The Fine Structure of the Rod-Bipolar Cell Synapse in the Retina of the Albino Rat*‡§

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PLATES 217 TO 222

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ABSTRACT

The fine structure of the rod-bipolar synapse is described and illustrated. Each rod spherule possesses a large, single, oval or elongate mitochondrion approximately 0.5 × 2.0 microns. Surrounding the mitochondrion are elements of agranular endoplasmic reticulum. The bipolar dendrite projects into the lower pole of the spherule and usually terminates in two lobes separated by a cleft. The plasma membranes appear dense and thicker in the region of the synapse. In the rod spherule cytoplasm, contiguous with the plasma membrane is a dense, slightly concave arciform structure, the *rod arciform density*, extending from the base of the bipolar bifid process through the cleft to an equivalent point on the opposite side. Also within the spherule, and external (towards the sclera) to the rod arciform density, is a parallel, dense, thin lamella, the *rod synaptic lamella*. This is approximately 25 mμ in thickness and 400 mμ in width at its widest extent. This half-moon-shaped plate straddles the cleft between the two lobes of the bipolar process. The lamella appears to consist of short regular rodlets or cylinders 5 to 7 mμ in diameter, oriented with their long axes perpendicular to the plane of the lamella. Minute cytoplasmic vesicles found in the cytoplasm of both the rod spherule and the bipolar terminal are most abundant near the rod synaptic lamella.

INTRODUCTION

The organization of the cellular elements which comprise the layers of the vertebrate retina has engaged the interest of many workers for over 100 years. Within this time span, advances in our knowledge can be said to have occurred in rather definitive stages. Numerous early studies correctly presented the form, disposition, and topography of the retinal elements in various animals, but these investigations were unable to demonstrate how the tissue components were arranged within this cellular complex, especially within the plexiform layers (23). With the introduction of silver-impregnation techniques and their application to neurohistological problems in general, much additional information was provided which elucidated, in part, the neuronal connections and synaptic relationships of the retinal cells and which permitted the subdivision of the internuncial cells into distinct types on morphological criteria (21, 20). In this respect, it seems that the use of the silver methods in studies of the retina during this period served to overemphasize the importance of establishing the pathways over which impulses may travel, to the detriment of revealing the cytological features which characterize these cells at their regions of synapse.

Within the last decade, the developments in cytochemistry and electron microscopy have stimulated cytologists to reexamine the structural rela-

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‡ The substance of this report was presented as a demonstration at a meeting of the American Society of Zoologists in Storrs, Connecticut, August 27 and 28, 1956; and it has appeared in abstract (12).

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tionships of the retinal elements. Significant contributions to our understanding of vertebrate photoreceptor synapses have come from the electron microscope studies by Sjöstrand (24, 26–28) of the guinea pig retina and by De Robertis and Franchi (7) of the rabbit retina. The present report extends the electron microscopic observations of the rod-bipolar synapse to the rat, a species previously unexplored by this technique. The results of this study provide new information about the fine structure of this synapse and clarify the relationships of some of the elements within the synaptic terminal of the rod which have not been dealt with in such detail by earlier works.

Materials and Methods

The eyes of adult albino rats of the Sprague-Dawley (Hisaw) strain were used. The animals were killed in the daytime by the administration of illuminating gas or by decapitation. The eyes were removed from their orbits, the frontal segments were cut off, and the lenses were expressed from the humoral cavity by gentle pressure. In most cases, the remaining tissue was immersed in cold (3 to 5°C.) buffered 1 per cent osmium tetroxide (14) at pH 7.4–7.7 for 30 to 60 minutes. In others, a few drops of the cold fixative were placed in the humoral cavity; this darkened the retina within a few minutes and permitted the excision of portions of the retina alone. After fixation, the tissue was washed briefly in distilled water and dehydrated over a period of 1 to 1½ hours in increasing concentrations of methanol (25, 50, 75, 95, and 100 per cent). The material was infiltrated with *n*-butyl methacrylate monomer (3 changes in 90 minutes) and embedded in prepolymerized methacrylate at 60°C. for 18 to 24 hours. Sections were cut with a glass knife on a Servall microtome at a thickness of 25 mμ to 50 mμ and picked up on carbon coated copper grids. The grids were examined without removing the plastic in an RCA electron microscope, model EMU 2e.[1] Micrographs were taken on Kodak contrast lantern slide plates at magnifications of 3,000 to 16,000 and enlarged, photographically, as desired.

