

ABSTRACT

John Parsons Chandler. RETINAL FINE STRUCTURE OF THE WESTERN PLAINS
GARTER SNAKE *THAMNOPHIS RADIX HAYDENI* KENNICOTT. (Under the direction
of Dr. Charles E. Bland) Department of Biology, November 1974.

The retinal morphology of *Thamnophis radix haydeni* Kennicott was described from light and electron microscopic observations. Numerous coated vesicles are located in the basal and apical cytoplasm of the pigment epithelial cells, and they are oftentimes continuous with the plasma membrane. This, with the basal infoldings and apical microvillous processes, suggests the presence of a transport mechanism in the pigment epithelium. Three types of photoreceptor are present in this afoveate retina, and they are the type A, B and C cones. The inner segments of the type A and type B chief cones contain large ellipsoids composed of tightly packed mitochondria. These mitochondria contain matrix lipid droplets. An oil droplet is frequently found in the apical inner segment cytoplasm of these cells. The type B accessory cone contains a paranuclear body. Photoreceptor synaptic terminals are predominantly of the cone type, and each cell typically has a conducting fiber. The inner plexiform layer contains ribbon, conventional and reciprocal synapses.

RETINAL FINE STRUCTURE OF
THE WESTERN PLAINS GARTER SNAKE
THAMNOPHIS RADIX HAYDENI KENNICOTT

A Thesis
Presented to
the Faculty of the Department of Biology
East Carolina University

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts in Biology

by
John Parsons Chandler

November 1974

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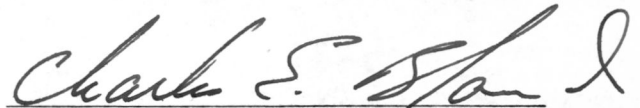
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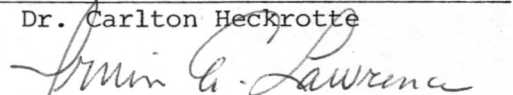
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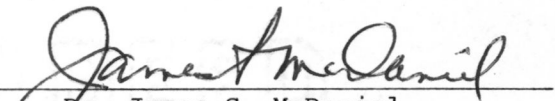
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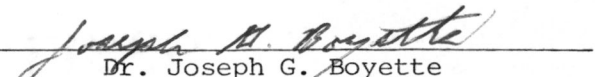
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DEDICATION

This thesis is dedicated to my grandfather, Doctor A. M. Dunlap, M.D. (1884-1973), who was, and continues to be, an inspiration to me.

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INTRODUCTION

The western plains garter snake, *Thamnophis radix haydeni* Kennicott (Colubridae), occurs throughout the midwest and has diurnal habits. Underwood (1970) stated that *Thamnophis* sp. possesses an all-cone retina containing photoreceptor cells typical of snakes with diurnal habits (Walls, 1942). The extent to which *T. r. haydeni* uses vision in food-getting is unclear, for observations in the laboratory indicate that olfaction is used to a great extent.

Retinal fine structure of few snakes have been described, and they are two colubrids, *Elaphe climacophora* (Yamada et al., 1966) and *Heterodon platyrhinos* (Underwood, 1970), one viperid, *Vipera berus* (Underwood, 1970) and one hydrophiid, *Pelamis platurus* (Hibbard and Lavergne, 1972).

The purpose of the present study is to describe the retinal fine structure of *T. r. haydeni* and relate these findings to what has been described in other vertebrate retinae.

REVIEW OF LITERATURE

Retinal fine structure of only four species of snake have been described, and although each of these reports (Yamada et al., 1966; Underwood, 1970; Hibbard and Lavergne, 1972) is only a partial description of each retina, the findings presented can be related to extensive reports of retinal morphology in other vertebrates.

The pigment epithelium has been described in the frog (Porter and Yamada, 1960), turtle (Yamada, 1961), chicken (Matsusaka, 1967), human (Yamada et al., 1958; Fine, 1961; Spitznas and Hogan, 1970), sheep (Leure-duPree, 1968), rabbit, mouse (Yamada et al., 1958), and albino rat (Wislocki and Ladman, 1955; Dowling and Gibbons, 1962).

Matsusaka (1967) reported a possible intracytoplasmic transport channel for nutrients in the chicken pigment epithelial cells. He found coated vesicles forming at the basal surface and they resembled those involved in protein transport in the mosquito oocyte (Roth and Porter, 1964). Basal infoldings and basal location of elongate mitochondria (Wislocki and Ladman, 1955) resemble those found in other epithelia known to be involved in transport activity, notably the kidney tubule cell (Pease, 1956). The coated vesicles and basal infoldings indicate that the retinal pigment epithelium is involved in transport.

The pigment epithelial cells are active in the metabolism and secretion of carotenoids involved with the visual pigment cycle (Wald, 1936). It is not known what visual pigment, or pigments, occur within the snake retina, but it is likely that there are similarities between this species and others which have been studied. The mammoth of the

Mongolian gerbil, though a protein hormone-producing cell, is active in secretion also. Intranuclear inclusions have been found in these cells (Nakayama and Nickerson, 1972; Nickerson, 1973) and their occurrence may be related to a disruption of their secretory function.

Droz (1961) reported that in the rods of rats and mice new protein is synthesized in the inner segment and moves to the outer segment, where he thought that it was catabolized. Young (1965) reported that new protein which is synthesized in the inner segments in frog, mouse and rat forms a band in the outer segment base which passes to the apex and then disappears. He suggested that the pigment epithelial cells may play a role in the disposal phase of outer segment renewal. The production of new discs is accompanied by concurrent removal of a comparable number of discs from the apical tip of the outer segment, resulting in the maintenance of a relatively constant number of discs in the rod outer segment (Young, 1967). Dowling and Gibbons (1962) reported the presence of lamellated structures in rat pigment epithelial cells which resembled outer segment fragments and speculated that outer segment fragments may be phagocytized by the pigment epithelium. Young and Bok (1969) identified these inclusions as outer segment fragments and called them phagosomes. Young and Droz (1968) demonstrated that new cone outer segment protein is synthesized in the inner segment. However, the distribution pattern within the outer segment is different from that in rods. Rather than forming whole new discs at the outer segment base, new protein is distributed randomly throughout existing discs, resulting in maintenance of the cone-shaped outer segment. The rod outer segment renewal system involving phagocytosis has been found

in frog (Young and Droz, 1968), monkey (Young, 1971), mouse (Droz, 1961), and albino rat (Droz, 1961; Young, 1965), all of which contain rod-type photoreceptors.

The photoreceptor synaptic terminals of *E. climacophora* (Yamada et al., 1966) and *P. platurus* (Hibbard and Lavergne, 1972) are similar to those of other vertebrates, e.g., toad (Carasso, 1957), lizard (Pedler and Tansley, 1963), turtle (Lasansky, 1971), pigeon (Cohen, 1963), human (Missotten, 1962; Fine, 1963), rabbit (De Robertis and Franchi, 1956), guinea pig (Sjöstrand, 1958) and rat (Ladman, 1958). All synaptic terminals described in snakes have been of the cone pedicle type and contain several triads. The synaptic organization of the snake photoreceptor pedicle has not been determined, but it is likely to correspond with those found in such diverse species as the fish (Stell, 1964, 1965), cat (Boycott and Kolb, 1973) and rat (Leure-duPree, 1974). The central element of the triad in these species has been found to be a bipolar cell dendrite and the lateral elements are processes of horizontal cells.

The inner plexiform layer was first described by Villegas (1960), and Missotten (1961) identified ribbon structures similar to the synaptic ribbons of the outer plexiform layer within one type of process. Kidd (1962) reported the presence of spine, conventional and serial synapses in addition to the ribbon synapse within the inner plexiform layer of the cat and pigeon. Fine (1963), reasoning that the ribbon was a structure characteristic of presynaptic elements, e.g. photoreceptor synaptic terminals, defined the bipolar cell synaptic terminals as the ribbon-containing elements of the inner plexiform layer. Dowling and Boycott (1966) reported the presence of the reciprocal synapse and

further described the processes of the inner plexiform layer, thus establishing the criteria by which neural elements of the inner plexiform layer are currently identified. Chandler (1974) reported the presence of conventional, ribbon, reciprocal and serial synapses in the inner plexiform layer of members of the genus *Thamnophis*.

Retinal oil droplets have been reported in all groups of vertebrates except snakes (Walls, 1942) and this lack of photoreceptor oil droplets has been accepted as an unique characteristic of ophidian retinae. Tansley and Johnson (1956) demonstrated that the inner segment in *Tropidonotus natrix natrix* condenses light passing through the long axis of the photoreceptor to the outer segment and they attributed it to internal reflection of light within the inner segment. Yamada et al. (1966) described small intramitochondrial lipid droplets within the inner segment in *E. climacophora* and speculated that they might act as a single large oil droplet, resulting in condensation of light as was found by Tansley and Johnson.

Underwood (1970) reported light microscopic observations of the photoreceptor cell types present in many species of snake. Although he presented no micrographs or diagrams, he stated that the genus *Thamnophis* possesses what Walls (1942) called the pattern for diurnal snakes, i.e., type A, B and C cones. Underwood also presented two electron micrographs each of portions of the double cones of *H. platyrhinos* and *V. berus*. The nuclear region of *H. platyrhinos* contains a paranuclear body. The ellipsoid mitochondria of the chief cell of *V. berus* contain granules and a matrix material which is more electron-opaque than that of the accessory cell mitochondria.

Yamada et al. (1966) described the retinal pigment epithelial and photoreceptor cells of *E. climacophora*. The pigment epithelial cells resemble those of other species. They found both single and double cones. Though there were no oil droplets, they speculated on the function of the small intramitochondrial lipid droplets in the ellipsoid. The photoreceptor synaptic terminals in this species are large and complex.

Hibbard and Lavergne (1972) found only one photoreceptor cell type in the retina of *P. platurus* and the ellipsoid mitochondria contain glycogen granules. The photoreceptor synaptic terminals of this species are complex also.

The present study expands the small body of information about snake retinal morphology and provides a wider basis for comparison of snake retinae to those of other vertebrates.

MATERIAL AND METHODS

Adult male western plains garter snakes, *T. r. haydeni*, were obtained from commercial suppliers (Lin's Aquarium, Cologne, Minnesota, and General Aquatics, Inc., Osseo, Minnesota) and maintained in the laboratory on a diet of ground fish and beef.

Tissue was fixed using either immersion or perfusion techniques. Fixation by immersion was accomplished by the following procedure. After decapitation under normal lighting conditions, eyes were removed and placed in chrome-osmium fixative (Dalton, 1955). The eyes were sectioned at the equator after one hour of fixation, and the cornea, lens and vitreous were discarded. The posterior portion of the globe, with retina attached, remained in cold (4°C) fixative overnight.

Perfusion was accomplished using the following procedure. After intraperitoneal injection of Nembutal, the snake was periodically turned onto its back, and when it was no longer able to pronate itself it was assumed that a surgical level of anesthesia had been reached. The heart of the supine animal was located by observing the rhythmic movement of the ventral surface over the beating heart. The heart was exposed through a mid-ventral incision approximately 4 cm long. All major posterior arteries and veins were clamped, and either the right atrium or a major anterior vein was cut. Perfusates were placed four feet above the animal, and the ventricle was cannulated with a 20 gauge needle. After flushing with buffer, the anterior vascular system was perfused with one of the following fixatives: 3% or 7% glutaraldehyde in Sorenson's phosphate buffer (Gomori, 1955); or 1/2 strength

Karnovsky's fixative. Perfusion lasted 5 to 10 minutes and usually required 50 to 100 ml of fixative. Following perfusion, the eyes were removed, placed in a dish of fixative, and sectioned at the equator. The posterior half of the globe was cut into narrow strips which were left in the fixative for an additional 10 minutes to 3 hours. Tissues were washed in the appropriate buffer and post-fixed in buffered 1% OsO_4 for between 8 minutes and 1 hour and then rinsed in buffer.

All tissues were dehydrated in a graded ethanol series (30%, 50%, 70%, 95%, 100%) with final dehydration in propylene oxide. Immersion-fixed retinæ were embedded in Epon 812 (Ladd), and perfusion-fixed retinæ were embedded in Araldite 6005 (Fullam).

Thick sections 0.5 to 2.0 μm thick and ultrathin sections 500 Å thick were cut on either a Sorvall MT-2B or Reichert OMU-2 ultramicrotome. Thick sections were cut with glass knives, stained with methylene blue-azure II (Richardson et al., 1960) and photographed with a Reichert Zetopan light microscope equipped with a Kam VBX camera. Ultrathin sections were cut with a diamond knife and mounted on uncoated copper grids. The sections were double-stained with uranyl acetate (Watson, 1958) and either lead citrate (Reynolds, 1963) or lead hydroxide (Millonig, 1961) and viewed with either a Hitachi HS-8 or Philips 300 transmission electron microscope.

Fixation with 3% glutaraldehyde in Sorenson's phosphate buffer or Dalton's chrome-osmium fixative was superior to that produced by all other fixatives used in this investigation. The most significant factor involved in obtaining good fixation was gentle handling of the tissue during preparation, especially in the early phases when

fixation was not yet complete.

RESULTS

Pigment Epithelium Layer

Pigment Epithelial Cell

Light microscopy. The pigment epithelium consists of a single layer of low cuboidal cells which rests on a basal lamina (Figs. 1, 2). The apical surface of each cell has numerous microvilli which pass vitread between the photoreceptors to the level of the mid-point of the inner segments (Figs. 1-3). Each cell has a large, centrally located nucleus. Pigment granules occur predominantly in the apical microvillous processes. A row of apicolateral desmosomes, Verhoeff's membrane, is located at the ventricular surface of the pigment epithelium and may be seen in fortuitous sections.

Electron microscopy. The basal surface of the pigment epithelial cell is characterized by extensive infoldings of the plasma membrane (Fig. 4). With the exception of a few ribosomes and profiles of tubular endoplasmic reticulum, the region of infolding is devoid of cytoplasmic organelles.

The basal lamina appears as a thin continuous sheet of moderately electron-opaque material with a fine granular appearance (Fig. 4). It conforms to the contours of the basal surfaces of the pigment epithelial cells, but does not enter the infoldings of the basal plasma membrane.

At the apex of the cell long microvillous processes extend between and ensheath each photoreceptor cell outer segment, and some extend between the inner segments (Fig. 5). The cytoplasm of these extensions

is finely granular and of medium electron-opacity. They contain pigment granules and profiles of tubular endoplasmic reticulum.

Frequently, apicolaterally, there is an extensive junctional complex (Fig. 6). Near the ventricular, or free, surface of two adjoining cells, the intercellular space narrows and the two unit membranes appear to fuse into a single linear density, the zonula occludens (ZO). This is less than 1 μ m in length, and there is no condensation of the adjacent cytoplasm. Deep to the ZO is the zonula adherens (ZA), characterized by an intercellular space of approximately 200 A and a dense fibrillar appearance of the adjacent cytoplasm. A third component of the complex is the macula adherens (MA). It is easily distinguished from the ZA by the sharply defined limits of membrane specialization and the presence of a homogeneous layer of cytoplasmic density beneath the plasma membranes, which are separated by an intercellular space of approximately 220 A. Verhoeff's membrane is formed by the extensive junctional complexes which encircle the cells.

Another modification of the plasma membrane of both the basal and apical surfaces is the appearance of pockets, or invaginations, resulting from the formation of pits, or caveolae (Fig. 4). The membrane of this flask-like invagination is thickened and is further specialized in that it has an ill-defined, radially striated layer of material on its outer surface. Circular profiles of coated vesicles with morphology similar to that of the caveolae are found throughout the cell, but are located predominantly near the basal and apical plasma membranes.

The nucleus is usually centrally located (Fig. 7). It has an

eccentric nucleolus and marginal condensation of chromatin on the inner surface of the nuclear envelope. A multivacuolar inclusion 2 μm in diameter was found in one cell (Fig. 8). Parallel tubular vacuoles appear to encircle a central vacuole 1.25 μm in diameter. The tubule diameter varies from 400 A centrally to 500 A at the periphery.

Smooth endoplasmic reticulum constitutes the major cytoplasmic component of the pigment epithelial cell (Figs. 4-8), and forms an elaborate network of tubular and vesicular profiles which ramify throughout the cytoplasm from the basal zone up to, and including, the microvilli. The membranes of the rough endoplasmic reticulum are continuous with those of the tubular smooth endoplasmic reticulum and are randomly distributed throughout the cytoplasm, except in the microvilli and the basal infoldings. Free ribosomes occur throughout the cytoplasm, but predominantly in the perinuclear area. Scattered Golgi profiles are found throughout the cytoplasm (Fig. 9), but their exact limits are often difficult to define in a cytoplasm so rich in smooth-surfaced membranes.

Mitochondria are generally ovoid in shape but may be elongate and finger-like (Figs. 4-7, 9). They are most frequently found adjacent to the convoluted basal margin and along the lateral surface of the cell. They have numerous tubular cristae embedded in a dense granular matrix which contains from one to fifteen electron-opaque granules 500 to 600 A in diameter. Few mitochondria are devoid of these granules.

Ovoid pigment granules 0.5 to 1.0 μm wide and 0.5 to 1.1 μm long occur within the cell body, and they tend to be longer, up to 3 μm , when in the microvillous processes (Figs. 4, 5). There are few pigment

granules in the basal half of the cell and they are found predominantly in the microvilli. Clusters of up to 26 pigment granules are found in the apical cytoplasm (Fig. 10), and there are no other cytoplasmic organelles between these granules. Developing pigment granules (Fig. 11) are also seen in the apical cytoplasm.

Moderately electron-opaque membrane-bound bodies 0.5 to 3.5 μm in diameter have a granular matrix and are found in the apical cytoplasm (Figs. 4, 5, 9). These are presumed to be lysosomes and are oftentimes associated with membrane-bound lipid droplets of similar size which occupy concavities in the opaque bodies (Fig. 9). Lipid droplets which are not associated with dense bodies may have a marginal opacity.

Photoreceptor Cell Layer

Type A Cone

Light microscopy. The large single cone corresponds to the type A cone of Walls (1942) and has an inner segment approximately 18 μm tall and 9 μm wide, and an outer segment 7 μm tall and 3 μm wide at its base (Figs. 1, 2). The ellipsoid nearly fills the cytoplasm of the inner segment and has a refringent area which is displaced toward the outer segment.

Oil droplets 2 to 5 μm in diameter are located in a scleral concavity of the ellipsoid in some type A cones and type B chief cells (Fig. 3).

Foot processes extend from the perinuclear regions of the photoreceptors into the outer plexiform layer at an angle of about 30° and end in an enlarged synaptic terminal, or pedicle. The vitreal surface of the pedicle is characterized by numerous invaginations.

Electron microscopy. The outer segment of the type A cone consists of a stack of 200 to 250 flattened saccules enclosed by the scleral portion of the plasma membrane. They are 200 A thick with an intersaccular space of 100 A (Fig. 12). Approximately the basal third of the saccules are continuous with the plasma membrane.

The tip of the outer segment is in close apposition to the pigment epithelium and often lies in an apical concavity of the epithelial cell (Fig. 13). There are, however, no membrane specializations between the photoreceptor and pigment epithelial cells.

The only cytoplasmic connection between the outer and inner segments is a narrow cylinder of cytoplasm containing the connecting cilium (Fig. 14). The cilium arises from a basal body in the apicolateral cytoplasm of the inner segment and passes through the narrow cytoplasmic bridge to the outer segment cytoplasm where the microtubular elements diverge and pass sclerad through approximately one third of the length of the outer segment. The cytoplasmic bridge is devoid of organelles other than the microtubular elements of the cilium.

Arising from the apicolateral surface of the inner segment and projecting sclerad are 15 to 20 calycal processes (Fig. 12) which lie parallel to the long axis of the photoreceptor along the outer segment plasma membrane.

The inner segment is ovoid and is sessile on the external limiting membrane (Fig. 12), which means that there is no myoid stalk between the ellipsoid region and the external limiting membrane. The ellipsoid, which occupies most of the cytoplasm, is composed of two morphologically distinct types of mitochondria. The inner, medullary mitochondria are

closely packed to the exclusion of all other cytoplasmic organelles. The matrix of these mitochondria contains lipid inclusions 0.1 to 0.2 μm in diameter, which are larger and more numerous in the central portion of the ellipsoid (Fig. 15). The outer, cortical mitochondria are long and filamentous with their long axes parallel to that of the photoreceptor cell. These mitochondria contain smaller, electron-opaque granules in their matrix.

The cytoplasm surrounding the ellipsoid is wider on the side where the connecting cilium is located (Fig. 12). This area typically contains many microtubules which appear to traverse the length of the inner segment from the basal body of the connecting cilium to the nuclear area. This cytoplasm also contains many free ribosomes and several lysosome-like structures.

Oil droplets, varying in size from 2 to 5 μm , are occasionally encountered in the type A photoreceptor and are located in the scleral cytoplasm in a concavity of the ellipsoid (Fig. 12). They are spherical and bounded by a single membrane which is entirely extramitochondrial, and frequently there are electron-opaque deposits lining the inner surface of the membrane (Fig. 16).

The basolateral plasma membrane of the inner segment gives rise to microvillous processes which extend into the interreceptor spaces toward the level of the external limiting membrane (Fig. 12). These microvilli interdigitate with similar projections from neighboring photoreceptors and surrounding Müller cells.

At the level of the external limiting membrane the plasma membranes of the photoreceptor and Müller cells form junctional complexes, a major

component of which is the zonula adherens (ZA). Microtubules which cross the narrow cytoplasm at the external limiting membrane appear to insert into the ZA (Fig. 17).

The type A photoreceptor nucleus lies just vitread to the level of the external limiting membrane and is ultrastructurally unremarkable (Fig. 18). The scleral perinuclear cytoplasm generally has several Golgi profiles. Free ribosomes are abundant, and microtubules which lie roughly parallel to the long axis of the photoreceptor are generally present. Lysosome-like inclusions with a diameter of 0.1 to 0.25 μm are frequently found in the scleral perinuclear cytoplasm and are occasionally found in association with Golgi profiles.

Morphologically there appears to be only one type of photoreceptor synaptic terminal, the pedicle, which is roughly pyramidal in shape with the base toward the inner layers (Fig. 19). At the electron microscopic level all pedicles appear to be morphologically similar, and they will be considered in this section.

The pedicle apex is joined to the photoreceptor perikaryon by a conducting fiber 1.5 μm in diameter which passes through the scleral portion of the outer plexiform layer at approximately a 30° angle regardless of location of the cell within the retina. The synaptic terminal expands at its base to a diameter of 15 to 20 μm . The base is slightly concave with many invaginations and also has many surface contacts. Two adjacent pedicles may contribute segments of one concavity (Fig. 20). Adjacent pedicles appear to be completely isolated laterally by Müller cell cytoplasm, and no interreceptor contacts have been found.

The major cytoplasmic components of the synaptic terminal are synaptic vesicles, synaptic ribbons, and tubular smooth endoplasmic reticulum. Endoplasmic reticulum is sparse in the pedicle and is, in general, apicocentrally located (Fig. 21) but may occur in the lateral cytoplasm as well. The bulk of the pedicle cytoplasm is occupied by synaptic vesicles, which appear as circular profiles with a diameter of 200 to 500 A. The synaptic ribbon is a flattened pentalaminar structure which lies in close proximity to the apex of a basal invagination, the entire structure being a synaptic complex, or triad.

Occupying each of the many invaginations are three processes from other cells (Fig. 22). It is presumed that the central element is a bipolar cell dendrite and the two lateral elements are horizontal cell processes. The central process extends approximately 1 μm into the pedicle and the lateral processes extend 1.5 μm on each side of the vertically compressed central element. This arrangement leaves an elongated ridge of receptor cell cytoplasm between the two lateral processes along which lies the arciform density. The synaptic ribbon lies perpendicular to the surface of the central process within this cytoplasm (Fig. 23). The synaptic ribbon is 0.25 μm tall, 1.0 μm long and approximately 250 A thick, and it is surrounded by a regularly spaced, single layer of synaptic vesicles.

Type B Cone

Light microscopy. The type A cone is often accompanied by a more slender photoreceptor to form a double cone, the type B cone of Walls (1942) (Figs. 1, 2). The chief (type A) and accessory cells are closely

apposed along the length of their inner segments, and their outer segments arise at the same horizontal plane. The outer segments are not, however, apposed along their lengths to their apices. The ellipsoid of the accessory cell, which is 5 μm long and 2 μm wide, lies entirely sclerad to the level of the midpoint of the chief cell inner segment. It is the lightest staining ellipsoid of the three in this retina. It is connected with the nuclear area by a slender myoid stalk. The scleral perinuclear area of the accessory cone is characterized by a paranuclear body which occupies a concavity of the nucleus.

Electron microscopy. The chief cell of the type B cone is morphologically identical to the type A cone (Fig. 24).

The outer segment of the accessory cell of the type B cone is of similar dimensions as that of the chief cell (Fig. 25), and their bases are apposed at the same level of the receptor cells (Fig. 24).

The inner segment is markedly different from that of the chief cell. It is slender and lies closely apposed to the inner segment of the chief cell (Fig. 26). The ellipsoid is approximately 2 μm wide and 5 μm long (Fig. 24) and is located in the scleral cytoplasm of the inner segment. The ellipsoid region is connected to the nuclear area by a slender myoid stalk, of which the major cytoplasmic inclusions are microtubules and free ribosomes (Fig. 26). The mitochondria of the accessory cell ellipsoid are easily distinguished from those of the chief cell because they have a less electron-opaque matrix and the cristae are more sheet-like (Figs. 16, 24-26). Electron-opaque intramitochondrial granules are present, and lipid inclusions are rarely found in the accessory cell mitochondria. The basolateral surface of

the myoid stalk gives rise to microvillous processes which interdigitate with those of neighboring photoreceptors and Müller cells (Fig. 26).

