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Morphogenesis of Photoreceptors in the Chick Retina: An Electron Microscopic Study

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MORPHOGENESIS OF PHOTORECEPTORS IN THE CHICK RETINA: AN ELECTRON MICROSCOPIC STUDY

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ABSTRACT

An electron microscopic investigation of the morphogenesis of photoreceptor cells in the domestic fowl (Gallus gallus) retina is presented in this study. The fine structural observations illustrate photoreceptor development ranging from five to twenty-one days of incubation and one to two days post-hatching. Retinal tissues were fixed in buffered aldehyde fixative, post-fixed in buffered 2% osmium tetroxide and otherwise conventionally prepared for electron microscopy.

The neural (sensory) retina of the chick is largely occupied by undifferentiated cells within the outer neuroblastic layer from the 5th to 9th days of incubation. This layer is later divided into the inner and outer nuclear layers as a result of the establishment of the outer plexiform layer. The ellipsoid cells of the outer nuclear layer will develop as the photoreceptors of the chick retina. Numerous centrioles are conspicuous within these cells at the level of the outer limiting membrane.

The first indication of differentiation in the photoreceptor proper occurs during the 9th to 11th days, as the apical cytoplasm of the future receptor cells extends beyond the outer limiting membrane. These bulbous cytoplasmic projections occupy the optic ventricle and represent the forming inner segments with their characteristic ellipsoid. By the 16th

day, the inner segments are extensively elongated. They are divided into the following two definitive portions: (1) the ellipsoid which occupies the distal one-third and (2) the myoid which represents the proximal two-thirds.

During the 17th day, irregularly arranged membranous discs are observed within the forming outer segments. This portion of the photoreceptor proper develops as a modified cilium which originates from a basal body within the ellipsoid region of the inner segment. The membranous discs are closely associated with the microtubules of the "connecting cilium" and many are continuous with cylindrical tubules measuring 240 to 400 A in diameter. The discs (135 A thick) become oriented at right angles to the plasmalemma by the 19th day. Some of the membranous discs are continuous with the plasmalemma. Groups of discs are found within the pigment epithelium during the second day post-hatching. This observation indicates that the pigment epithelium actively participates in the removal of membranous discs.

During the early stages of photoreceptor development, the basal portions of the receptor cells are not very conspicuous, with the exception of a large nucleus. By the 16th day basal arborizations begin to develop. Extensive synaptic terminals are plentiful and extend into the outer plexiform layer by the 19th day. They contain numerous synaptic vesicles and synaptic ribbons. Both conventional and invaginated

ribbon) synapses occur between the photoreceptors and secondary neurons (bipolar and horizontal cells).

This study indicates that the morphogenesis of photoreceptors in the chick retina corresponds well with that in other vertebrate species. Moreover, the basic fine structure of the mature photoreceptor correlates favorably with that in a number of vertebrates, including amphibians, other avian species and numerous mammals.

CHAPTER I

INTRODUCTION

The vertebrate eye develops as a peripheral extension of the developing central nervous system (CNS). The paired evaginations known as the optic vesicles soon invaginate to form the optic cups. As a result, the retina becomes a double layered epithelium, the surface cells of which are joined by desmosomes (maculae adherentes). These intercellular junctions form the following fenestrated "membranes": (1) the outer limiting membrane (OLM) which represents junctions between the peripheral cells of the neural retina and (2) Verhoeff's membrane which consists of junctions between the pigmented epithelial cells (Fine, 1961). The free surfaces of these cells face an embryonic lumen which is continuous with the lumen of the neural tube (CNS). It is often referred to as the "optic ventricle" (Cohen, 1963 b). It remains as a potential space in the adult vertebrate retina (Fine, 1961). The outer layer of the optic cup will develop as the pigment epithelium, whereas the inner layer represents the neural (sensory) retina (Copenhaver, et al., 1971). The external portion of the developing neural retina, located nearer to the sclera, represents the outer neuroblastic layer. This

expansive layer contains irregularly arranged undifferentiated cells. Following progressive cellular division and migration, the outer neuroblastic layer becomes divided into two nuclear zones (Mann, 1950; Duke-Elder and Cook, 1963; Patten, 1968). These zones are recognized as the inner and outer nuclear layers (INL, ONL), separated by the outer plexiform layer (OPL). The ONL represents the last two generations of the "germinal" nuclei within the outer neuroblastic layer (Weysse and Burgess, 1906). These cells are destined to become the future photoreceptors of the neural retina. The cell bodies of the photoreceptors will remain within the ONL, while a basal synaptic terminal will expand into the OPL. The photoreceptors proper will extend beyond the OLM into the optic ventricle, forming the rod and cone layer of the neural retina.

Thorough reviews on the light microscopic morphology of the vertebrate retina are offered by Polyak (1941), Wolff (1948), and Duke-Elder and Wybar (1961). With the advent of electron microscopy, the vertebrate retina has undergone extensive study. Reviews on the fine structure of the vertebrate retina are given by Cohen (1963 b), Dowling (1970), Young (1970, 1971 a), Hebel (1971), and Hogan, et al. (1971). The fine structural morphology of mature photoreceptors (rod and cone cells) is well substantiated in several vertebrate species through the observations of numerous investigators. These species include the mudpuppy, Necturus maculosus (Brown, et al., 1963; Dowling and Werblin,

1969), newt, Triturus viridescens (Dickson and Hollenberg, 1971, 1972; Keefe, 1971), leopard frog, Rana pipiens (Nilsson, 1964 a, 1964 b, 1964 c; Young, 1967; Cohen, 1968; Dowling, 1968), water frog, R. esculenta (Young, 1968, 1969 a; Young and Droz, 1968), domestic pigeon, Columba livia (Cohen, 1963 a), domestic fowl (Villegas, 1960; Morris and Shorey, 1967), domestic mouse, Mus musculus (Cohen, 1960; Young, 1967), domestic rat, Rattus norvegicus (Ladman, 1958; Pedler and Tilly, 1967; Young, 1967), American gray squirrel, Sciurus carolinensis (Cohen, 1964), thirteen lined ground squirrel, Citellus tridecemlineatus (Hollenberg and Bernstein, 1966), guinea pig, Cavia cobaya (Sjöstrand, 1949, 1953 a, 1953 b; Pedler and Tilly, 1967), domestic rabbit, Oryctolagus cuniculus (De Robertis, 1956 a, 1956 b; De Robertis and Franchi, 1956; De Robertis and Lasansky, 1958), domestic dog, Canis familiaris (Shively, et al., 1970; Hebel, 1971), Rhesus monkey, Macaca mulatta (Villegas, 1960; Cohen, 1961 a, 1961 b, 1965 a, 1965 b; Dowling, 1965; Dowling and Boycott, 1966; Young, 1971 b, 1971 c) and man, Homo sapiens (Cohen, 1965 a, 1965 b; Dowling, 1970; Spitznas and Hogan, 1970; Uga, et al., 1970). These reports indicate that the fine structural morphology of photoreceptors from various vertebrates is essentially comparable. Note Figure 1 which diagrammatically depicts the typical fine structure of a mature photoreceptor (cone cell). All of the terminology given is applicable to both

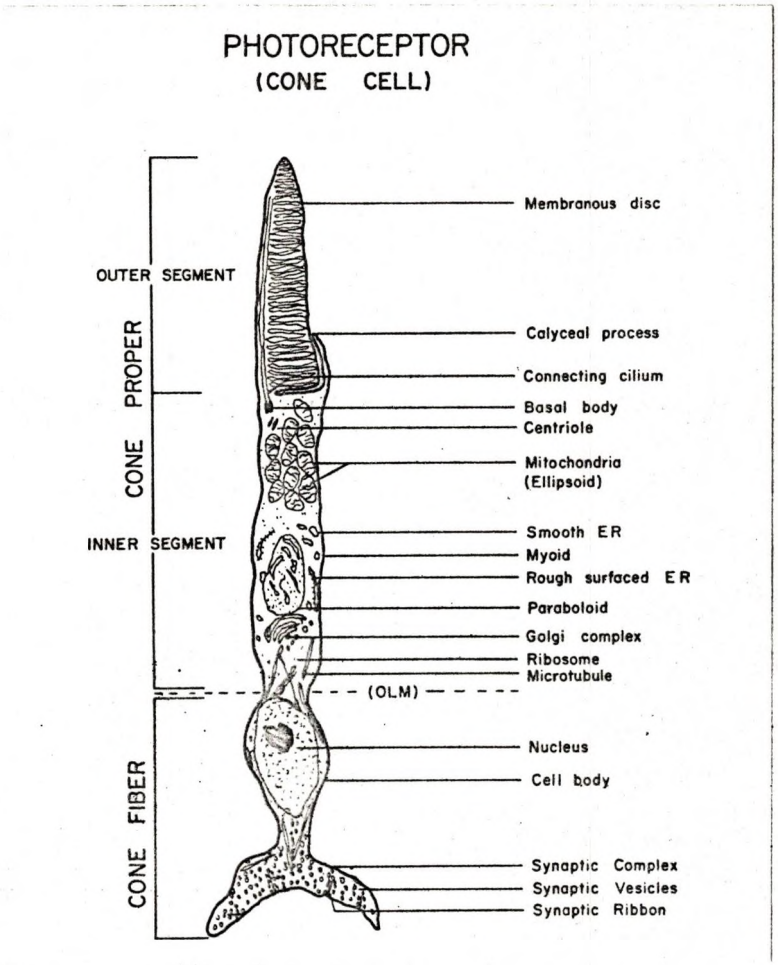


Fig. 1.--Mature photoreceptor in the chick retina. All vertebrate photoreceptor cells possess this basic structure with the exception of the paraboloid, which is not found in mammalian retinas. The terminology given applies to both rods and cones.

rod and cone receptor cells. The fine structure of vertebrate photoreceptor components may be summarized as follows.

Inner Segment

The inner segment represents the proximal one-half of the elongated photoreceptor proper, which extends beyond the OLM to the

level of the outer segment (Polyak, 1941). It is divided into the following two regions: (1) the ellipsoid and (2) the myoid. The ellipsoid occupies approximately the distal one-third of the inner segment, and consists primarily of dense accumulations of mitochondria which are usually oriented parallel to the long axis of the inner segment (Sjöstrand, 1953 b; Hogan, et al., 1971, and others). Frequently, a large lipid droplet is observed in the distal part of the inner segment of cones (Cohen, 1963 b; Copenhaver, et al., 1971). The surrounding cytoplasm contains free ribosomes, a few membrane bound vesicles and occasional microtubules. A basal body is located eccentrically at the distal end of the inner segment (Villegas, 1964). Often, another centriole is present, positioned perpendicularly to the basal body. Both of these organelles consist of nine concentrically arranged triplets of microtubules which are characteristic of centriole structure (Brown, et al., 1963; Fawcett, 1966). A "connecting cilium" is continuous with the basal body and extends distally into the outer segment. The morphology of the cilium is typical, with the exception of the two central microtubules which are absent (De Robertis, 1956 a). Frequently, "ciliary rootlets" are observed originating from the two centrioles and extend toward the vitreous into the myoid. These filaments possess a regular pattern of periodicity measuring about 700 A (Sjöstrand, 1953 b; Cohen, 1960; Orzalesi and Bairati, 1964). Narrow, elongated cytoplasmic processes of the distal inner segment extend along the outer segment for varying

distances. These are usually referred to as "calyceal" processes (Cohen, 1961 b, 1963 b, 1964) or occasionally as "dendrites" (Brown, et al., 1963; Dickson and Hollenberg, 1971). The cell membranes of the inner and outer segments are uninterrupted. Continuity between the inner and outer segments is by means of the modified cilium which includes a small stalk of cytoplasm and the cell membrane.

The myoid represents the proximal two-thirds of the inner segment, extending toward the vitreous from the ellipsoid to the level of the OLM. The cytoplasm of the myoid is directly continuous with that of the photoreceptor cell body which lies within the ONL. The myoid contains numerous ribosomes often in the form of polyribosomes, vesicles of smooth endoplasmic reticulum, cisterns of rough surfaced endoplasmic reticulum and numerous microtubules which are primarily oriented with the long axis of the inner segment. Numerous vesicles of the Golgi apparatus are observed in the proximal portion of the myoid (Cohen, 1963 b; Hogan, et al., 1971). The ciliary rootlets which traverse the myoid are often closely associated with vesicles of the smooth endoplasmic reticulum and Golgi apparatus (Cohen, 1963 b; Uga, et al., 1970). A structure known as the "paraboloid" is observed within the cytoplasm of the myoid. It is peculiar to retinal photoreceptors and is usually reported to be present in cones, although it is occasionally observed in rods (Morris and Shorey, 1967; Dickson and Hollenberg, 1971; Keefe, 1971). The paraboloid is present in the retinas of numerous

vertebrates including fish, reptiles, amphibians and birds. However, it has not been observed in the mammalian retina. The paraboloid is round to oval in shape, and consists of glycogen granules interspersed between vesicles of smooth endoplasmic reticulum (Cohen, 1963 b; Meller and Breipohl, 1965). However, cisterns of rough surfaced endoplasmic reticulum have been observed to be continuous with the vesicles at the periphery of the paraboloid (Carasso, 1960; Yamada, 1960). Meller and Breipohl (1965) refer to the paraboloid as a specialized portion of the smooth endoplasmic reticulum and indicate that it may be involved with glycogen storage or glycolysis (Dickson and Hollenberg, 1972).

Individual inner segments are separated from one another by elongated villous processes of the Müller cells (Meller and Glees, 1965). In addition, fine processes of the bipolar cells commonly referred to as "Landolt's clubs," penetrate into the optic ventricle and further isolate individual inner segments (Cohen, 1963 b; Keefe, 1971).

Outer Segment

The outer segment represents the distal half of the photoreceptor proper. It extends toward the sclera from the inner segment to interdigitate with the pigment epithelium. Microvilli of the pigmented epithelial cells extend inward and isolate outer segments from one another (Bairati and Orzalesi, 1963). In addition to these villous processes, Spitznas and Hogan (1970) report that "broad cytoplasmic

sheets" extend from the pigment epithelium and envelop individual outer segments in the human retina. Both types of processes contain pigment granules, and each plays an important role in the normal relationship between the neural retina and the pigment epithelium (Spitznas and Hogan, 1970).

The "connecting cilium" is eccentrically positioned in the outer segment. As it extends into the outer segment, it undergoes a transition from the normal ciliary doublets to singlets of microtubules (Cohen, 1965 a), which however, account for very little of the volume of the outer segment.

The fine structural morphology of the main portion of the outer segment is unique. It consists of stacks of membranous discs or saccules which are positioned perpendicularly to the cell membrane. Each disc consists of two unit membranes which are continuous at their periphery (Hogan, et al., 1971). Considering membrane thickness and the slight "intradisc space," the total thickness of the membranous discs averages from 140 to 150 A (Sjöstrand, 1953 a, 1961; Moody and Robertson, 1960; Young, 1969 b; Cohen, 1964; Hogan, et al., 1971; Korenbrot, et al., 1973). These measurements vary according to photoreceptor type (rod or cone), species, and techniques of fixation. The number of discs present in an outer segment varies from 600 to 1,200 depending on photoreceptor type and species (Hogan, et al., 1971). The relationship between the membranous discs and the plasmalemma varies considerably. In

primates, the discs of both rods and cones are usually completely separate from the cell membrane (Young, 1969 b; Cohen, 1965 a; Hogan, et al., 1971). However, in many lower vertebrates, the discs are continuous with and appear to form as invaginations of the cell membrane (Nilsson, 1964 c; Young, 1969 a, 1971 c; Leeson, 1971, and others). As a result of this continuity, the "intradisc space" is continuous with the extracellular space (Cohen, 1968). Rods generally display this continuity with the cell membrane at the basal portion of the outer segment, whereas it is observed throughout the cone. These continuities become progressively fewer towards the distal end of the cone outer segment. When observed in cross section, the discs of rods are lobulated while those of cones have a smooth spherical shape.