Other eyes were fixed in Bensley's acetic-osmic-bichromate mixture (2), which is recommended for the preservation of mitochondria, dehydrated and embedded in paraffin and sectioned at 2 μ. These sections were stained by Altmann's acid-aniline fuchsin method for mitochondria or by a modified Bodian staining method (13) that, as we have found, reveals more clearly the large mitochondria in the retina.

[1] The microscope was fitted with a 250 μ condenser aperture and a 50 μ copper objective aperture housed in an externally centerable objective aperture assembly which was purchased from Canal Instruments Corporation, Bethesda, Maryland.

OBSERVATIONS

Light Microscopy:

The topography of the retina of the albino rat as seen in Fig. 1 conforms to the earlier histological descriptions of the rodent retina by a number of workers (23, 21, 1, 8, 11, 9, 20, 31). The details of structure of the outer plexiform layer visible with the light microscope in preparations for mitochondria are shown in Figs. 2 and 3. All that is visible at such magnifications are extremely large mitochondria in the zone immediately inside (toward the vitreous) the outer (rod) nuclear layer.

Electron Microscopy:

General.—Sections through the outer plexiform layer, where the synapses between the rods and bipolar cells occur, show numerous rod spherules invaginated by the dendritic terminals of the bipolar cells (Figs. 4 and 5). Each rod spherule contains a single, large mitochondrion. The synapse appears as a complex structure at the base of the spherule. As part of this structure the dendritic terminal of the bipolar cell, which penetrates the spherule for a distance of about 1 μ, is narrow at its base and usually ends in two lobes with a deep cleft between (Figs. 13, 14, and 17). A dense, laminar structure, referred to here as the "synaptic lamella," is found in the cytoplasm of the rod spherule close by the synapse. In sections, this synaptic lamella appears either as a single band or occasionally as two dense bands, one on each side of the synapse. Small vesicles fill the remaining cytoplasm of the spherule and appear to be similar to the synaptic vesicles described by others (17, 6, 19, 18, 22, 27, 29, 32, 30).

Rod-Spherule Mitochondrion:

The large, single mitochondrion is probably the most striking component of the spherule. In Figs. 6 and 7, several features of its internal structure are shown. It is usually oval or elongate in profile and measures approximately 0.5 × 2.0 microns. With such dimensions, it is surprising to find that this organelle has not been described before. The mitochondrion is bounded by a double membrane, the inner one of which is in continuity with internal projections (cristae), which in favorable places can be traced across the body of the mitochondrion and appear to make contact with the inner membrane of the opposite side. The cristae seem to be lamellar in form, although they

may appear discontinuous in profile (Fig. 6). In sections parallel to their plane of orientation (Fig. 7), the cristae assume a reticulate pattern.

Endoplasmic Reticulum:

Profiles of moderately large, vesiculate structures are found in the rod spherule, usually close to the mitochondrion (Figs. 6 to 8). These appear to be similar to the agranular endoplasmic reticulum that De Robertis and Franchi (7) described in the rabbit spherule, and they resemble the organelles that Palade (16) found in seminal epithelia and which he also identified as elements of the ER. In occasional sections, these vesiculate profiles form flattened cisterns (Fig. 8). Apparent continuity between the walls of the endoplasmic reticulum and the membranes bounding the synaptic vesicles within the spherule cytoplasm is occasionally observed (Fig. 7,*).