Another characteristic structure of the accessory cell is the paranuclear body, a dense aggregation of mitochondria located in a concavity of the scleral surface of the nucleus (Fig. 26). The paranuclear body does not generally extend sclerad beyond the external limiting membrane, but there is, on occasion, a single mitochondrion which extends several micrometers beyond the level of the external limiting membrane in the middle of the myoid stalk (Figs. 27, 28). The paranuclear body is roughly spherical and approximately 5 μm in diameter. The nucleus of the accessory cell is noteworthy only with respect to its close association with the paranuclear body.

Type C Cone

Light microscopy. The third type of photoreceptor is shorter than the type A and B cones, and it corresponds morphologically to the type C cone of Walls (1942) (Fig. 2). The inner segment is 15 μm long and 5 μm wide and is nearly filled by the ellipsoid. The outer segment is approximately 3 μm wide at its base and tapers slightly as it passes sclerad. It does not contact the pigment epithelium, but extends to within a few micrometers of the epithelial cells.

Electron microscopy. The type C cone extends to within 2 to 3 μm of the pigment epithelium (Fig. 29), but the inner segment is considerably shorter than those of the other photoreceptors (Fig. 30). Because of this, the outer segment is proportionately longer in the type C cone. The base of the outer segment is about 3 μm wide and tapers slightly as

it passes toward the pigment epithelium. The outer segment is surrounded by 15 to 20 regularly arranged calycal processes which extend several micrometers toward the pigment epithelium (Fig. 31).

The ellipsoid occupies nearly all of the cytoplasm of the inner segment and is sessile on the external limiting membrane (Fig. 30). The ellipsoid of the type C photoreceptor has a cortex and a medulla. The cortex is thinner than that of the type A cone, the former being 1 to 2 mitochondria thick, the latter being 2 to 3 mitochondria in thickness. The medullary mitochondria of the type C cone are large, approximately 1.0 μm in diameter, and tightly packed to the exclusion of all other cytoplasmic organelles. Both types of mitochondria contain numerous electron-opaque granules 300 to 350 A in diameter within their matrices (Figs. 29-32). The basolateral surface of the inner segment gives rise to microvillous processes which interdigitate with those of adjacent cells (Fig. 27). The nuclear area contains smooth and rough endoplasmic reticulum, free ribosomes, and Golgi profiles (Fig. 33).

Inner Nuclear Layer

Horizontal Cell

Light microscopy. The horizontal cells are located in a single layer in the outer portion of the inner nuclear layer (Fig. 1). Their nuclei are 5 μm in diameter with a homogeneous nucleoplasm and their perikarya are larger than those of the bipolar and Müller cells. There is a single thick scleral process which branches as it reaches the outer plexiform layer.

Electron microscopy. Each horizontal cell is generally separated

from adjacent horizontal and bipolar cells by processes from Müller cells (Fig. 34). The plasma membrane surrounding the perikaryon has no specialized contacts with adjacent cells in the form of either desmosomes or synapses. Synaptic specializations with the photoreceptors have already been discussed.

The apical portion of the perikaryon contains mitochondria, Golgi profiles, scattered smooth and rough endoplasmic reticulum and free ribosomes (Fig. 34). Most sections of mitochondria contain 1 to 6 granules within their matrix. Microtubules are the major cytoplasmic component of the horizontal cell processes in the outer plexiform layer, but they do not appear to enter the perikaryon. The nucleus is approximately 5 μ m in diameter with a homogeneous distribution of chromatin (Fig. 34).

Lateral elements of the triad contain synaptic vesicles and are presumed to be horizontal cell processes (Fig. 35). The presumed bipolar cell dendrites are devoid of synaptic vesicles.

Bipolar Cell

Light microscopy. The bipolar cell has two processes arising from opposite poles of the cell body (Fig. 1). Bipolar cell nuclei are ovoid in shape and lie within a thin layer of cytoplasm.

Electron microscopy. Except at synapses, each bipolar cell and its processes are generally isolated from neighboring neurons by glial elements (Fig. 34). Because the courses of the bipolar cell processes are so tortuous, it is impossible to follow them to their synaptic contacts in either the outer or inner plexiform layers using the methods employed

in the present study. It is, however, possible to identify bipolar cell processes by various morphological criteria, and these are described elsewhere.

The scleral perikaryon contains a cluster of several mitochondria and Golgi profiles. There are scattered profiles of rough endoplasmic reticulum and free ribosomes. Microtubules oriented in a sclero-vitreous axis are also present (Fig. 36). The nucleus is ovoid with a diameter of 5 μm and has a homogeneous distribution of chromatin with an eccentric nucleolus 0.5 μm in diameter.

Amacrine Cell

Light microscopy. The amacrine cell perikarya are located at the vitreous surface of the inner nuclear layer (Fig. 1). Their nuclei are centrally located within a large perikaryon, and there is a single process which extends into the inner plexiform layer.

Electron microscopy. Amacrine cell bodies are usually separated from adjacent cells by cytoplasmic processes of bipolar or Müller cells (Fig. 37). When they lie apposed to other amacrine perikarya the plasma membranes make no specialized contacts with each other (Fig. 38). The cell processes within the inner plexiform layer, however, make numerous conventional synaptic contacts with other cell processes and these will be discussed in the section on the inner plexiform layer.

The perikaryon contains mitochondria, scattered profiles of rough endoplasmic reticulum and free ribosomes. There is a large Golgi apparatus, and centrioles may occur in the vitreous cytoplasm. Microtubules are not present within the perikaryon (Fig. 38). The nuclear

envelope is oftentimes invaginated. The nucleoplasm is homogeneous and frequently contains a nucleolus 0.5 μm in diameter.

Müller Cell

Light microscopy. The Müller cell is a neuroglial element of the retina and sends processes from its cell body sclerad to the external limiting membrane and vitread to the inner surface of the retina. The cell body tends to be fusiform in shape, filling the space between neurons (Fig. 1). The nucleus also tends to be fusiform. This nuclear area tapers both apically and basally, and the processes appear to ramify between the neural elements.

Just sclerad to the photoreceptor nuclei the apical processes expand to fill the spaces between photoreceptors. At this level there are extensive junctional complexes between photoreceptors and Müller radial fibers. The apical limits of the Müller cells appear as a discontinuous dense line called the desmosome layer, or external limiting membrane, although it is not a true membrane.

The basal extensions pass through the inner plexiform and ganglion cell layers as thick processes, giving the inner layers a vertically striated appearance. After passing the nerve fiber bundles they expand to form end feet which line the inner surface of the retina and rest on a basal lamina.

Electron microscopy. The cytoplasm of the Müller cell body contains mitochondria, Golgi profiles, microtubules and small amounts of rough endoplasmic reticulum (Fig. 39). The slender apical process has scattered rough endoplasmic reticulum, microtubules, free ribosomes and

an occasional mitochondrion (Fig. 40). The apical expansion frequently contains numerous mitochondria (Fig. 41) and occasionally gives rise to a cilium which projects into the ventricular space (Fig. 42). The specialized apical surface gives rise to numerous microvilli which extend into the ventricular space and interdigitate with the microvilli of the photoreceptors (Figs. 41, 42).

The basal process contains many microtubules and in the basal expansion there are large numbers of mitochondria (Fig. 43). The lateral plasma membrane of the end feet form tight junctions. In thin section, these areas appear curved or wavy, and these junctions may be macular in shape. There is no submembrane density associated with this specialization in either cell. A basal lamina is associated with the inner surface of the Müller cell processes.

Inner Plexiform Layer

Synaptic Processes

Light microscopy. The inner plexiform layer is the neuropil where synaptic contact between bipolar, amacrine and ganglion cells occurs (Fig. 1). In avascular retinae the inner plexiform layer is normally devoid of cell bodies, however, scattered cells do occur within this layer in this species (Fig. 2). The routine staining procedures used do not allow determination of the type of cell, as any cellular processes which may be present cannot be traced.

Electron microscopy. The inner plexiform layer is composed of discrete membrane-bound processes (Fig. 44). It is generally impossible to trace the individual cell from which a particular element has arisen;

however, the processes can usually be identified as to cell type by means of morphological criteria of the cytoplasmic components within the processes.

The major cytoplasmic component of the bipolar cell process is the synaptic vesicle. They are present in all parts of the process, but are particularly numerous in the synaptic regions (Fig. 44). The most distinctive feature of the bipolar process is the synaptic ribbon. Ribbons approximately 0.5 μm long are located at areas of synaptic contact. There are also generally 1 to 10 mitochondria in each synaptic area. Bipolar cell processes are generally larger than those of amacrine and ganglion cells. The presence of a synaptic ribbon is the single most important criterion for positive identification of a bipolar cell process where the element cannot be traced directly to the cell body.

Amacrine cell processes are generally smaller than those of bipolar cells and may measure up to 2 μm in diameter. They contain synaptic vesicles in a concentration comparable to bipolar cell processes and frequently contain several mitochondria (Fig. 44). These processes may be more positively distinguished from bipolar processes when they are either a postsynaptic element of a ribbon synapse or the presynaptic element of a conventional synapse.

Ganglion cell processes are distinguished by the presence of ribosomes and the scarcity, or absence, of synaptic vesicles (Fig. 45). These processes are often postsynaptic elements of synaptic dyads.

There are three types of synapses in the inner plexiform layer, and they are the conventional, the ribbon and the reciprocal synapses.

The conventional synapse is characterized by areas of membrane density of two neuronal elements for approximately $0.5\ \mu\text{m}$ along their apposed membranes. The presumed presynaptic element contains a localized aggregation of synaptic vesicles in the cytoplasm just below the area of membrane specialization (Fig. 45). Though both membrane elements are dense, the presumed presynaptic membrane exhibits greater density than the presumed postsynaptic membrane. There is no aggregation of synaptic vesicles at the postsynaptic membrane.

The ribbon synapse (Fig. 45) is characterized by a specialized association between three neuronal processes forming a dyad. The bipolar axon, containing the ribbon, is presumed to be presynaptic to two other neuronal elements, the membranes of which exhibit specialized density where the three are juxtaposed. The ribbon bisects the angle between the two other elements, and each of these may be either an amacrine or ganglion cell process.

A bipolar and an amacrine cell may form a reciprocal synapse, a relationship in which an amacrine cell process which is postsynaptic at a ribbon synapse becomes presynaptic to the same bipolar cell through a conventional synapse (Fig. 46). The conventional and ribbon synapses usually occur within $0.5\ \mu\text{m}$ of each other.

Ganglion Cell Layer

Ganglion Cell

Light microscopy. The ganglion cells form a single layer in the inner portion of the retina (Fig. 1). Their dendritic processes extend into the inner plexiform layer and their axons proceed within the nerve

fiber bundles to the optic disc where they form the optic nerve.

The ganglion cell nucleus is the largest of the retinal nuclei and is centrally located in a large perikaryon. The cytoplasm contains Nissl bodies which may extend into the primary dendrite.

Electron microscopy. Each ganglion cell is usually separated from adjacent ganglion cells and the nerve fiber bundles by Müller cell processes. There are no membrane specializations at areas of inter-ganglion cell contact (Fig. 47). The lateral surfaces of the perikaryon which are in contact with the Müller cells occasionally contain submembrane cisternae approximately 0.5 μm in length (Figs. 47, 48).

The perikaryon is approximately 10 μm in diameter and contains a centrally located nucleus approximately 7 μm in diameter (Fig. 48). The nucleoplasm consists of homogeneous chromatin and an eccentric nucleolus 0.5 μm in diameter. The perikaryon also contains numerous mitochondria and a well developed Golgi apparatus. Nissl bodies are present in the perikaryon and may extend into the dendrite (Fig. 47). The presence of ribosomes in a synaptic process within the inner plexiform layer is one of the criteria for identification of ganglion cell dendritic processes.

Nerve Fiber Bundle

Light microscopy. The nerve fiber bundles lie vitread to the ganglion cell perikarya and are separated from one another by thick basal processes of the Müller cells (Fig. 1). The bundles are small in the peripheral retina and increase in size as they proceed to the optic disc due to the increase in numbers of ganglion cell axons that join

the nerve fiber bundles centrally. The fibers within the bundles are unmyelinated.

Electron microscopy. Axons within the nerve fiber bundles contain microtubules and mitochondria approximately 0.5 μm in diameter and 0.5 to 2.0 μm in length (Fig. 49).

DISCUSSION

The pigment epithelium of the western plains garter snake is similar in many respects to that of the frog (Porter and Yamada, 1960), turtle (Yamada, 1961), rat snake (Yamada et al., 1966), chicken (Matsusaka, 1967), human (Yamada et al., 1958; Fine, 1961; Bairati and Orzalesi, 1963; Spitznas and Hogan, 1970), sheep (Leure-duPree, 1968), rabbit, mouse (Yamada et al., 1958) and albino rat (Wislocki and Ladman, 1955; Dowling and Gibbons, 1962).

In the present study the nucleus of one pigment epithelial cell contained a vacuolated inclusion similar to what has been found in the nuclei of mammothrophs of the female Mongolian gerbil (Nakayama and Nickerson, 1972). In the gerbil the early developing structure of this inclusion, and its function, are unknown at present. It was suggested that the inclusion may arise in response to either cytoplasmic degeneration or some type of cellular stimulation. Nickerson (1973) found that it appears in response to administration of estrogen, yet he could not find the early developing stages. This nuclear inclusion was heretofore unknown in the retina. Though the mammothroph produces a protein hormone and the pigment epithelial cell is active in carotenoid metabolism, they are both active secretory cells, and the nuclear inclusions may be related to this function.

The absence of capillaries within this retina presents problems of nutrient supply to the middle layers. The more abundant vascular supply is from the choriocapillaris (cf. Fig. 1) and in order for nutrients to reach the neural retina they must pass through the pigment epithelium.

As in other epithelia, there are apicolateral junctional complexes which presumably limit passage of material from one surface of the epithelium to the other via the extracellular space. Several morphological features indicate the presence of a transport mechanism through the cytoplasm and they are: basal infoldings; apical microvilli; caveolae; and coated vesicles. The basal infoldings and apical microvilli are a common feature of epithelia engaged in transport (Pease, 1956), and the presence of mitochondria near the infolded basal surface is consistent with previous findings in such epithelia. Caveolae in this retina, frequently present at the blind terminus of the basal infoldings and at the bases of the apical microvilli, are similar to those involved in protein transport in the mosquito oocyte (Roth and Porter, 1964). Coated vesicles with surface structure similar to that of the caveolae are found throughout the cytoplasm and may represent a mechanism of transport from one surface of the pigment epithelium to the other. Matsusaka (1967) described similar structures in the chicken retina and speculated that they may be involved in a transport mechanism. The prominence of caveolae and coated vesicles in the western plains garter snake may be indicative of a need for an extensive transport system in this avascular retina.

A cytoplasmic inclusion which is present in the pigment epithelium of many species, but not in *T. r. haydeni*, is the phagosome. It has been well established through the work of Droz (1961), Dowling and Gibbons (1962), Young (1965, 1967, 1971), Young and Droz (1968), and Young and Bok (1969), that the pigment epithelium is involved in the outer segment renewal system of rods. However, the renewal of cone

outer segments does not appear to involve morphological changes in the pigment epithelial cells. Structurally, the only class of photoreceptor found in this species is the cone, and no phagosomes are found in the pigment epithelial cells. These findings lend support to the hypothesis that cone outer segment renewal does not involve phagocytosis of outer segment fragments by the pigment epithelial cells.

The photoreceptor cells of this species are of three types, and they all conform to the morphological criterion for cones that requires continuity between intradisc spaces with the extracellular space. According to the nomenclature of Walls (1942), the three photoreceptors in the western plains garter snake are the type A, B and C cones. This is the pattern for snakes with diurnal habits, and Underwood (1970) stated that *Thamnophis* sp. possesses this combination of photoreceptors.

Reports of snake photoreceptor morphology have been limited to one hydrophiid, *P. platurus* (Hibbard and Lavergne, 1972), two colubrids, *E. climacophora* (Yamada et al., 1966) and *H. platyrhinos* (Underwood, 1970) and one viperid, *V. berus* (Underwood, 1970). The inner segment of the large single cones of *T. r. haydeni*, *P. platurus* and *E. climacophora* all contain a large ellipsoid which nearly fills the inner segment cytoplasm. The ellipsoid mitochondria of *P. platurus* contain glycogen granules in a uniform distribution pattern. Intramitochondrial lipid inclusions in the ellipsoid of *E. climacophora* are larger in the central mitochondria. The ellipsoid of *T. r. haydeni* resembles that of *E. climacophora* with respect to the size of the ellipsoid, conformation of mitochondria and presence of intramitochondrial inclusions. Yamada et al. (1966) suggested that since snake photoreceptors do not have large

oil droplets, the ellipsoid, with such numerous intramitochondrial lipid droplets, might act as a single large oil droplet. Tansley and Johnson (1956) reported that the inner segment of the cone in *T. n. natrix* condenses light passing through the long axis of the photoreceptor. This condensation may occur due to the effect of the ellipsoid as postulated by Yamada et al. (1966).

Some of the type A cones and type B chief cells contain a large extramitochondrial oil droplet which resembles those occurring in other species (Cohen, 1963; Pedler and Tansley, 1963). Walls (1942) and Underwood (1970) stated that the lack of such photoreceptor oil droplets was an unique characteristic of the ophidian retina. Their function in this retina is not known.

Underwood's (1970) single micrograph of the ellipsoids of a type B cone from *V. berus* shows the mitochondria of the chief cell to contain refringent granules which may be comparable to those found in the colubrid type A and type B chief cell ellipsoid mitochondria. The accessory cell mitochondria of both *V. berus* and *T. r. haydeni* have a less electron-opaque matrix than those of the chief cell. In addition to the ellipsoid, a second cluster of mitochondria occurs in the accessory cells. This paranuclear body reportedly occurs only in the accessory cell of the ophidian double cone (Walls, 1942) and is located just sclerad to the nucleus. The only previously published electron micrograph of the paranuclear body (Underwood, 1970) shows the mitochondria to be tightly packed with a less electron-opaque matrix than those of the ellipsoid. This agrees with what has been reported in the present study.

The photoreceptor synaptic terminals of *T. r. haydeni* are mainly of the pedicle type, which is characteristic of cones. They have an extensively invaginated concave vitreal surface into which many cell processes enter. There may be as many as seven synaptic ribbons in a single section and it is presumed that each pedicle may contain up to fifteen ribbon synapses. The structure of the synaptic pedicle of both *P. platurus* and *E. climacophora* appear to be similar to that of the present species. The arrangement of cell processes from horizontal and bipolar cells at the ribbon synapse of these three species is not known. There have been consistent findings regarding synaptic structure in a wide range of species, and there is reason to assume that the arrangement of the snake photoreceptor triad is similar to those of other vertebrates. Through the combined descriptions of the fish (Stell, 1965), amphibian (Carasso, 1957), turtle (Lasansky, 1971), human (Missotten, 1962), rabbit (De Robertis and Franchi, 1956), guinea pig (Sjöstrand, 1958), and rat (Ladman, 1958; Leure-duPree, 1974), the basic morphological characteristics of the photoreceptor synaptic terminal are known.

The photoreceptor synaptic terminal of this species resembles that of the pigeon (Cohen, 1963) in several respects. The base of the terminal in both species is concave and this depression is filled with profiles of cellular processes. Frequently there are basal concavities which are formed by two adjacent synaptic terminals, each photoreceptor forming a portion of the arc. It is possible that these two pedicles in the western plains garter snake are from the chief and accessory cells of the type B cone, and this arrangement could permit pooling of information from both photoreceptors of the type B cone.

The pedicles of *E. climacophora* (Yamada et al., 1966) and *T. r. haydeni* are of comparable size, and their basal concavities are filled with similar tortuous processes from other cells. These processes have not been traced to their cells of origin; however, there is no reason to doubt that the arrangement of postsynaptic processes at the triad are different from that in such diverse species as the shark (Stell, 1964, 1965), cat (Boycott and Kolb, 1973) and rat (Leure-duPree, 1974). Through electron microscopic and Golgi-EM techniques these investigators have demonstrated that the central element of the triad arises from a bipolar cell and the lateral elements are horizontal cell processes.

The processes of the inner plexiform layer (Villegas, 1960; Missotten, 1961) and the synapses which they form (Kidd, 1962; Fine, 1963; Dowling and Boycott, 1966) have been described. In the present study an attempt has been made to identify the cellular processes of the inner plexiform layer without interpreting the synaptic organization of this retina. Only the conventional, ribbon and reciprocal synapses have thus far been found in the present species. The inner plexiform layer of a closely related species, *Thamnophis sauritus*, contains, in addition, the serial synapse (Chandler, 1974), and a more thorough search may reveal its presence in *T. r. haydeni*.

Figure 1. The retina can be divided into five major layers and they are the pigment epithelium layer (PEL), photoreceptor cell layer (PCL), inner nuclear layer (INL), inner plexiform layer (IPL) and ganglion cell layer (GCL). A, type A cone; AC, amacrine cell; B, type B cone; BC, bipolar cell; ChC, choriocapillaris; GC, ganglion cell; HyC, hyaloid capillary; HC, horizontal cell; MC, Müller cell body; MCA, Müller cell apical process; MCB, Müller cell basal process; NFB, nerve fiber bundle. 770 X.

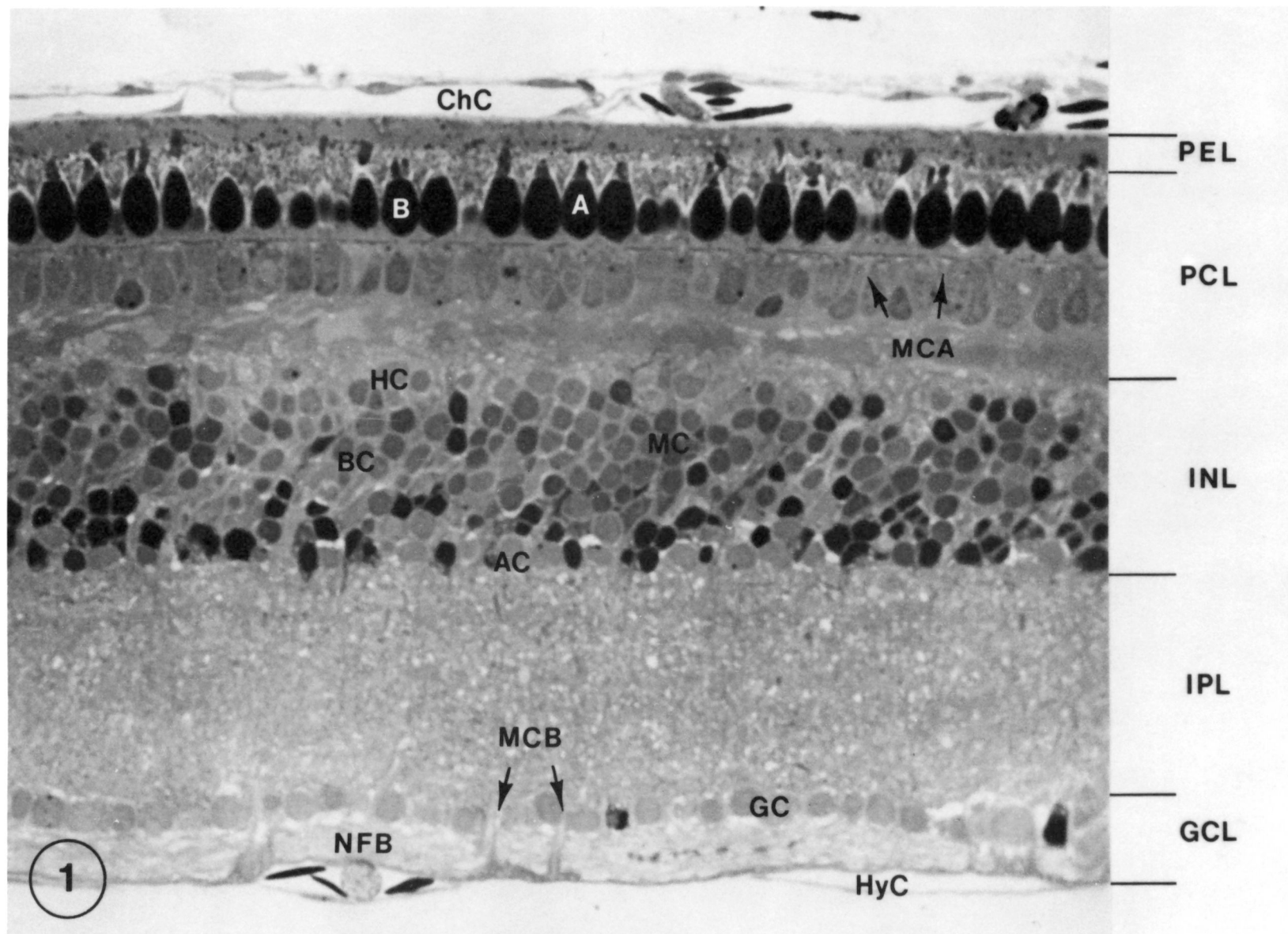


Figure 2. The three types of photoreceptor cells are the type A cone (A), type B cone (B) and type C cone (C). Cells may occur within the inner plexiform layer (arrows). 830 X.

Figure 3. Oil droplets (arrows) occur in both the type A cone (A) and the chief cell of the type B cone (Bc). Type C cones (C) are also present among the microvillous processes of pigment epithelial cells. 2000 X.