Through the use of radioactively labeled amino acids, it is evident that there is constant renewal of the membranous discs in rods of vertebrate retinas (Droz, 1963; Young, 1967, 1968, 1969 a, 1970, 1971 b, 1971 c; Young and Droz, 1968). Mature cones, on the other hand, do not form new discs (Young, 1971 a), but evidence suggests that they may regain this capacity when injured (Kroll and Machemer, 1968). During the renewal process, discs are formed at the base of the outer segment by invaginations of the cell membrane. Once contact is lost with the plasmalemma, the discs are pushed toward the sclera by newly formed discs. After reaching the apical end of the outer segments, groups of discs are "shed" and engulfed by the pigment epithelium. These

phagosomes then undergo progressive digestion within the cytoplasm of the pigmented epithelial cells (Porter and Yamada, 1960; Yamada, 1961; Bairati and Orzalesi, 1963; Moyer, 1969; Young and Bok, 1969; Ishikawa and Yamada, 1970; Spitznas and Hogan, 1970; Marshall and Ansell, 1971; Young, 1971 b, 1971 c).

Photoreceptor Fibers, Cell Body and Synaptic Terminal

The remainder of the photoreceptor which lies on the vitreous side of the OLM, consists of three portions: (1) the outer fiber which extends from the cell body to the OLM, (2) the cell body containing the nucleus, and (3) the inner fiber which ends as a synaptic terminal within the outer plexiform layer (OPL; Polyak, 1941). The extent of the outer and inner fibers varies considerably among numerous vertebrates. The nucleus occupies nearly the entire cell body. Often the nuclei of cone cells protrude through the OLM and into the proximal portion of the myoid. The number and position of rod and cone nuclei within the ONL vary among species.

The cytoplasm of the outer fiber contains numerous ribosomes, microtubules predominantly arranged parallel with the long axis of the cell, and vesicles of Golgi and smooth endoplasmic reticulum. The sparse cytoplasm surrounding the nucleus contains a few ribosomes and microtubules (Hogan, et al., 1971).

The inner fiber of photoreceptors contains irregularly arranged microtubules, ribosomes and numerous synaptic vesicles. The synaptic vesicles usually range in size from 300 to 600 A in diameter (Evans, 1966) which is the size range generally associated with most vesicles containing neurotransmitter substances (Dickson and Hollenberg, 1971). The synaptic terminations of the inner fibers are commonly referred to as rod "spherules" and cone "pedicles." These structures synapse with dendrites of bipolar cells and cytoplasmic processes of horizontal cells. The cell bodies of these cell types lie within the inner nuclear layer (INL). The entire "synaptic complex" consists of the following three elements: (1) the presynaptic membrane (synaptic terminal), (2) the cellular contacts (synapse), and (3) the postsynaptic membrane (bipolar and horizontal cells; Hogan, et al., 1971). Where synapses occur, there are always two common observations: (1) synaptic vesicles and (2) increased density of either the pre- or postsynaptic membrane or both (Cohen, 1965 b). Two general types of synapses occur between photoreceptors and bipolar and horizontal cells. They are either conventional (superficial) or "invaginated" (ribbon) synapses (Dowling, 1970). The conventional synapse which is observed throughout the vertebrate nervous system, usually consists of an aggregation of synaptic vesicles in the presynaptic cytoplasm and an increased density of either the pre- or postsynaptic membranes or both, depending on the species and photoreceptor types (Dowling, 1968; Dickson and

Hollenberg, 1971, and others). Moreover, only one postsynaptic element is present at these synapses (Dowling, 1970). Synaptic ribbons are not generally associated with this type of synapse, although they are observed (Dowling and Werblin, 1969).

The invaginated or ribbon synapse incorporates a specialized structure known as the "synaptic ribbon" or "synaptic lamella" (Lasansky, 1969). It is unique to special sensory receptor cells (Fine, 1963), for it is found only in the photoreceptor and bipolar cells of the retina (Dowling and Boycott, 1966), and in the hair cells of the cochlea (Smith and Sjöstrand, 1961) and vestibular apparatus (Hogan, et al., 1971). The synaptic ribbon averages 350 A in width (Matsuska, 1967) and is an electron dense band or bar within the presynaptic cytoplasm. It is adjacent to the plasmalemma and is usually oriented perpendicularly to it. The synaptic ribbon is surrounded by synaptic vesicles in the form of either an irregular cluster or arranged in parallel rows on each side of the ribbon. Frequently, a dense globule is observed interposed between the synaptic ribbon and the plasmalemma. This structure was first observed by Ladman (1958) and is known as the "arciform density." In contrast to conventional synapses, the ribbon synapse has multiple postsynaptic elements. The bipolar and horizontal cells make synaptic contact with the photoreceptors by means of a structural "triad." Two horizontal cell processes extend deeply into the synaptic terminal and are positioned laterally, one on each side of the synaptic ribbon, while

a bipolar dendrite is located centrally and more superficially (Dowling, 1965). Ribbon (invaginated) synapses are much more numerous in cones than in rods.

Intercellular contacts occur between individual photoreceptors at various levels on the vitreous side of the OLM. These contacts may represent desmosomal junctions, since the photoreceptors are epithelial in origin. Synaptic specializations are generally not associated with these contacts, although increased densities of the plasmalemma are reported (Cohen, 1964). These interreceptor contacts are observed in the retinas of several vertebrate species (Sjöstrand, 1958; Cohen, 1964, 1965 b; Nilsson, 1964 b; Dowling and Boycott, 1966; Hogan, et al., 1971, and others). To date, there is not enough evidence to support the idea that these intercellular contacts are functional synapses.

Morphogenesis of Photoreceptors

Fine structural studies of the developing vertebrate retina are few, in comparison to those which concern the adult retina. The morphogenesis of photoreceptors, as revealed by electron microscopy, is described for amphibians (Carasso, 1958, 1959, 1960; Nilsson, 1964 c), the domestic fowl (Meller, 1964; Meller and Breipohl, 1965; Sheffield and Fischman, 1970), domestic mouse (De Robertis, 1956 b, 1960; Tokuyasu and Yamada, 1959; Caley, et al., 1972), domestic rat (De Robertis, 1960; Weidman and Kuwabara, 1968), domestic cat, Felis domestica (Tokuyasu

and Yamada, 1959), the Rhesus monkey (Samorajski, et al., 1965; Keefe, et al., 1966), and man (Yamada and Ishikawa, 1965).

The differentiation of neurons in the vertebrate retina begins at the center of the optic cup and progresses towards the periphery (Weysse and Burgess, 1906; Noell, 1958; Fujita and Horii, 1963; Aguire, et al., 1972). Morphogenesis of the photoreceptors occurs at different rates among vertebrate species. Once the ONL is established the inner segment begins to develop as a large bulbous process. It extends beyond the OLM and into the optic ventricle (Sheffield and Fischman, 1970). The inner segment possesses mitochondria, ribosomes, cisterns of rough surfaced endoplasmic reticulum, and vesicles of Golgi and smooth endoplasmic reticulum. In essence, the inner segment contains all of the "metabolic machinery" necessary for the synthesis of proteins required for the manufacture of membranous discs (Nilsson and Crescitelli, 1970). A primitive cilium appears at the distal end of the forming inner segment and projects into the optic ventricle (Keefe, et al., 1966). The cilium enlarges and numerous vesicles and tubules are observed within the cytoplasm. These are referred to as "morphogenetic material" (De Robertis, 1956 b, 1960), "ciliary vesicles" (Tokuyasu and Yamada, 1959) and smooth endoplasmic reticulum (Weidman and Kuwabara, 1968). These irregularly arranged vesicles enlarge, flatten out and assume a final disposition perpendicular to the cell membrane, forming the membranous discs of the outer segment. De Robertis (1960)

reports that the tubular filaments of the cilium also contribute to this membrane synthesis.

Infoldings of the cell membrane at the distal end of the developing outer segment are observed. These coalesce with the tubular and vesicular structures, and as a result aid in the synthesis of membranous discs (Tokuyasu and Yamada, 1959). In the leopard frog tadpole, the membranous discs of the photoreceptors develop exclusively from invaginations of the plasmalemma at the basal surface of the outer segments (Nilsson, 1964 c). This process occurs on the side opposite from the connecting cilium. Older discs are then pushed toward the sclera by newly formed ones. In cones, many discs remain continuous with the cell membrane, whereas in rods most of them are pinched off. This phenomenon has been observed in the adult vertebrate retina during the renewal of discs in rod outer segments (Young, 1967, 1971 c).

During regeneration of photoreceptor outer segments in vertebrates with retinal damage, the membranous discs reform by means of irregularly arranged membrane bound vesicles and tubules. Eventually, flattened saccules (discs) become reoriented at right angles to the cell membrane. These observations were made during retinal detachment due to vitamin A deficiency (Dowling and Gibbons, 1961), experimental retinal detachment (Kroll and Machemer, 1968) and retinal damage by visible light (Kuwabara and Gorn, 1968; O'Steen, et al., 1972). These regneration patterns

agree with earlier fine structural observations of normal photoreceptor development.

The presence of developing membranous discs in the outer segment does not insure a functional retina. ERG's are not obtained in the developing retina until the following mature synaptic components are present: (1) synaptic vesicles, (2) synaptic ribbons and (3) invaginated (ribbon) synapses (Nilsson and Crescitelli, 1970).

Most of the fine structural studies in the vertebrate retina are performed on the adult retina. The mature morphology of photoreceptors cannot be fully appreciated or understood without first observing their development in the embryo. The highly complex structure of mature rods and cones is vastly simplified when observed during morphogenesis. Previous studies do not report on the development of the entire photoreceptor from its initial appearance to maturity. A detailed account of this developmental process is thus deemed necessary. The chick is chosen for this study because a complete developmental series is readily obtainable. It is the purpose of this paper to report, in detail, on the morphogenesis of photoreceptors in the chick retina.

CHAPTER II

MATERIALS AND METHODS

Fertile pullet eggs (G. gallus; White Leghorn strain) were incubated at 38° C for periods ranging from five to twenty-one days. Chicks aged from one to two days post-hatching were also used. In all, fifty-five chicks were sacrificed at one day intervals. Each was staged according to the Hamburger-Hamilton series (Hamilton, 1952).

Eyes were excised and immersed in cold cacodylate-buffered aldehyde fixative (pH 7.4; Karnovsky, 1965) for two to three hours. After halving each eye along the choroid fissure and removing the vitreous and sclera, equatorial bands of retinal tissue were obtained. The tissue was then washed in buffer and post-fixed in cacodylate-buffered 2% osmium tetroxide (Karnovsky, 1965). After a buffer wash, the tissue was dehydrated in ascending grades of ethanol and pure propylene oxide. Once the equatorial bands of tissue were passed into Epon 812 (6:4 ratio; Luft, 1961), they were progressively cut into small pieces, from the iris to the fundus. Each piece was embedded separately and oriented for cross-sectional microtomy. After hardening, blocks were chosen from the mid-portion of each band of tissue. Thick sections

were stained with 1% toluidine blue solution at high pH. These sections were used for the purpose of light microscopic identification and photomicrography in a Zeiss Photoscope. Thin sections were mounted on membrane covered (Formvar) grids and stained with uranyl acetate (Greenlee, et al., 1966) for one hour and lead citrate (Venable and Coggeshall, 1965) for fifteen minutes. All tissues were examined in a Philips EM-200 electron microscope.

CHAPTER III

OBSERVATIONS

The following paragraph offers a brief resume of photoreceptor morphogenesis in the chick from five to twenty-one days and one to two days post-hatching, as summarized in Figure 2. A thorough description of this developmental process will follow.

The external portion of the neural retina in the chick represents the outer neuroblastic layer during the 5th to 9th day of incubation (Figure 3). By the 11th day a definitive outer plexiform layer (OPL) is present, dividing the outer neuroblastic layer into outer and inner nuclear layers (ONL, INL). The ONL is typically two to three cell layers in thickness. From the 9th to 11th day, cytoplasmic extensions of the developing photoreceptors (within the ONL) migrate beyond the level of the outer limiting membrane (OLM) and project into the optic ventricle. These spherical to oval projections represent the forming photoreceptor inner segments (Figure 4). By the 16th day the ONL is for the most part, one to two cell layers thick (Figure 5). Numerous arborizations are formed at the bases of the developing receptor cells. These synaptic terminals contain a few synaptic vesicles and occasional synaptic ribbons. The

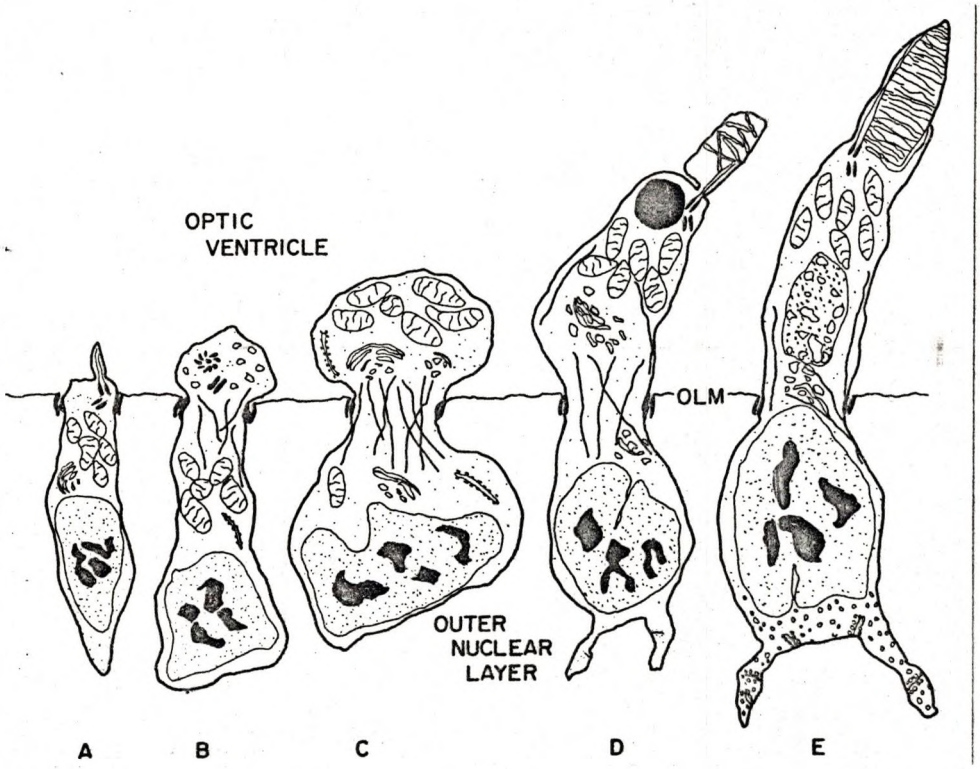


Fig. 2.--Morphogenesis of photoreceptors in the chick retina. The chronology given is as follows: (A) 7th day (Figures 3, 8, 9), (B) 11th day (Figures 4, 10), (C) 14th day (Figures 11-14), (D) 16th day (Figures 5, 6, 15, 16, 17, 24) and (E) 19th day (Figures 7, 19, 31).

photoreceptor inner segments elongate extensively by the 17th day (Figure 6). During this stage, irregularly arranged membranous discs and their associated connecting cilia are observed in forming outer segments. As a result of this progressive photoreceptor growth, the neural retina becomes farther removed from the pigment epithelium, although the rods and cones remain closely associated with it. Mature outer segments with stacked membranous discs, oriented at right angles to the plasmalemma,

are present in the rod and cone layer during the 19th day (Figure 7). The ONL is two to three cell layers thick from the 21st day to one and two days post-hatching. Numerous synaptic complexes are observed between the photoreceptors and bipolar and horizontal cells within the OPL. Throughout development, numerous glial elements (Müller cells) are interspersed among the photoreceptors within the ONL.

Inner Segment

External to the OLM, the optic ventricle is initially a very narrow potential space due to the close apposition of the neural (sensory) retina to the pigment epithelium. It is further obliterated by microvilli from the bordering cells of both the neural retina and the pigment epithelium. At this time (5th to 8th day) the cells of the outer neuroblastic layer are embryonic cells showing little differentiation. The future photoreceptor cells stain more densely than the glial elements (Müller cells). The developing photoreceptors are typically polarized with respect to organelle placement within their cytoplasm. The apical portions of these cells (next to the optic ventricle) possess numerous mitochondria and free ribosomes, in addition to occasional cisterns of rough surfaced endoplasmic reticulum and vesicles of the Golgi. The basal portions of the photoreceptors (nearer to the vitreous) are almost exclusively occupied by the nucleus plus some free ribosomes. Desmosomal junctions are present between photoreceptor and Müller cells at the

outer extent of the neural retina. These intercellular junctions form the fenestrated OLM (Figure 8). Centrioles are very conspicuous at the level of the OLM and are associated occasionally with a cilium that projects into the optic ventricle (Figures 8, 9).

