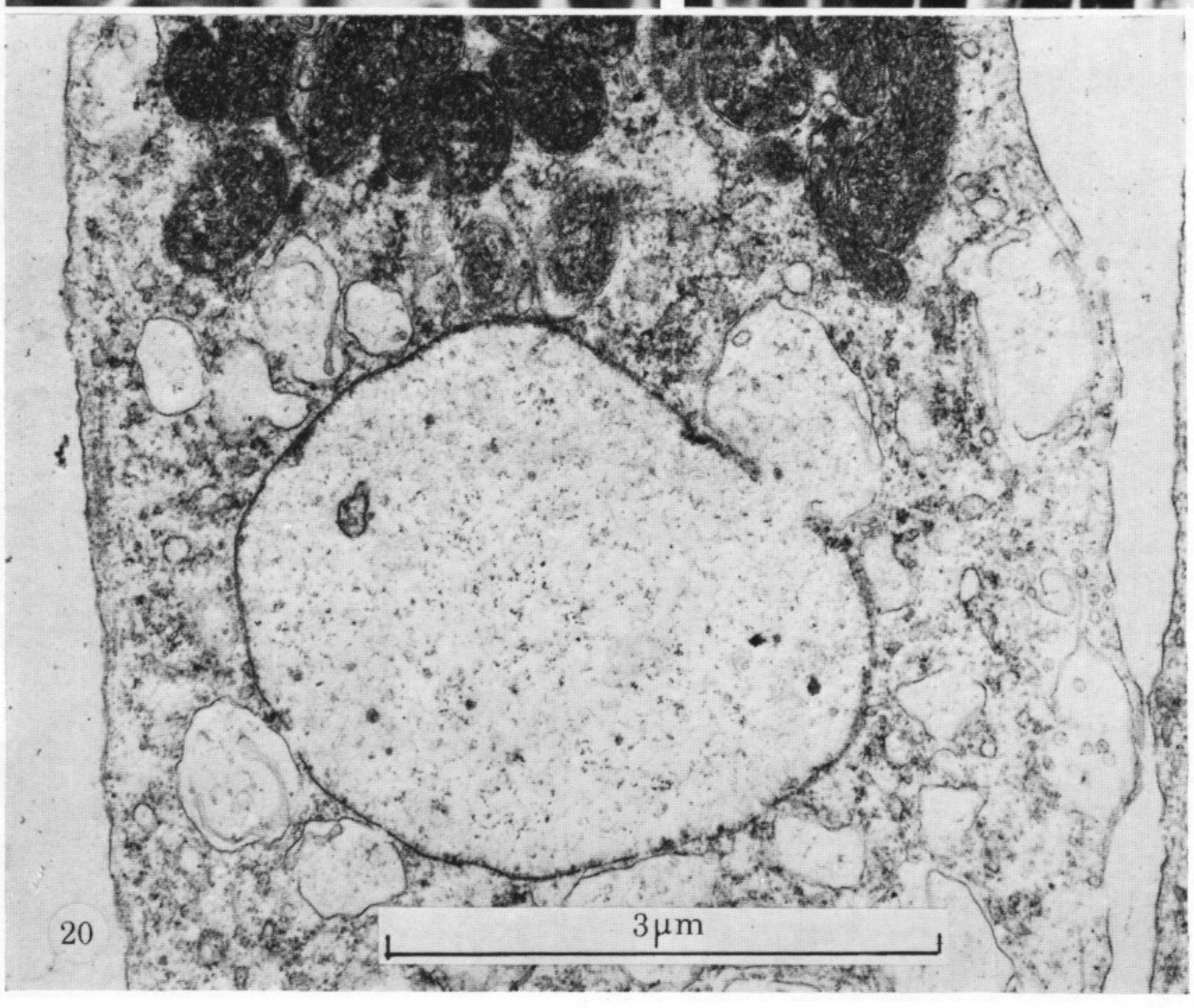
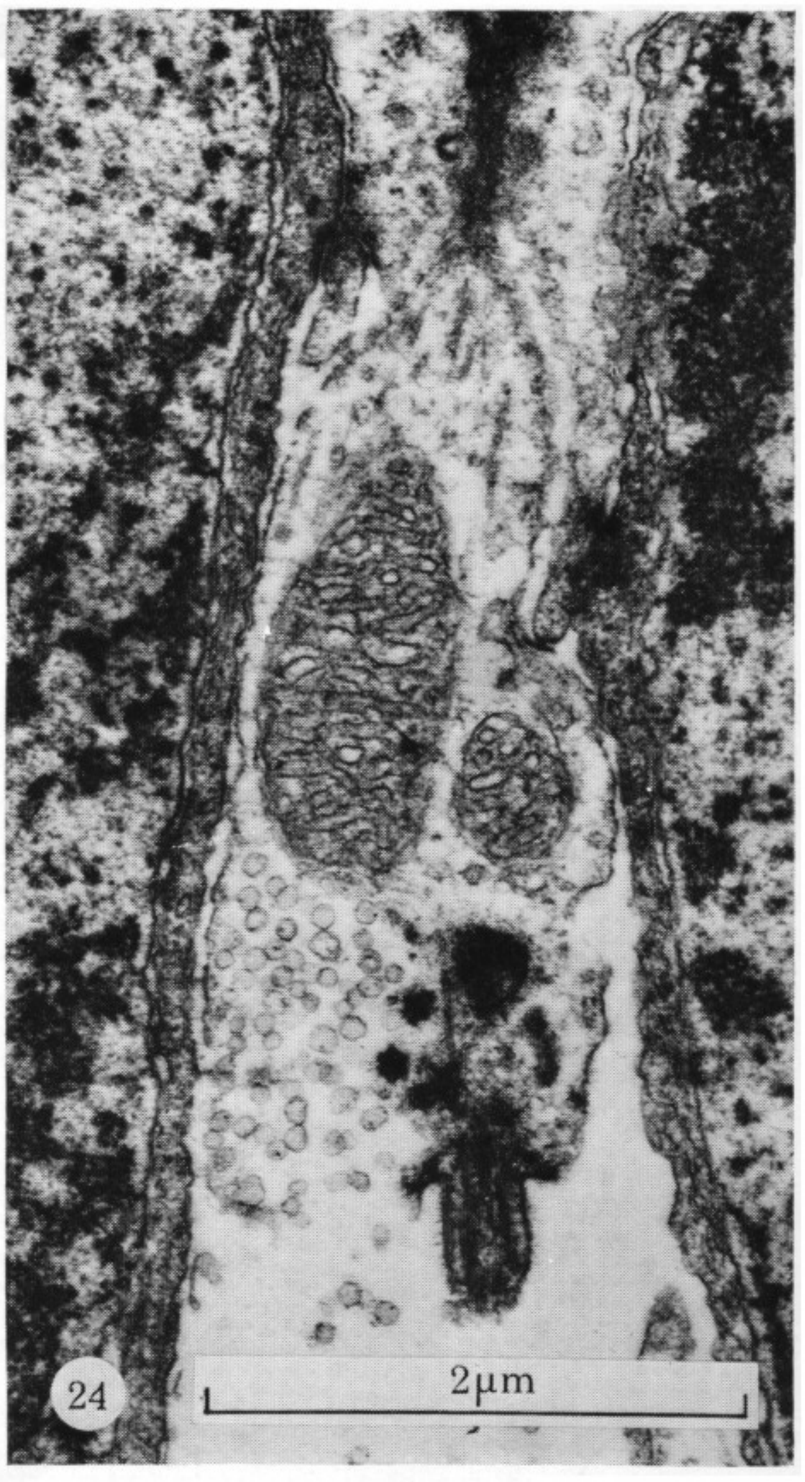


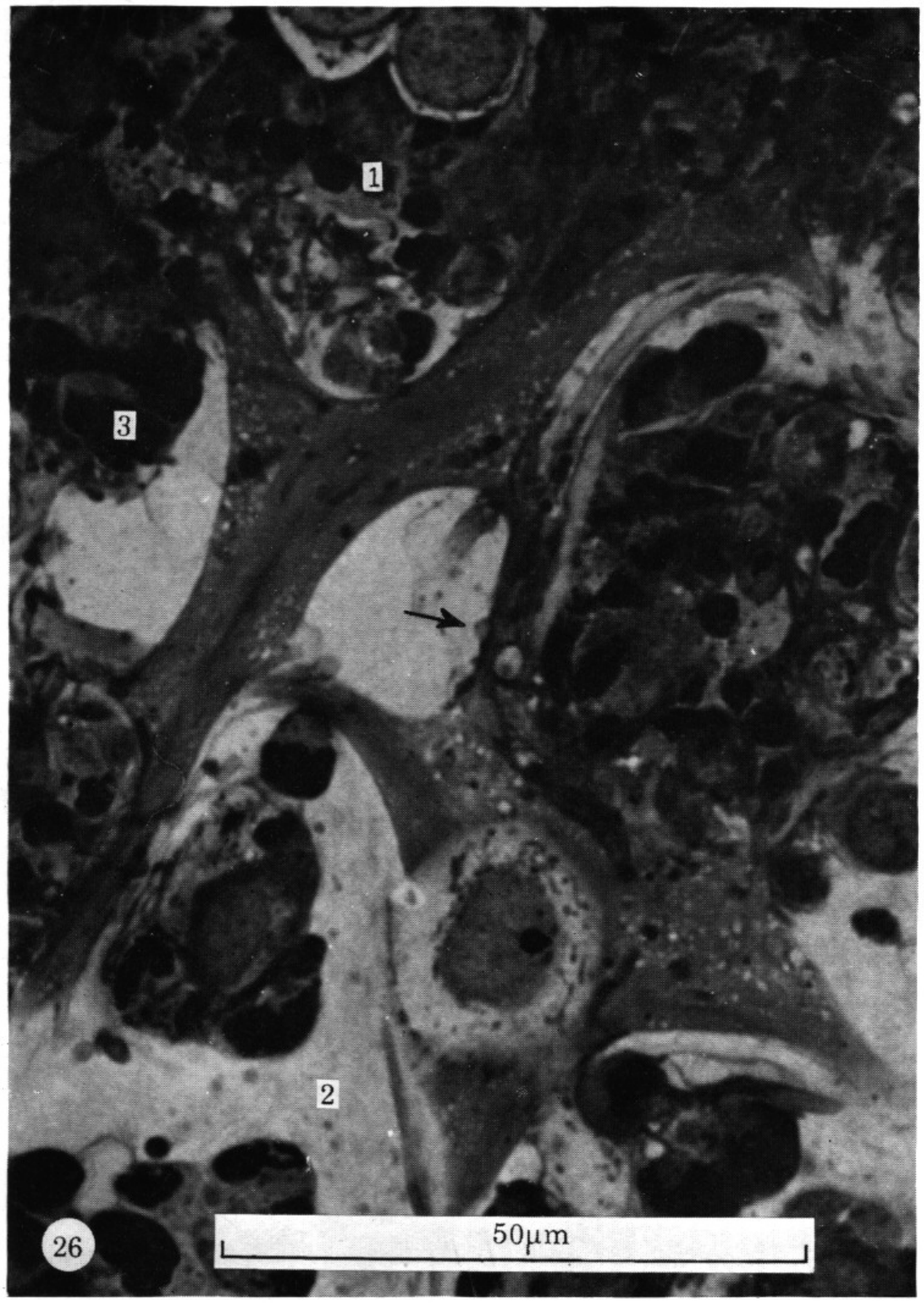








FIGURES 19, 20; 22–24. For legends see facing page