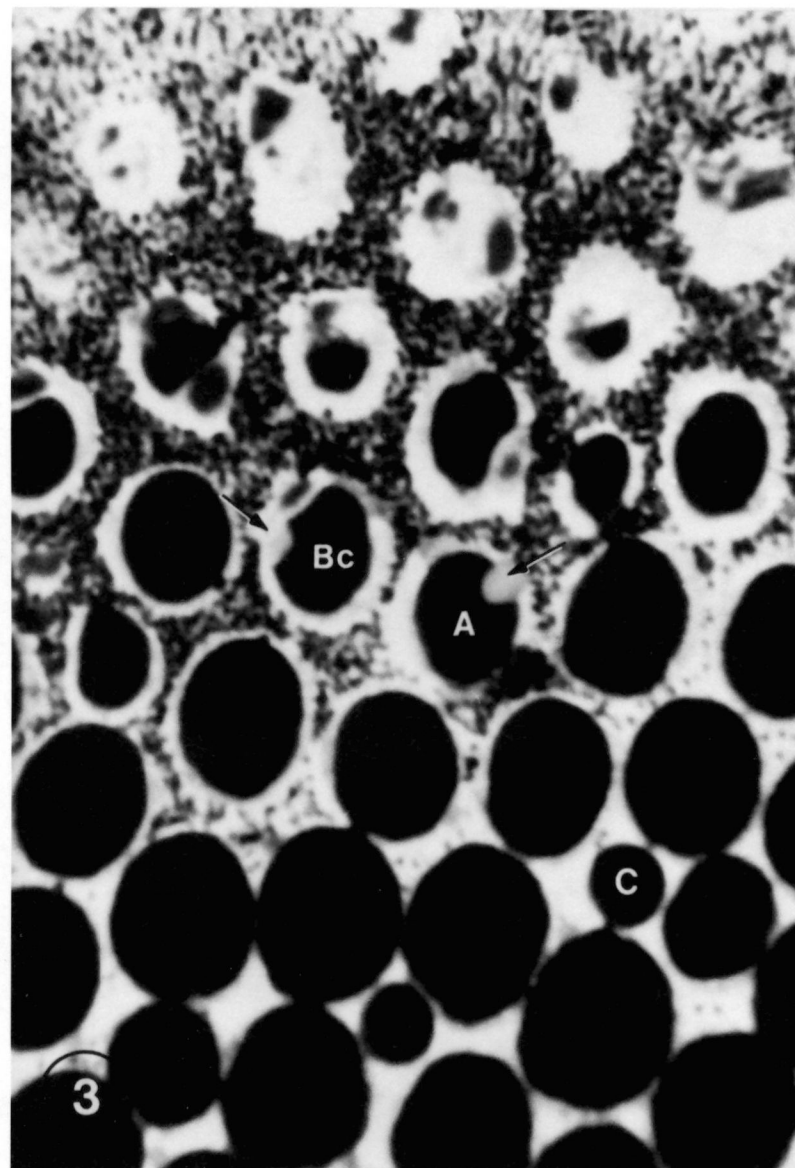
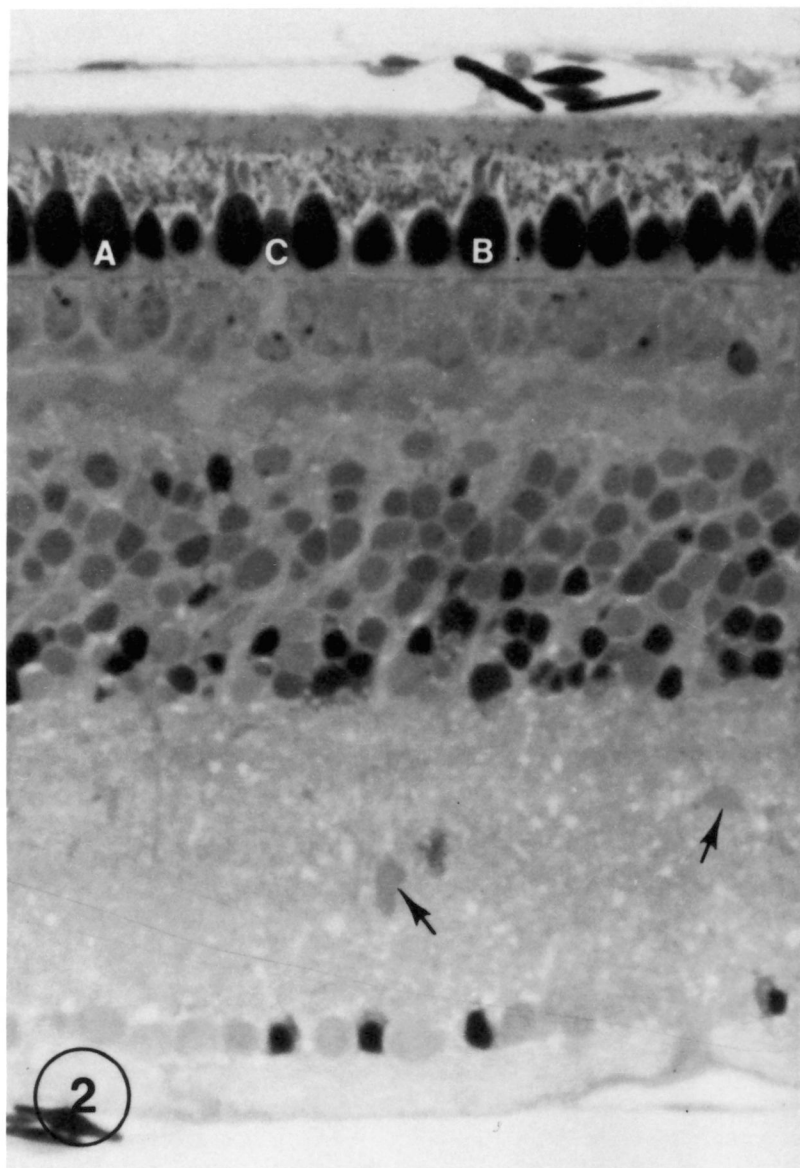


Figure 4. The pigment epithelial cells rest on a basal lamina (BL). The basal surface is invaginated and the apical surface gives rise to microvillous processes (MP). Mitochondria (M) are generally located in the basal and lateral cytoplasm. Caveolae (C) arise from the basal and apical surfaces and coated vesicles (CV) with similar membrane structure occur throughout the cytoplasm. Smooth endoplasmic reticulum (SER) is the major cytoplasmic component. Lysosomes (L) occur mainly in the apical cytoplasm. Pigment granules (PG) occur in the apical cytoplasm and in the microvillous projections. 28,000 X.

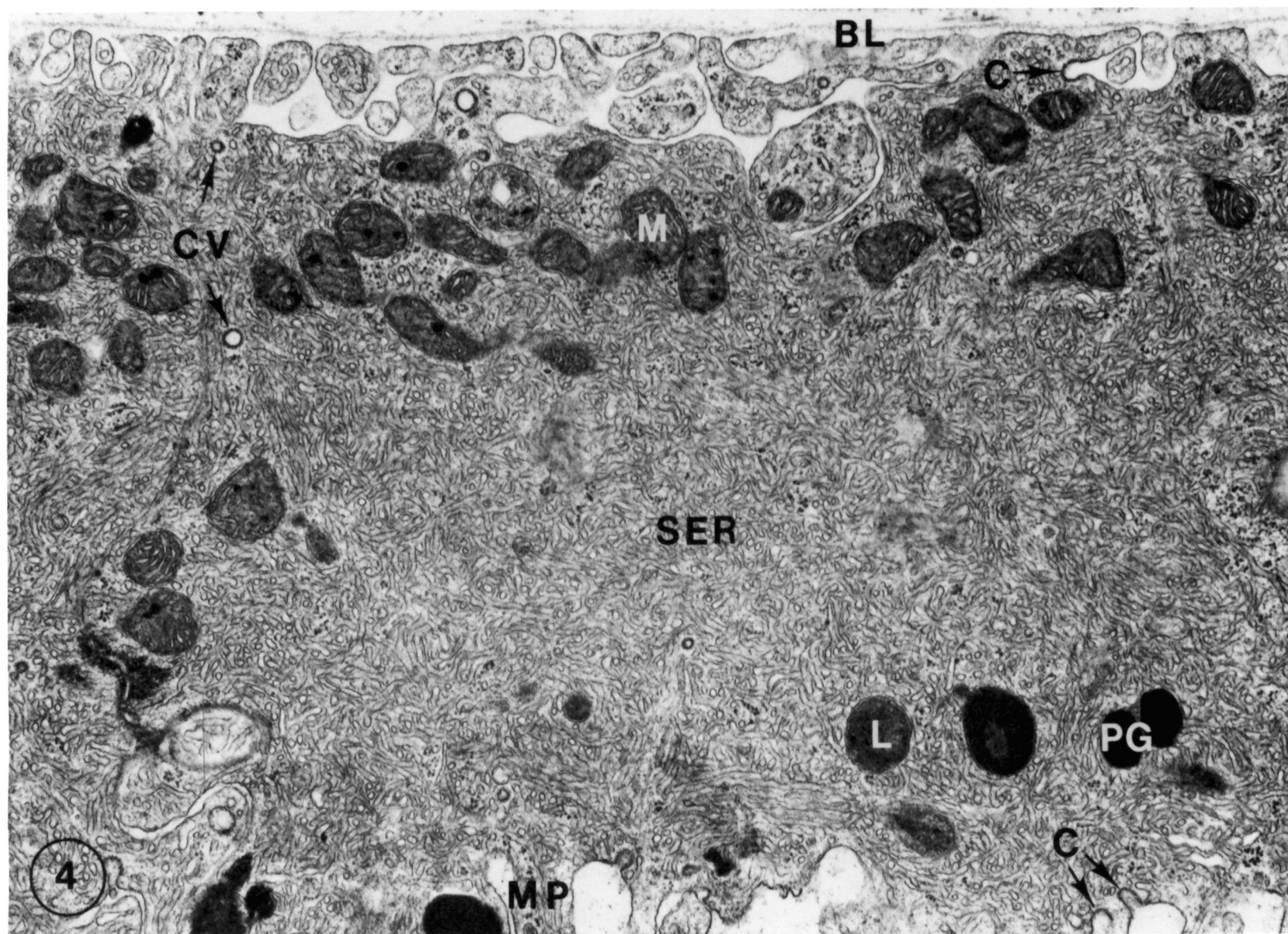


Figure 5. Apical microvillous processes (MP) of the pigment epithelial cells extend between photoreceptor cells (PC). Pigment granules (PG) generally occur in the microvillous processes. Mitochondria (M) frequently occur in the lateral cytoplasm. Lysosomes (L) may be associated with lipid droplets. Lipid droplets (LD) may also occur unassociated with lysosomes. Smooth endoplasmic reticulum (SER) occurs throughout the pigment epithelial cell. 11,000 X.

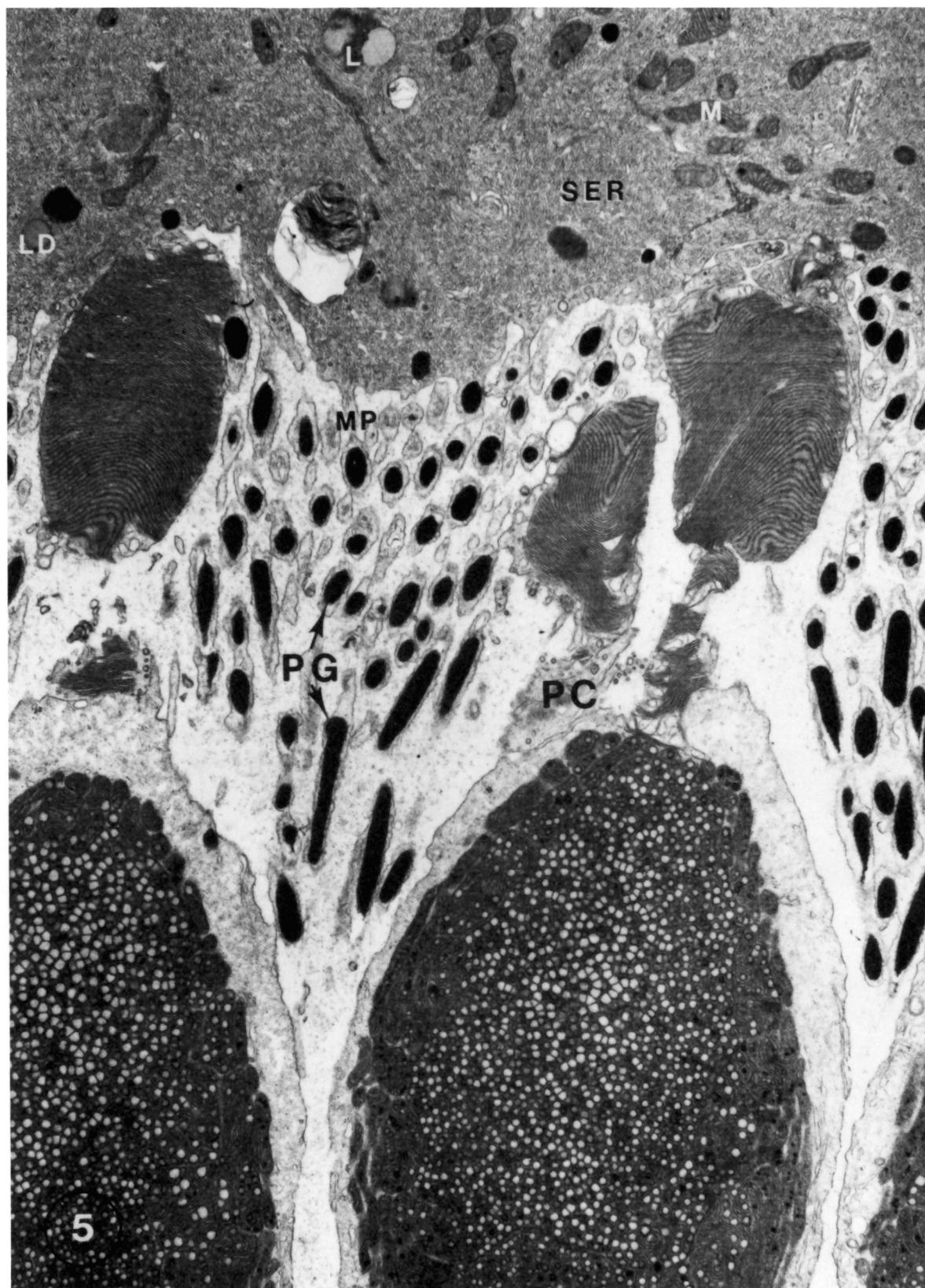


Figure 6. The apicolateral plasma membranes of pigment epithelial cells form junctional complexes consisting of a zonula occludens (ZO), a zonula adherens (ZA) and a macula adherens (MA). Mitochondria (M) occur laterally and smooth endoplasmic reticulum (SER) occupies much of the cytoplasm. 30,000 X.

Figure 7. The pigment epithelial cell nucleus (N) is generally centrally located. Mitochondria (M) occur basally, and smooth endoplasmic reticulum (SER) occupies much of the cytoplasm. 12,000 X.

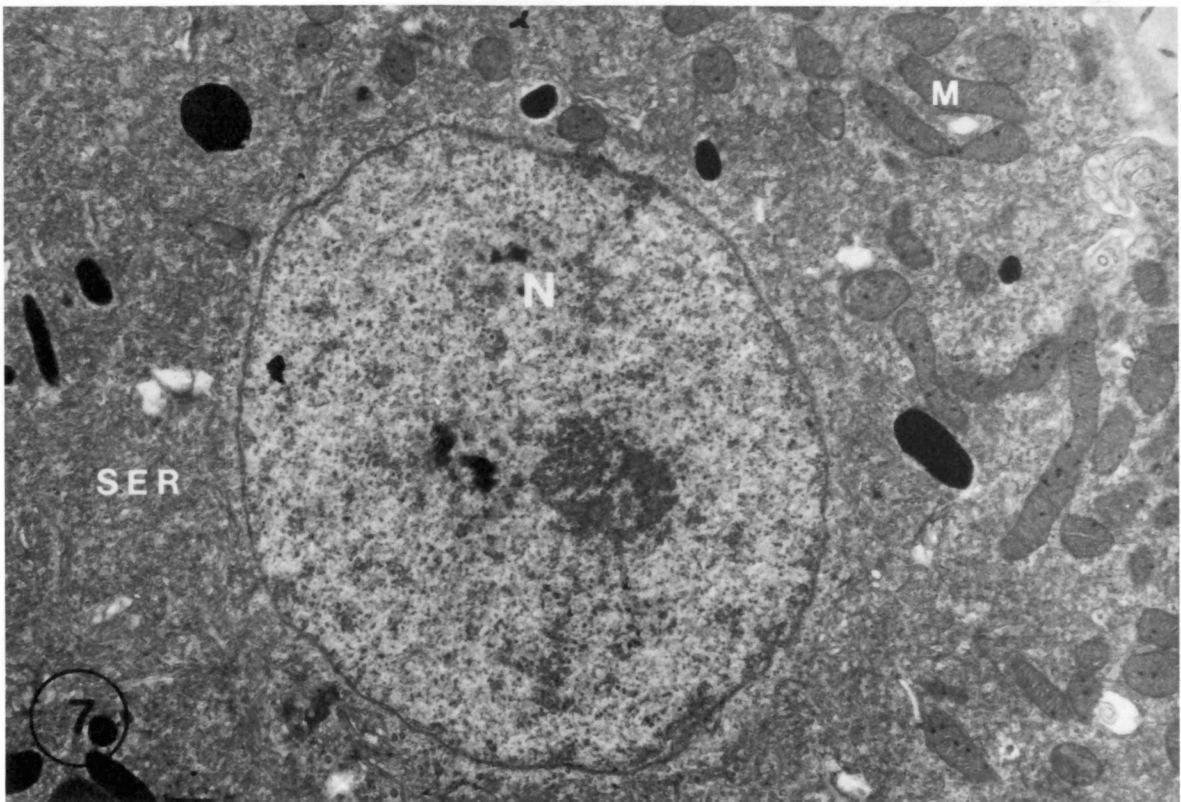
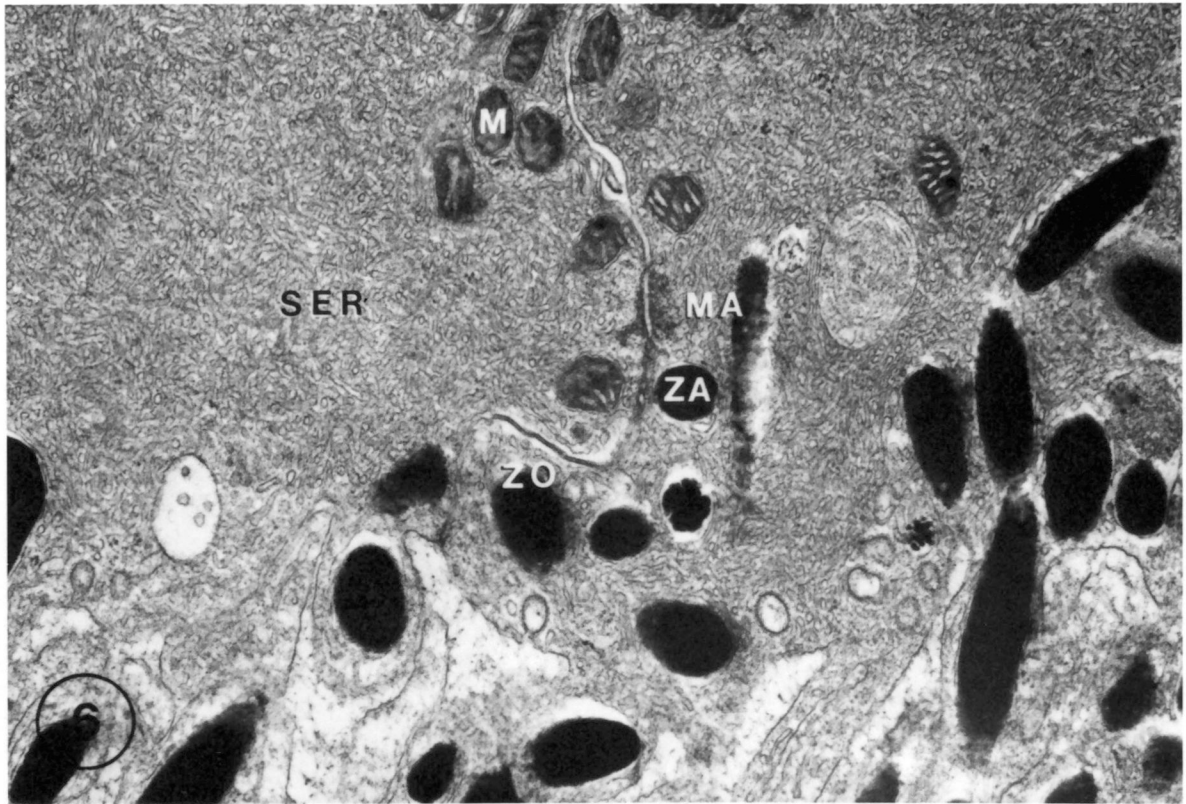


Figure 8. The pigment epithelial cell nucleus may contain a multi-vacuolar inclusion consisting of tubular vacuoles (TV) and a central vacuole (V). Smooth endoplasmic reticulum (SER) fills much of the cytoplasm. Ribosomes (R) occur predominantly in the perinuclear region. 16,000 X.

Figure 9. Golgi profiles (G) are often difficult to identify due to the abundance of smooth-surfaced tubular membranes in the pigment epithelial cell. Mitochondria (M) occur in the basal cytoplasm. Lysosomes (L) tend to occur in the apical cytoplasm. 12,500 X.

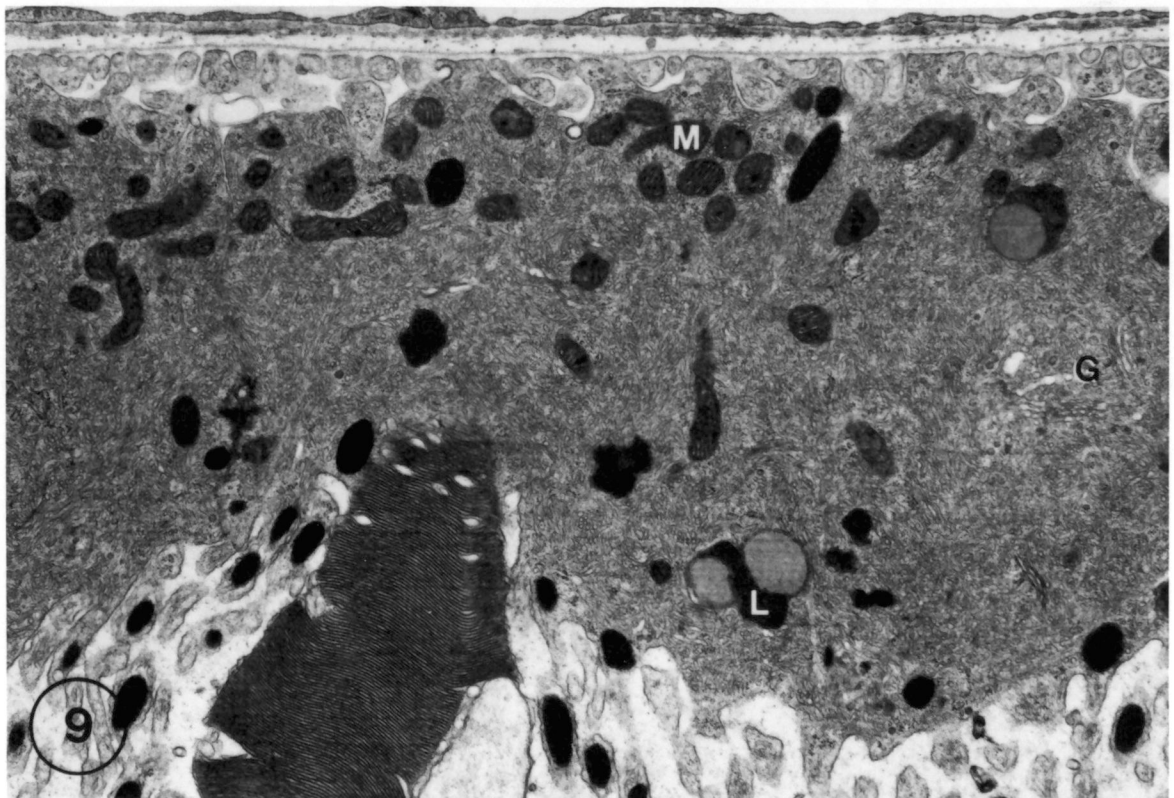
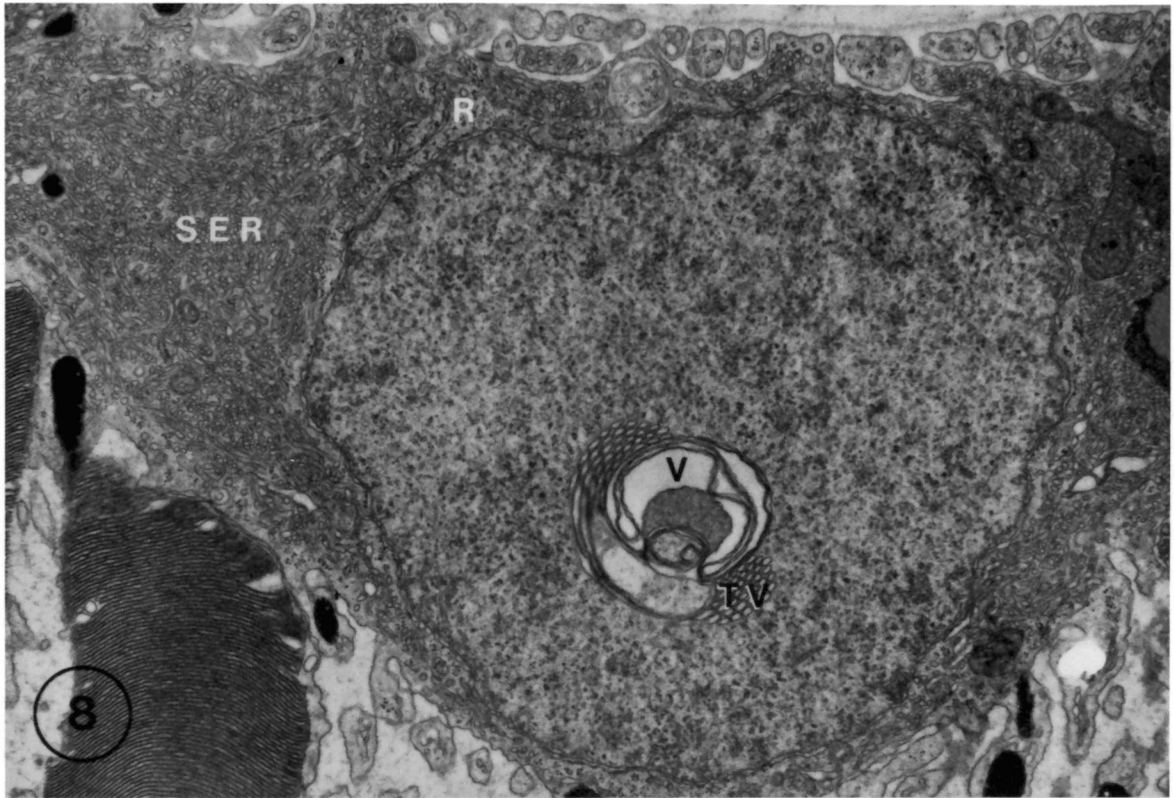


Figure 10. Pigment granules (PG) may occur in the apical cytoplasm of pigment epithelial cells in groups of up to 26 granules. 36,000 X.