Synaptic Membranes:

The dentritic terminal of the bipolar cell penetrates into a deep invagination at the base of the rod spherule. Along the zone of contact the two synaptic elements are in close apposition, but not in continuity. A space of approximately 20 to 30 mμ separates the plasma membrane of the spherule from that of the dentritic terminal. Usually, but not invariably, both membranes are more electron-opaque in the region of close apposition than around the rest of the spherule and dendrite (Figs. 9, 13, 14, and 17). These closely apposed, often differentiated parts of the respective cell membranes can be regarded as synaptic membranes. The increased density of the synaptic membranes makes them appear slightly thicker than the other cytoplasmic limiting membranes. Similar observations on the synaptic membranes have been reported by De Robertis and Franchi (7) for the rabbit and by Sjöstrand (26–28) for the guinea pig.

Rod Synaptic Lamella:

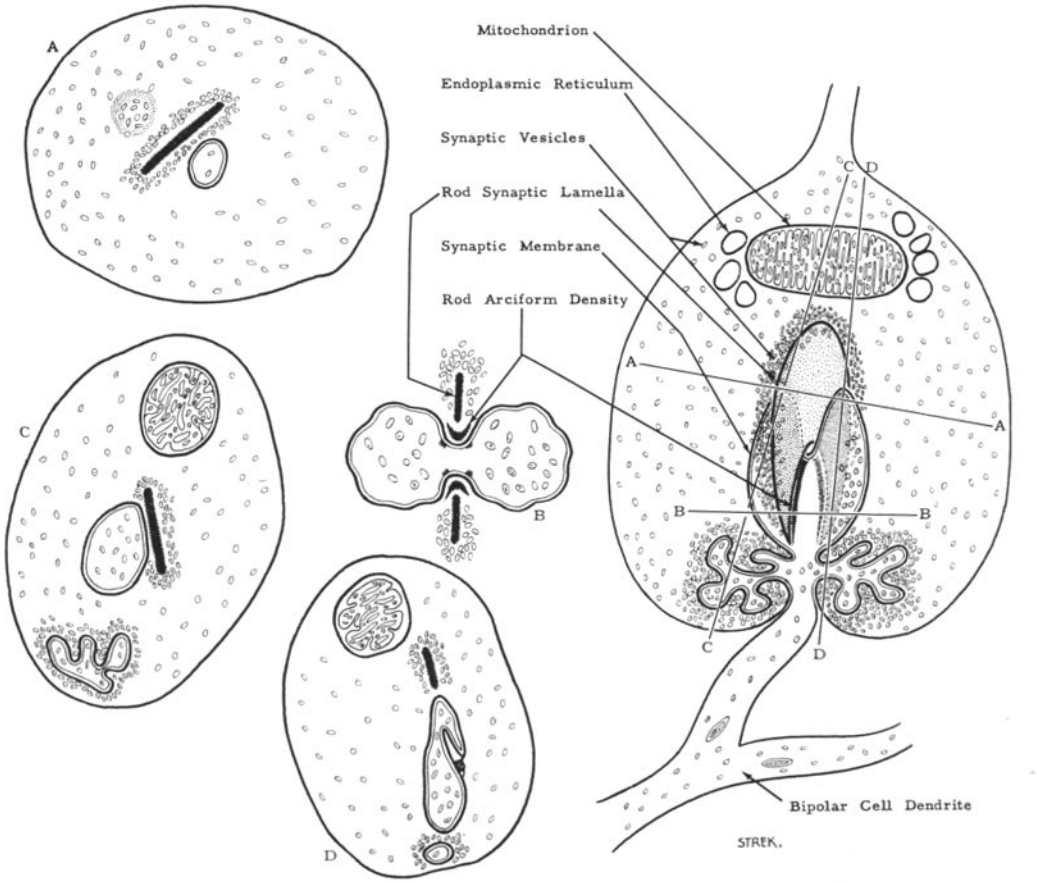
A characteristic component in the rod-spherule cytoplasm is an extremely electron-opaque structure that appears either as a single band or, occasionally, as two bands. Study of many spherules has led to the impression that these are sections of a thin lamellar structure that straddles the invaginating bifid dendrite of the bipolar cell at the synaptic union within the rod spherule. The term *rod synaptic lamella* (RSL) has been applied to identify this structure. Figs. 10 to 16 show a representative series of thin sections through various levels and in different planes of the synaptic junction of the rod and bipolar cells, and they illustrate the extent and form of the rod synaptic lamella. These micrographs form the basis for Text-fig. 1. Thus, in Fig. 10 and Text-fig. 1 *A*, the plane of section passes almost transversely through the middle of the spherule, touches the bifid ending of the enclosed dendrite, and shows the lamella oriented in the same plane as the dendritic terminal furrow. This representation corresponds to the sectioned plane *A-A* in the composite reconstruction of the spherule in Text-fig. 1. In Figs. 11 and 16 and Text-fig. 1 *B*, the transverse section passes nearer the base of the invaginated spherule. The rod synaptic lamella now takes the appearance of two bands and corresponds to the plane of section designated *B-B* in Text-fig. 1. Text-fig. 1 *C* is taken from Fig. 12 and illustrates the appearance of an oblique section to the long axis of the spherule. Here the lamella is seen as a single band next to only one profile of the bifid dendrite of the bipolar cell. The inner[2] extent of the lamella is shown to good advantage in Fig. 13, where it appears to terminate at the region just proximal to the point where the dendrite of the bipolar cell forms its bifid process. This inner extent of the lamella is also shown at the right of Fig. 14. The distance to which the lamella protrudes within the spherule cytoplasm can be ascertained from Figs. 13 and 14.