From the 9th to 11th day, the ONL becomes established as a result of the appearance of the outer plexiform layer (OPL). In many instances, the apical cytoplasm of the developing photoreceptor cells may be observed protruding beyond the level of the OLM into the optic ventricle. Numerous centrioles (diplosomes) are found within these cytoplasmic projections (Figure 10).

A large portion of the apical cytoplasm of the photoreceptors continuously migrates through the OLM, forming extensive oval cytoplasmic processes during the 12th to the 15th day (Figure 11). The optic ventricle is expanded considerably by the differentiating receptor cells. In addition, numerous microvilli from the pigment epithelium and the Müller cells extend into this space. The bulbous projections of the photoreceptors contain cellular organelles which occupy the future inner segments. A large cluster of mitochondria is very conspicuous in the distal portion of the inner segment; an observation which relates to the developing ellipsoid. The remaining cytoplasm contains numerous ribosomes, vesicles of the Golgi apparatus and smooth endoplasmic reticulum, and cisterns of rough surfaced endoplasmic reticulum (Figure 12). Occasional centrioles are also observed (Figure 13). Not all of these constituents

are present in every section of the inner segment. Rather, one or two elements may predominate depending on the plane of tissue section. At the level of the OLM, the photoreceptors are constricted and numerous microtubules occupy this region, oriented primarily with the longitudinal axis of the cell (Figure 14).

By the 16th to 17th day, the optic ventricle shows expansion due to further growth of the inner segments. A larger number of microvilli are present in the optic ventricle. Many are from the pigment epithelium and contain mature pigment granules. Several of the inner segments now have an elongated shape (Figures 15, 16). Most of the supranuclear organelles have migrated into the elongating inner segments. Often there is a polarization of organelles within the inner segments. From distal (scleral) to proximal (toward the vitreous), these include: a cilium, basal body and centriole, an occasional lipid droplet, the ellipsoid (mitochondria) and vesicles of the Golgi apparatus and smooth endoplasmic reticulum. Ribosomes, cisterns of rough surfaced endoplasmic reticulum and microtubules are scattered throughout the inner segments (Figures 15, 16). Many rounded and oval inner segments are still observed, possessing the typical organelles which vary according to the plane of tissue section (Figure 17). Occasional nuclei bulge out into the myoid region of the inner segments. At the base of the inner segments the OLM stains more densely than in previous stages. Vertical desmosomal junctions are numerous between photoreceptors and Müller

cells, in addition to horizontal densities within the glial elements (Figure 18). Numerous centrioles are also observed within the glial cells at the level of the OLM.

The optic ventricle continues to enlarge during the corresponding growth of the receptor cells. Mature photoreceptors are observed within the rod and cone layer from the 19th to the 21st day and from one to two days post-hatching. The developing inner segments have continued to elongate and now are morphologically differentiated into two primary regions (Figure 1). The accumulation of mitochondria in the distal one-third represents the ellipsoid, whereas the proximal two-thirds of the inner segment is known as the myoid. At the distal end of the ellipsoid are located the previously mentioned cilium and associated basal body, centriole and frequent lipid droplet (Figure 19). Often calyceal processes extend from the ellipsoid, along the sides of the outer segment (Figure 1). The myoid primarily consists of vesicles of Golgi and smooth endoplasmic reticulum, ribosomes and cisterns of rough surfaced endoplasmic reticulum. In many myoid regions a peculiar organelle, the paraboloid, is often initially observed during the 19th day (Figures 20, 21). It frequently occupies the proximal two-thirds of the myoid. It is oval to ellipsoidal in shape and consists of glycogen granules interspersed between vesicles of smooth endoplasmic reticulum and Golgi (Figure 20). Free ribosomes, cisterns of rough surfaced endoplasmic reticulum and microtubules are found throughout the cytoplasm of the inner segments.

By the 19th day, the inner segments of individual photoreceptors are isolated from one another by extensive bundles of microvilli originating from the Müller cells (Figure 22). In addition, villous processes containing pigment granules often extend from the pigment epithelium as far inward as the distal end of the inner segment (Figure 23).

Outer Segment

Cilia which project from receptor cells are often observed within the optic ventricle. It is not until the 17th day that cilia are associated with the membranous discs of the forming outer segments. Initially, the discs are irregularly arranged and it is very difficult to discern their relationship with one another or with the plasmalemma of the outer segment (Figures 24, 25). More discs are added as growth proceeds. After their initial appearance, the discs become intimately associated with the ciliary microtubules on one side and with numerous membrane bound cylindrical tubules on the other (240 to 400 A diameter). In many instances direct continuity of the disc membranes with those of the tubules are observed (Figures 26-30). By the 19th day and thereafter, mature outer segments are present with stacks of membranous discs (135 A thick) oriented at right angles to the plasmalemma (Figure 31). Occasionally direct continuities are observed between the membranous discs and the cell membrane, particularly at the base of the outer segment. These appear to be invaginations of the plasmalemma (Figure 32).

The connecting cilium, including a small amount of cytoplasm and the cell membrane are the only source of continuity between the outer and inner segments (Figures 26, 33). By the second day post-hatching, the outer segments are isolated from one another by the villous process of the pigment epithelium. During this stage, numerous groups of disc membranes are found within the cytoplasm of the pigmented epithelial cells (Figures 34, 35). This observation suggests a constant turnover of membranous discs; their degradation thus occurring in the pigment epithelium.

Photoreceptor Fibers, Cell Body and Synaptic Terminal

Throughout the early stages of development, the cell bodies of the photoreceptors do not possess very conspicuous characteristics. A nucleus with one to several nucleoli occupies most of the cytoplasm. Although numerous free ribosomes and microtubules are observed in this area, the majority of the supranuclear organelles (Golgi apparatus, smooth and rough surfaced endoplasmic reticulum and mitochondria) migrate beyond the OLM into the forming inner segments. As a result, by the 16th to 17th day, individual nuclei sometimes project a short distance into the inner segments. At this time arborizations form at the basal ends of the photoreceptors, extending inward to the OPL (Figures 36, 37). These structures contain numerous microtubules (270 A diameter) synaptic vesicles (360 A diameter) and occasional small

synaptic ribbons which are surrounded by a cluster of synaptic vesicles (Figures 38-40). By the 19th day and thereafter, numerous synaptic vesicles, synaptic ribbons (320 A thick) and synapses are associated with the synaptic terminals of the photoreceptors. The synaptic ribbons which lie within the cytoplasm of the photoreceptor synaptic terminal, are often perpendicular to the membrane and are surrounded by either a cluster or parallel rows of synaptic vesicles, one on each side (Figures 41-45). Occasionally a dense globular structure is interposed between the synaptic ribbon and the receptor cell plasmalemma. This globular structure represents the "arciform density" (Figure 46). Bipolar dendrites and horizontal cell processes make two types of synapses with the photoreceptor synaptic terminals: (1) conventional synapses (superficial contacts; Figures 41, 42) and (2) ribbon (invaginated) synapses (Figures 42-46). There are also frequent conventional synapses between the secondary neurons (Figures 41, 43, 44, 46).

By the second day post-hatching no further changes are observed in the photoreceptors of the chick retina. A mature functional retina exists as is exemplified by the presence of well developed outer segments and synaptic complexes.

CHAPTER IV

DISCUSSION

This extensive study of photoreceptor morphogenesis in the chick retina reports on numerous stages of development, from the initial presence of undifferentiated cells to the mature photoreceptor. From the observations obtained, it is evident that the overall pattern of photoreceptor development in the neural retina of the chick is comparable to that reported in other vertebrate species, including the leopard frog (Nilsson, 1964 c; Nilsson and Crescitelli, 1970), domestic mouse (De Robertis, 1956 b, 1960; Tokuysau and Yamada, 1959; Caley, et al., 1972), domestic rat (De Robertis, 1960; Weidman and Kuwabara, 1968), domestic cat (Tokuyasu and Yamada, 1959); Rhesus monkey (Samorajski, et al., 1965; Keefe, et al., 1966) and man (Yamada and Ishikawa, 1965). Moreover, the fine structural morphology of mature photoreceptors in the chick retina corresponds with that in numerous vertebrates, as reported by others (Sjöstrand, 1961; Cohen, 1963 b; Dowling, 1970; Young, 1969 b, 1970, 1971 a; Hogan, et al., 1971). However, few of the previous embryological studies have emphasized photoreceptor development in its entirety. Therefore, it seems pertinent to consider in detail

the stages of this continuous developmental process for comparison with the observations of others in various vertebrate species. Many of the observations in this study complement those of other investigations, but certain of the observations amplify and add to those observed previously.

Inner Segment

In the chick embryo, centrioles are observed within the apical cytoplasm of the photoreceptors at the level of the outer limiting membrane (OLM) during the very early stages of development. All vertebrate retinas examined to date have displayed this particular characteristic (De Robertis, 1956 b, 1960; Tokuyasu and Yamada, 1959; Nilsson, 1964 c; Weidman and Kuwabara, 1968; Sheffield and Fischman, 1970; and others). The centrioles are often observed in pairs, commonly referred to as a "diplosome." After the protrusion of photoreceptor cytoplasm beyond the OLM, the centrioles are constantly observed within the inner segment throughout all subsequent developmental stages. Since these organelles are commonly observed in the chick and other vertebrates, they must be of important functional significance. It is now established that the "connecting cilium" develops from one of the centrioles, which is then referred to as the basal body. In turn, the outer segment of the photoreceptor proper develops as a result of the differentiation of this primitive cilium (De Robertis, 1956 b, 1960; Tokuyasu and Yamada, 1959;

Nilsson, 1964 c; Samorajski, et al., 1965; Keefe, et al., 1966; Weidman and Kuwabara, 1968; Caley, et al., 1972).

In addition to centrioles, the apical cytoplasm of the early photoreceptors possesses numerous mitochondria and vesicles of the Golgi and smooth endoplasmic reticulum. In the chick, as in many other vertebrates, these organelles migrate beyond the OLM, into the forming inner segments. Thus, the first indication of the development of the photoreceptor proper is the appearance of primitive bulbous inner segments within the optic ventricle (De Robertis, 1956 b, 1960). Once established, the inner segments expand and elongate, resulting in the continuous enlargement of the optic ventricle (Caley, et al., 1972). In the chick, two definitive regions are readily recognized within the inner segment during the 16th day. The ellipsoid occupies the distal one-third, whereas the myoid represents the proximal two-thirds of the inner segment. The paraboloid is observed within the myoid of the inner segment by the 19th day. Most investigators report the paraboloid as being peculiar to the cone cell, although it is occasionally observed in rods (Morris and Shorey, 1967; Dickson and Hollenberg, 1971). In this study, the paraboloid is observed in both rods and cones. However, in cones the glycogen granules are associated with vesicles of smooth endoplasmic reticulum and Golgi, whereas in rods no vesicles are present.

Abundant amounts of Golgi are frequently observed within the myoid region of the inner segment. This organelle plays a very important

functional role in the photoreceptor cell. It is now established that the proteins synthesized within the Golgi are transported, via the connecting cilium, to the outer segment where they are incorporated into newly formed membranous discs (Young, 1967, 1968; Young and Droz, 1968).

Numerous microtubules are also present within the proximal portion of the myoid, especially at the level of the OLM. Since microtubules are often interpreted as cytoskeletal elements which play a significant role in maintaining cell shape and in ciliary movement (Fawcett, 1966), it seems likely that they contribute to the physical stability of the photoreceptor proper. It is also suggested that they may be involved with transport of materials through the cytoplasm of the photoreceptor.

Considering all of the morphological aspects, the fine structure of the inner segments in the chick retina corresponds well with studies in other vertebrate forms (Cohen, 1963 b).

Outer Segment

The appearance of a cilium extending from a basal body within the ellipsoid region of the inner segment (16th day) always precedes the formation of the outer segment during the development of the photoreceptor proper (De Robertis, 1956 b, 1960; Tokuyasu and Yamada, 1959; Nilsson, 1964 c; Samorajski, et al., 1965; Keefe, et al., 1966; Weidman and Kuwabara, 1968; Caley, et al., 1972). Irregularly arranged membranous discs are observed within the forming outer segment during the

17th day in the chick. By the 19th day the discs are arranged perpendicularly to the plasmalemma. During their formation, the discs are closely associated with the microtubules of the connecting cilium, and many illustrate continuity with cylindrical tubules measuring 240 to 400 A in diameter. These tubules are not continuous with the ciliary microtubules or the plasmalemma. These observations are not unlike those noted in other vertebrates in which the origin of the membranous discs is traced to vesicles or tubules. De Robertis (1956 b) first suggested that the membranous discs are derived from small tubules and vesicles (50 to 70 A in diameter) which are found in the cytoplasm of the primitive cilium in the domestic mouse. These structures are collectively referred to as "morphogenetic material." Vesicles and tubules (300 to 400 A in diameter) are also observed within developing outer segments of various amphibian larvae, and it appears as though they contribute to disc formation (Carasso, 1958, 1959). Vesicles are also observed in immature outer segments in the domestic cat (Tokuyasu and Yamada, 1959) and rat (Weidman and Kuwabara, 1968). The vesicles are referred to as "ciliary vesicles" and smooth endoplasmic reticulum, respectively. Moreover, when outer segments are damaged resulting from retinal detachment (Dowling and Gibbons, 1961; Kroll and Machemer, 1968) and visible light (Kuwabara and Gorn, 1968; O'Steen, et al., 1972), regeneration of the membranous discs occurs by means of irregularly arranged vesicles and tubules. In all of the previously cited investigations, the

membranous discs are initially randomly arranged, followed by repositioning perpendicularly to the plasmalemma of the outer segment.

In more mature outer segments in the chick, some of the membranous discs are observed to be continuous with the plasmalemma. As a result the "intradisc space" is continuous with the extracellular space. In the domestic mouse and rat, De Robertis (1960) notes continuities between the plasmalemma and the developing discs on the side of the outer segment opposite from the cilium, in addition to the "morphogenetic material." In the domestic cat (Tokuyasu and Yamada, 1959), numerous infoldings of the plasmalemma are observed at the tip of the outer segment. These make contact and become continuous with the "ciliary vesicles," and eventually give rise to the flattened membranous discs. In the leopard frog tadpole, discs develop perpendicularly to the long axis of the outer segment as invaginations of the plasmalemma. No vesicles are observed prior to disc formation (Nilsson, 1964 c). Although, where discontinuities are observed in the discs, rows of vesicles are present. During the renewal of discs in the outer segments of the adult vertebrate retina, the discs also form as invaginations of the plasmalemma at the base of the outer segment (Young, 1967, 1971 c). Even though the morphology of the outer segment in rods and cones differs somewhat, the membranous discs of all photoreceptors show the same principle of membrane formation (De Robertis, 1960; Nilsson, 1964 c).

From the observations of this study and others, it seems apparent that the membranous discs of the outer segment form by (1) coalescence of vesicles and tubules, (2) invaginations of the plasmalemma or (3) a combination of both. Both of these processes are observed in the neural retina of the chick. It seems reasonable that both means of disc formation are compatible and could possibly occur concurrently during this extensive developmental process.

The microtubules of the cilium extend for varying distances within the forming outer segment. Although, De Robertis (1956 b) suggests that the membranous discs are directly connected with the microtubules of the cilium, no published micrographs have shown this to be the case. The cilium is located eccentrically within the forming and mature outer segment. This study corresponds with previous observations which illustrate that the lower portion of the cilium does not differentiate, but rather, remains in the adult as the only connection between the inner and outer segments. Thus, it is referred to as the "connecting cilium."

Groups of membranous discs are eventually observed in the pigment epithelium of the chick retina. It is now an established fact that membranous discs are shed from the outer segments and engulfed by the pigment epithelium. During this study in the chick and in other vertebrates, these phagosomes then undergo digestion within the cytoplasm of the pigmented epithelial cells (Bairati and Orzalesi, 1963; Young and Bok, 1969; Ishikawa and Yamada, 1970; Young, 1971 b, 1971 c). At the same

time, continual renewal of the discs occurs at the base of the photoreceptors, resulting in an outer segment size which remains relatively constant.

Photoreceptor Fibers, Cell Body and Synaptic Terminal

The outer fiber of the photoreceptor which lies between the cell body and the OLM, varies in its appearance depending on the position of the cell body. In the chick, numerous receptor cell bodies lie just beneath the OLM. As a result, the outer fiber is virtually non-existent. In others, the cell body is farther removed from the OLM and the outer fiber is long and attenuated. These cells are usually identified as rod cells, although this is not a consistent diagnostic feature among all vertebrates. These observations agree well with those in other vertebrate forms (Polyak, 1941). Microtubules are found within the outer fiber and continue into the myoid, adding to the stability of the photoreceptor. The Golgi apparatus often migrates into the early forming inner segments, and eventually becomes a prominent feature in the myoid region rather than in the outer fiber of the mature receptor cell.