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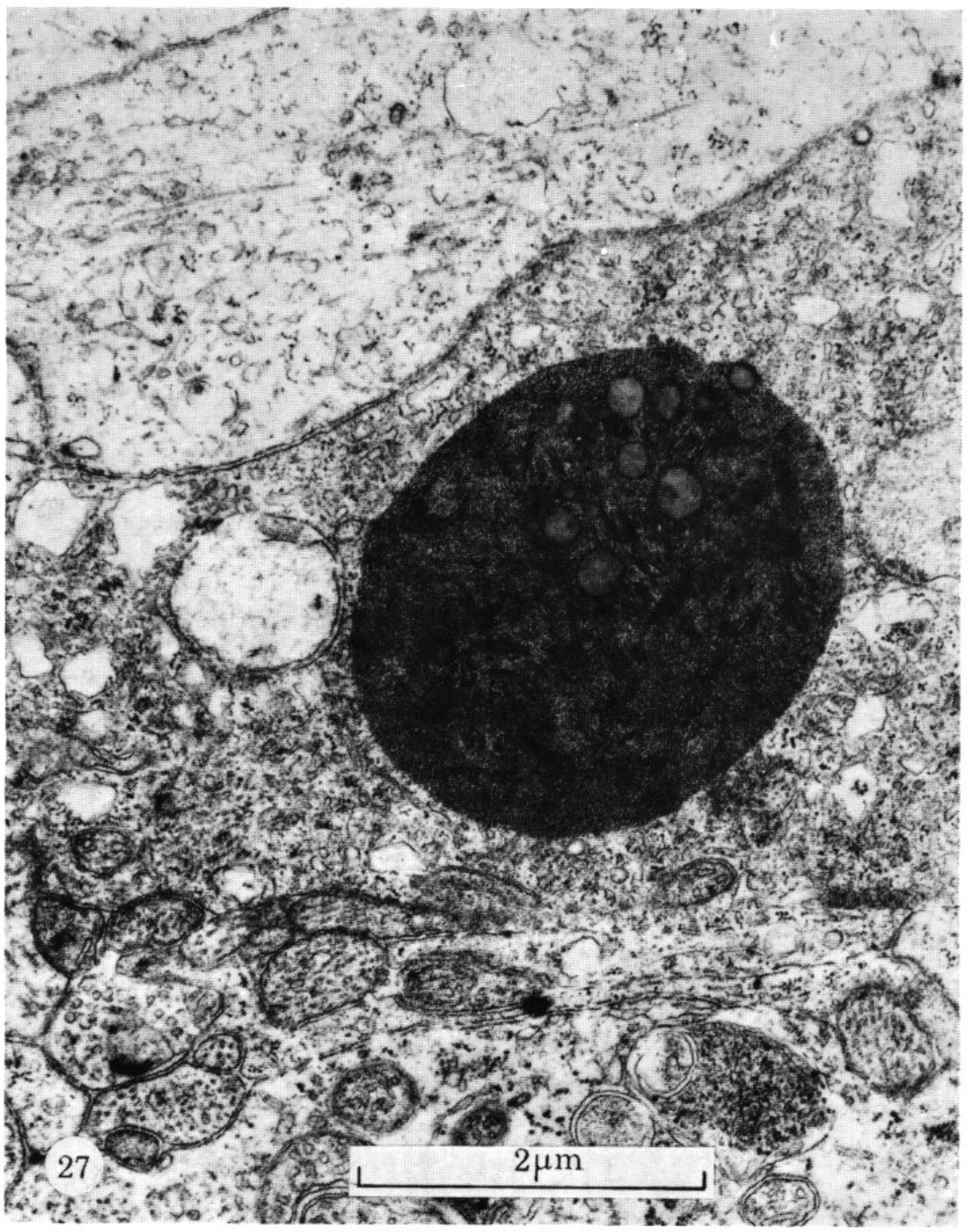

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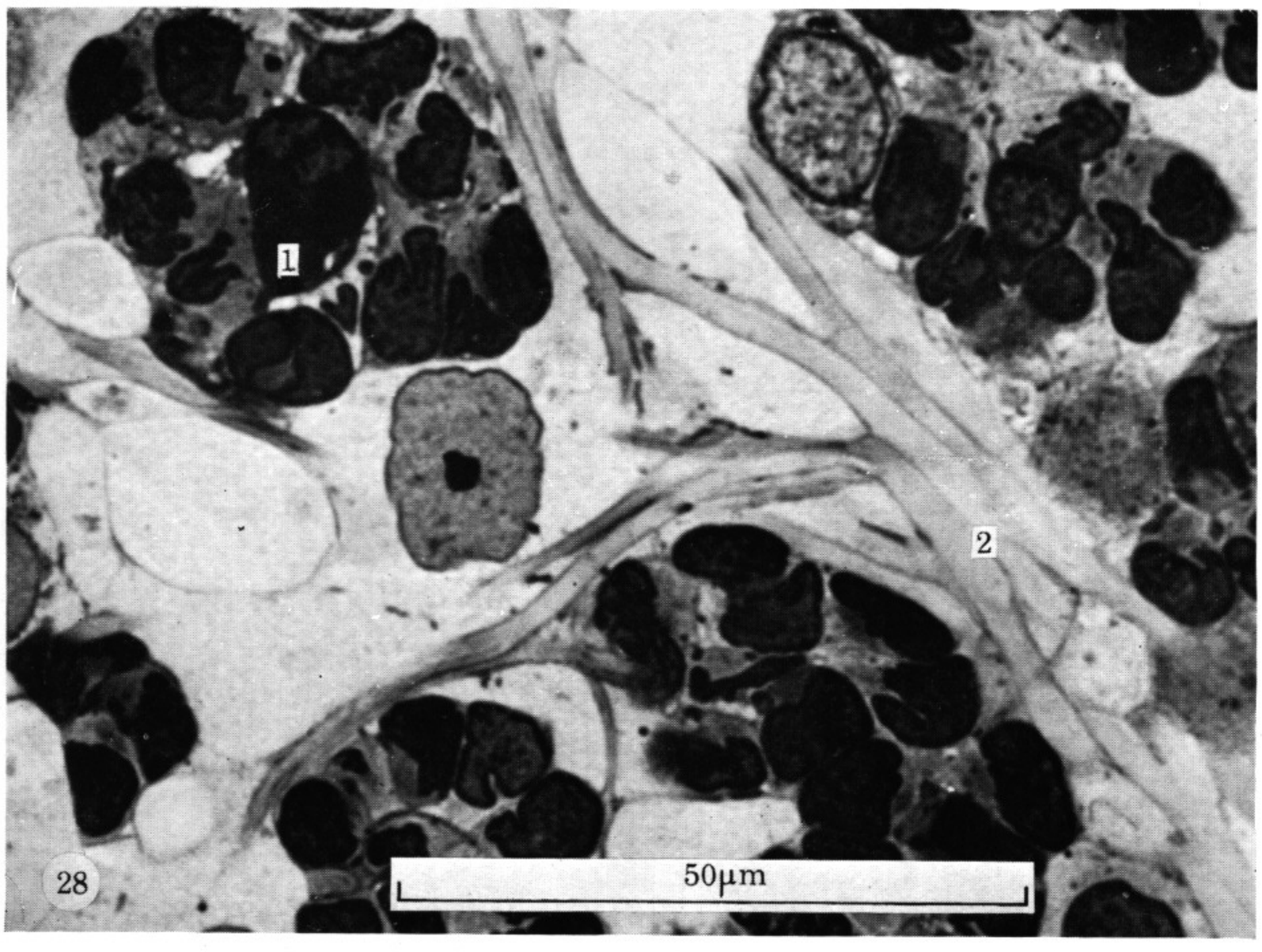