Figure 11. Developing pigment granules (DPG) occasionally occur in the apical cytoplasm. 43,000 X.

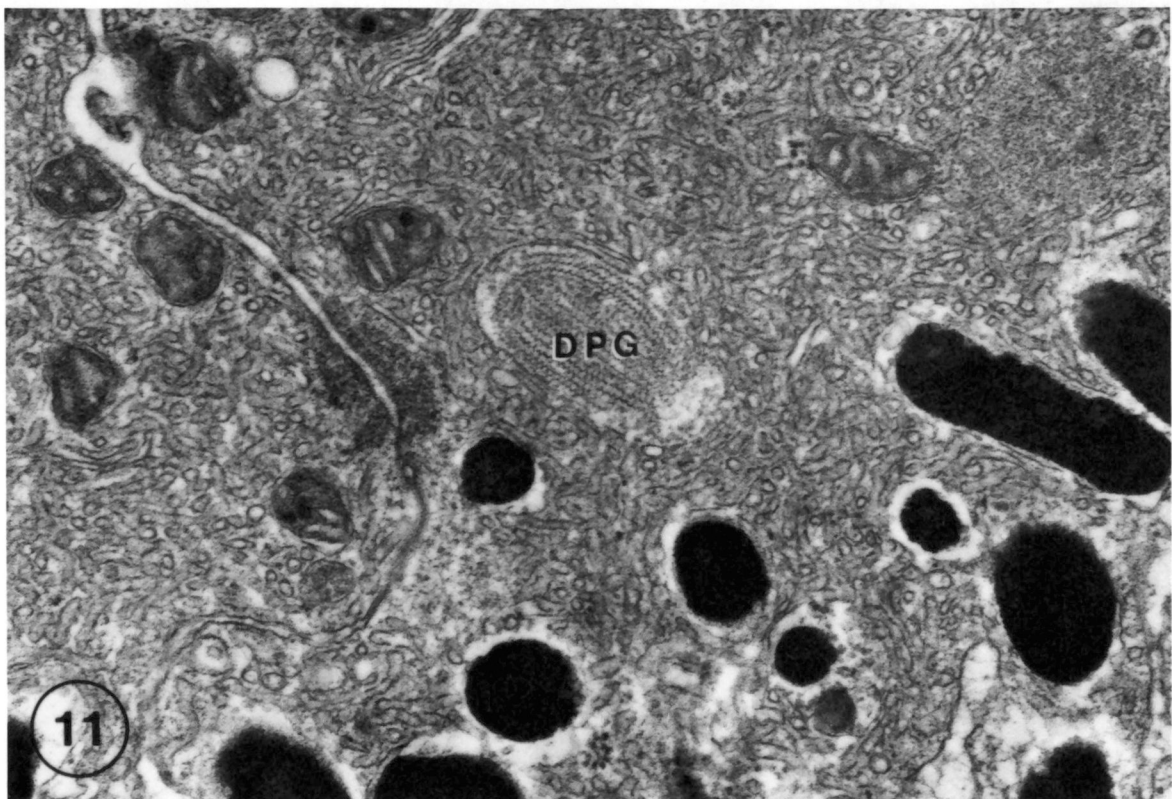
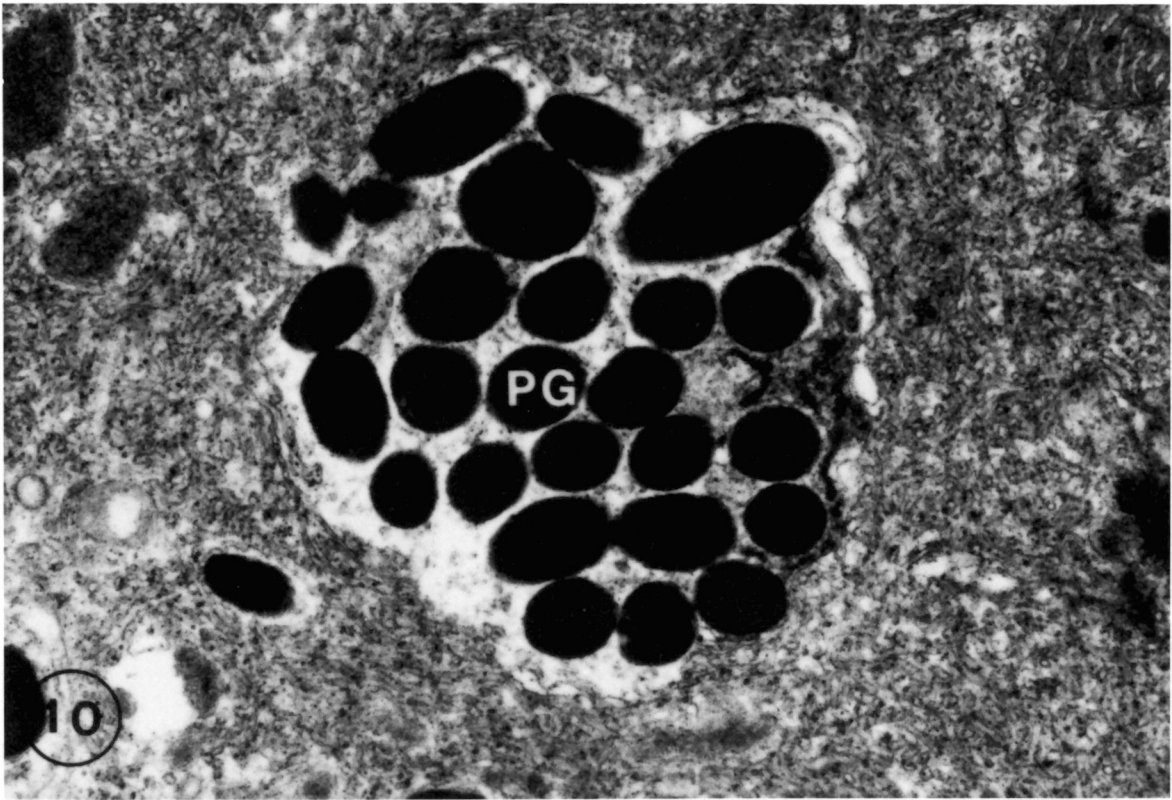


Figure 12. The type A cone consists of an outer segment (OS) containing flattened saccules and an inner segment which is nearly filled by the ellipsoid (E). The apical plasma membrane of the inner segment gives rise to calycal processes (CP) and the plasma membrane at the base of the inner segment gives rise to microvillous processes (MP) which interdigitate with similar processes from adjacent photoreceptors and Müller cells. The inner segment cytoplasm on the side where the connecting cilium (CC) arises is wider than that around the rest of the ellipsoid. An oil droplet (OD) may occur in the apical cytoplasm in the inner segment. 10,000 X.

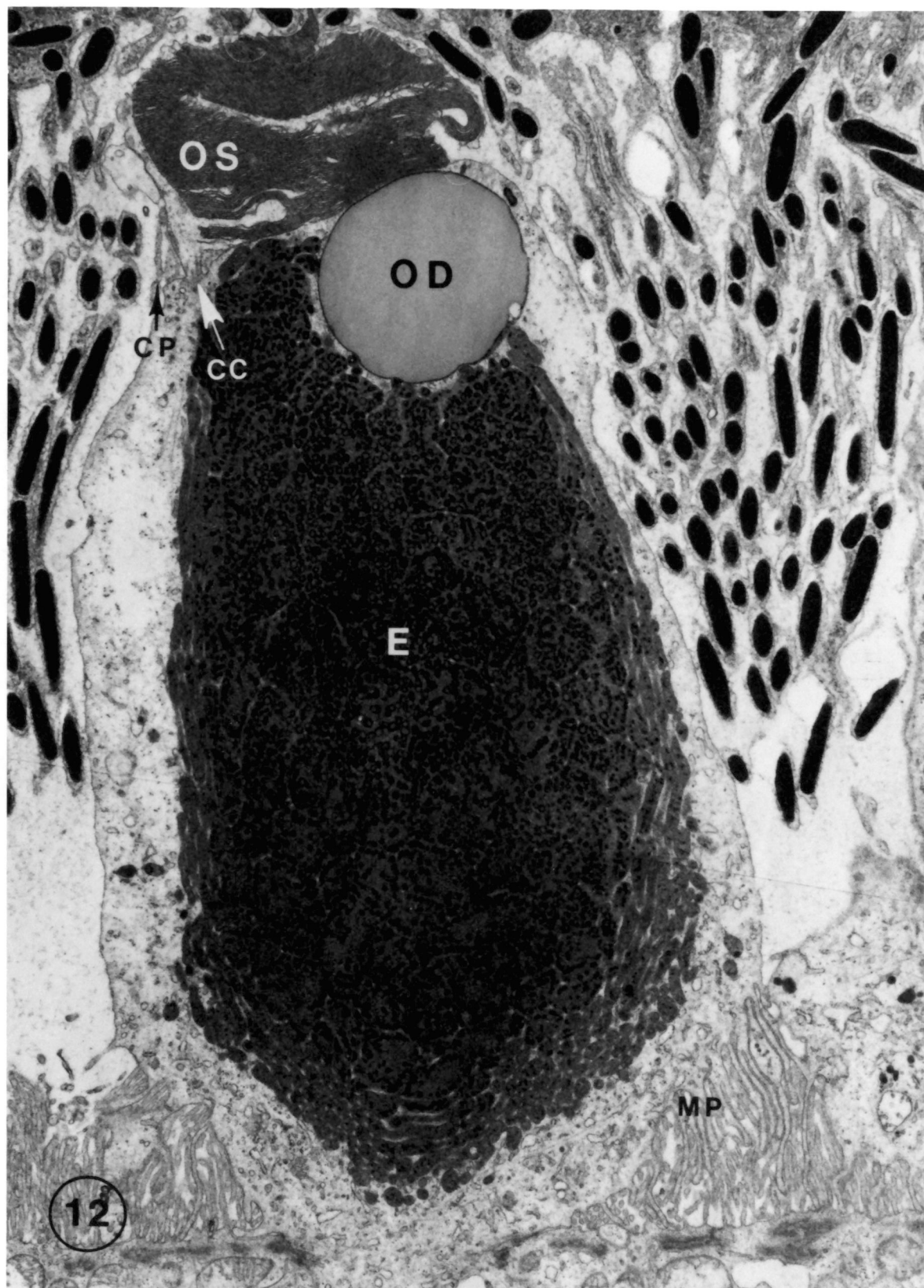


Figure 13. Photoreceptor cell outer segments (OS) frequently lie in indentations of a pigment epithelial cell (PE). 34,500 X.

Figure 14. The only cytoplasmic connection between the outer segment (OS) and the inner segment (IS) is the connecting cilium, which arises from a basal body (BB). 30,000 X.

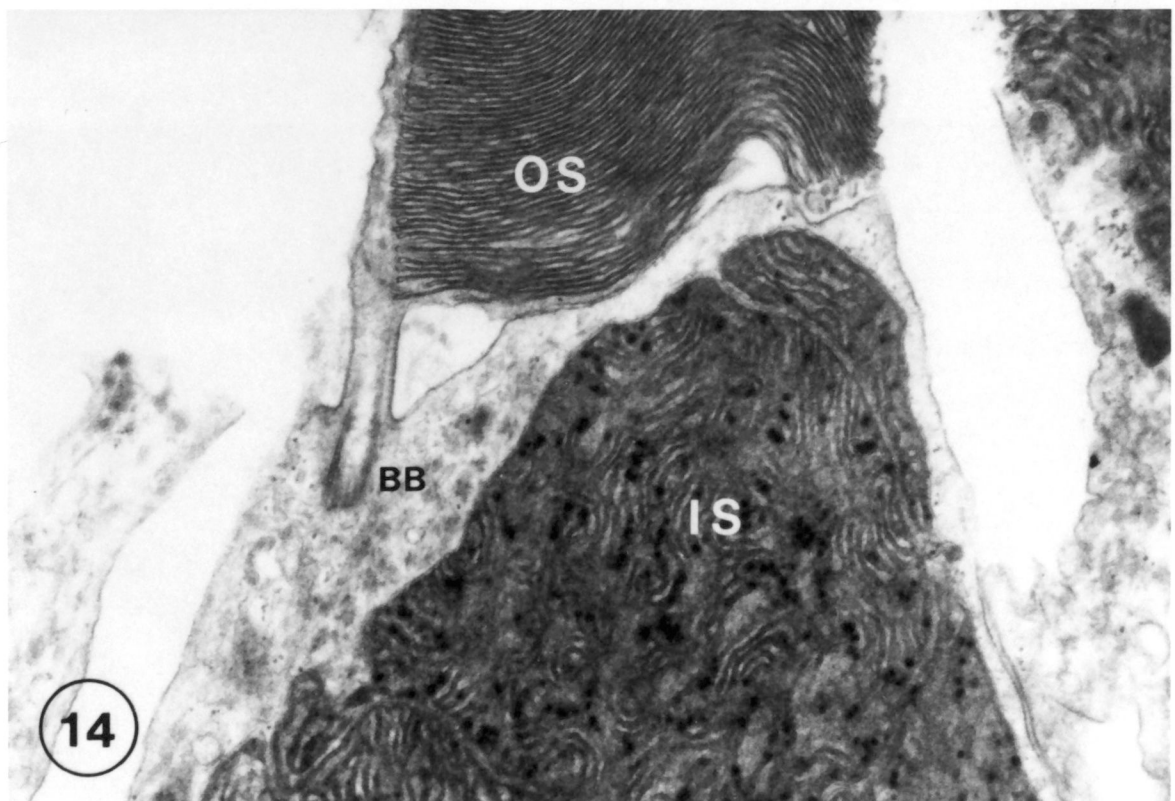
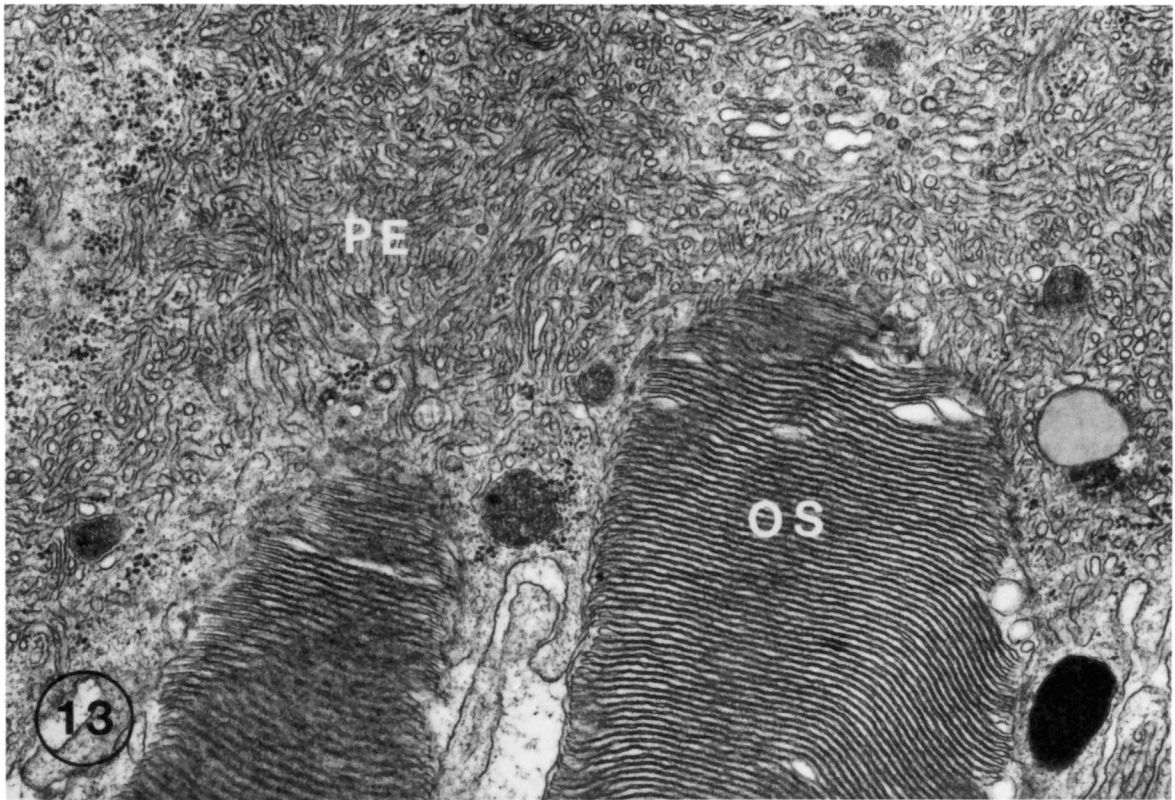


Figure 15. The ellipsoid of the type A cone contains a layer of small cortical mitochondria (CM) around the larger medullary mitochondria (MM). The cortical mitochondria contain small electron-opaque matrix granules and the medullary mitochondria contain larger lipid droplets. 9000 X.

Figure 16. The chief cell (Bc) of the type B cone occasionally contains an oil droplet (OD). The ellipsoid mitochondria of the accessory cell (Ba) of the type B cone contain electron opaque granules (arrow) and a less electron-opaque matrix than the mitochondria of the chief cell. 13,000 X.

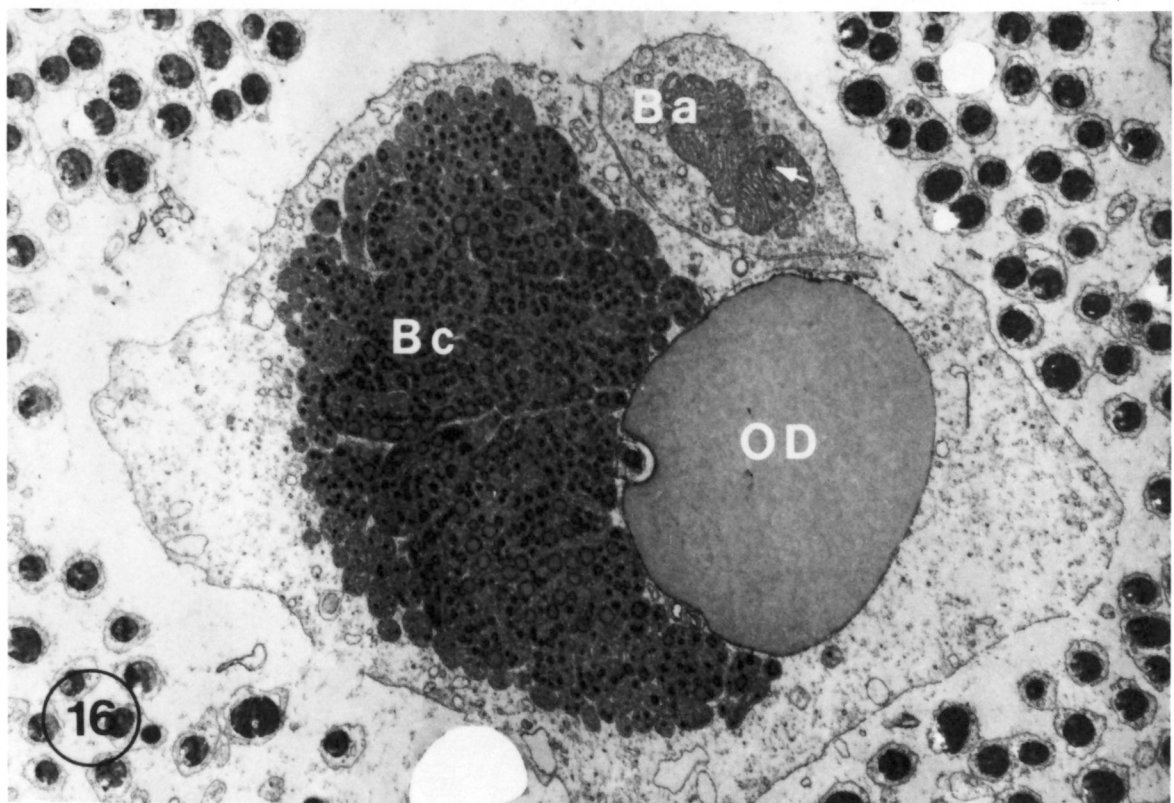
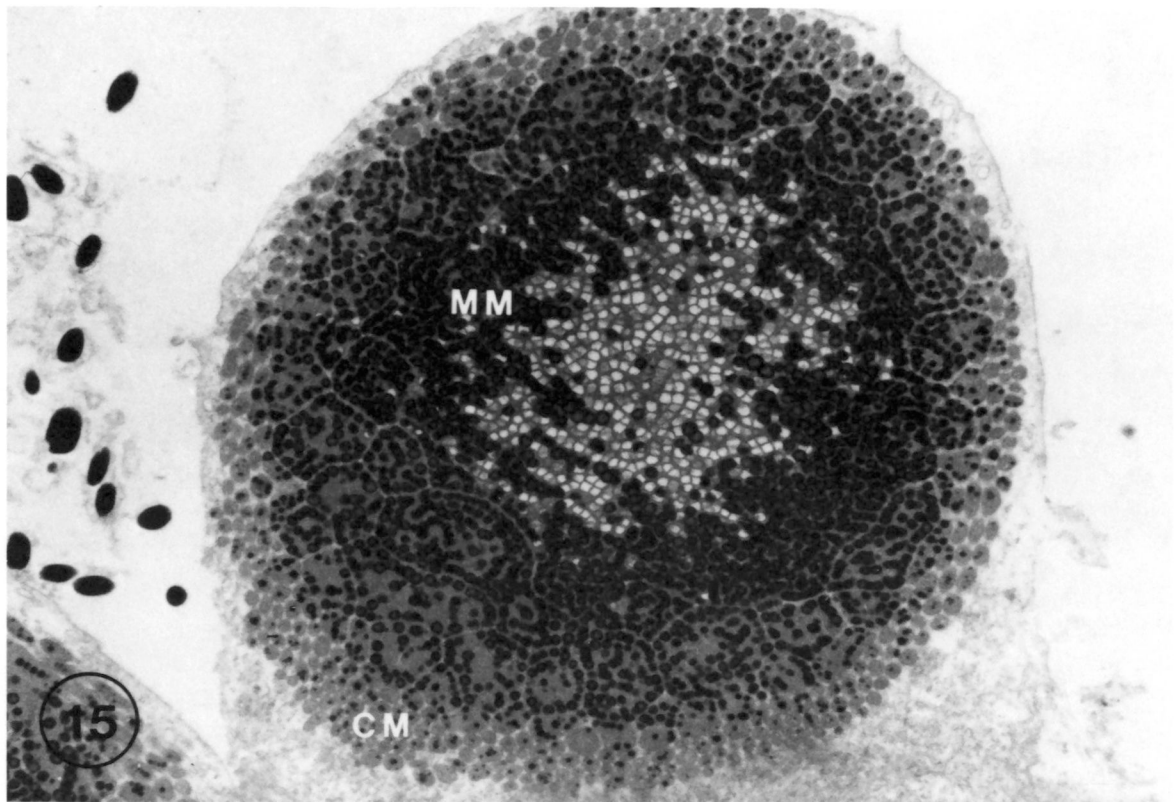


Figure 17. At the level of the external limiting membrane (ELM) the photoreceptor cells (PC) and Müller cells (MC) form junctional complexes consisting principally of the zonula adherens (ZA). Microtubules (MT) which cross the narrow cytoplasm of the photoreceptor cell appear to enter the zonulae adherentes. 43,000 X.