The rod synaptic lamella appears to be half-moon-shaped. It is approximately 25 mμ in thickness and approximately 400 mμ in width in the region of the maximal expansion of the crescent. At high magnifications, the lamella appears to consist of short, regular dense rodlets or filaments approximately 5 to 7 mμ in diameter, disposed with their long axes perpendicular to the plane of the lamella. Separating adjacent dense rodlets are spaces of approximately the same dimensions (Figs. 15 and 18).

Rod Arciform Density:

In the cytoplasm of the rod spherule, between the synaptic lamella and the membranes that constitute the rod spherule–bipolar cell synaptic junction, is a thin, linear, arciform aggregation of dense material that extends from the base of the

[2] In describing the relationship of elements within retinal structures, the term "inner" designates the position of such elements nearer the vitreous, whereas "outer" indicates a location nearer the sclera.

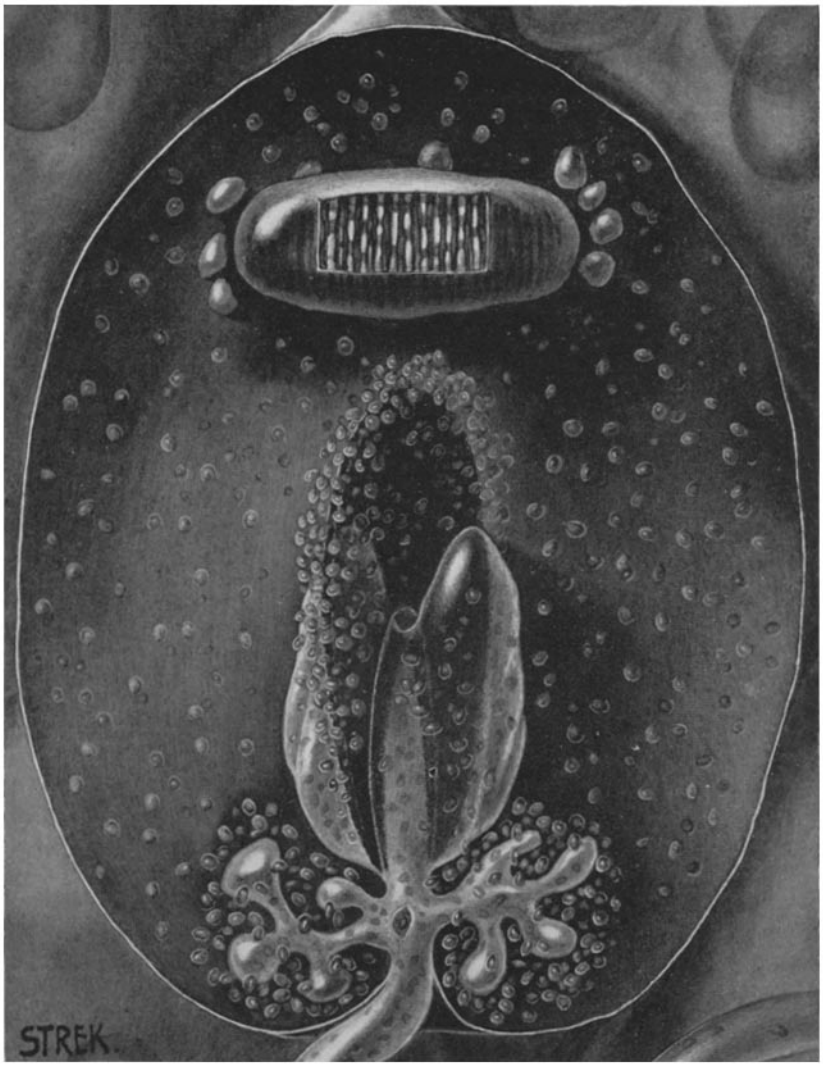


TEXT-FIG. 1. These diagrammatic views were compiled from a series of electron micrographs (Figs. 6 to 16), and are regarded as representing the structural organization of the rod spherule–bipolar cell synapse. *A* is the profile which would be obtained if the composite three-dimensional diagram at the right were sectioned through plane *A-A*. Profiles *B*, *C*, and *D* would be the result of sections through planes *B-B*, *C-C*, and *D-D*, respectively. (This composite three-dimensional diagram, as well as that in Text-fig. 2, is not drawn strictly to scale. In both, the proportions of the dendrite-synaptic lamella component are slightly enlarged when compared to the indicated size of the mitochondrion.)