In all vertebrates including the chick, the cell bodies of developing and mature photoreceptors are largely occupied by the nucleus (Cohen, 1963 b). Microtubules are present in the surrounding cytoplasm which is void of any other conspicuous organelles.

The inner fiber develops as the synaptic terminal of a given photoreceptor (Hogan, et al., 1971). These structures form below the nucleus at the basal portion of the rods and cones. They begin to appear by the 16th day in the chick retina. During their initial appearance, few synaptic vesicles are present and only occasional synaptic ribbons are observed. By the 19th day, both conventional and invaginated (ribbon) synapses are observed. These two principal types of synaptic contacts are observed in all vertebrate retinas (Dowling, 1970). Components of mature synaptic complexes include: synaptic vesicles, synaptic ribbons and the synaptic membranes. Nilsson and Crescitelli (1970) note that ERG's are not obtained in the developing retina of the leopard frog until these synaptic elements are present. In view of these findings, it seems apparent that by the 19th day, the chick possesses a fully functional retina based on the presence of well developed synaptic complexes.

A detailed account of the development of the inner and outer segments and the synaptic terminal is given in this study. The fine structural morphogenesis of photoreceptors in the chick retina appears to correspond with that in other vertebrate forms (De Robertis, 1956 b, 1960; Tokuyasu and Yamada, 1959; Nilsson, 1964 c; Keefe, et al., 1966; Weidman and Kuwabara, 1968; Caley, et al., 1972). The development of the photoreceptor proper is first indicated by the appearance of the inner segment. The outer segment differentiates from a primitive cilium. The membranous discs of the outer segment apparently develop through the

coalescence of tubules and as invaginations of the plasmalemma. Both forms of disc formation are observed in other vertebrate retinas (De Robertis, 1956 b; Nilsson, 1964 c, and others). During the growth of the outer segment, the synaptic terminal is also developing at the base of the photoreceptor, resulting in synaptic contact with secondary neurons within the outer plexiform layer (OPL).

This study indicates a need for additional research on developing photoreceptors, such as more detailed observations of membranous disc formation, the origin of synaptic ribbons and vesicles, microtubule functions and many others. It is evident that the vertebrate retina offers an excellent system for the study of cellular differentiation. Indeed, cellular morphology is vastly simplified when studied during development.

APPENDIX

LEGEND TO FIGURES

AD	arciform density	MY	myoid
BB	basal body	N	nucleus
BM	Bruch's membrane	NBL	outer neuroblastic layer
C	centriole	ONL	outer nuclear layer
CAP	capillary	OPL	outer plexiform layer
CL	cilium	OV	optic ventricle
D	desmosome	PE	pigment epithelium
ES	ellipsoid	PG	pigment granule
G	Golgi apparatus	PSY	postsynaptic neuron
GLY	glycogen	RC	photoreceptor cell
INL	inner nuclear layer	SER	smooth endoplasmic reticulum
IS	inner segment	SR	synaptic ribbon
MC	Müller cell	ST	synaptic terminal
MD	membranous discs	SV	synaptic vesicle
MT	microtubules	TB	cylindrical tubule
MV	microvilli		

Figures 3-7 are electron micrographs which illustrate a brief resume of the development of photoreceptors in the chick retina. A light micrograph (inset) shows the entire retina during the corresponding stage of development. The remaining figures are all electron micrographs. Figures 8-23 illustrate the development of the inner segment, whereas Figures 24-35 show the development of the outer segment. Figures 36-46 concentrate on the development of the synaptic terminal of the photoreceptor. All tissues were stained with uranyl acetate and lead citrate.

PLATE I

Fig. 3.--Outer neuroblastic layer of neural retina (7 days, stage 29). The outer limiting membrane (arrows) marks the separation betwee the neural retina and the pigment epithelium (PE). The optic ventricle i virtually obliterated due to the close apposition of the neural retina and pigment epithelium. The outer neuroblastic layer (NBL) has not yet divided into the outer and inner nuclear layers, as illustrated in the lig micrograph (inset). The future photoreceptor cells (RC) are ellipsoidal and stain more densely than the Müller cells (MC). The apical cytopla of the photoreceptor cells contains numerous mitochondria, free riboson Golgi and cisterns of rough surfaced endoplasmic reticulum. The basal portions of these cells are almost entirely occupied by the nucleus. 15,000X. Inset 300X.

PLATE I

PLATE II

Fig. 4.--Establishment of definitive outer nuclear layer (11 days, stage 37). The outer neuroblastic layer is now divided into the outer (ONL) and inner nuclear layers (INL). The outer plexiform layer (OPL) separates these two nuclear layers. The outer nuclear layer is from two to three cell layers thick. Beyond the level of the outer limiting membrane (arrows), bulbous expansions of the photoreceptors now extend into the enlarged optic ventricle (OV). These cytoplasmic projections represent the future inner segments. The pigment epithelium (PE) and a capillary (CAP) of the choriocapillaris are located above the optic ventricle. 8,000X. Inset 220X.

PLATE II

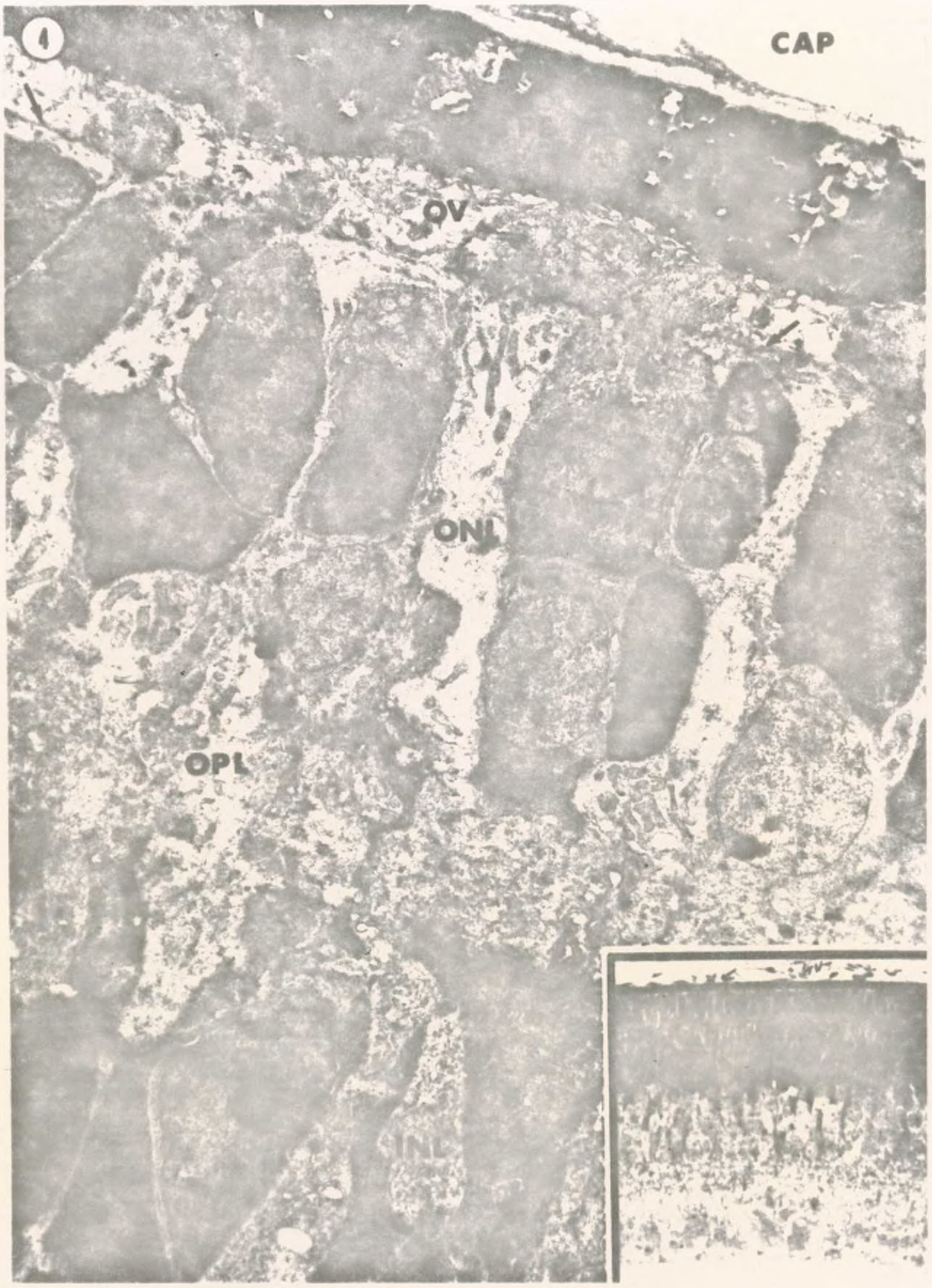


PLATE III

Fig. 5.--Developing photoreceptor cells (16 days, stage 42). The outer nuclear layer is for the most part, one to two cell layers thick. The developing basal arborizations of the photoreceptors represent the synaptic terminals (ST), which extend into the outer plexiform layer (OPL). In rods and cones, these are referred to as spherules and pedicles, respectively. Beyond the outer limiting membrane (arrows), the optic ventricle is expanded due to the growth of the inner segments (IS). Microvilli (MV) extend into the optic ventricle from the Müller cells and the pigment epithelium (PE). 6,500X. Inset 220X.

PLATE III

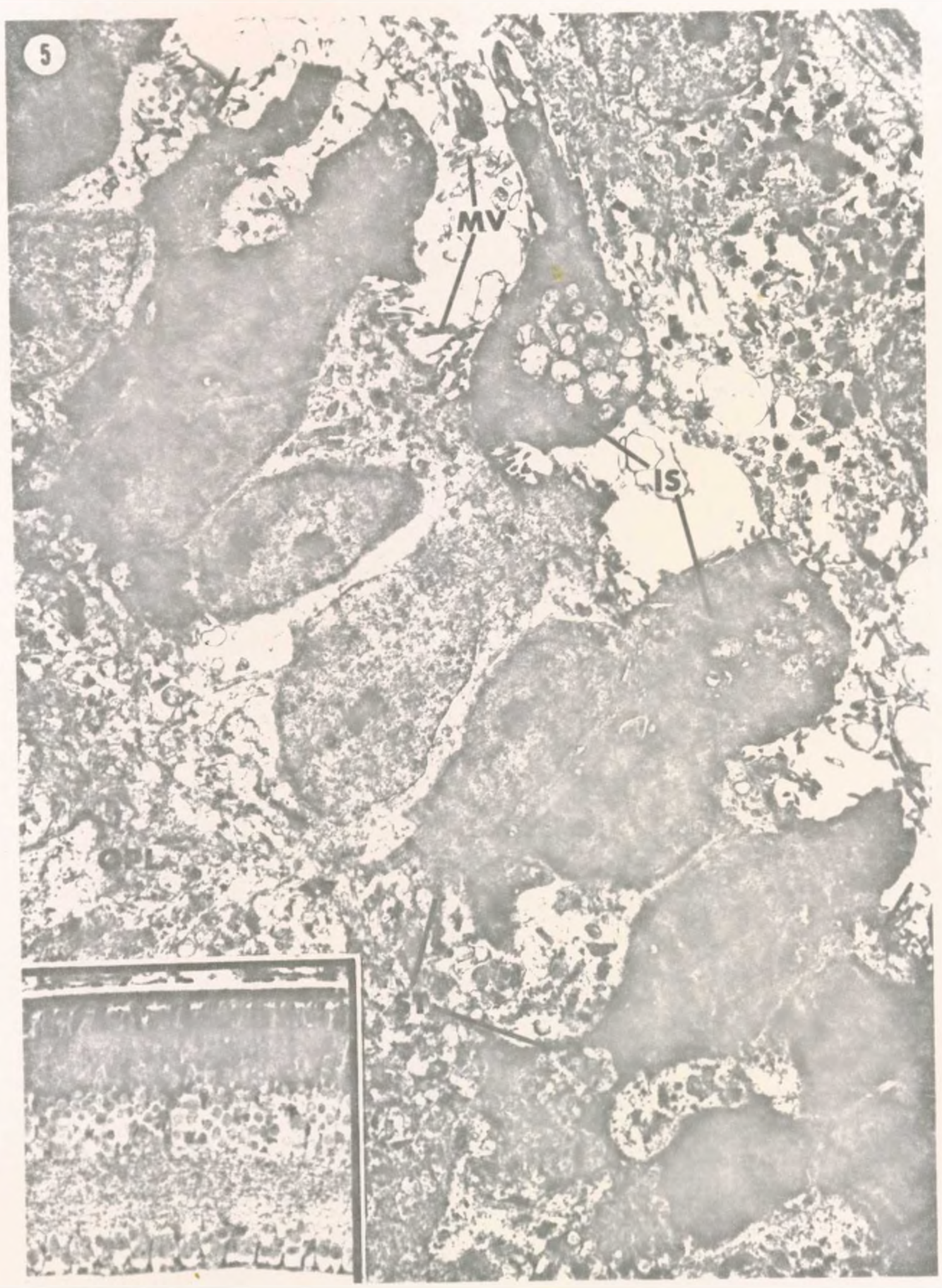


PLATE IV

Fig. 6.--Elongation of the inner segment (17 days; stage 43). The inner segments of the photoreceptor cells elongate, and as a result the pigment epithelium (PE) becomes farther removed from the neural retina. The ellipsoid (ES) is now very conspicuous in the distal one-half of the inner segment. The myoid region (MY) of the inner segment contains abundant Golgi (G). Note the marked increase of pigment granules within the pigment epithelium. 9,800X. Inset 220X.

PLATE IV

PLATE V

Fig. 7.--Mature rod and cone layer (19 days, stage 45). The inner and outer segments of the photoreceptors extend beyond the level of the outer limiting membrane (arrows) to the pigment epithelium (PE). Both mature and forming membranous discs (MD) are observed within the outer segments. The ellipsoid (ES) and paraboloid (P) are conspicuous within the inner segments. Numerous microvilli isolate individual photoreceptors within the optic ventricle. The outer nuclear layer (ONL) is one to two cell layers in thickness. Bruch's membrane (BM) and a capillary (CAP) of the choriocapillaris are located above the pigment epithelium. 5,600X. Inset 220X.