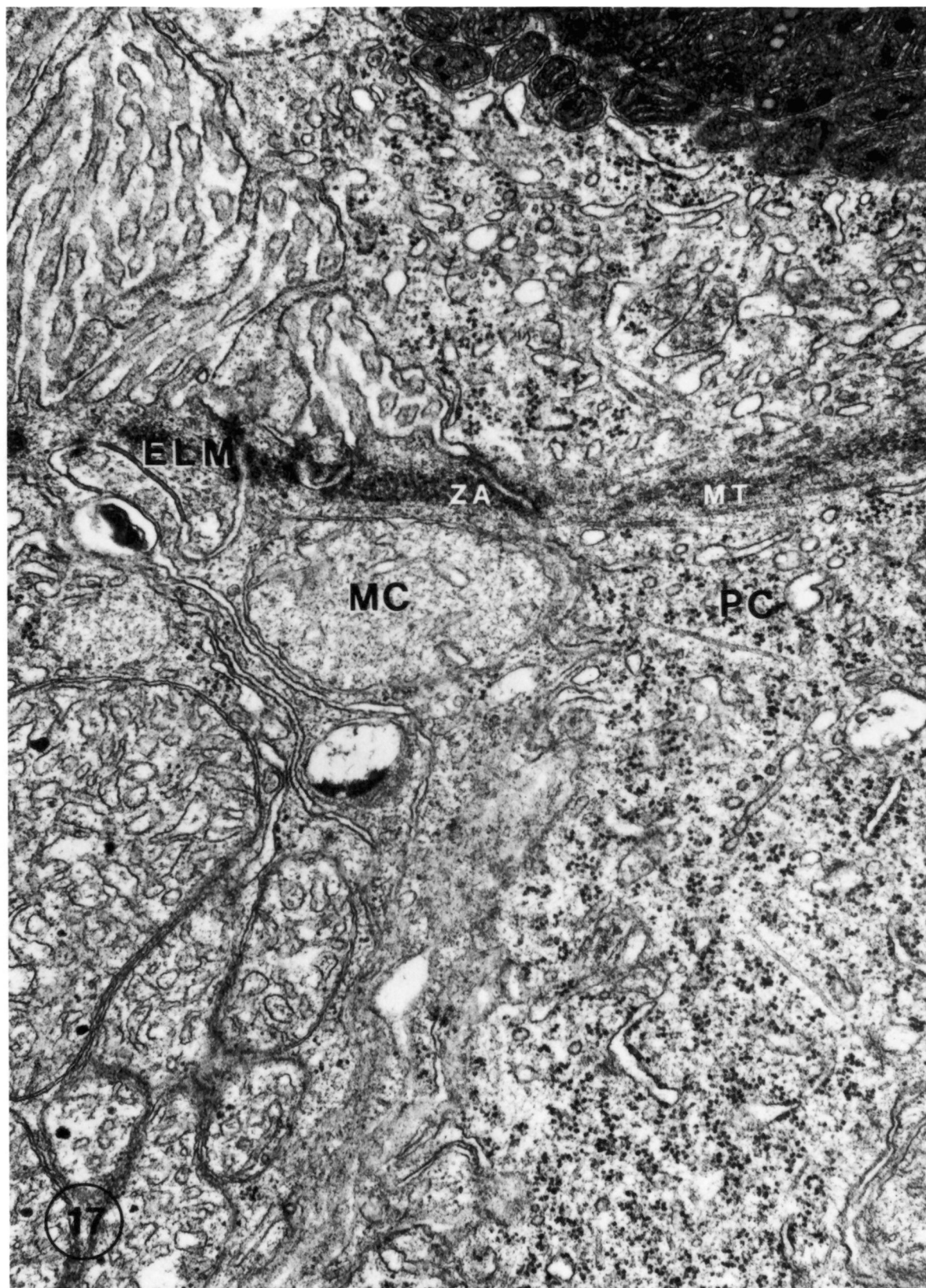


Figure 18. The type A cone cytoplasm contains a nucleus (N), Golgi profiles (G), microtubules (MT), and lysosomes (L). 15,000 X.



Figure 19. The pedicle (P) is connected to the photoreceptor perikaryon by a conducting fiber (CF). Surface contacts (SC) occur on the basal concavity. Pedicles are isolated laterally by Müller cell cytoplasm (arrows). 15,000 X.

Figure 20. Two pedicles (P) may each form a portion of a basal concavity, yet they are separated by Müller cell cytoplasm (arrows). 11,000 X.

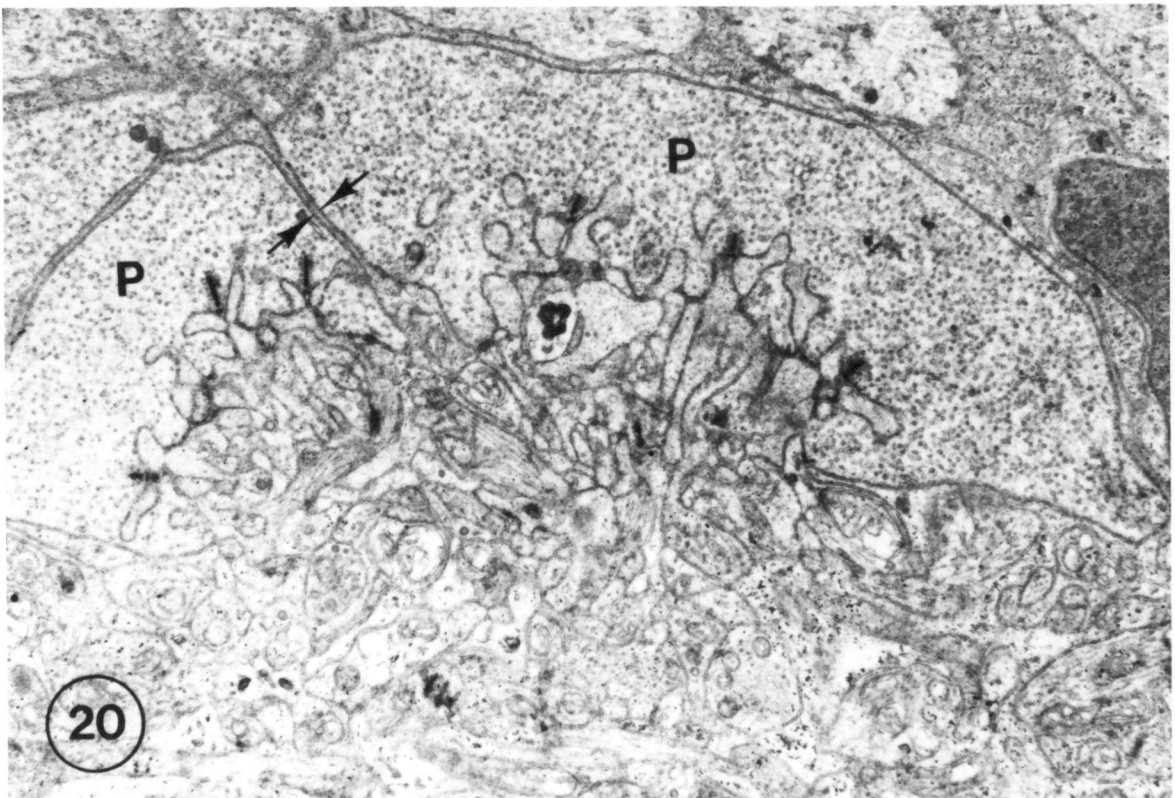
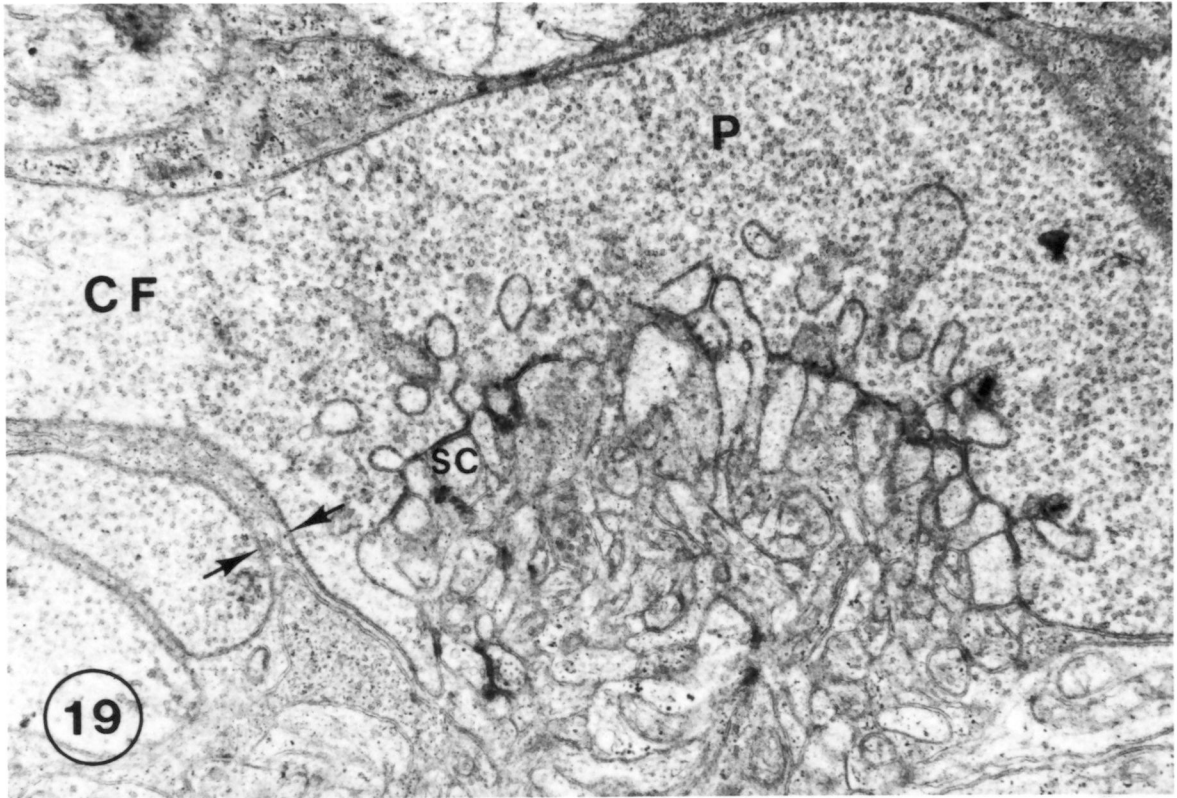


Figure 21. Smooth endoplasmic reticulum (SER) may occur apicocentrally in the pedicle. 10,000 X.

Figure 22. Three processes enter each basal invagination of the pedicle, constituting a triad. The lateral elements are presumed to be horizontal cell processes (HP) and the central element is presumed to be a bipolar cell dendrite (D). The arciform density (AD) lies between the two lateral elements and the synaptic ribbon (SR) bisects the angle between the lateral elements and is surrounded by a regularly arranged layer of synaptic vesicles. 21,000 X.

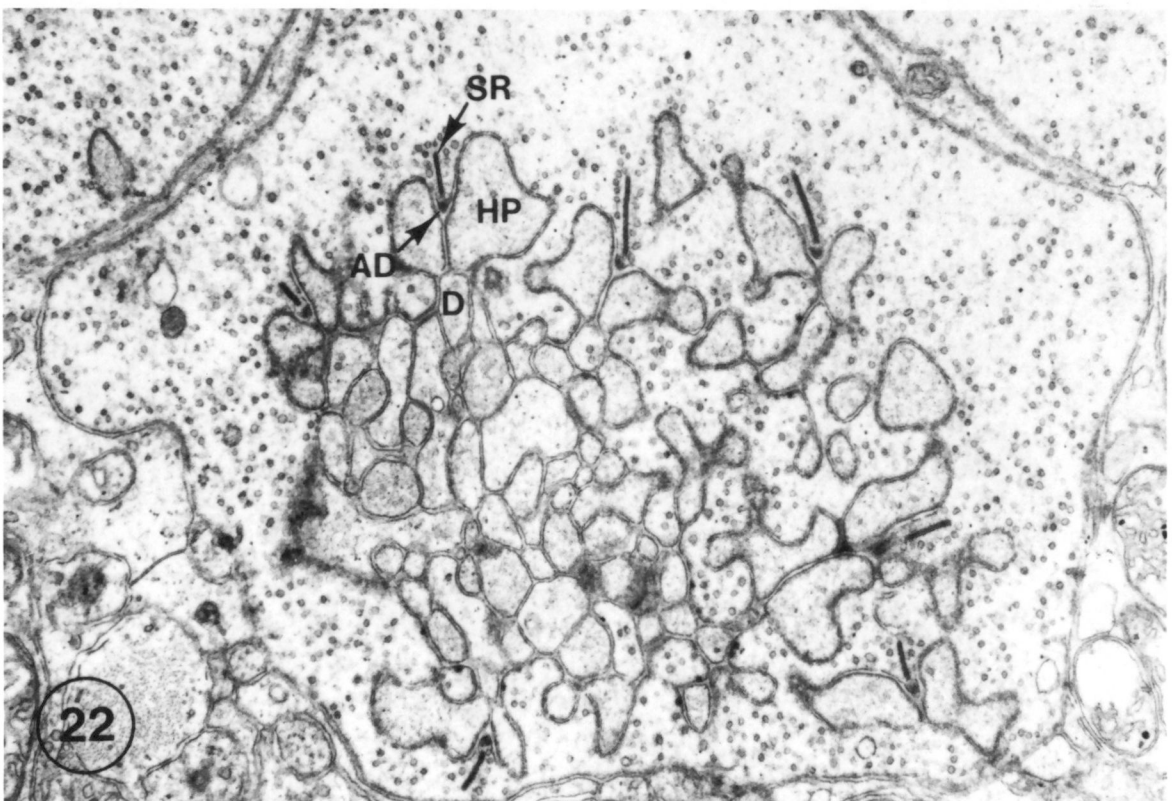
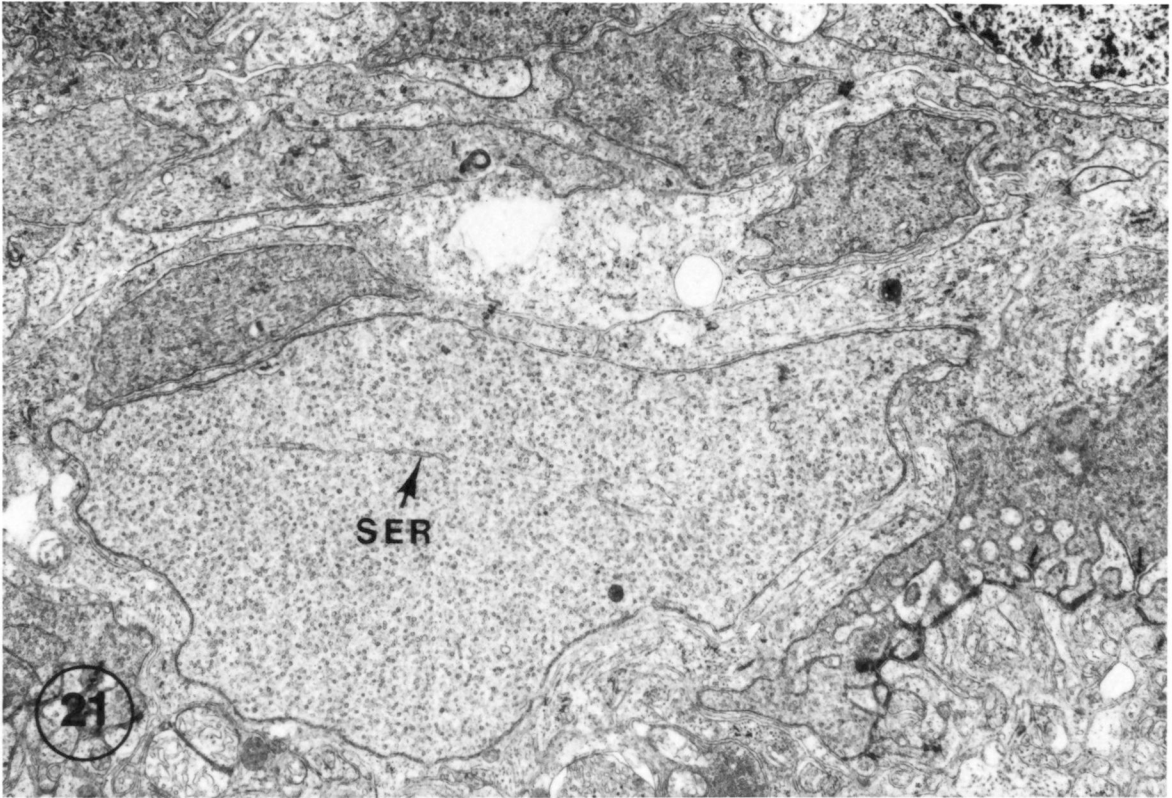


Figure 23. The synaptic ribbon (SR) bisects the angle between the lateral elements of the triad (HP). The arciform density (AD) lies beneath the synaptic ribbon between the lateral elements. 70,000 X.

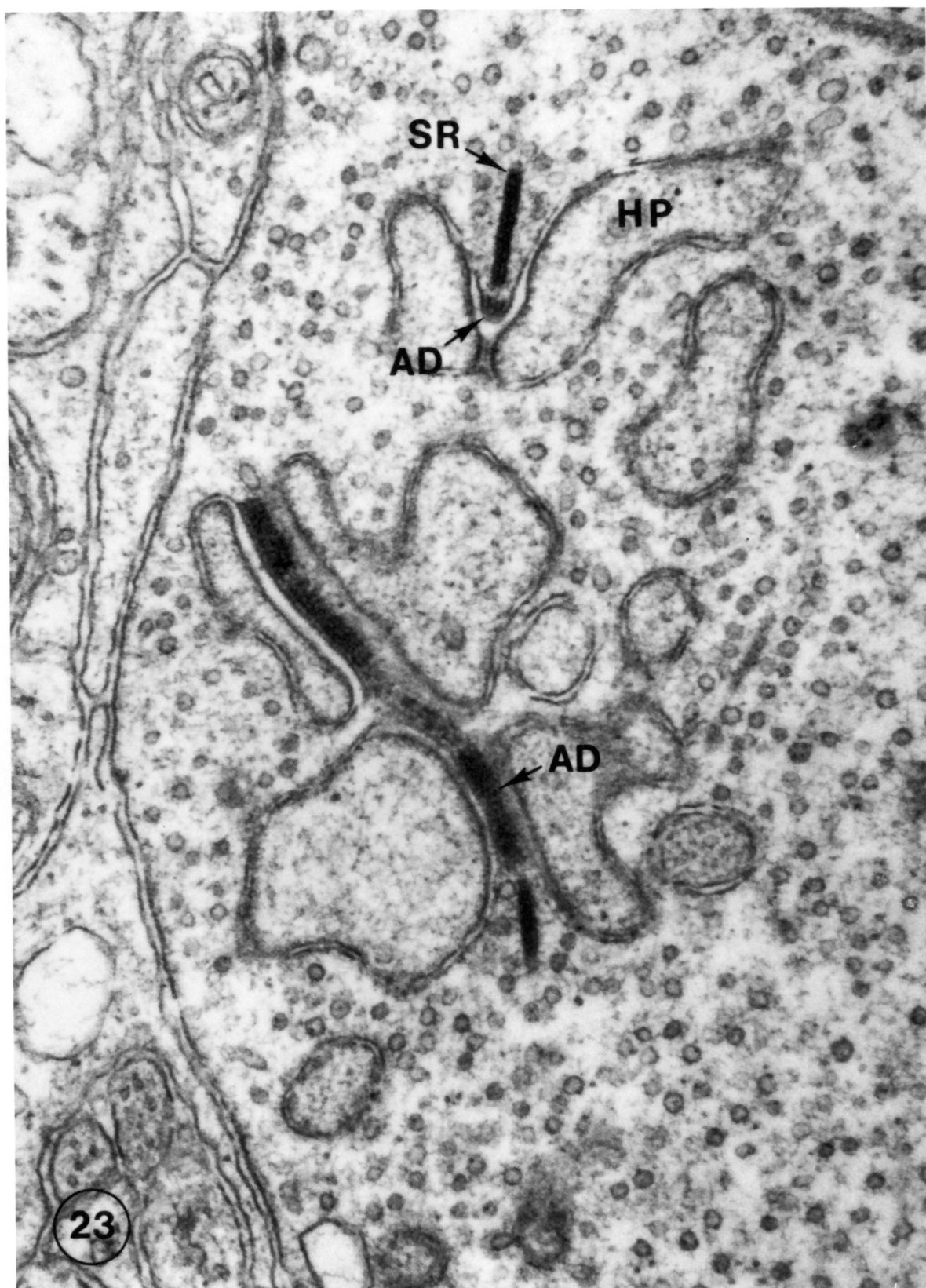


Figure 24. The type B chief cell (Bc) is morphologically identical to the type A cone (cf. Fig. 9). The accessory cell inner segment lies closely apposed to that of the chief cell and contains a smaller ellipsoid (E). These ellipsoid mitochondria contain a less electron-opaque matrix and fewer granules than those of the chief cell. The outer segments (OS) of the chief and accessory cells arise at the same horizontal plane. 12,000 X.

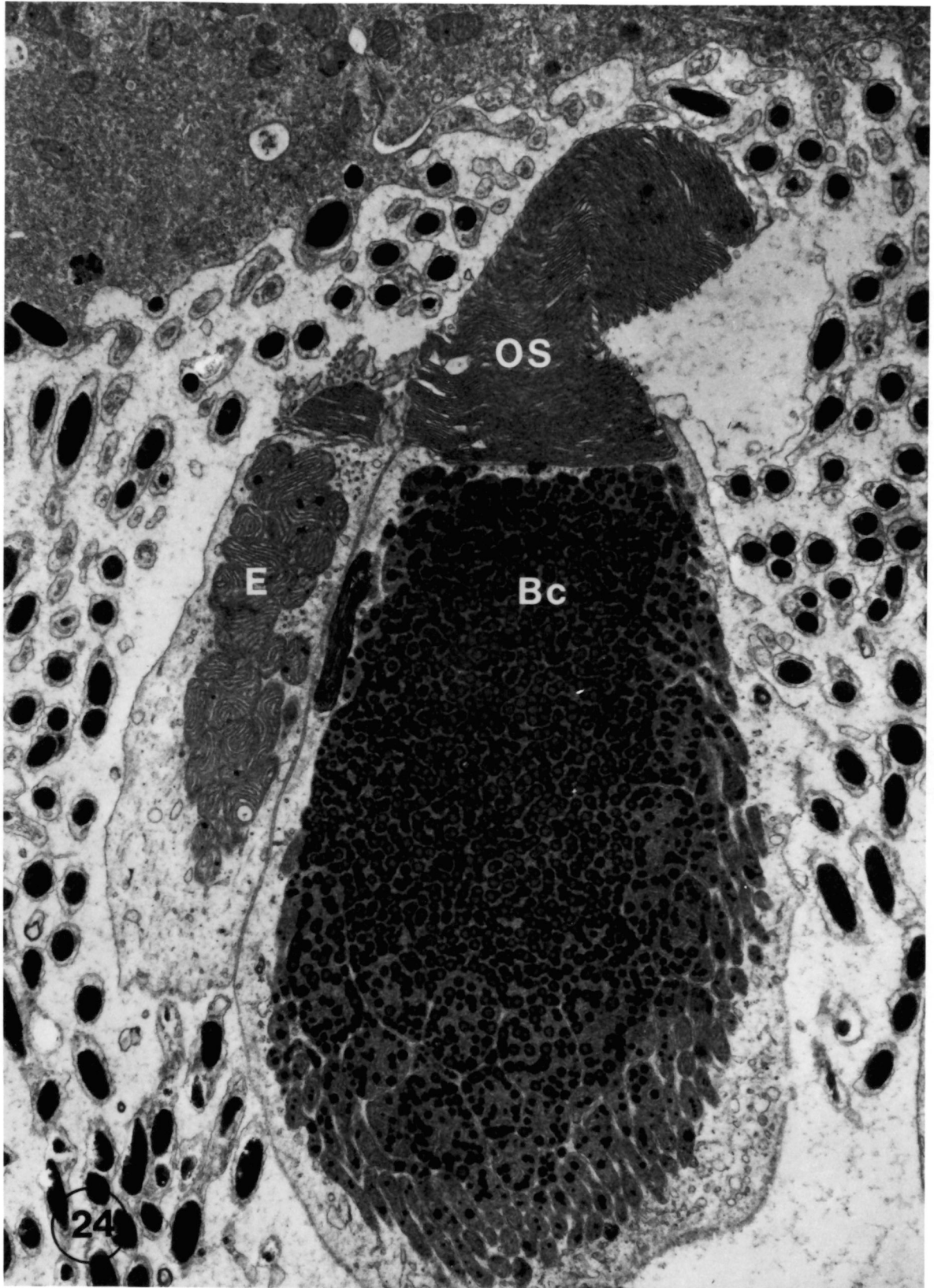


Figure 25. The outer segment (OS) of the accessory cell (Ba) is as tall as that of the chief cell (Bc) (cf. Fig. 21). Ellipsoid mitochondria of the accessory cell contain a less electron-opaque matrix than those of the chief cell. 14,500 X.

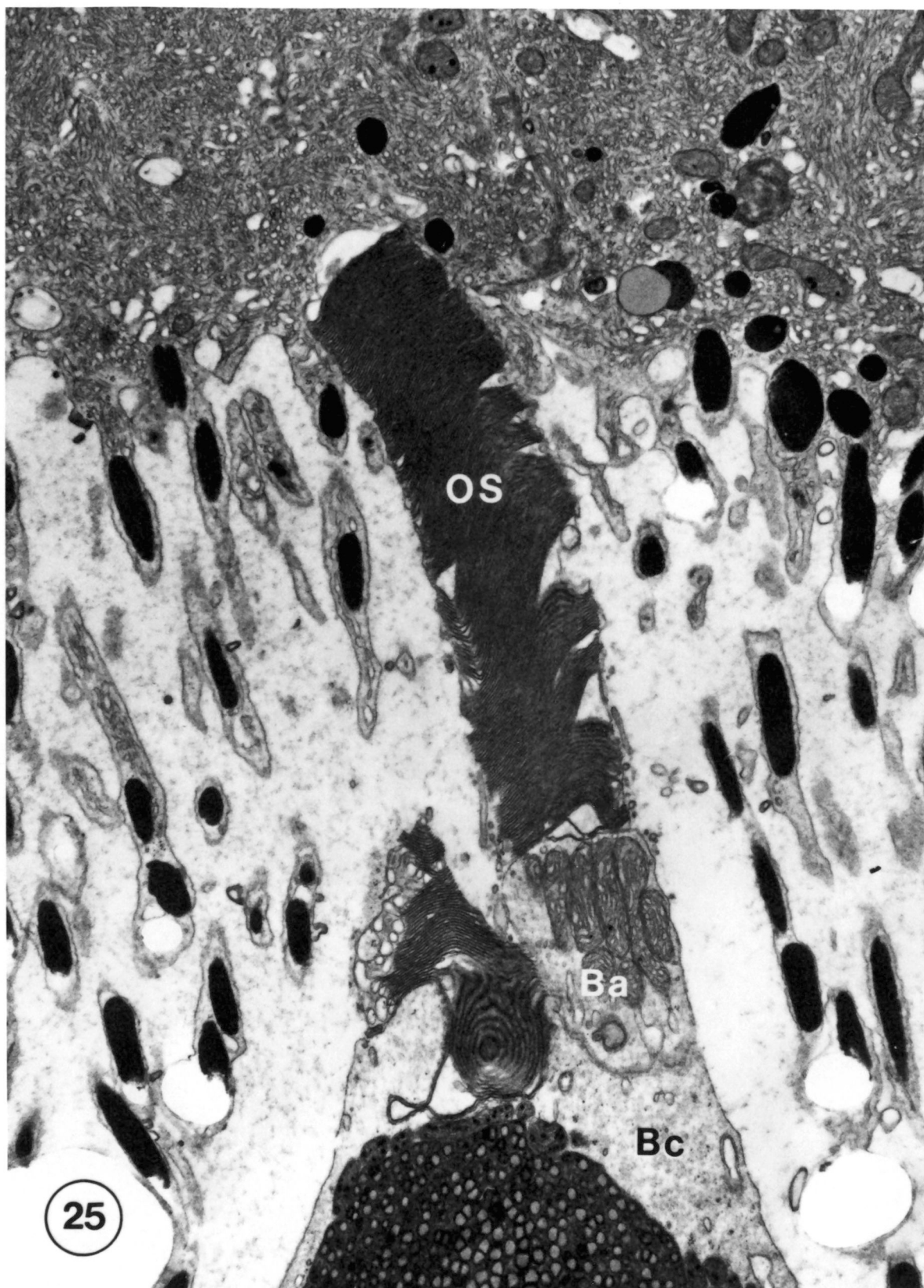


Figure 26. The inner segments of the type B chief (Bc) and accessory (Ba) cells lie closely apposed. The accessory cell ellipsoid (E) region is joined to the nuclear area by a myoid stalk (MS). The basal plasma membranes of both cells of the type B cone have microvillous processes (MP) which interdigitate with those of other photoreceptors and Müller cells (MC). A second aggregation of mitochondria occurs in the accessory cell as a paranuclear body (PNB) in the scleral perinuclear cytoplasm. 12,000 X.