bulbous process formed by the dendritic invagination of the bipolar cell through the cleft between its two lobes to an equivalent point on the opposite side (see Text-fig. 1). Thus, its course appears to follow that of the synaptic lamella; and, like the lamella, it is entirely contained within the spherule cytoplasm. This structure is here designated the *rod arciform density* (RAD) (Figs. 8, 11, 15, and 16). It is a dense, narrow band with two distinct curvatures: one along its long axis faces the synapse proper; the other along its short axis faces the lamella. The RAD is approximately 50 mμ in width between the margins of the concavity and approximately 20 mμ at its greatest thickness midway between the tips of the crescent (Fig. 15). An interval of 10 mμ intervenes between the rod-spherule plasma membrane and the arciform density.

Synaptic Vesicles:

Numerous small (25 to 50 mμ,) membrane-bounded vesicles are found in the rod-spherule cytoplasm. The vesicles appear most numerous immediately next to the rod synaptic lamella (Figs. 7 to 14, 16). In favorable sections, the membranes bounding the vesicles appear to be in continuity with the membranous investment of the endoplasmic reticulum (Fig. 7). Similarly, some of the bounding membranes of the vesicles can be observed to be in continuity with the spherule



TEXT-FIG. 2. A composite diagram of the rod spherule–bipolar cell synapse expressed as a halftone drawing, to illustrate some of the three-dimensional relationships which have been derived from the examination of many electron micrographs.

plasma membrane (Fig. 17), thereby rendering the vesicles extensions of the synaptic space. Contrary to the observations of De Robertis and Franchi (7), synaptic vesicles are consistently seen in the cytoplasm of the synaptic terminal of the bipolar member of the rod-bipolar synapse (Figs. 5, 7, 8 to 17).