PLATE V

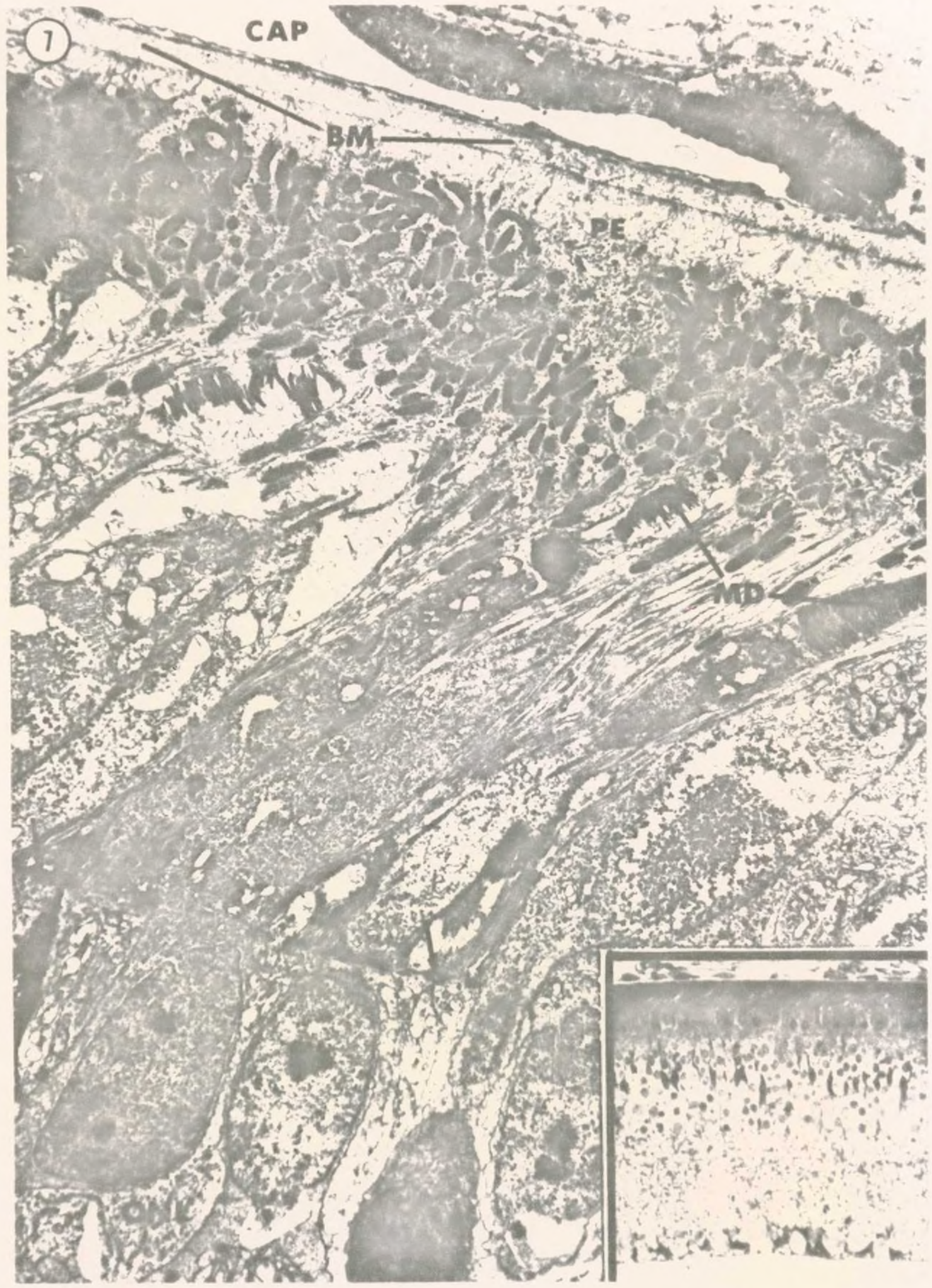


PLATE VI

Fig. 8.--The outer limiting membrane (7 days, stage 29). At the periphery of the neural retina, desmosomes (D) occur between adjacent photoreceptor and Müller cells. These intercellular junctions constitute the fenestrated outer limiting membrane. Centrioles (small arrows) are observed in the apical cytoplasm of both photoreceptor and glial cells. Note the basal body and associated cilium (large arrow) which project toward the pigment epithelium (PE). 18,000X.

Fig. 9.--Cilium and basal body (7 days, stage 29). During this early stage of development, a cilium (CL) projects into the optic ventricle. The ciliary doublets (microtubules) which make up the framework of the cilium are continuous with a basal body (BB). An associated centriole (C) is located beneath the basal body. 68,000X.

PLATE VI

PLATE VII

Fig. 10.--Centrioles (11 days, stage 37). The apical cytoplasm of the developing photoreceptor cells extends beyond the outer limiting membrane (large arrows), into the optic ventricle. Centrioles (small arrows) are observed within these cytoplasmic projections. The photoreceptor cells are constricted at the level of the outer limiting membrane. Microtubules (MT) which are oriented with the long axis of the cell are conspicuous in this narrow region. 36,000X.

PLATE VII

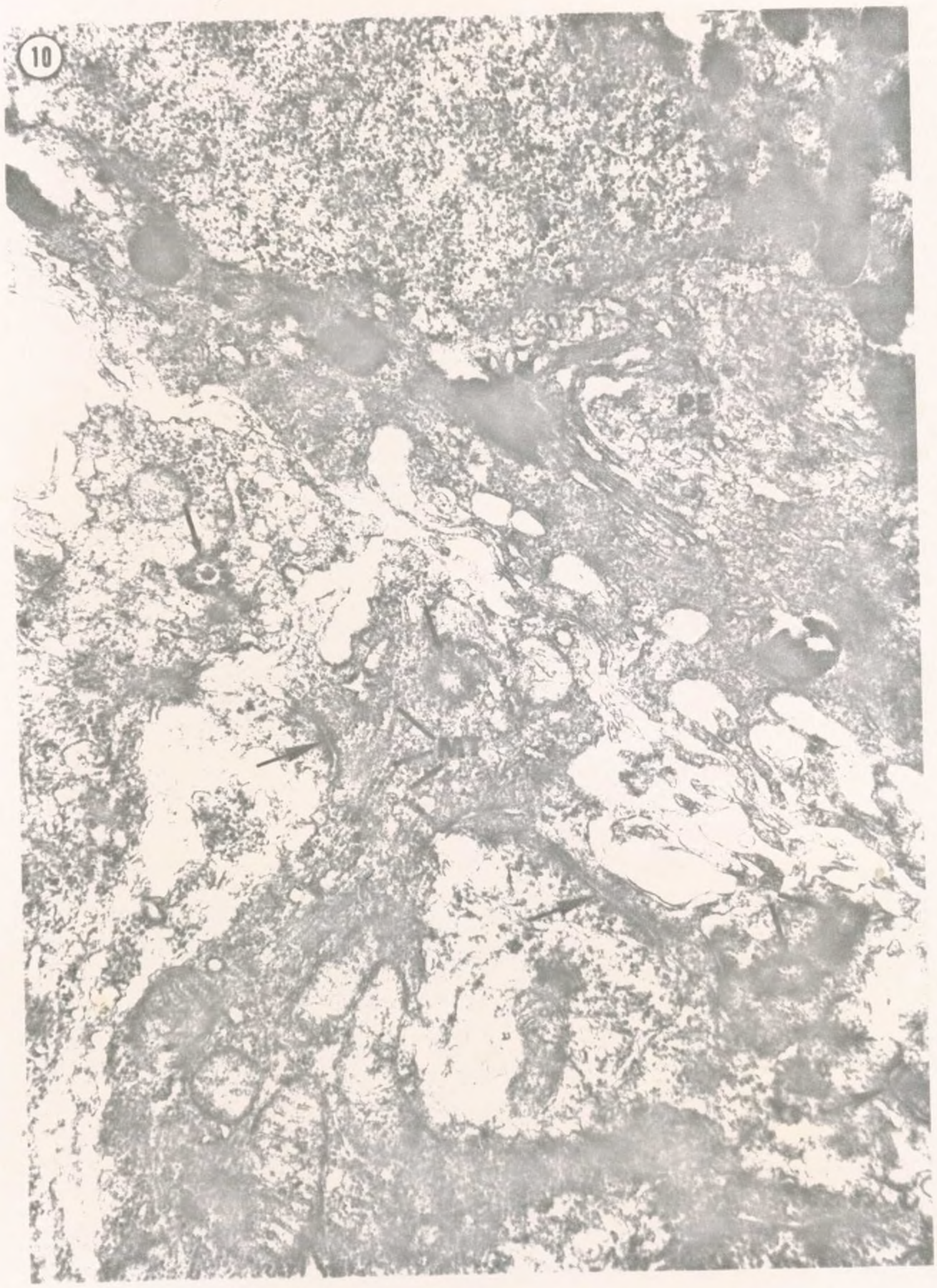


PLATE VIII

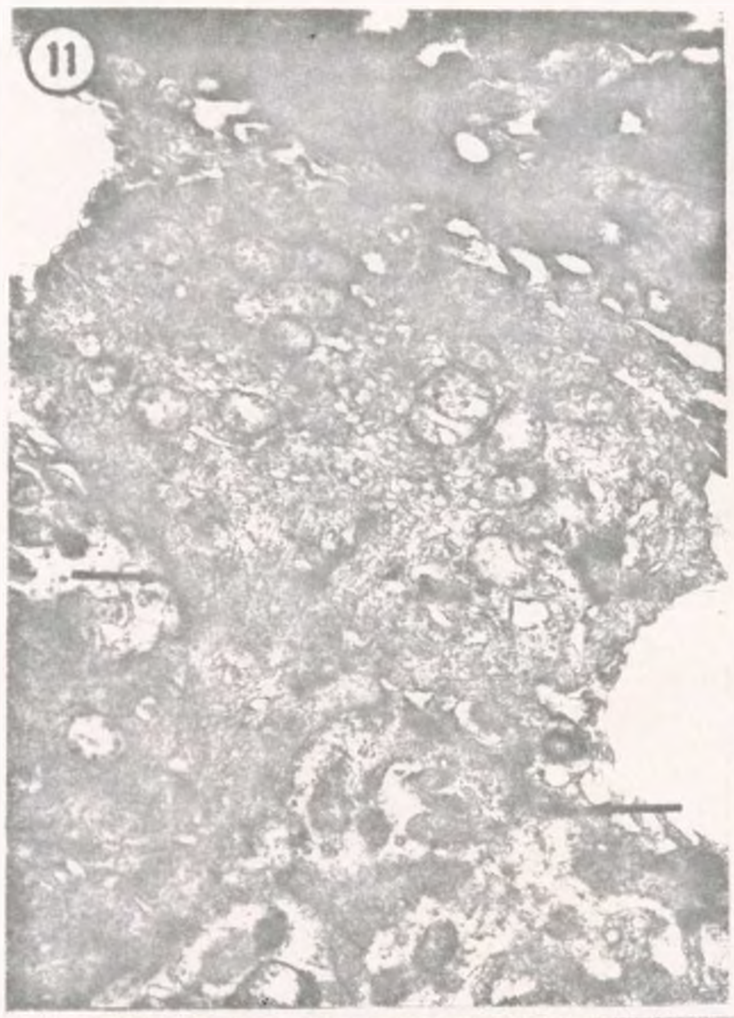
Fig. 11.--Bulbous process of developing photoreceptor (12 days, stage 38). Additional apical cytoplasm of the photoreceptors extends through the outer limiting membrane (arrows) with increasing age. The process contains numerous mitochondria, smooth endoplasmic reticulum, free ribosomes and microtubules. 15,000X.

Fig. 12.--Forming inner segment (14 days, stage 40). The illustrated organelle placement is suggestive of that found in better developed inner segments. An accumulation of mitochondria in the distal portion relates to the future ellipsoid. The remainder of the inner segment contains those organelles common to the myoid region, including numerous vesicles of the Golgi and smooth endoplasmic reticulum. The pigment epithelium (PE) is farther removed from the sensory retina. 15,000X.

Fig. 13.--Centriole within inner segment (13 days, stage 39). The fine structural morphology of the centriole (C) is typical, with nine concentrically arranged triplets of microtubules (MT), each averaging 270 A in diameter. The microtubules in the upper one-half of the centriole are tangent to the plane of tissue section due to a twisting effect. Note the surrounding smooth endoplasmic reticulum (arrows). 128,000X.

Fig. 14.--Constriction of photoreceptor (14 days, stage 40). Photoreceptors are constricted at the level of the outer limiting membrane (arrows). Numerous microtubules (MT) averaging 270 A in diameter are observed within this region of the photoreceptor. Mitochondria, Golgi and polyribosomes are abundant within the inner segment (IS). 19,000X.

PLATE VIII



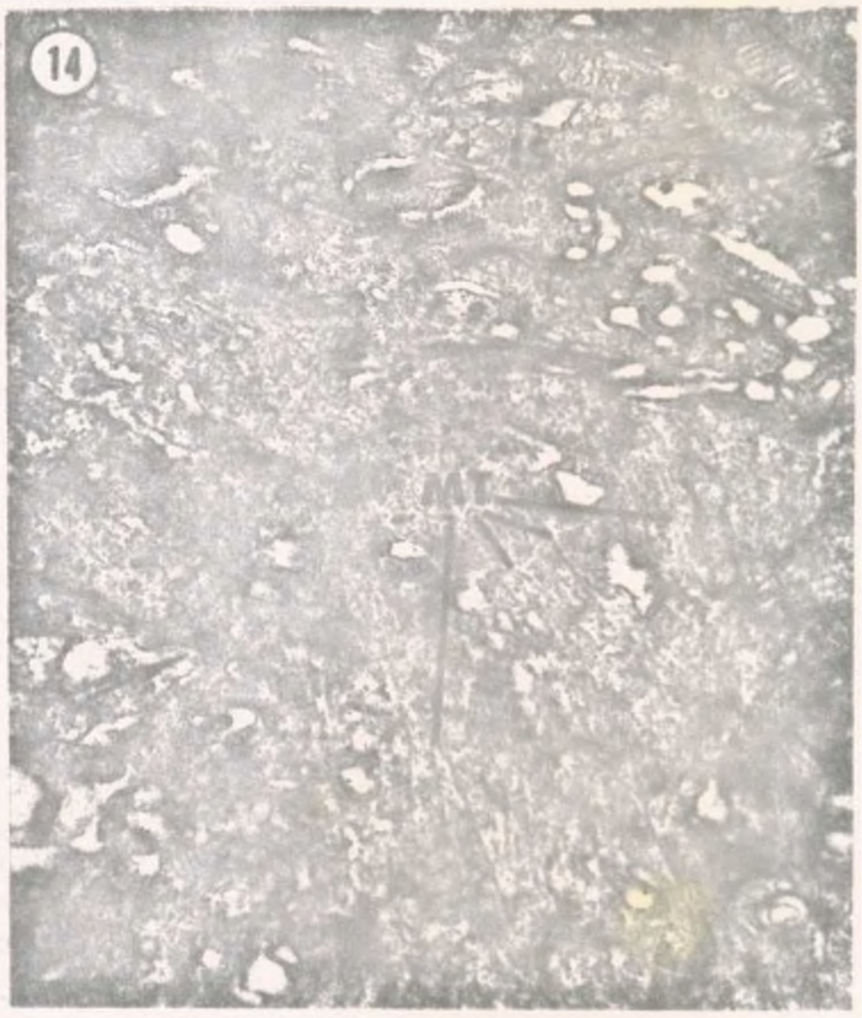


PLATE IX

Fig. 15.--Inner segment of photoreceptor (16 days, stage 42). By 16 days, the inner segments are more elongated. The distal end of the inner segment contains a basal body and associated cilium, a centriole and a lipid droplet. The ellipsoid (ES) is very conspicuous. The myoid (MY) possesses numerous ribosomes, cisterns of rough surfaced endoplasmic reticulum and microtubules. On the vitreous side of the outer limiting membrane (arrows), the Golgi apparatus (G) is conspicuous in the supranuclear region. 16,000X.

Fig. 16.--Inner segment of photoreceptor (16 days, stage 42). The myoid region differs from the previous one in Figure 15. The Golgi apparatus (G) dominates the cytoplasm of the myoid. Note the pair of centrioles (C) near the outer limiting membrane (arrows), within the cytoplasm of what appears to be a glial element or a Landolt's club (dendritic process of a bipolar cell). 15,000X.