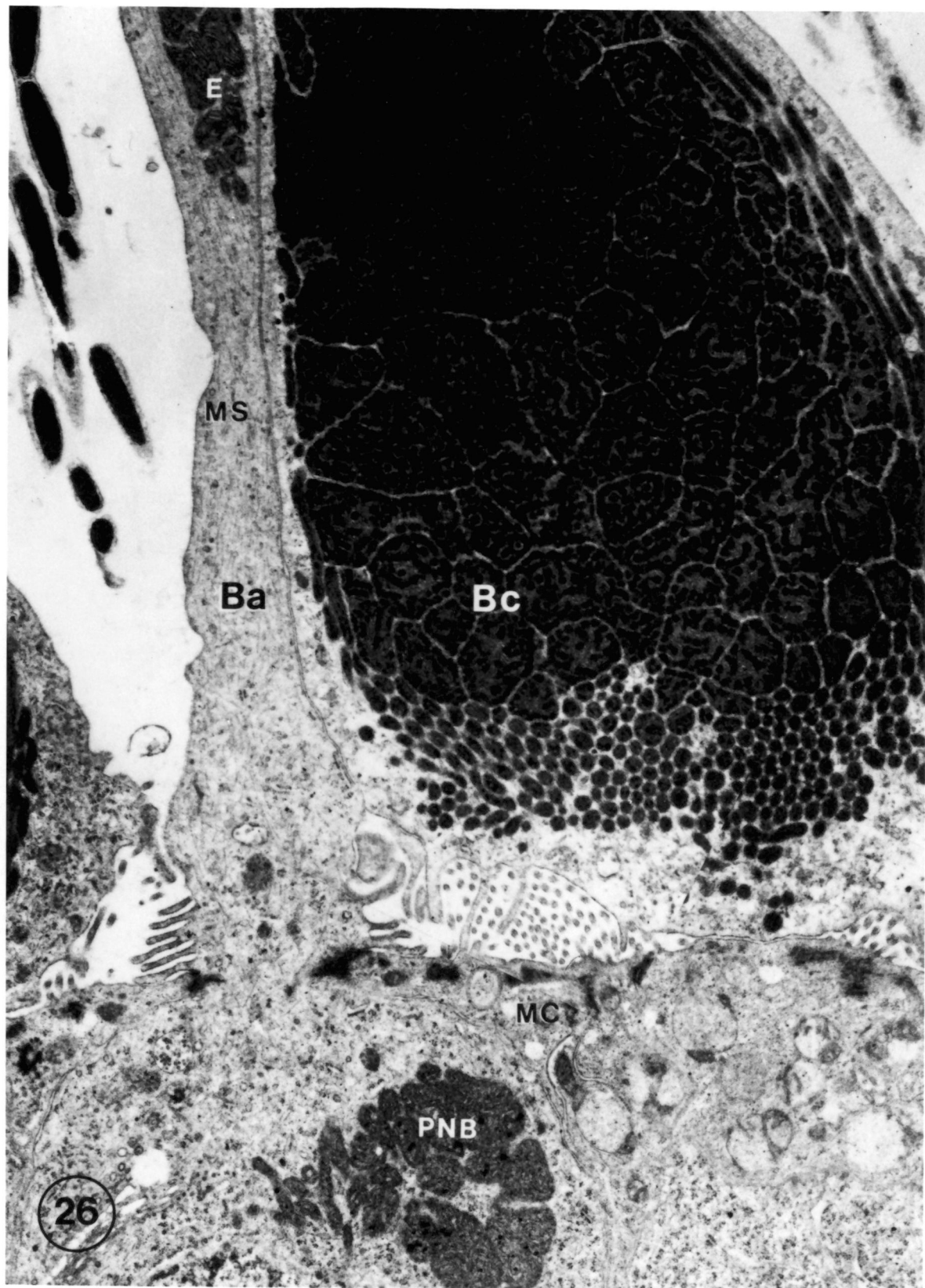


Figure 27. A single mitochondrion (M) may pass from the paranuclear body (PNB) beyond the external limiting membrane (ELM) into the myoid stalk (MS). 25,000 X.

Figure 28. A single mitochondrion (M) may pass from the paranuclear body (PNB) several micrometers into the myoid stalk (MS). 16,500 X.

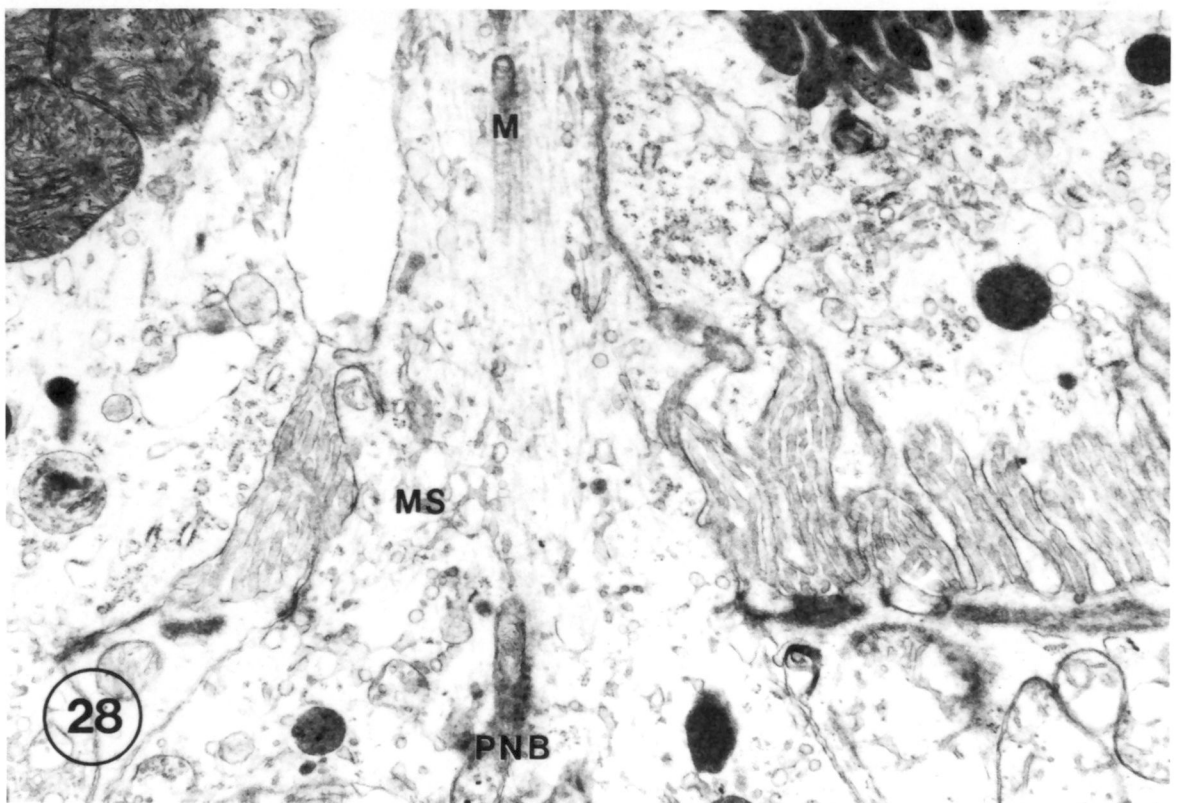
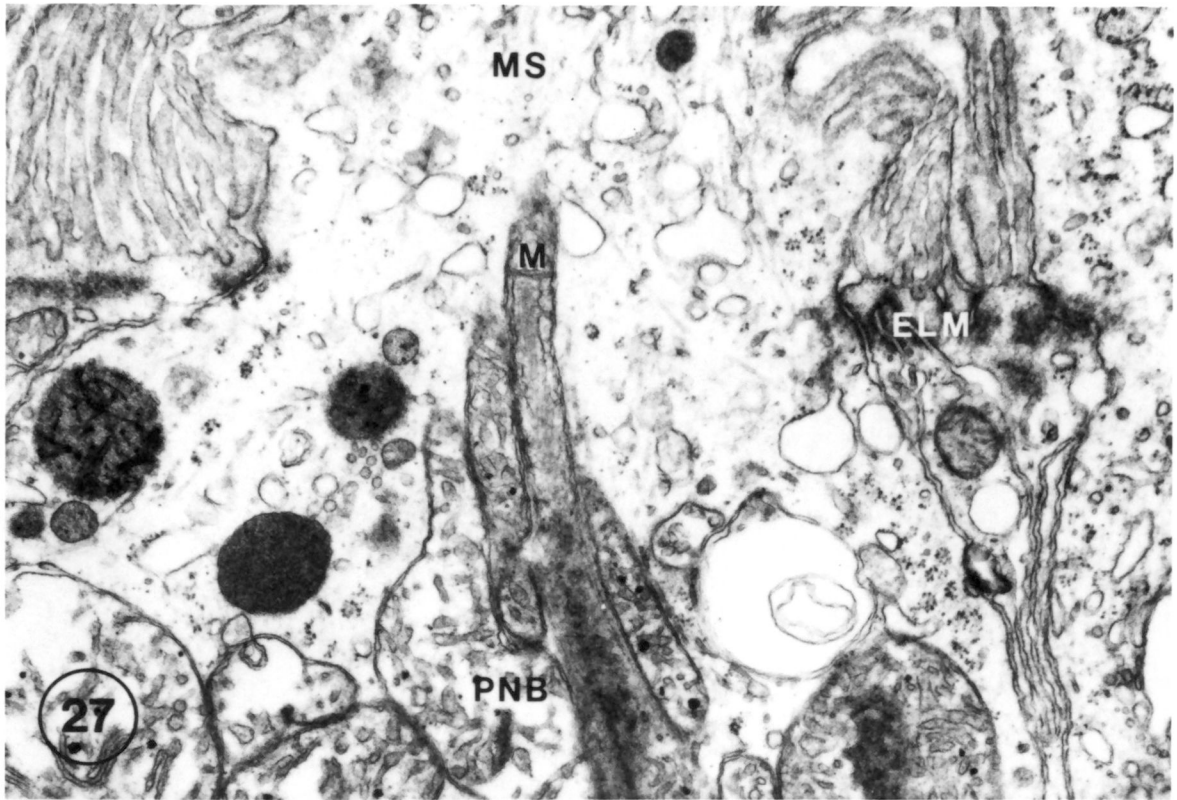


Figure 29. The outer segment (OS) of the type C cone does not reach the pigment epithelial cells (PE). 14,500 X.



Figure 30. The inner segment of the type C cone (C) is shorter than that of the type A cone (A). Calycal processes (CP) arise from the apical plasma membrane of the inner segment. The outer segment (OS) tapers slightly from its base. Microvillous processes (MP) of the basolateral plasma membrane of the inner segment interdigitate with those of other photoreceptors and Müller cells. 12,500 X.

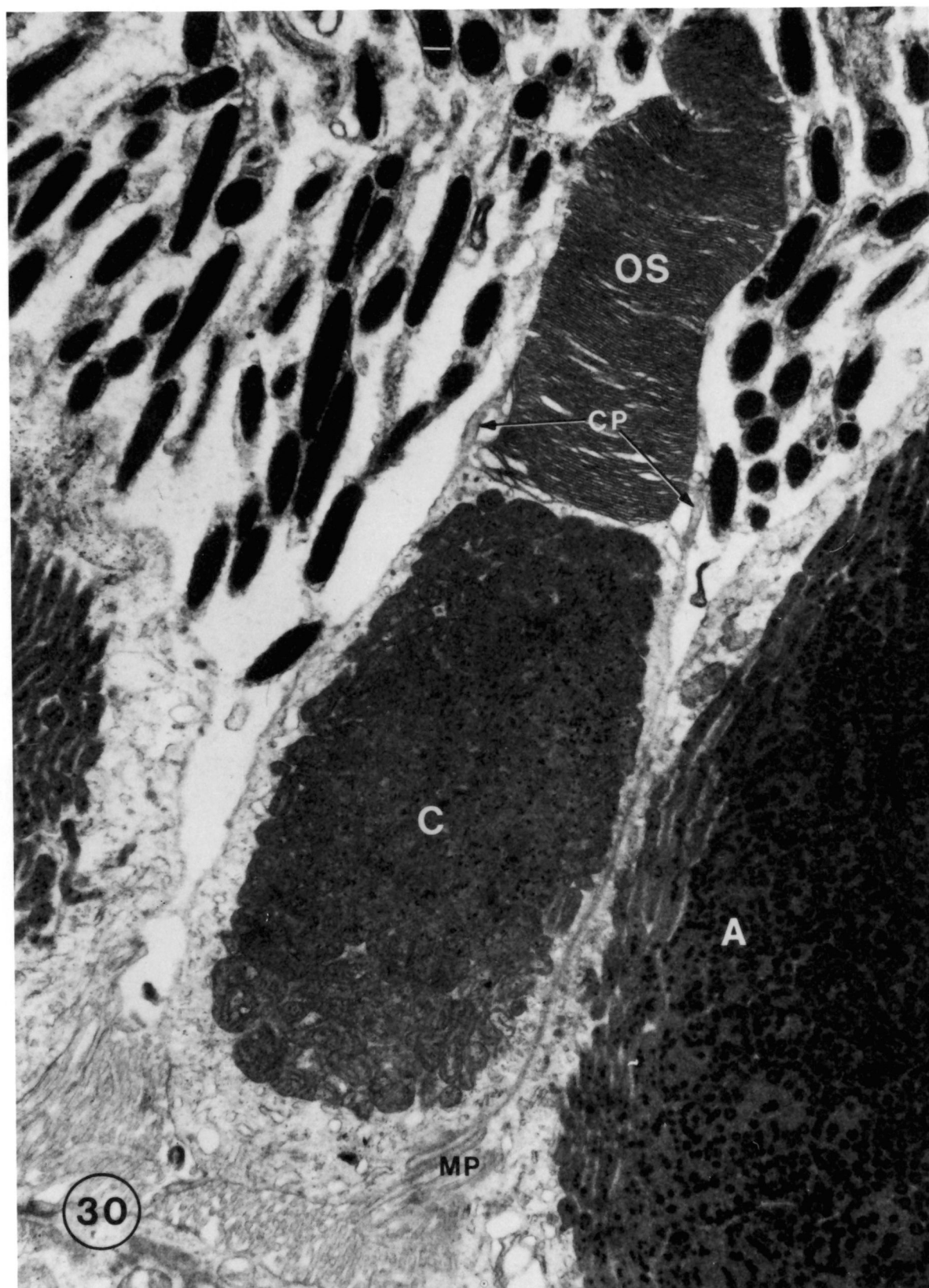


Figure 31. Electron micrograph of type A (A), B chief (Bc), B accessory (Ba) and C (C) cones at the level of the inner segments. The outer segment of a second type C cone (OS) is surrounded by calycal processes (arrows). 12,000 X.

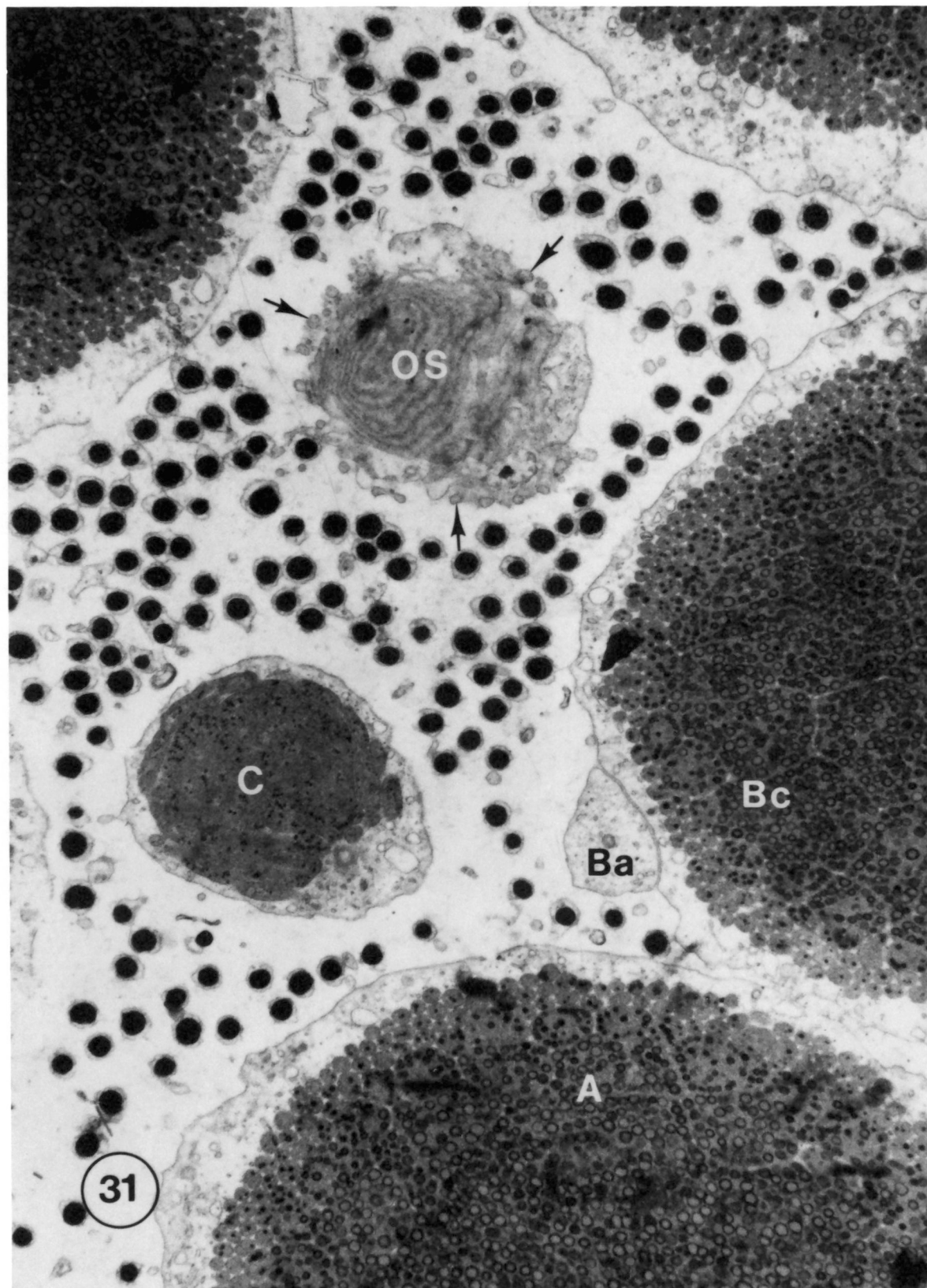


Figure 32. The mitochondria (M) of the type C cone contain electron-opaque granules (arrows) and shelf-like cristae. 35,000 X.

Figure 33. Sclerad to the nucleus (N) the cytoplasm of the type C cone contains endoplasmic reticulum (ER), Golgi profiles (G) and lysosomes (L). 17,000 X.

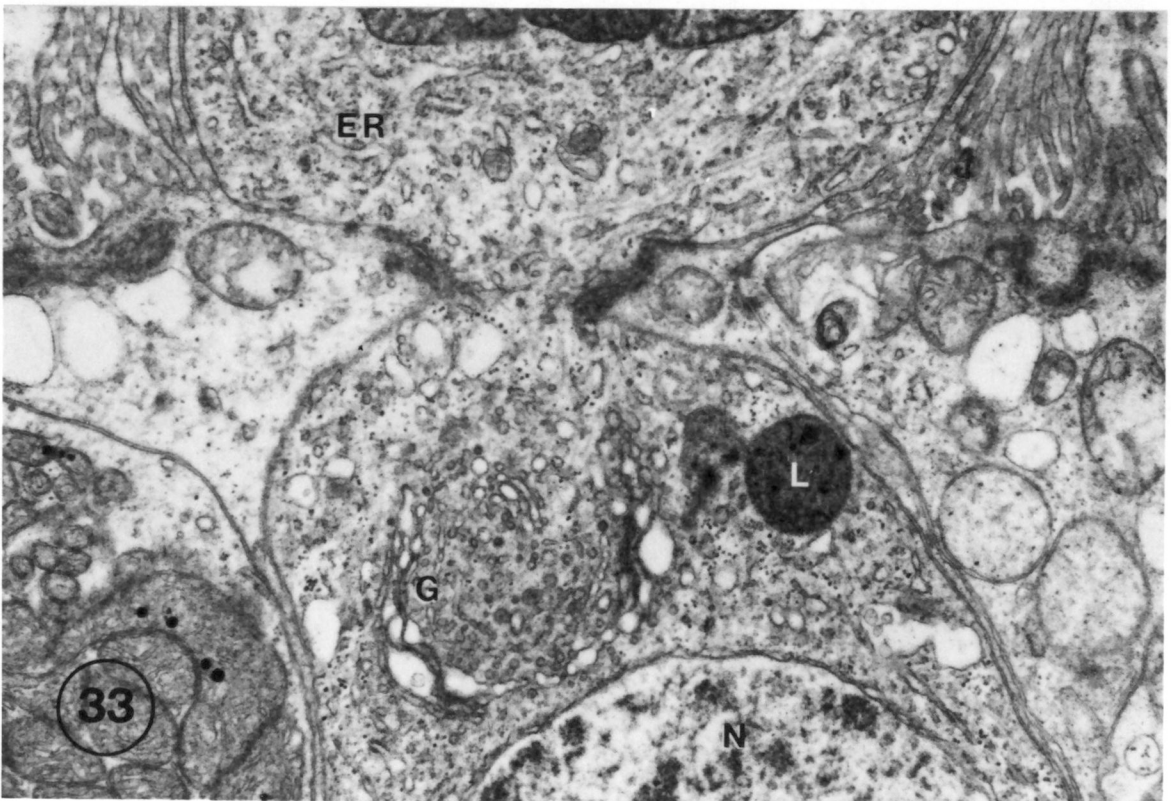
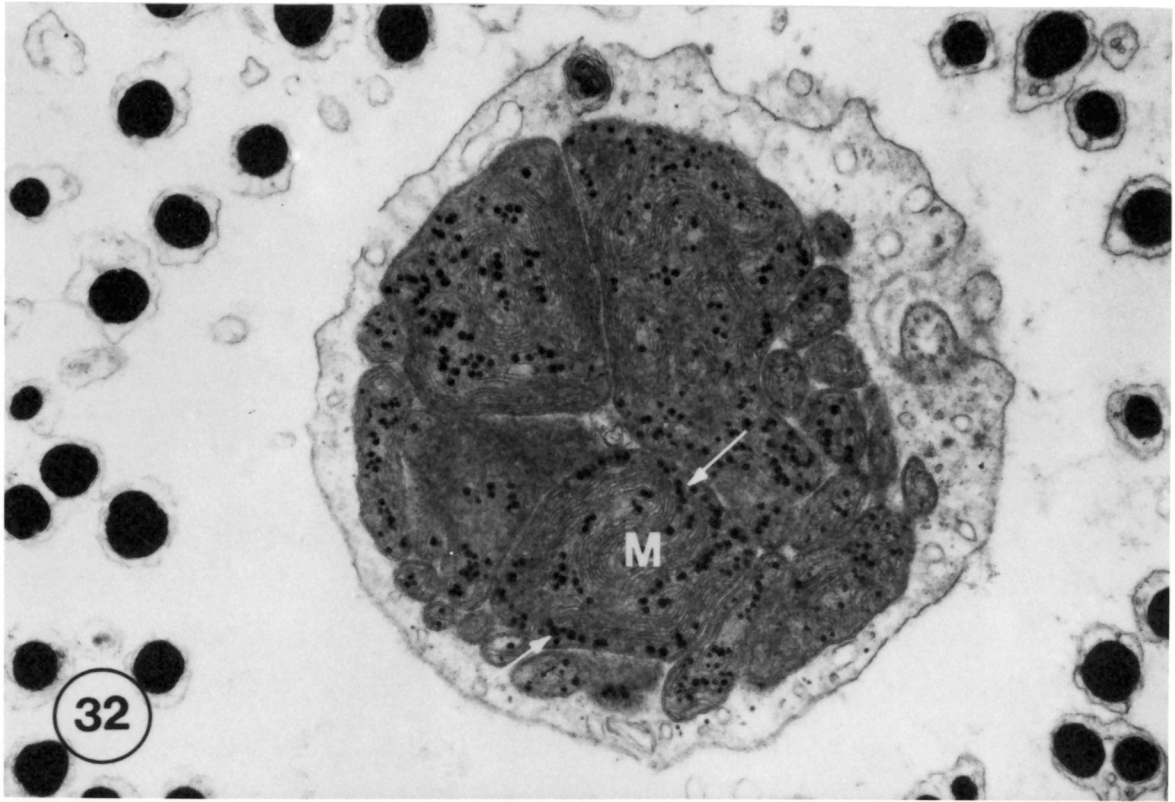


Figure 34. Each horizontal cell (HC) is generally separated from adjacent horizontal and bipolar (BC) cells by a Müller cell process (arrows). The apical cytoplasm of the horizontal cell contains mitochondria (M), Golgi profiles (G), rough endoplasmic reticulum (RER) and free ribosomes (circle). 10,000 X.