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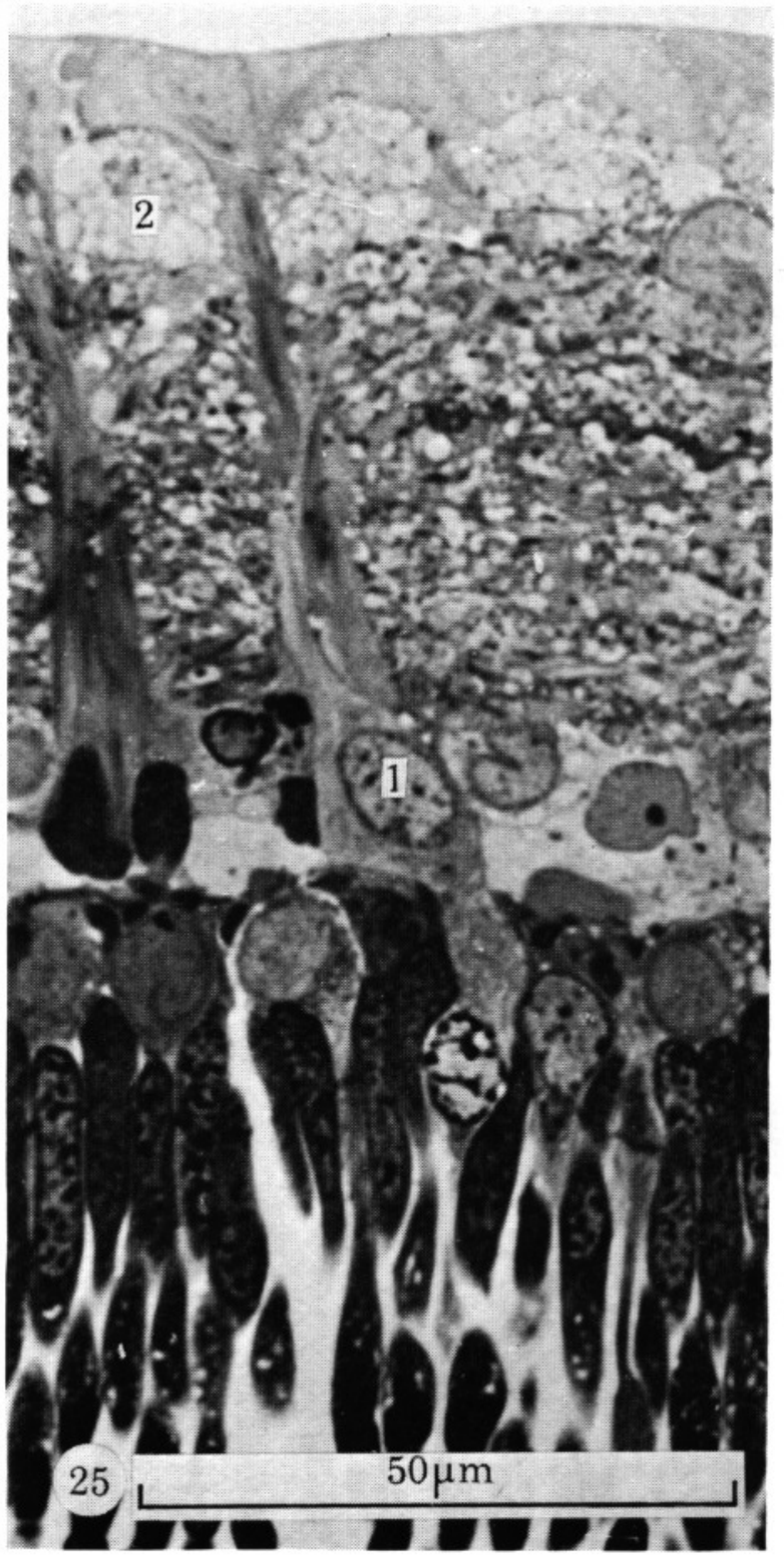

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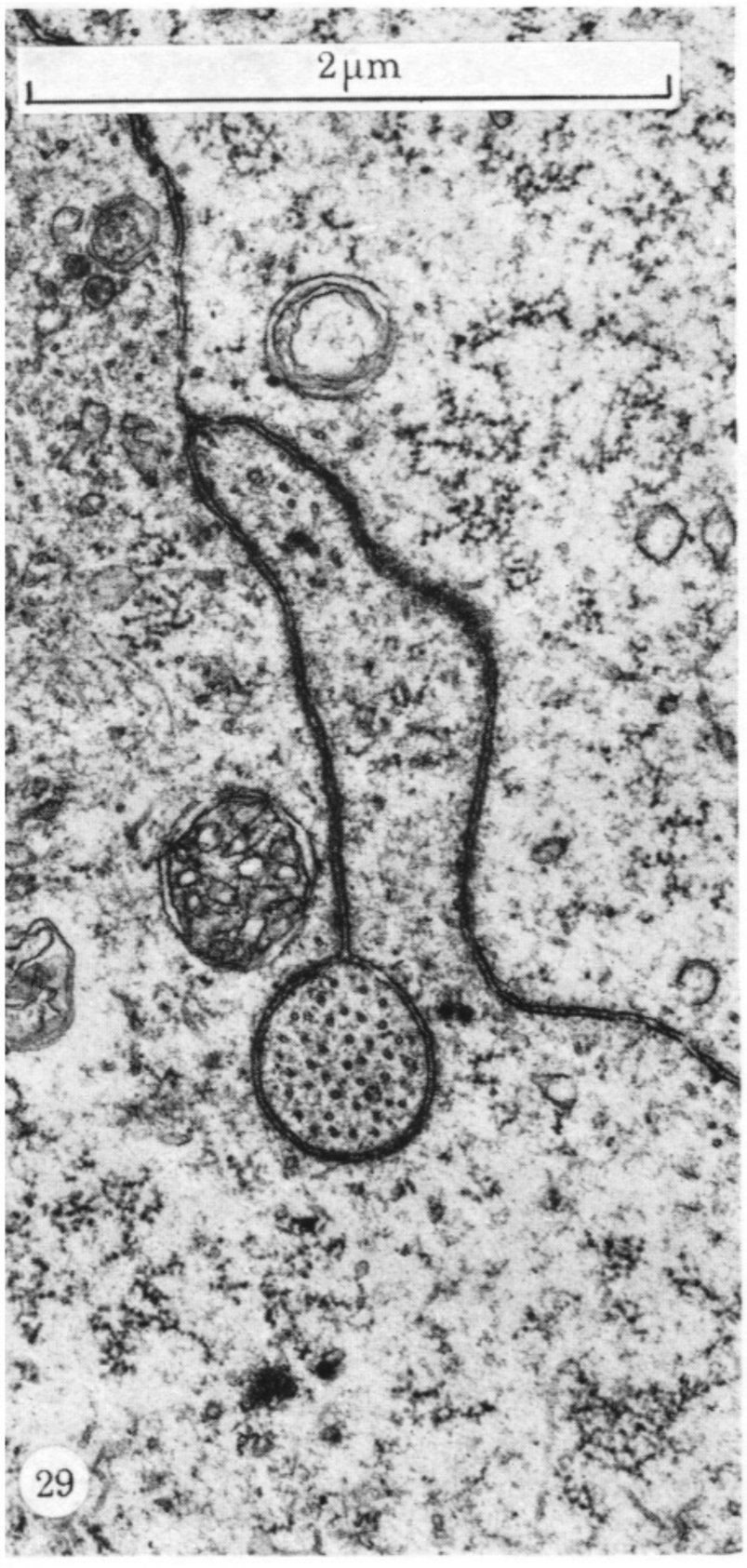