PLATE IX

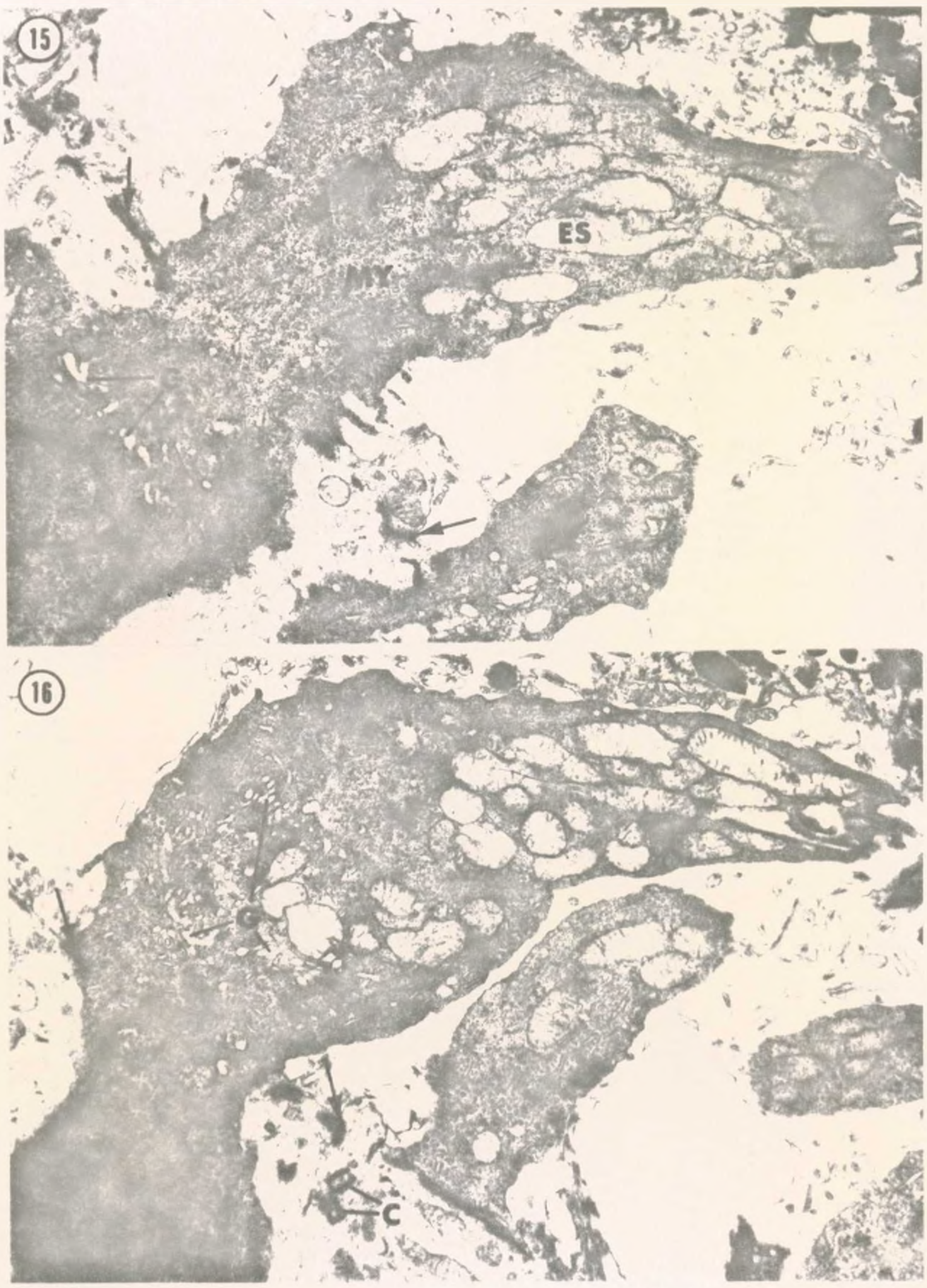


PLATE X

Fig. 17.--Developing photoreceptors (17 days, stage 43). Many rounded to oval inner segments are present within the optic ventricle. The ellipsoid and myoid are easily recognized in the inner segment near the center of the micrograph (arrow). Within the outer nuclear layer (ONL), the cell bodies of the photoreceptors are readily distinguished from the Müller cells which stain much lighter. The synaptic terminals of the receptor cells extend into the outer plexiform layer (OPL) to synapse with bipolar and horizontal cells. The pigment epithelium (PE) contains numerous pigment granules. 4,700X.

Fig. 18.--Organization of the outer limiting membrane (16 days, stage 42). The outer limiting membrane stains very densely and is comprised of vertical desmosomes (D) and horizontal dense bands (arrows). An inner segment projects into the optic ventricle near the center of the field. Note the microvilli (MV) which extend from the Müller cells. These processes are often referred to as the "fiber baskets of Müller." 30,000X.

PLATE X

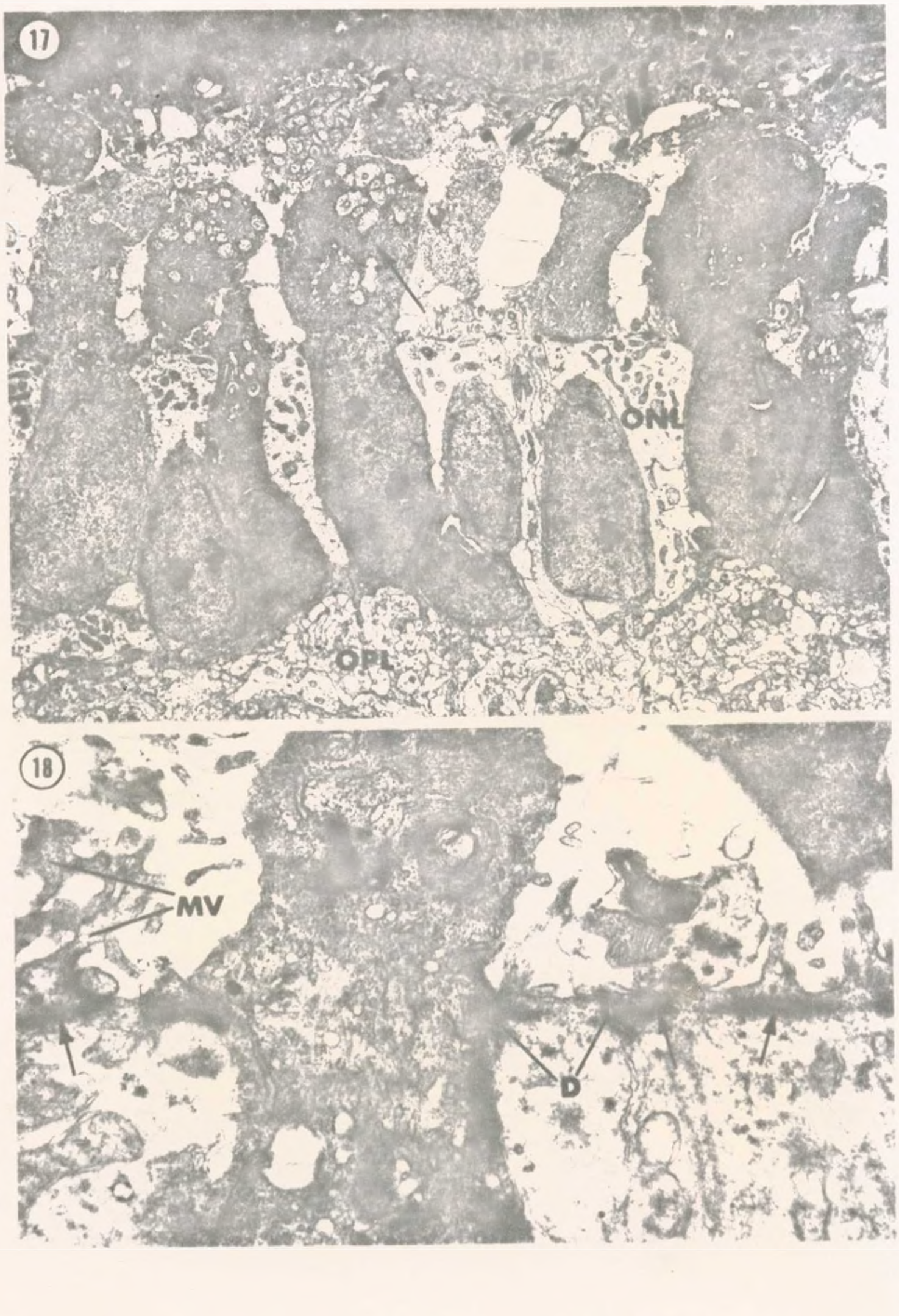


PLATE XI

Fig. 19.--The ellipsoid region (19 days, stage 45). A basal body with associated cilium and a centriole are present at the distal end of the ellipsoid region. A lipid droplet (small arrow) is also observed. The ellipsoid (ES) is located proximal to the lipid droplet. Note the forming membranous discs (MD) within the outer segment, which at this stage do not occupy it completely. A villous process from the pigment epithelium extends along one side of the photoreceptor proper (large arrow). 11,000X.

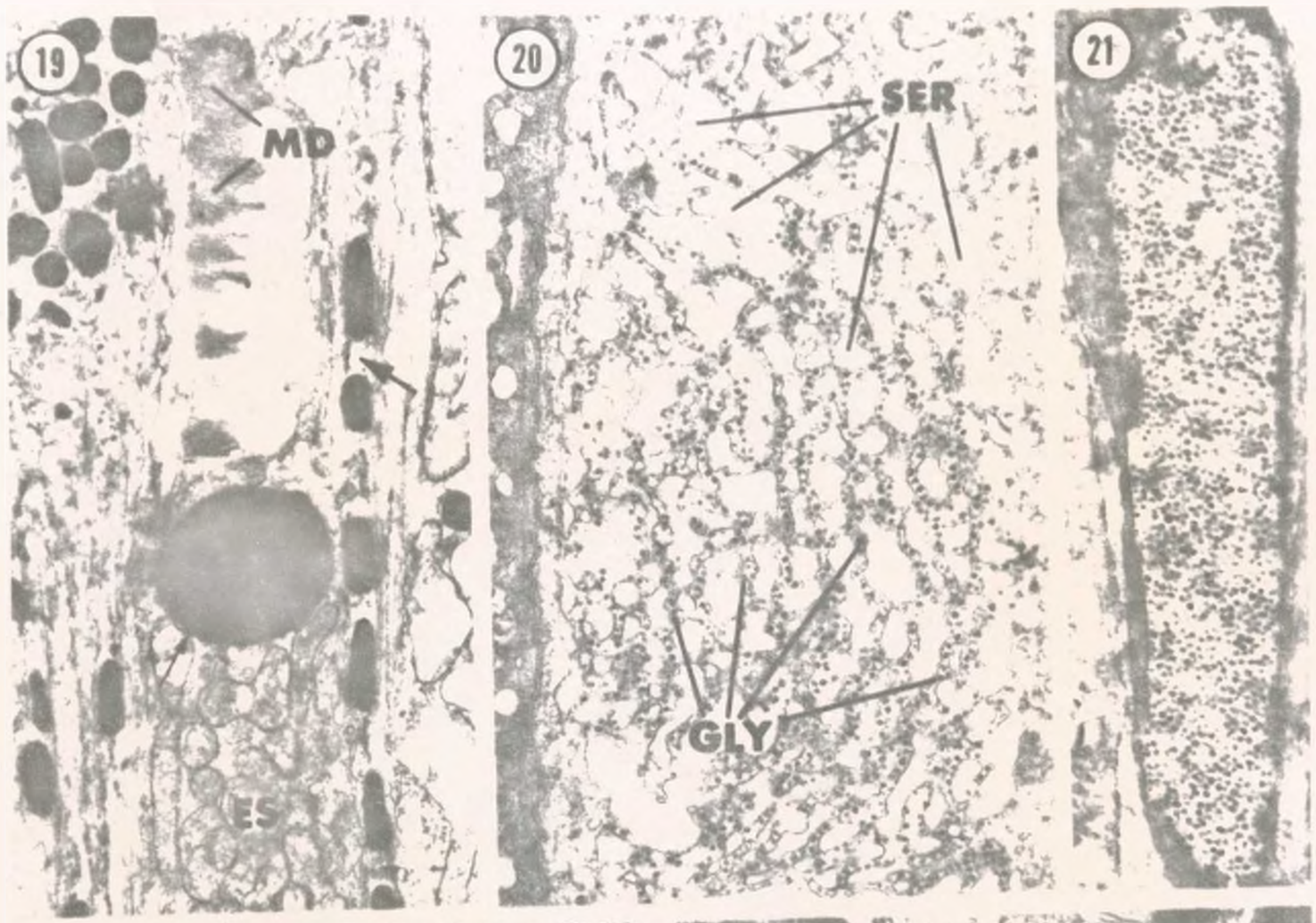
Fig. 20.--Paraboloid in cone cell (21 days, stage 46). This paraboloid occupies a large portion of the myoid. It consists of numerous vesicles of smooth endoplasmic reticulum (SER). These vesicles are indistinguishable from those of the Golgi complex which also occupies the myoid. The interstices between the vesicles contain abundant amounts of glycogen granules (GLY). These should not be mistaken for ribosomes or polyribosomes. 21,000X.

Fig. 21.--Paraboloid in rod cell (19 days, stage 45). The paraboloid of the rod cell is much more compact than that of the cone cell. It also occupies the myoid region of the inner segment. Glycogen granules are plentiful, but vesicles are not present. 18,000X.

Fig. 22.--Microvilli (21 days, stage 46). Numerous microvilli (MV) extend beyond the outer limiting membrane (arrows) from the Müller cells. These processes separate individual inner segments. Note the paraboloid on the right, whereas the myoid on the left contains smooth endoplasmic reticulum, polyribosomes and microtubules. 21,000X.

Fig. 23.--Microvilli of the pigment epithelium (20 days, stage 45). Microvilli containing pigment granules (PG) extend toward the vitreous as far as the distal end of the inner segment (IS). Well developed membranous discs (MD) are present within the outer segment. Note the calyceal process (arrow) extending along the surface of the outer segment. 28,000X.

PLATE XI



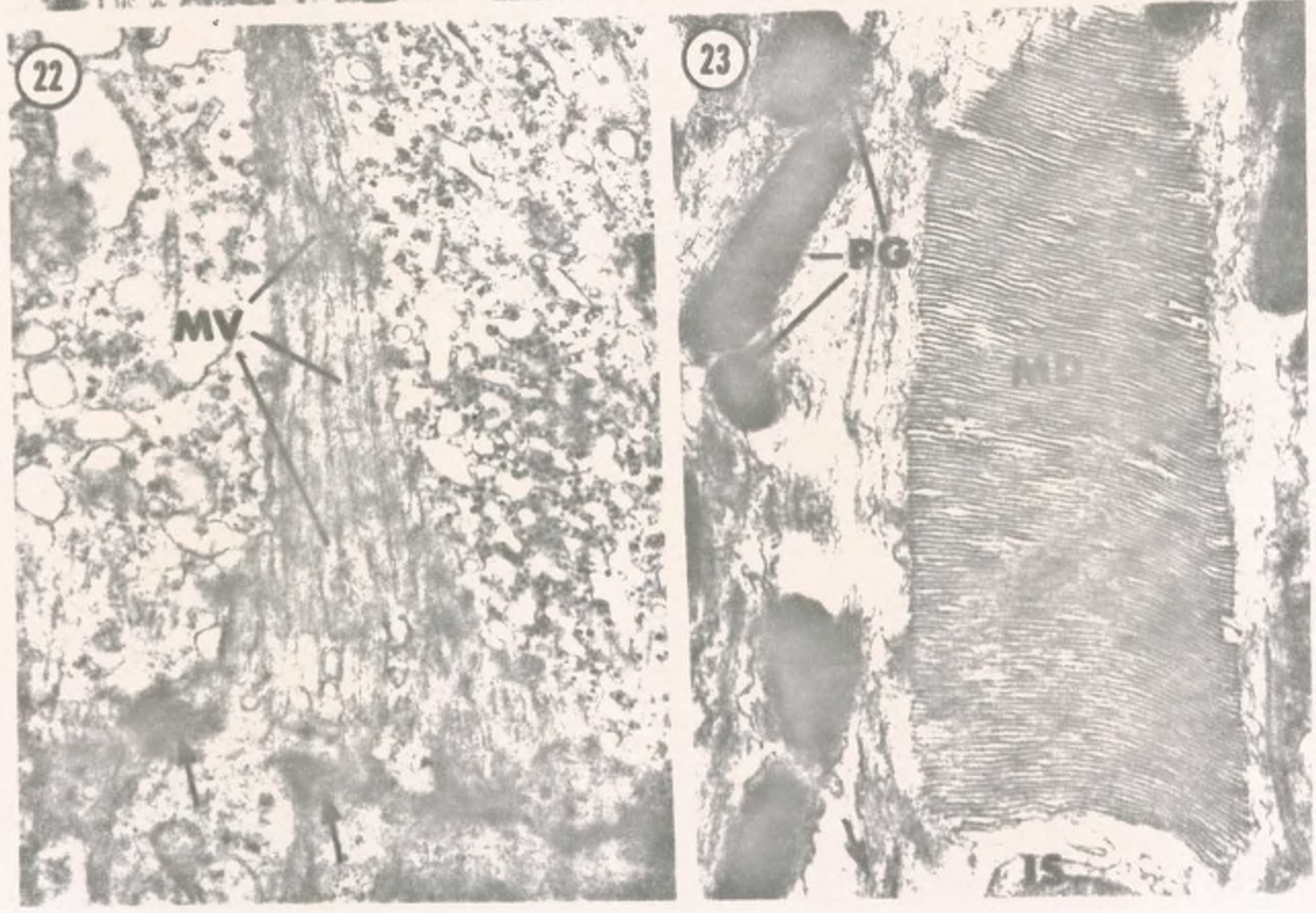


PLATE XII

Fig. 24.--Development of membranous discs (17 days, stage 43). A centriole and basal body are observed within the distal extent of the inner segment (IS). The "connecting cilium" (CL) projects into the optic ventricle. Irregularly arranged membranous discs (MD) are present within the outer segment. Initially, it is very difficult to discern the relationship of the discs with the plasmalemma or the cilium. 76,000X.