Figure 35. The lateral elements of the triad are presumed to be horizontal cell processes (HP) and may contain synaptic vesicles. The central element, presumed to be a bipolar cell dendrite (D), does not contain synaptic vesicles. 18,000 X.

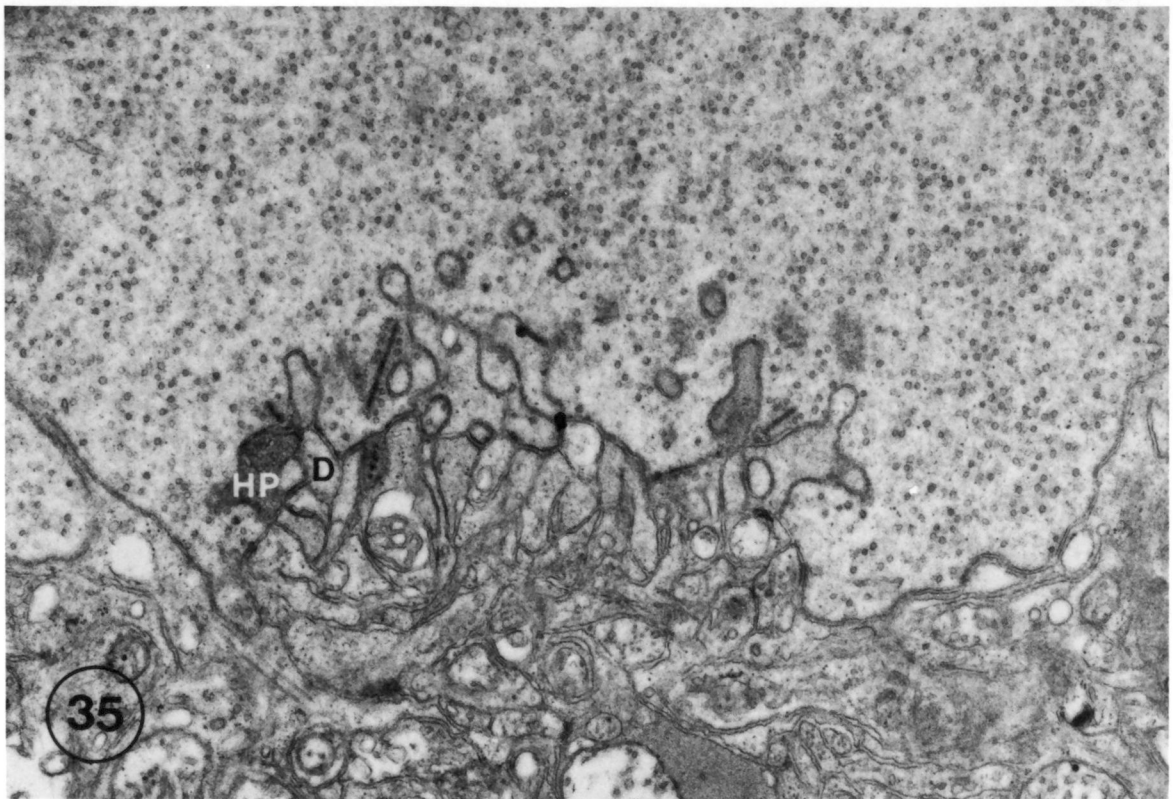
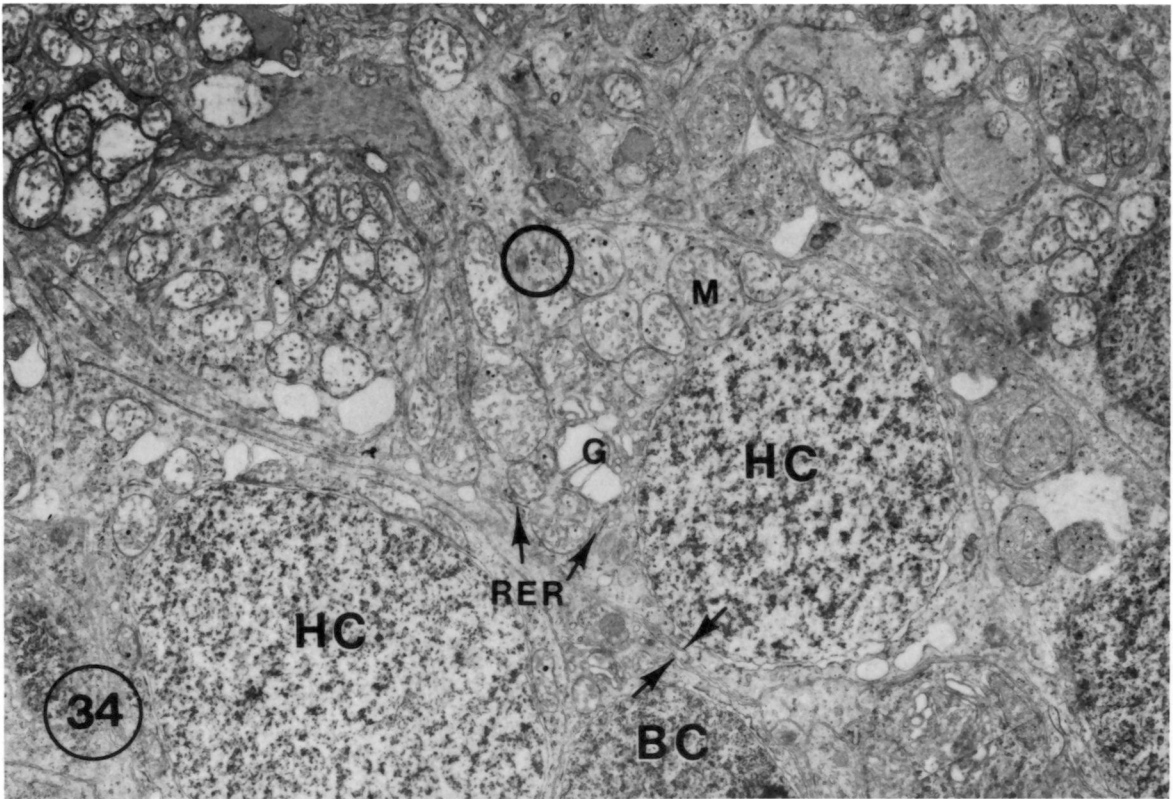


Figure 36. The scleral perikaryon of the bipolar cell (BC) contains mitochondria (M) and Golgi profiles (G). The nucleus is ovoid and frequently contains a nucleolus (Nl). 10,000 X.

Figure 37. Amacrine cells (AC) are usually separated from adjacent cells by processes of bipolar or Müller cells (arrows). 10,000 X.

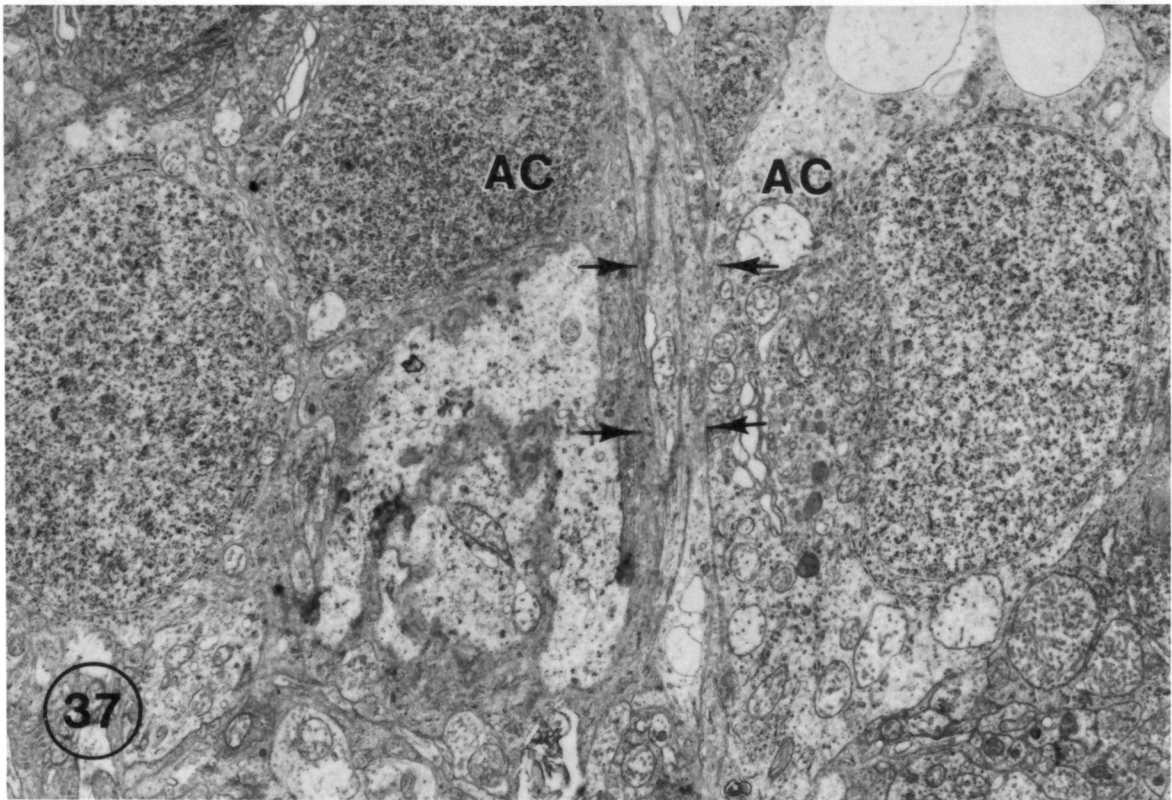
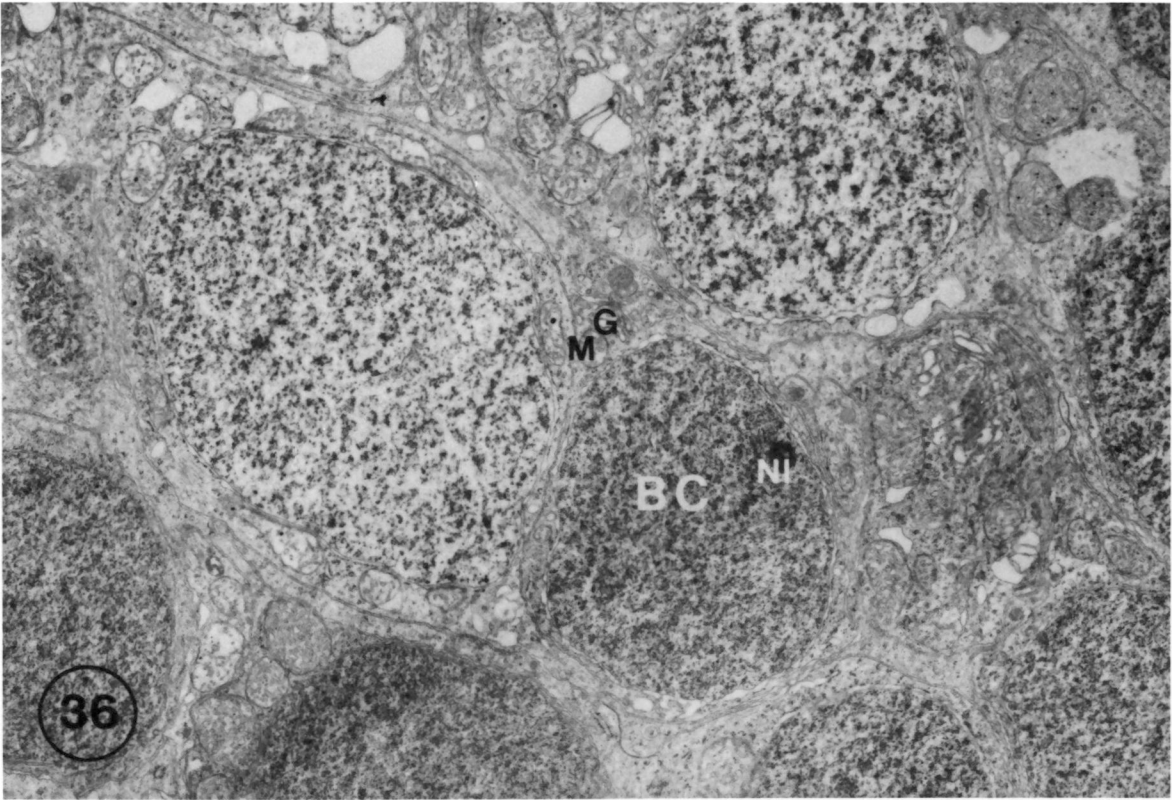


Figure 38. When amacrine cell perikarya (AC) are apposed, they form no specialized contacts. The perikaryon contains mitochondria (M), Golgi profiles (G), rough endoplasmic reticulum (RER), free ribosomes (circle), and occasionally a centriole (*) is seen. 15,000 X.

Figure 39. The cell body of the Müller cell (MC) is fusiform and contains a nucleus (N), mitochondria (M), Golgi profiles (G), microtubules (MT) and endoplasmic reticulum (RER). 12,500 X.

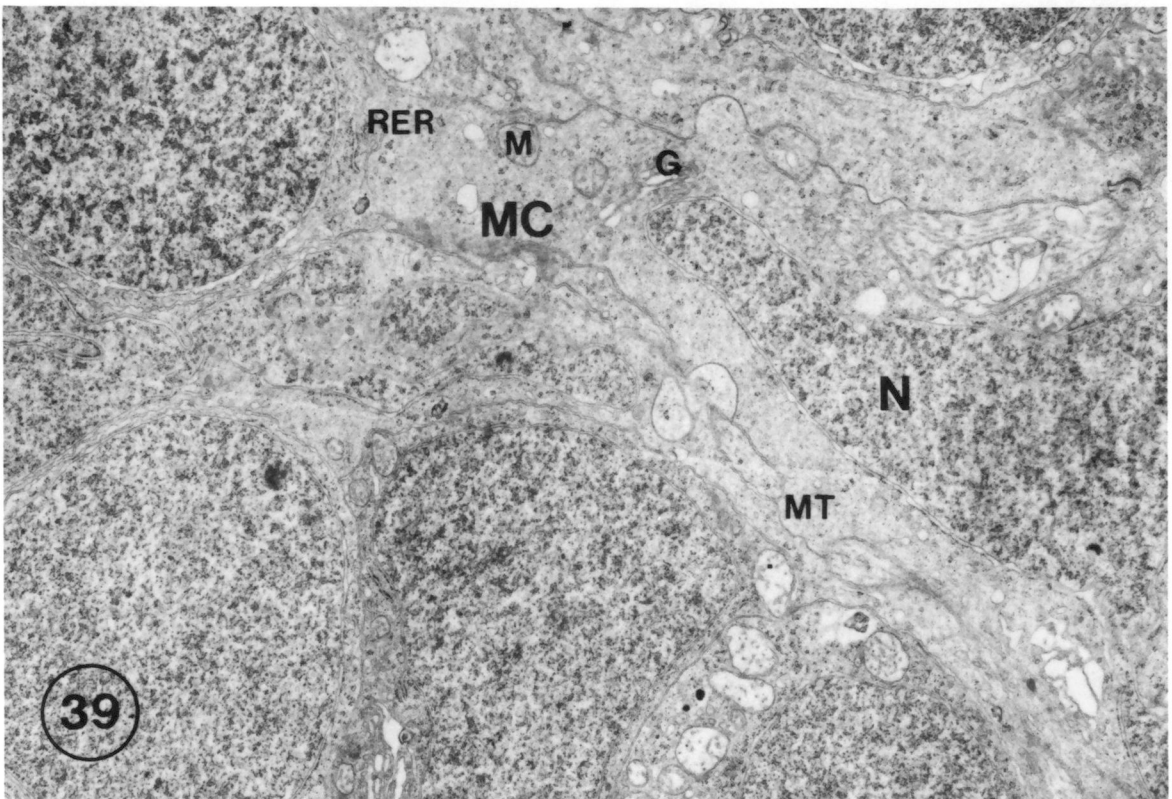
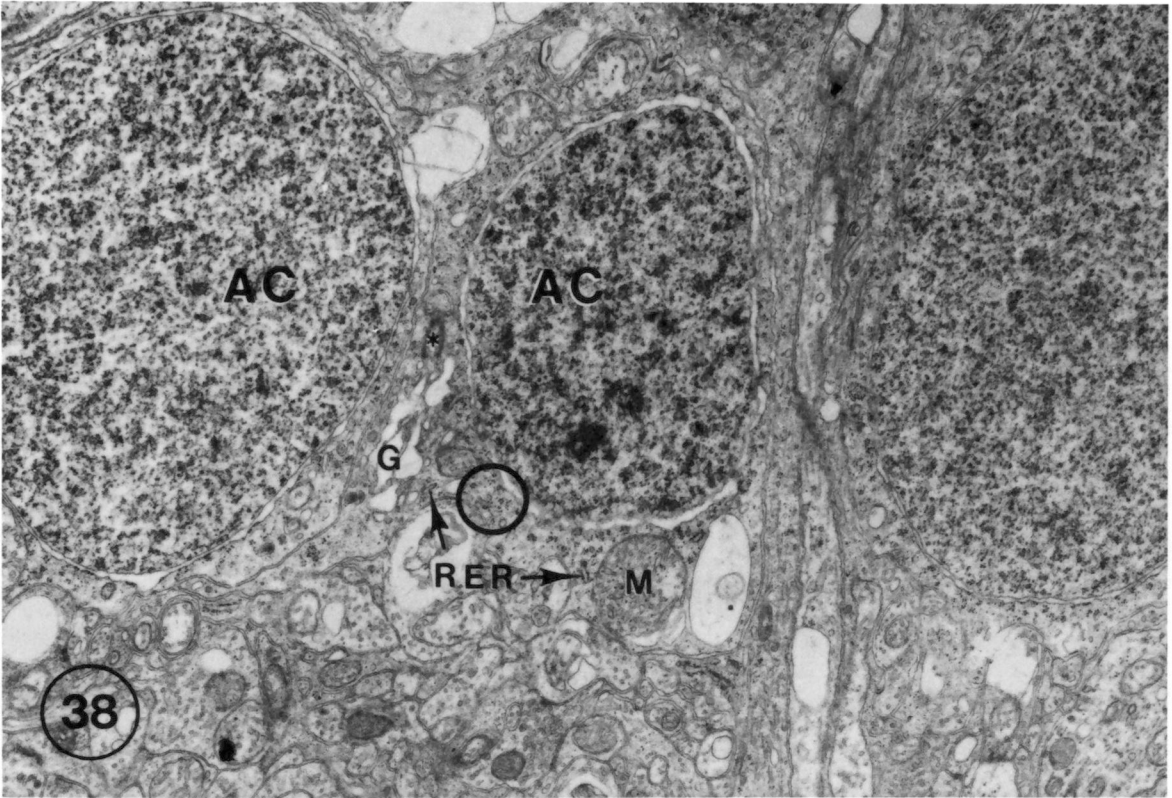


Figure 40. The slender apical process of the Müller cell (arrows) contains rough endoplasmic reticulum (RER), microtubules (MT) and mitochondria (M). 16,500 X.

Figure 41. The apical Müller cell process (MC) expands and fills the space between the photoreceptor cells. This expansion contains mitochondria (M) and the ventricular surface gives rise to microvillous processes (MP) which interdigitate with those of photoreceptor cells. 15,000 X.

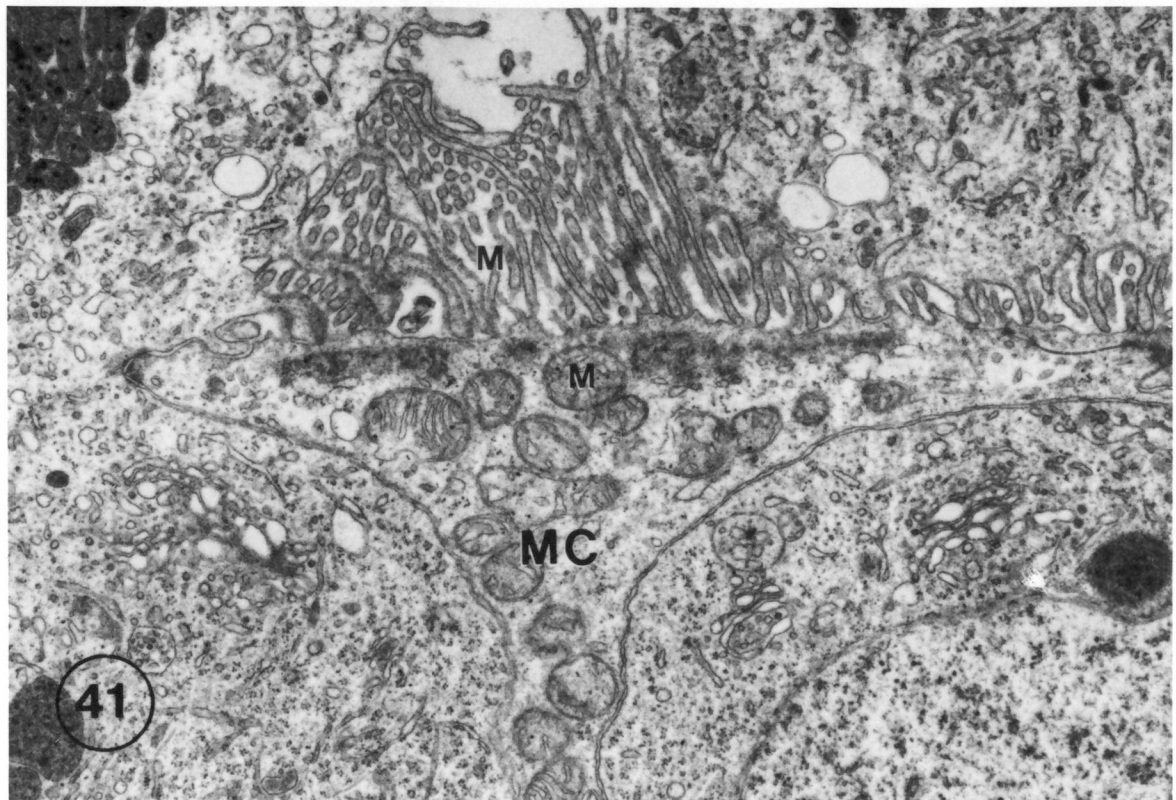
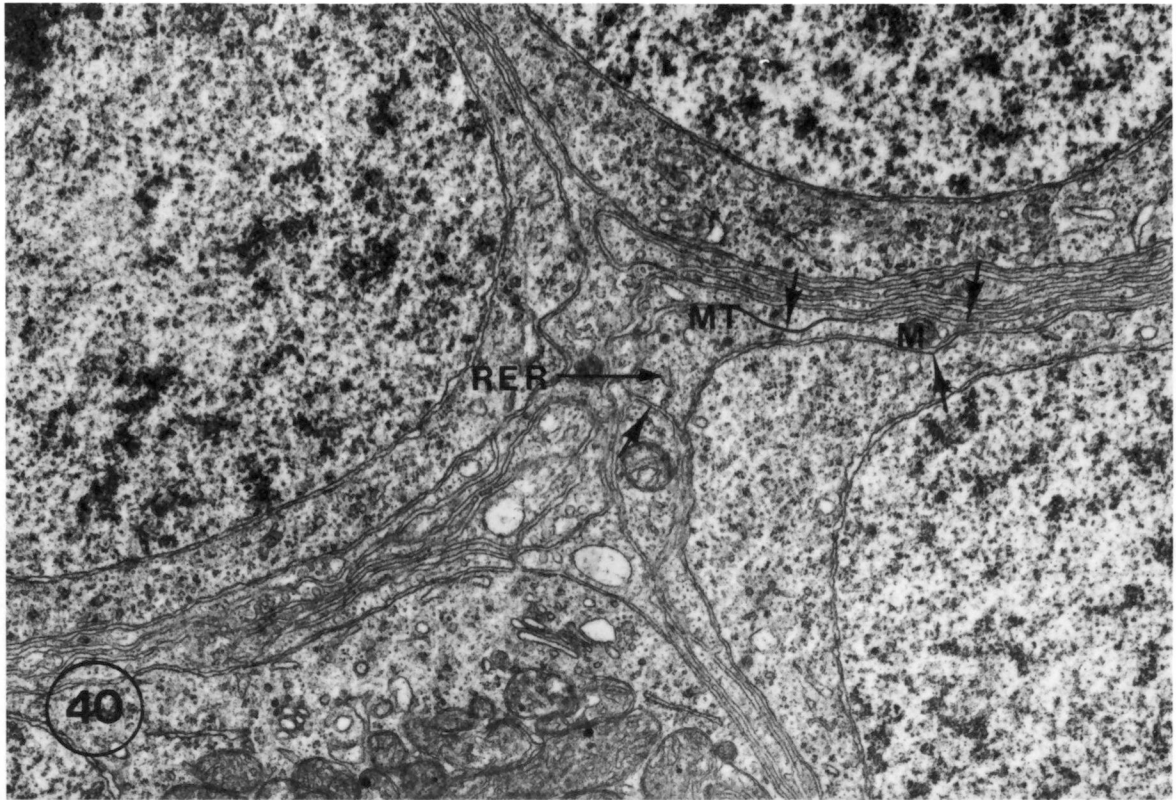


Figure 42. The apical surface of the Müller cell (MC) occasionally gives rise to a cilium (Cl). 13,500 X.