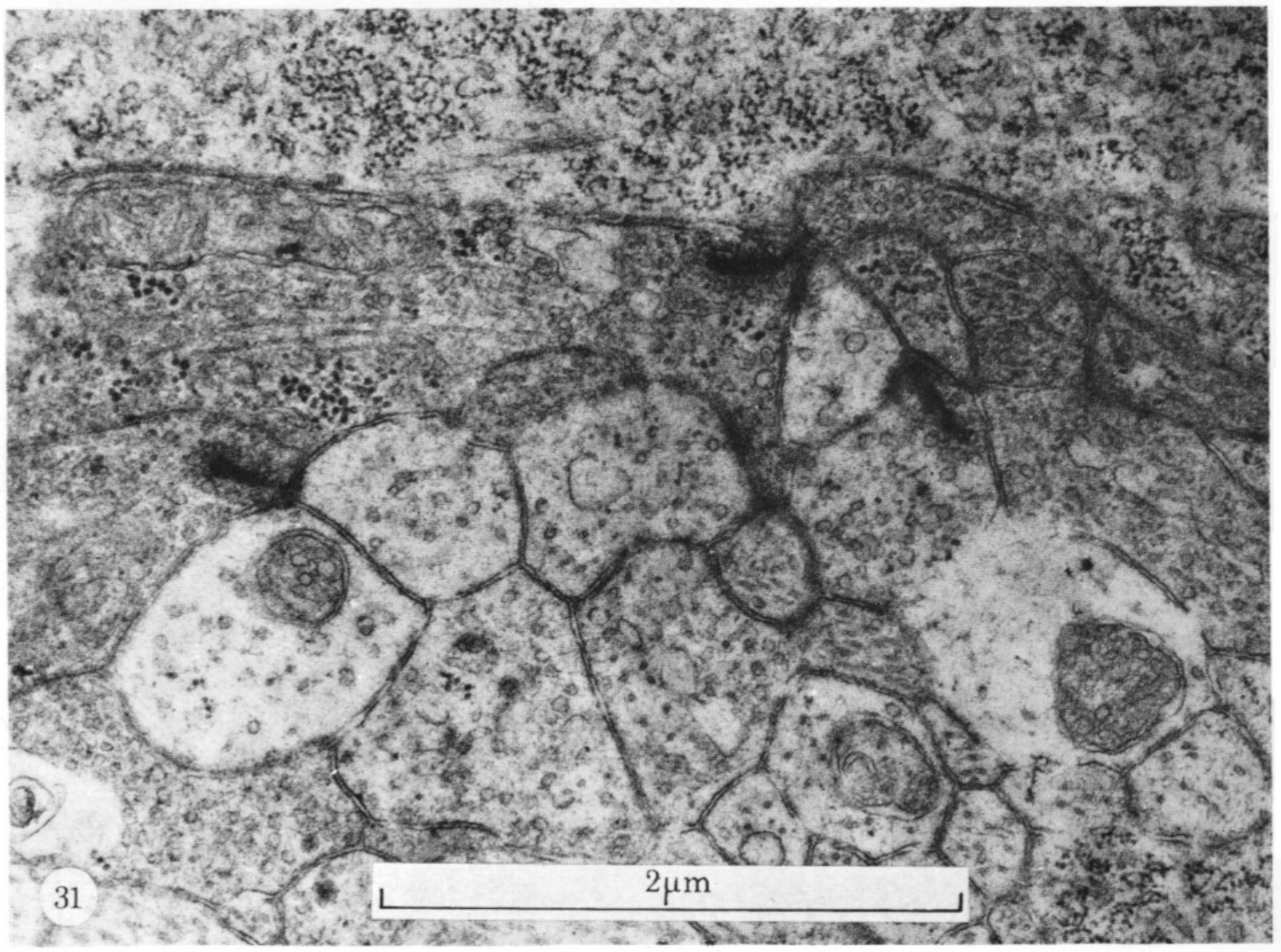

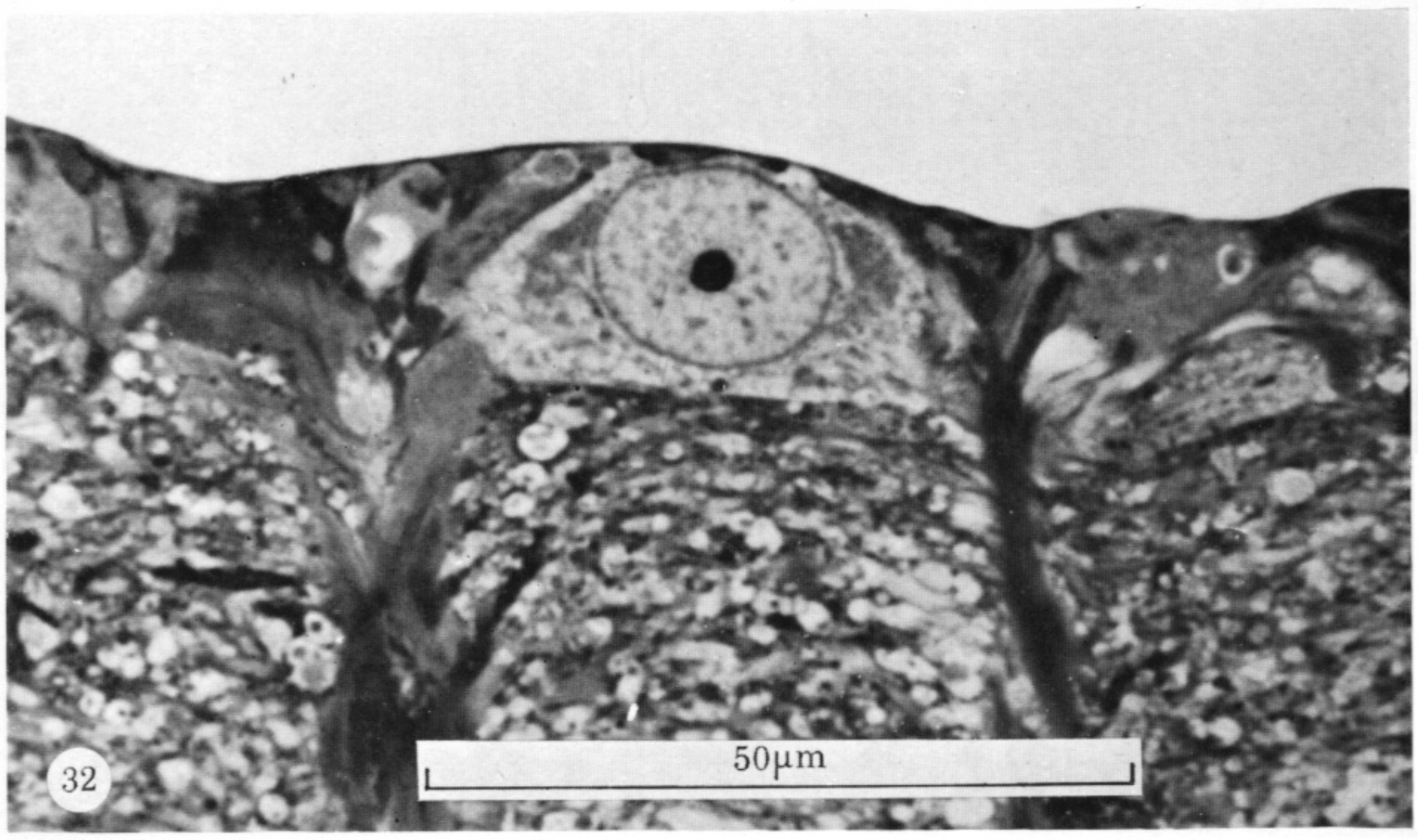

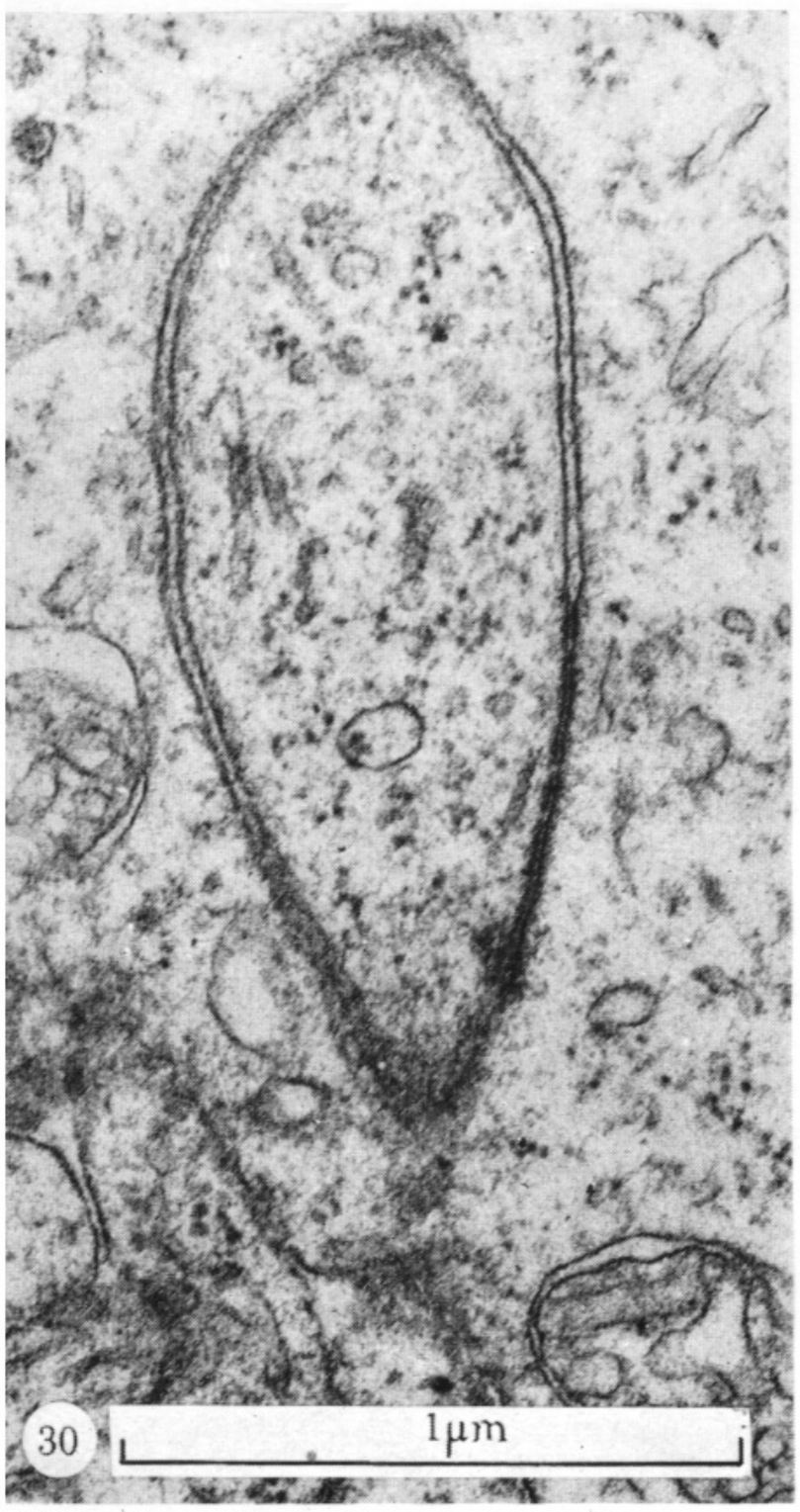

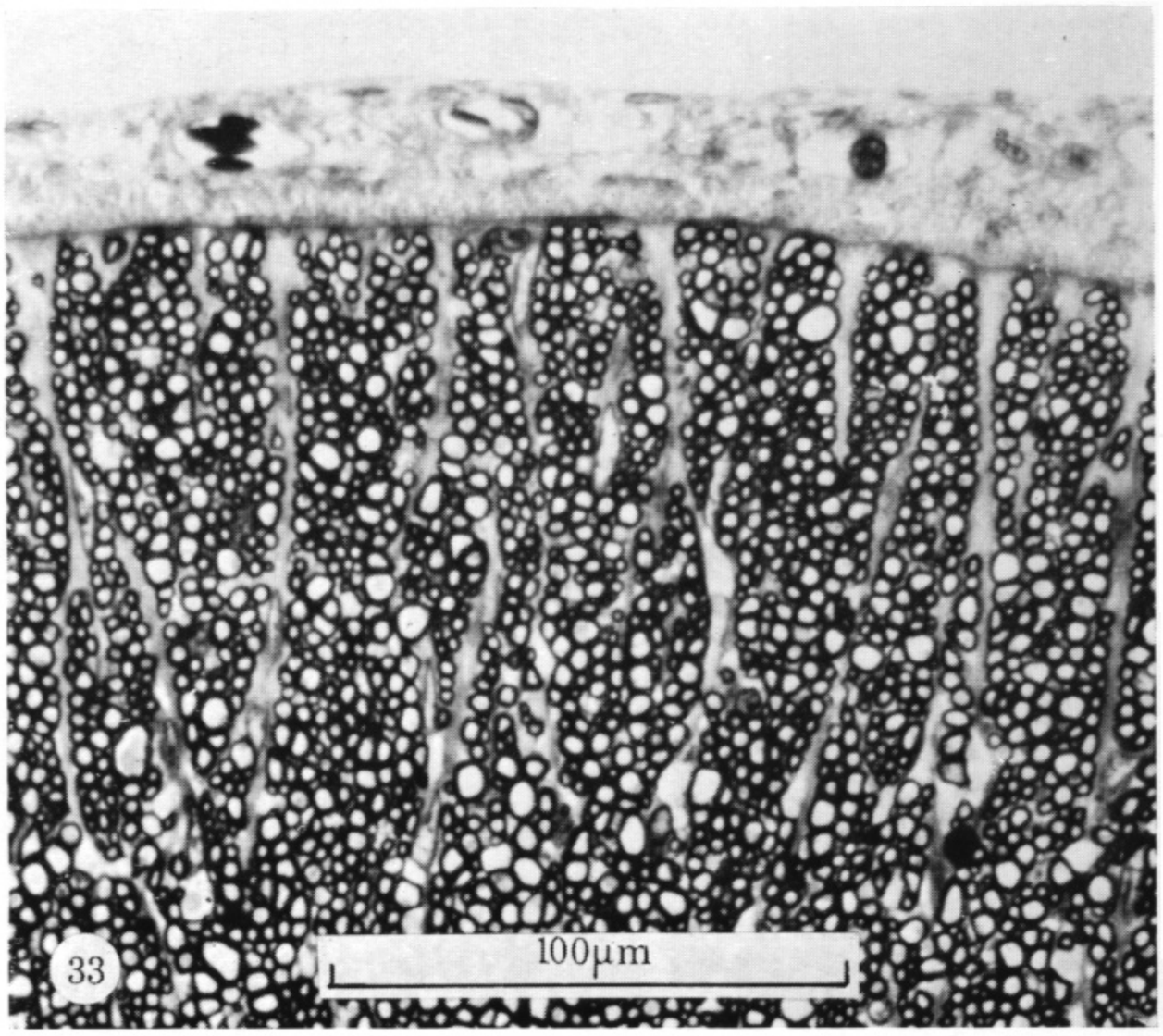