Fig. 25.--Membranous discs (17 days, stage 43). Adjacent inner (IS) and outer segments are present in the optic ventricle. A few membranous discs (MD) measuring 135 A in thickness are observed within the outer segment. A pigment granule (PG) is located within the pigment epithelium in the upper portion of the micrograph. 70,000X.

PLATE XII

PLATE XIII

Figure 26 represents a low magnification of a developing outer segment. It illustrates the intimate relationship between the connecting cilium, partially formed discs, cylindrical tubules and the plasmalemma. Moreover, it reveals the continuity between the inner and outer segments. Figures 27-30 represent portions of Figure 26 in greater detail. These micrographs reveal the continuity between the forming membranous discs and the cylindrical tubules which appear to contribute to disc formation.

Fig. 26.--Developing outer segment (21 days, stage 46). The lower part of the micrograph illustrates the distal end of the inner segment (IS). A small portion of the ellipsoid is present in addition to a centriole and basal body. A calyceal process (small arrows) extends from the ellipsoid along the margin of the outer segment. Continuity between the inner and outer segments is restricted to a small amount of cytoplasm, the connecting cilium and the plasmalemma (large arrows). The forming membranous discs (MD) are intimately associated with the cilium and cylindrical tubules (TB) within the outer segment. 43,000X.

Figs. 27-29.--Membranous discs and cylindrical tubules (21 days, stage 46). These micrographs illustrate the direct continuity between the membranous discs (135 A in thickness) and the cylindrical tubules (arrows) which measure from 240 to 400 A in diameter. Tubules are not observed to result as invaginations of the cell membrane. 54,000X.

Fig. 30.--Membranous discs and cylindrical tubules (21 days, stage 46). This micrograph illustrates more detail of Figure 29. Continuity between the flattened discs and the cylindrical tubules is clearly revealed (arrows). 62,000X.

PLATE XIII

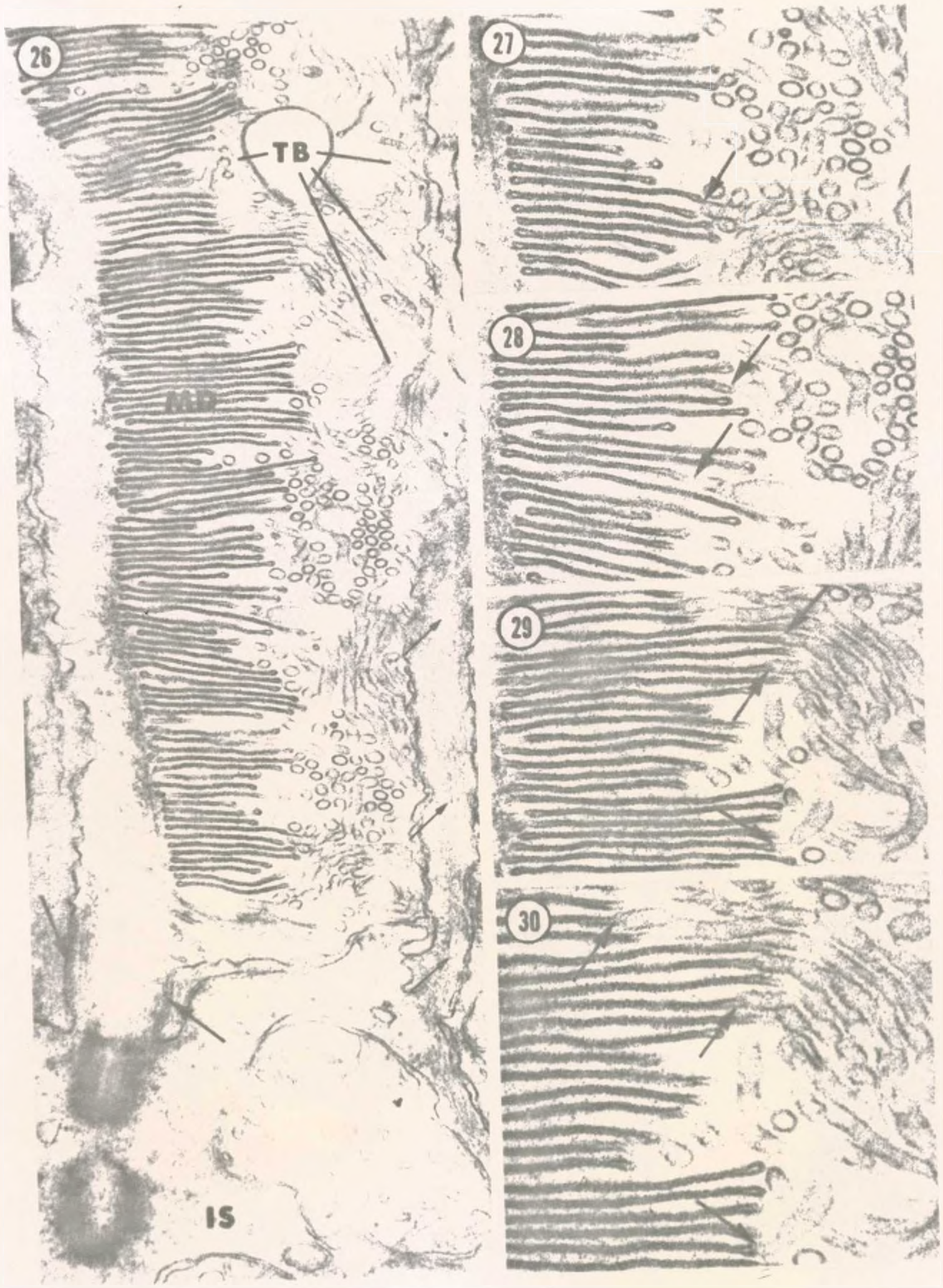


PLATE XIV

Fig. 31.--Mature outer segment (19 days, stage 45). The flattened membranous discs (135 A thick) are parallel to one another and are arranged at right angles to the plasmalemma. The discs now extend across the entire outer segment. Note the pigmented microvilli (MV) which isolate individual photoreceptors. The area within the rectangle is shown in the following figure. 30,000X.

Fig. 32.--Association of plasmalemma and membranous discs (19 days, stage 45). This micrograph illustrates in detail, the basal portion of the outer segment in Figure 31. The membranous discs are continuous with the plasmalemma of the outer segment (arrows). As a result, the "intradisc space" is confluent with the extracellular space. 35,000X.

Fig. 33.--The connecting cilium (21 days, stage 46). The ciliary doublets of the connecting cilium (CL) are illustrated in detail. They are in direct continuity with a basal body (BB) in the cytoplasm of the inner segment. An associated centriole lies beneath the basal body. The extracellular space is observed at the immediate margins of the connecting cilium (arrows). 57,000X.

PLATE XIV

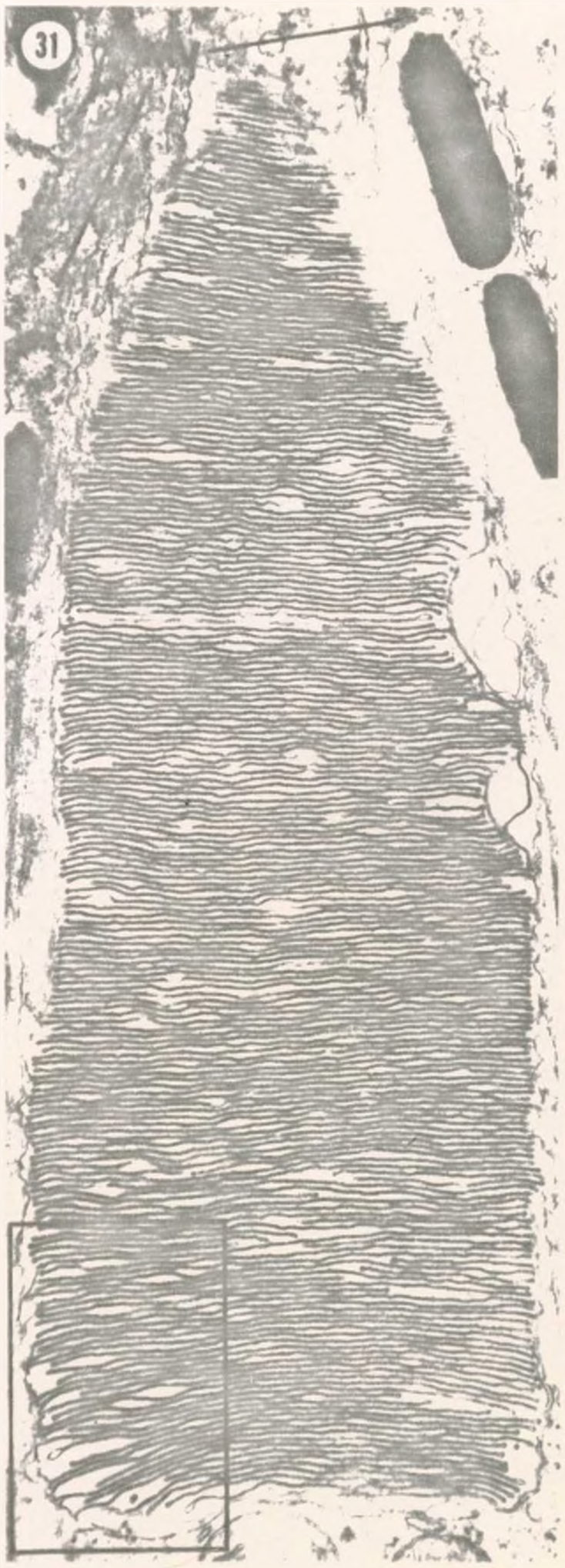


PLATE XV

Figures 34 and 35 illustrate groups of photoreceptor membranous discs within the pigment epithelium.

Fig. 34.--Membranous discs within pigment epithelium (2 days post-hatching). A large segment of membranous discs (MD) are present in the cytoplasm of a pigmented epithelial cell. The basal portion of this cell is highly convoluted (arrows). Note the desmosome (D) between the cells of the pigment epithelium. These form a fenestrated "membrane" known as Verhoeff's membrane. 30,000X.

Fig. 35.--Membranous discs within pigment epithelium (2 days post-hatching). Several groups of membranous discs (arrows) are illustrated among the pigment granules within the apical cytoplasm of a pigmented epithelial cell. 33,000X.

PLATE XV

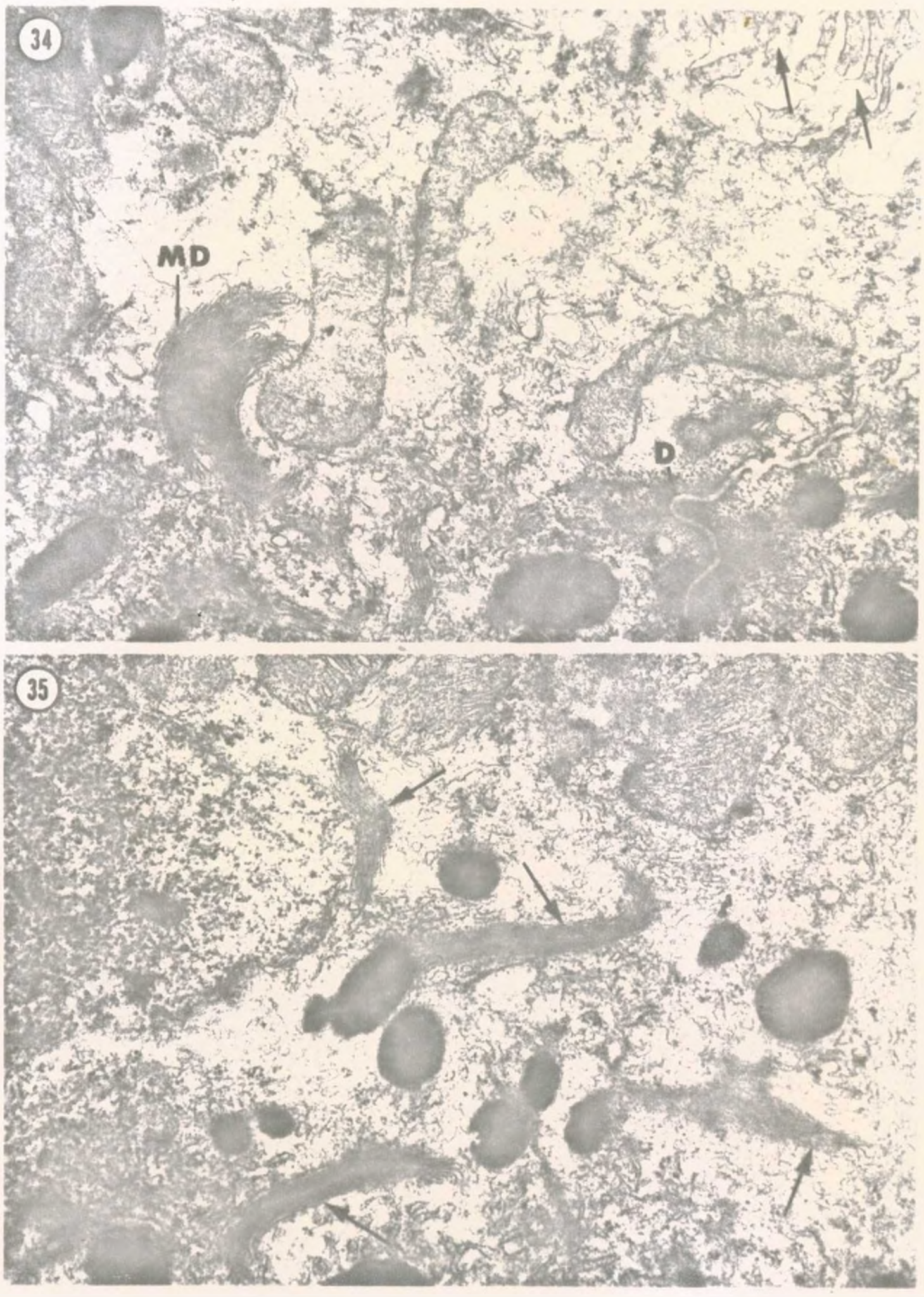


PLATE XVI

Fig. 36.--Developing rod cell (16 days, stage 42). Numerous vesicles of the Golgi apparatus (G) occupy the rod outer fiber which lies between the nucleus (N) and the outer limiting membrane (arrows). Arborizations are developing at the basal portion of the photoreceptors. These represent the synaptic terminal (ST) which extends into the outer plexiform layer (OPL). 8,100X.

Fig. 37.--Developing cone cell (16 days, stage 42). The cell body is almost entirely occupied by the nucleus (N). The developing synaptic terminal (ST) extends toward the vitreous, into the outer plexiform layer (OPL). 17,000X.