Figure 43. The basal Müller cell processes (MC) contain microtubules (MT) and mitochondria (M) and form lateral membrane specializations (arrows). A basal lamina (BL) is associated with the vitreal surface of the Müller cell processes. 12,000 X.

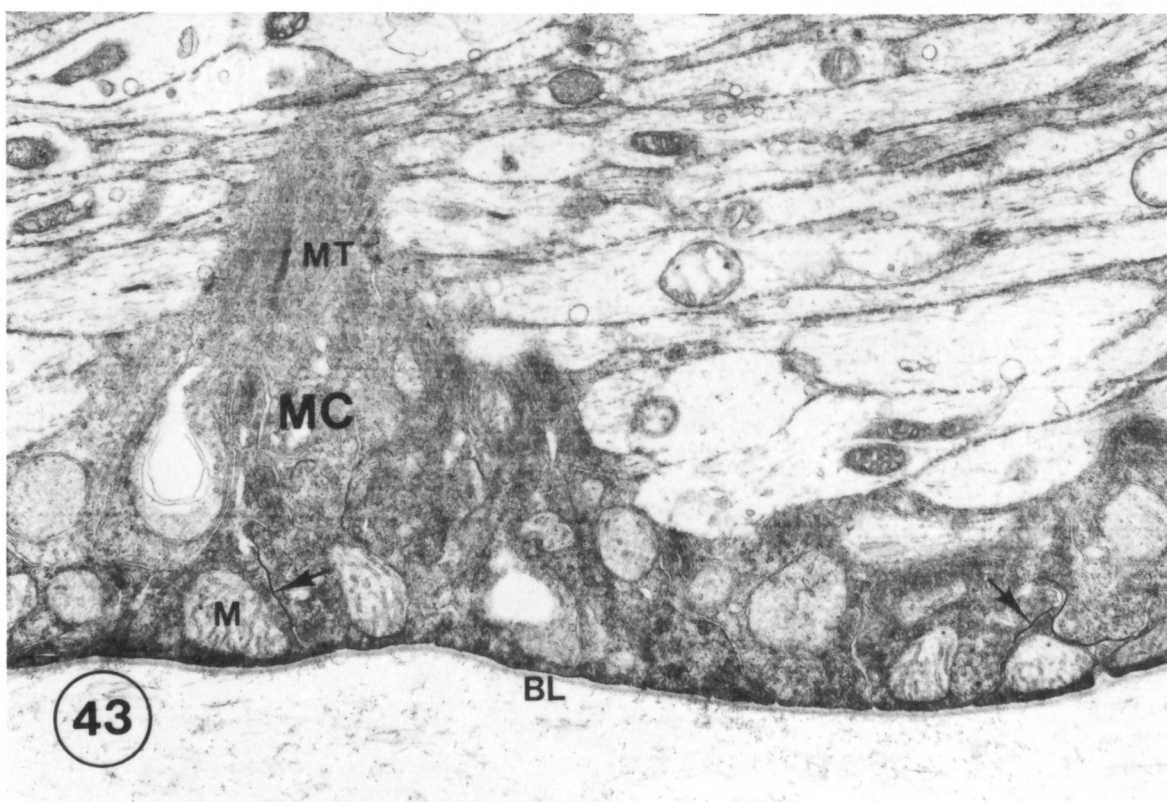
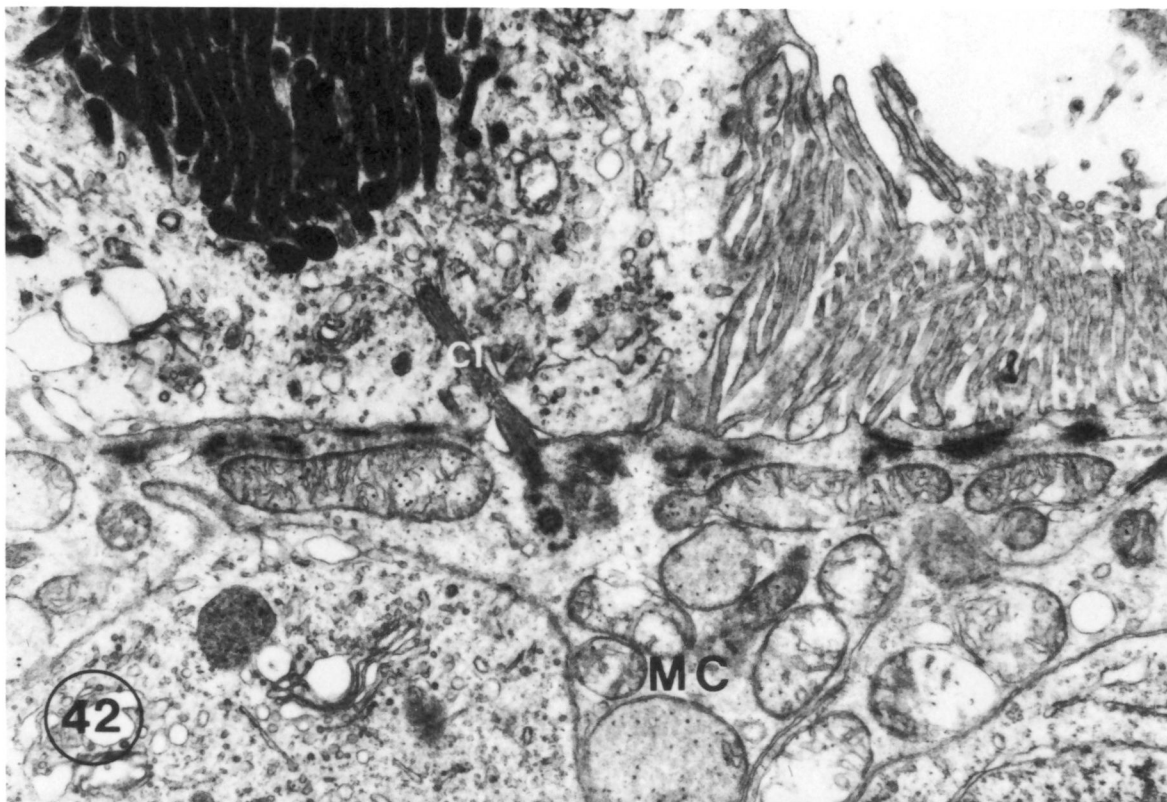


Figure 44. The inner plexiform layer contains membrane-bound cellular processes. Bipolar cell processes (BC) contain synaptic vesicles (SV), synaptic ribbons (SR) and generally a mitochondrion. Amacrine cell processes (AC) contain synaptic vesicles (SV) and mitochondria (M) and can be distinguished from bipolar processes when they are a post-synaptic element of a dyad. 48,500 X.

Figure 45. The conventional synapse (CS) is characterized by an aggregation of synaptic vesicles within the presumed presynaptic element at an area of membrane specialization of both elements. The ribbon synapse, or dyad, is a specialized association between three neuronal elements. The bipolar cell process (BC), containing the synaptic ribbon (SR), is presynaptic to two other elements, in this case an amacrine process (AC) and a ganglion cell process (GC). The ganglion cell process is distinguished by the presence of ribosomes (circle) and the scarcity or absence of synaptic vesicles. 35,000 X.

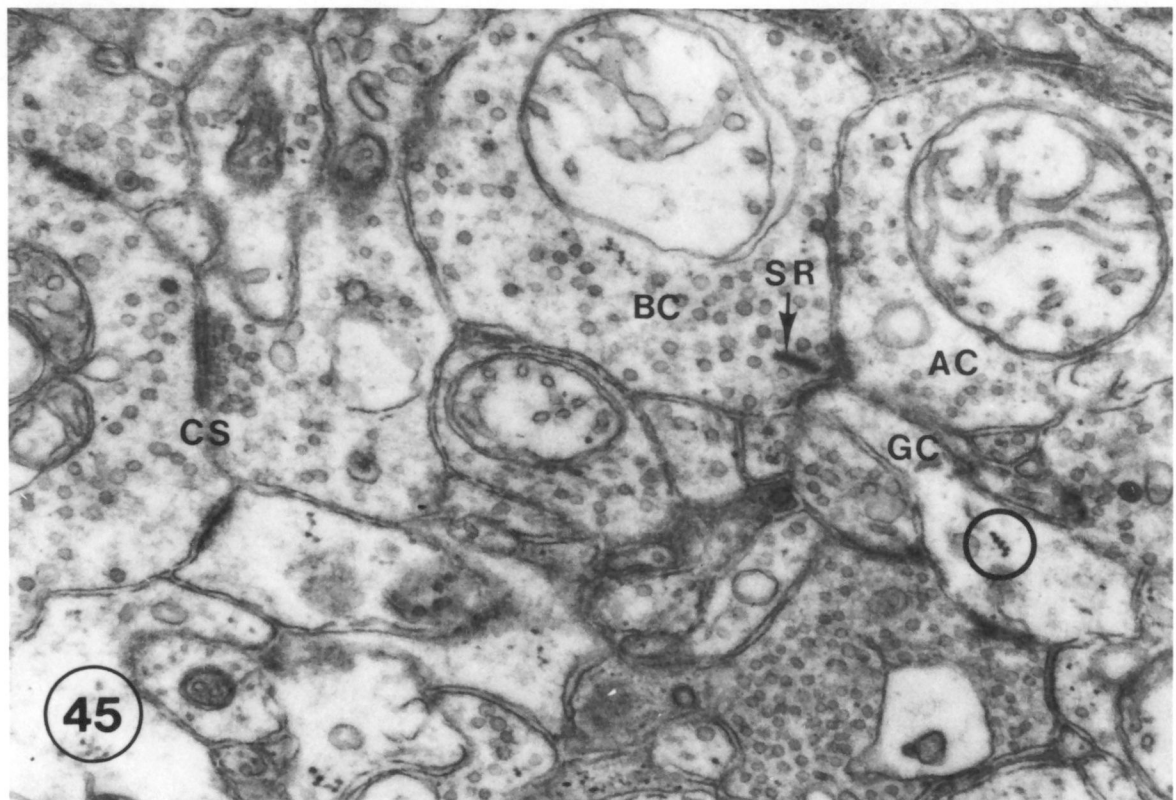
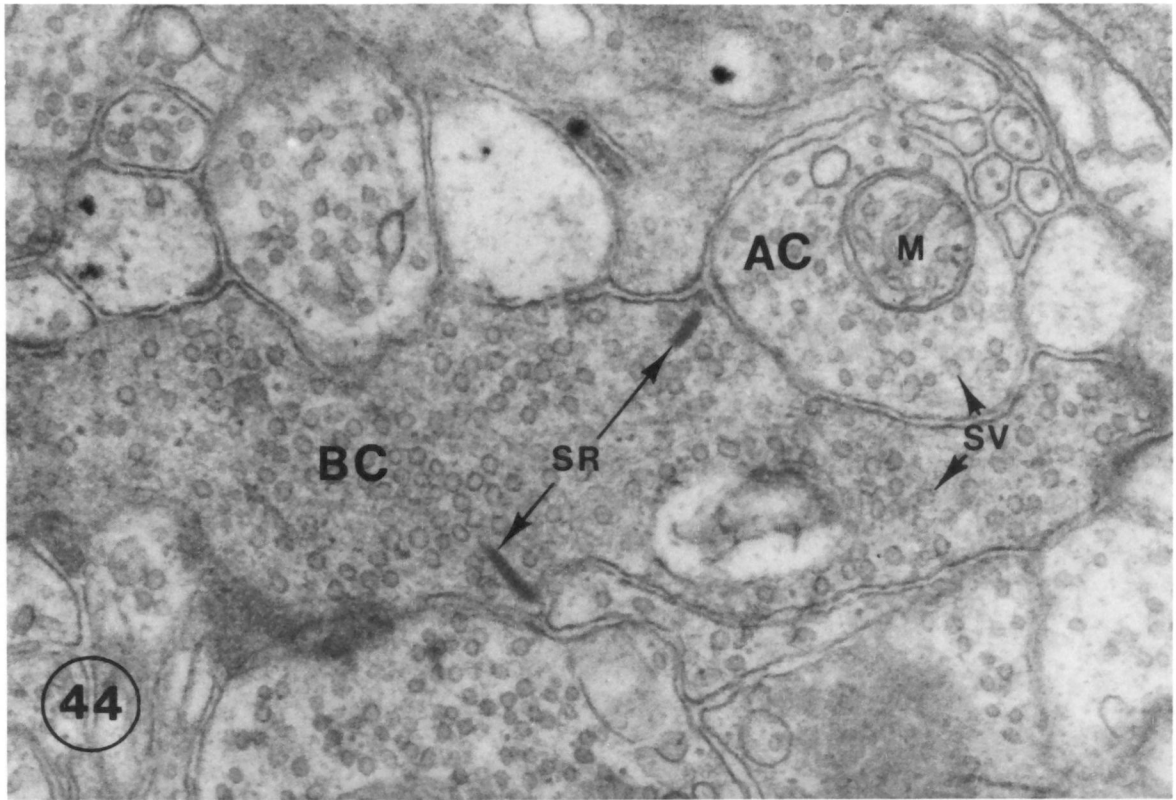


Figure 46. The reciprocal synapse is a specialized contact which consists of a ribbon synapse (RS) and a conventional synapse (CS). An amacrine cell process (AC) which is postsynaptic at a ribbon synapse becomes presynaptic to the same bipolar cell process (BC) through a conventional synapse. 39,500 X.

Figure 47. Ganglion cell perikarya (GC) are usually separated by Müller cell processes (MC). Nissl bodies (NB) may extend into the dendrite. The ganglion cell cytoplasm may contain submembrane cisternae (SC) at areas of contact with Müller cells. 15,000 X.

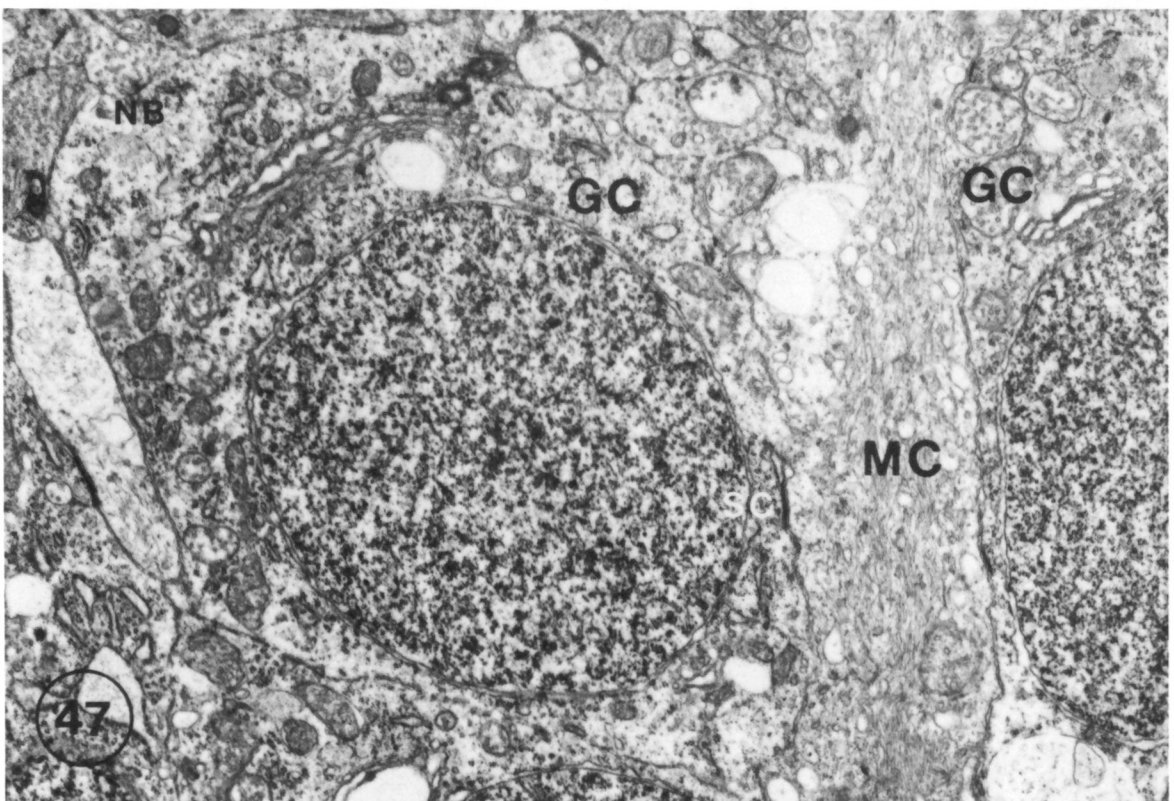
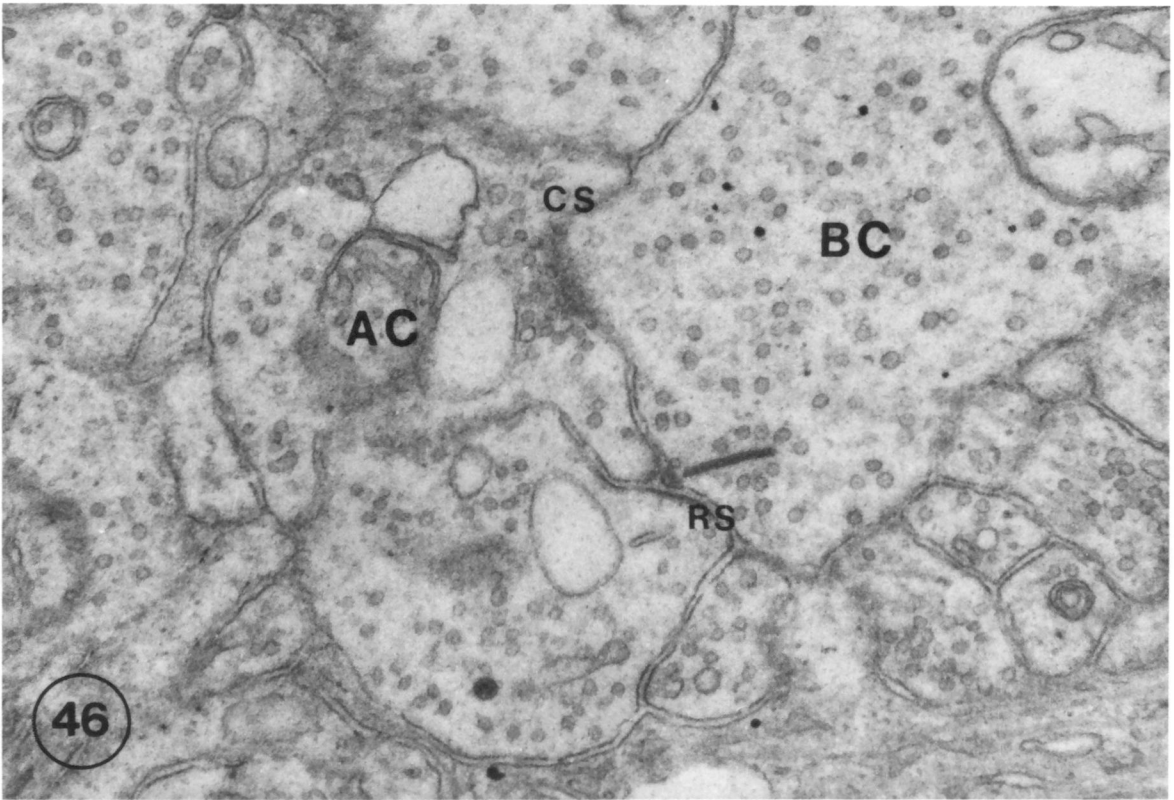
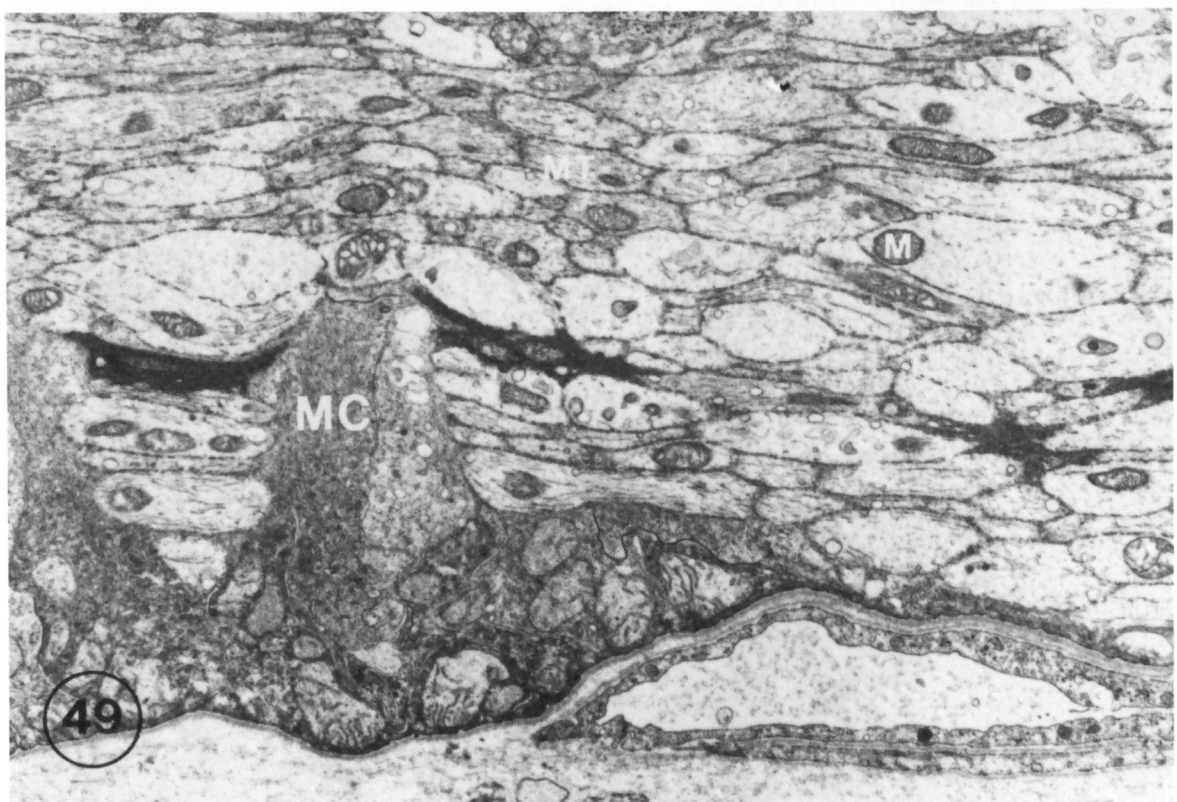
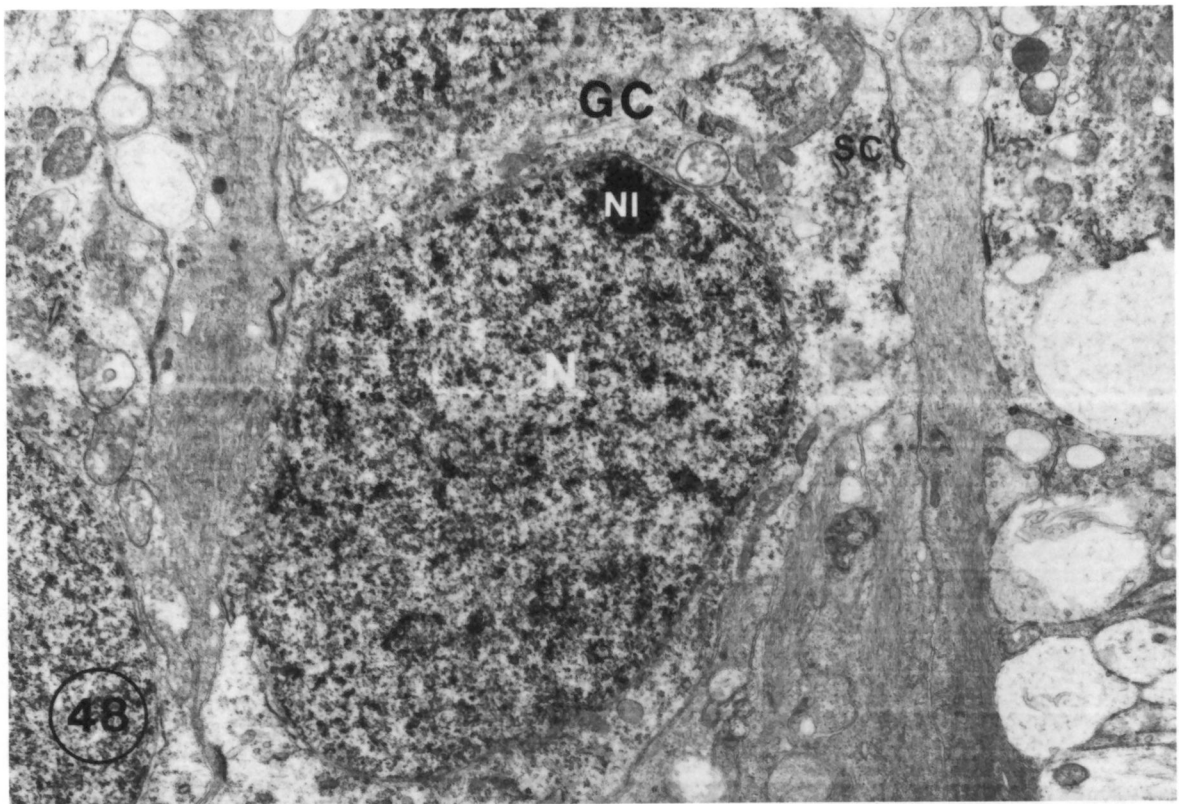


Figure 48. The ganglion cell perikaryon (GC) contains a nucleus (N) with an eccentric nucleolus (Nl). The ganglion cell cytoplasm may contain submembrane cisternae (SC) at areas of contact with Müller cells (MC). 10,000 X.

Figure 49. Nerve fiber bundles are separated by Müller cell processes (MC). Ganglion cell axoplasm contains microtubules (MT) and mitochondria (M). 12,500 X.



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