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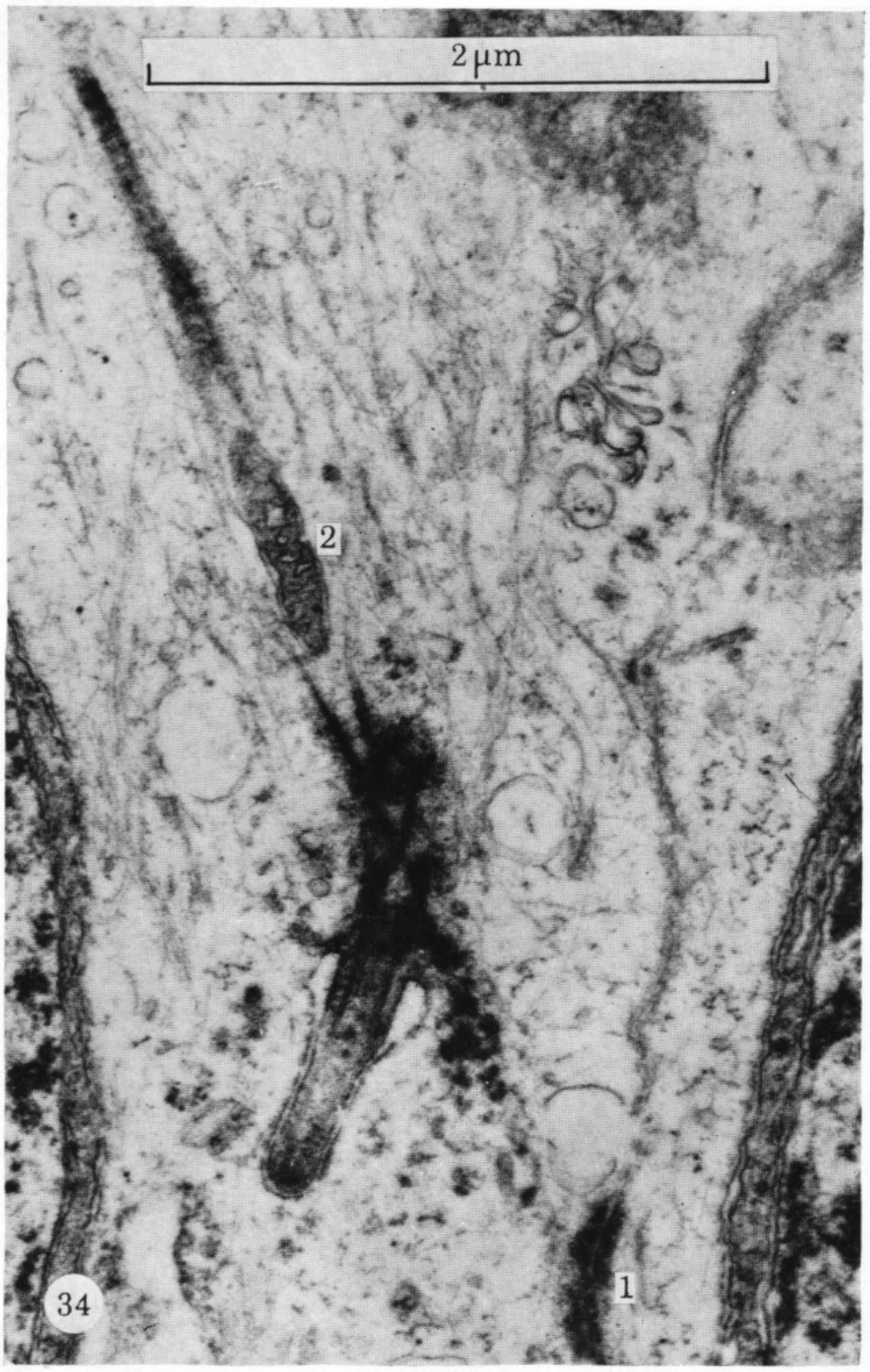

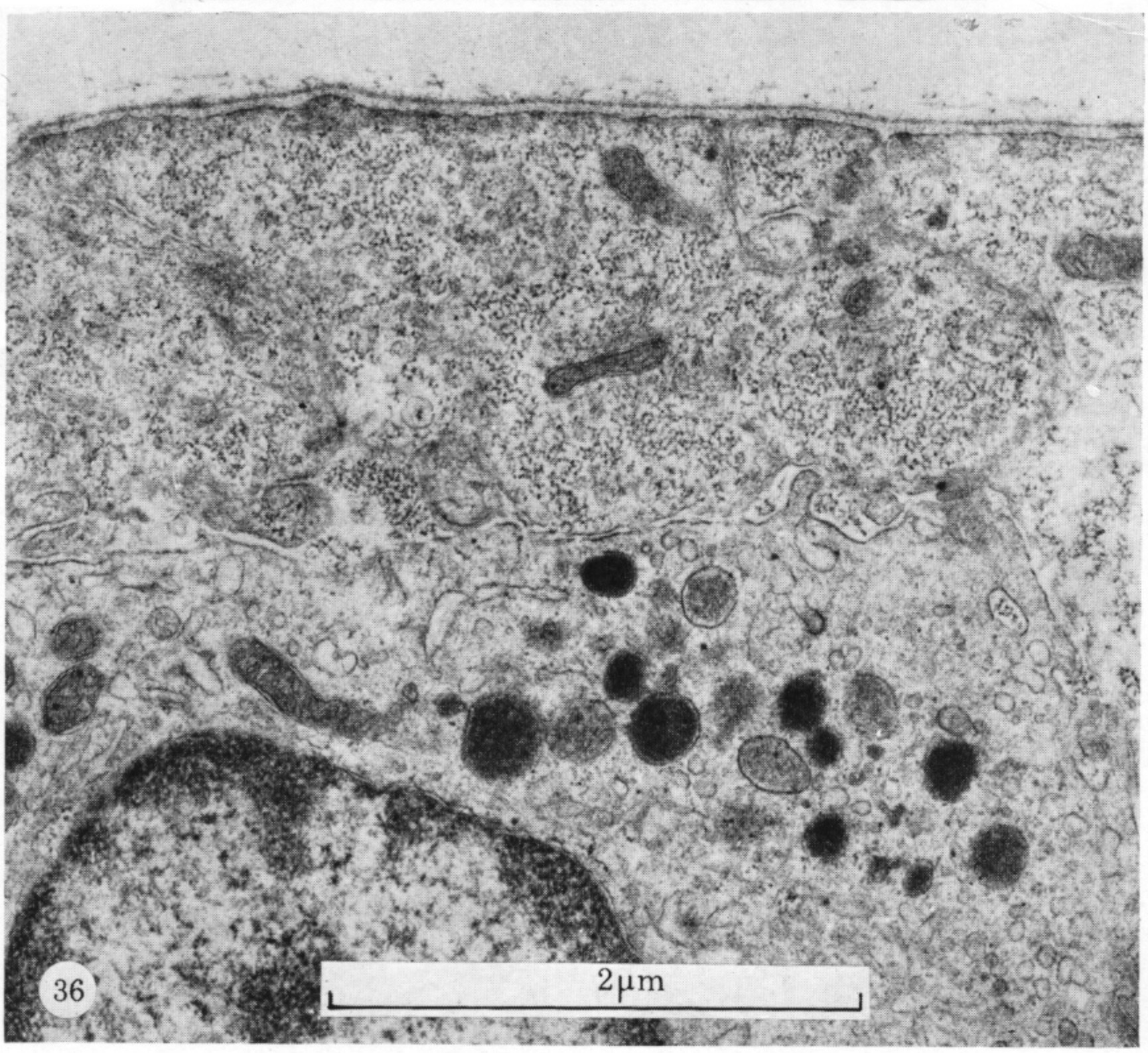

FIGURE 34. Radial fibre cilium. Radial fibre cytoplasm just vitread to the outer limiting membrane (1) contains a cilium complex. There are two centrioles, of which one is the basal body of the cilium. This centriole gives rise to striated rootlets, beside which alined mitochondria are located (2). The cilium appears to be buried in the cytoplasm.

FIGURE 36. Inner limiting membrane. The inner boundary of the retina is formed by radial fibre expansions with the closely adherent vitreous condensation. The cell beneath the radial fibre is tentatively identified as a microglial cell.