PLATE XVI

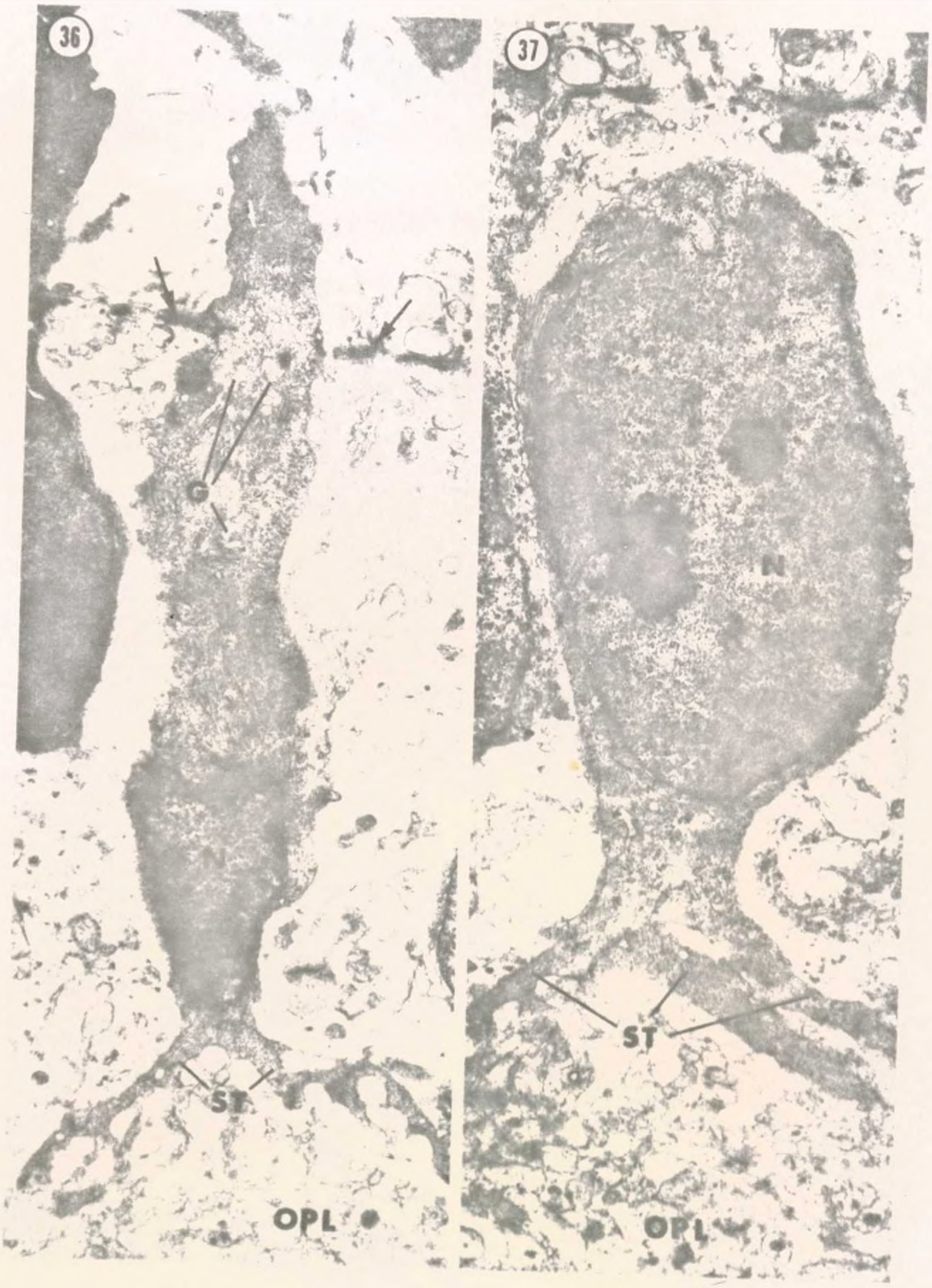


PLATE XVII

Fig. 38.--Microtubules within synaptic terminal (16 days, stage 42). Numerous microtubules (MT) are illustrated in the developing synaptic terminal at the base of the photoreceptor. Synaptic vesicles (SV) are abundant. A forming synaptic ribbon (arrow) is also present in the lower portion of the receptor cell. The nucleus (N) is at the upper left. 11,000X.

Fig. 39.--Synaptic ribbon (16 days, stage 42). This small synaptic terminal possesses a better developed synaptic ribbon (SR) which is surrounded by an array of synaptic vesicles (arrows). 64,000X.

Fig. 40.--Synaptic terminal (16 days, stage 42). The illustrated photoreceptor displays a more extensive synaptic terminal. Developing synaptic ribbons (arrows) with associated synaptic vesicles are present. A few microtubules are also observed within these cytoplasmic processes. The neuropile of the outer plexiform layer (OPL) surrounds the synaptic terminal. 25,000X.

PLATE XVII

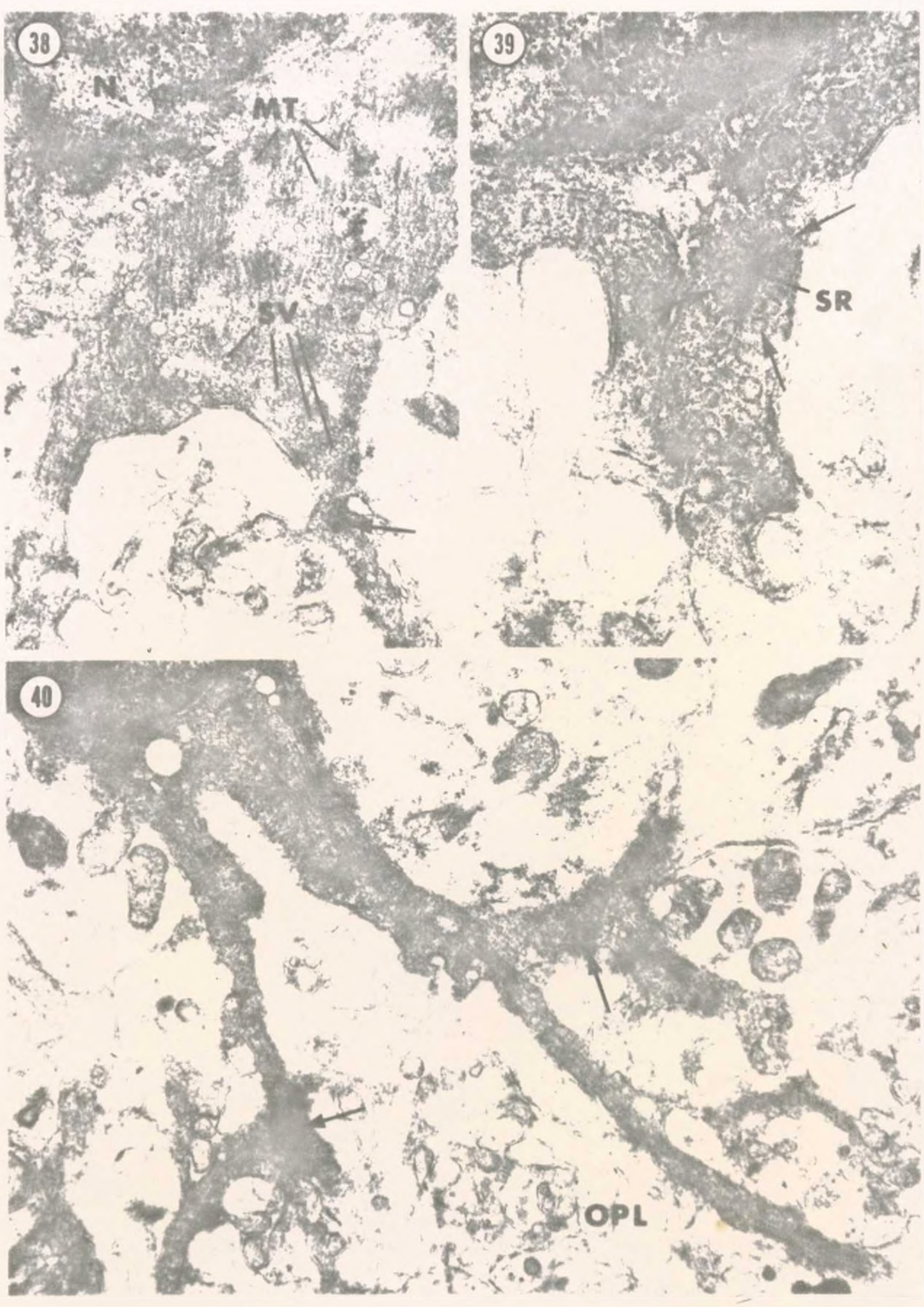


PLATE XVIII

Figures 41-44 illustrate the two types of synaptic arrangements observed in the photoreceptors of the chick retina. These are as follows: (1) the conventional (superficial) synapse and (2) the ribbon (invaginated) synapse.

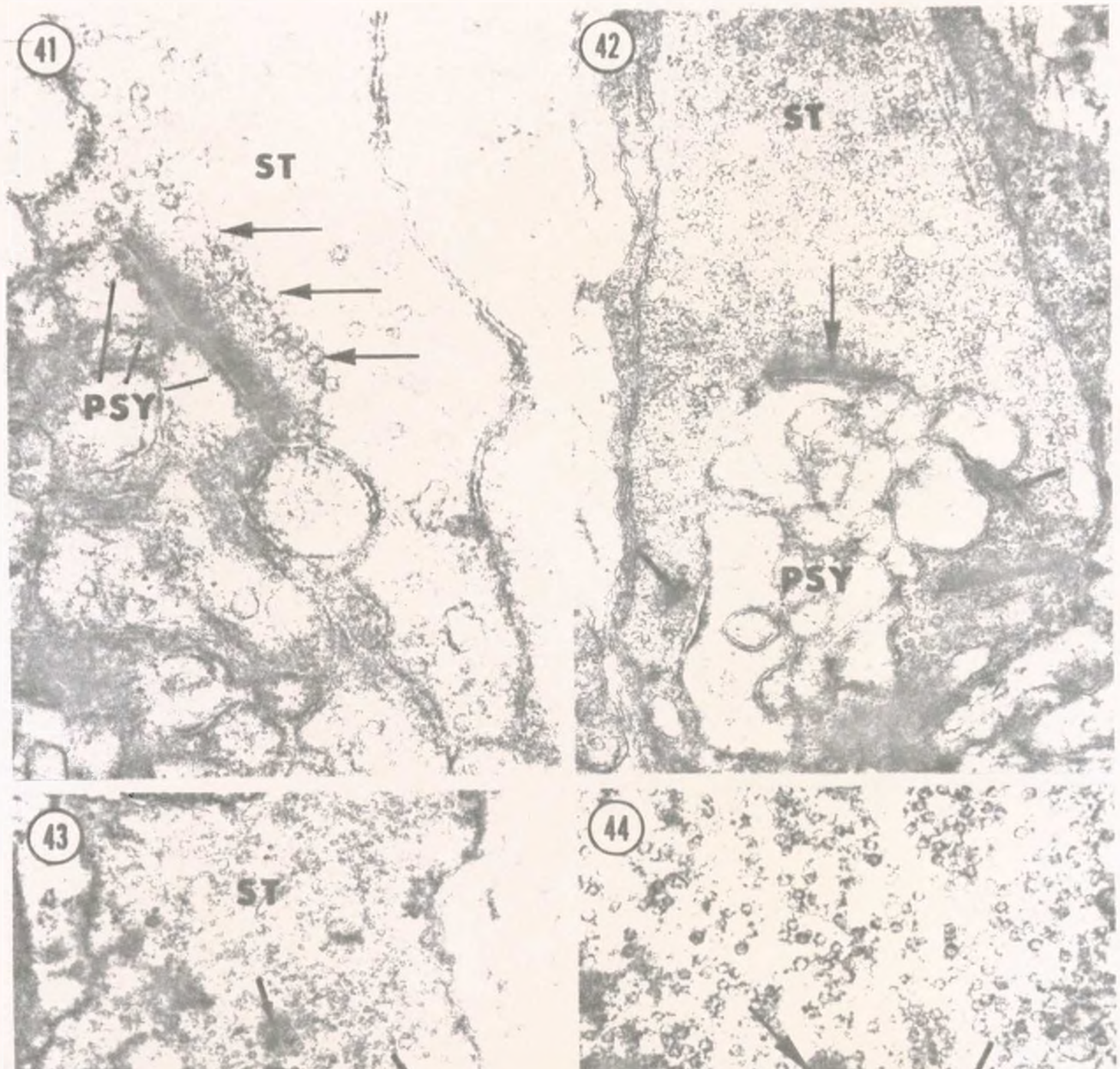
Fig. 41.--The conventional synapse (19 days, stage 45). The plasmalemma of the synaptic terminal (ST) thickens considerably, as do the cell membranes of the postsynaptic elements (PSY). An accumulation of synaptic vesicles (arrows) is associated with the presynaptic membrane. 63,000X.

Fig. 42.--Combined synapses (21 days, stage 46). Both conventional (large arrow) and ribbon synapses (small arrows) are illustrated in this synaptic terminal (ST). Synaptic vesicles virtually fill the entire structure. Numerous postsynaptic neurons (PSY) invaginate into the photoreceptor. 37,000X.

Fig. 43.--Ribbon (invaginated) synapses (19 days, stage 45). Several synaptic ribbons (arrows) with associated synaptic vesicles are present within the synaptic terminal (ST). They are usually perpendicular to, and closely associated with the presynaptic membrane. Postsynaptic elements are abundant within the outer plexiform layer (OPL). 21,000X.

Fig. 44.--Synaptic ribbons (20 days, stage 45). Synaptic ribbons appear as electron dense bands within the presynaptic cytoplasm. They are surrounded by either a cluster of synaptic vesicles (large arrow) or a parallel row of vesicles, one on each side (small arrows). 45,000X.

PLATE XVIII



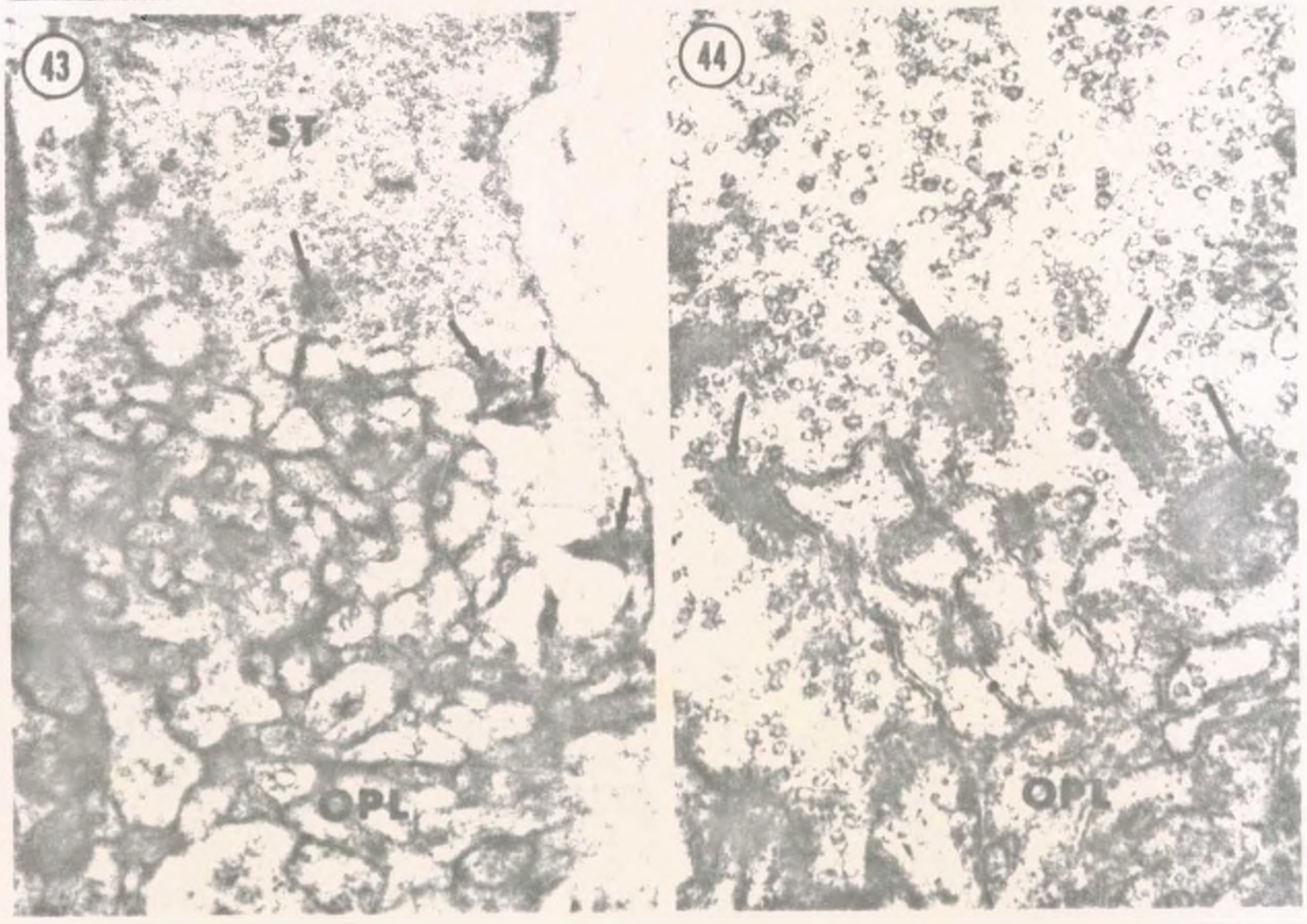


PLATE XIX

Fig. 45.--Synaptic ribbons (19 days, stage 45). This micrograph illustrates the synaptic ribbons (SR) in detail. They are 320 A thick and are surrounded by a parallel array of synaptic vesicles on each side (arrows). The synaptic vesicles average 360 A in diameter. 110,000X.

Fig. 46.--The arciform density (20 days, stage 45). A globular, electron dense body is interposed between the synaptic ribbon and the presynaptic membrane. This structure is known as the arciform density (AD). A "triad" of secondary neurons synapse with the photoreceptor cell. A conventional synapse occurs between the two lateral post-synaptic elements (arrow). The nucleus (N) of the receptor cell is at the upper left. 140,000X.

PLATE XIX

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