Reconstruction of the Rod Spherule:

The present conception of the three-dimensional appearance of the rod spherule and its constituents is presented in Text-fig. 2. The mitochondrion with its surrounding elements of endoplasmic reticulum occupies that part of the spherule closest to the nucleus of the rod cell. The rod-bipolar synapse and its associated structures, the synaptic lamella and the arciform density, are found in the inner part of the spherule. Synaptic vesicles are distributed throughout the cytoplasm, but tend to be concentrated in the region of the synapse and next to the rod synaptic lamella. The lateral outfoldings of the dendritic membrane figured at the base of the spherule in Text-fig. 2 do not appear to be a constant feature of the synapse;

they are not found in some cases, in which their absence cannot be explained by an unfavorable orientation of the sections.

There is appreciable variation in the form and disposition of the components, in the same retina and in retinas from similar animals. These differences suggest that these are dynamic structures which undergo continuous changes in life.

DISCUSSION

Many of the constituents of this synaptic connection appear to be common to other mammalian retinas as described by recent investigations. For example, in sections of the rod terminal spherule, profiles of a dense bar or rod-like structure have been recognized in the guinea pig by Sjöstrand (22, 23, 25) and in the rabbit by De Robertis and Franchi (7). In the rat, this rodlet appears to be a sectioned part of an extremely electron-opaque lamella, designated the rod synaptic lamella; its disposition in the spherule and its relation to the synapse have been characterized. Contrary to the conclusions of De Robertis and Franchi and of Sjöstrand, the lamella appears to be confined wholly within the spherule cytoplasm and to be neither an extension of the synaptic membranes nor in contact with them.[3]

Profiles of similarly dense structures are found as constituents of the cone spherules of the rat (unpublished observations of the author). The three-dimensional pattern of the cone synaptic lamella is thought to be similar to that found in the rod spherule although reconstructions have not yet been made. In addition, dense bands, apparently profiles of lamellar structures, have been identified in sections of the rod spherules of the opossum and woodchuck, as well as in the cone spherules of the woodchuck and grey squirrel.

A new cytoplasmic structure that follows the concave rim of the rod synaptic lamella in the spherule cytoplasm has been described and designated as the rod arciform density.

Specializations of the type described above have not been observed at other sensory bipolar synapses (4, 5, 29, 30, 32). They appear to be peculiar to the retina. What significance they have for the transmission of impulses in this particular sense organ remains to be determined.

Another constituent of the spherule cytoplasm is the large, single mitochondrion. Of all the species that have been investigated to date, this organelle is found only in rat and mouse rod spherules. Its structure seems to be similar in general to mitochondria of non-neural tissues (15, 25); however, in contrast to the usual small mitochondria of neural tissue, this spherule mitochondrion appears to be unique in its large size and its topographical relationship in the rod-bipolar synapse. Since such a mitochondrion has not been found in other predominantly rod retinas, namely guinea pig (24) and opossum, its functional significance in the spherules of the rat and mouse retinas remains to be elucidated.

Synaptic vesicles are found in great numbers in the cytoplasm of the rod spherule and to a lesser extent in the invaginated bipolar dendrite. De Robertis and Franchi (7) reported that in the rabbit the synaptic vesicles were restricted to the spherule cytoplasm. Apparently, there is species variation, for, in some species other than the rat, I likewise have found vesicles limited to the spherule. For example, in the opossum, the vesicles are also present in the dendrite of the bipolar cell, resembling in this connection the situation in the rat; whereas, in the woodchuck, few if any synaptic vesicles were found in the invaginating dendrite.[4] Furthermore, I have found that differences in the tonicity of the fixative as well as the length of time of fixation seem to be important factors in the preservation of synaptic vesicles *in situ*. It could very well be that closer attention will have to be directed to the determination of optimal conditions of fixation before the evaluation of retinal structures from a comparative species approach can be made with consistent reproducibility.

Many investigators have pointed out the possible correlations between the information obtained from biophysical investigations of neuromuscular transmission and the morphological observations of various synaptic junctions reported by electron microscopists (10). The biophysical evidence indi-

[3] After this manuscript was sent to press, a paper by Nina Carasso (Comptes Rendus de l'academie des Sciences, 1957, **245**, 216) on the fine structure of the photoreceptor cell synapses in the retina of the toad tadpole was called to my attention. The author describes and illustrates single and multiple synaptic lamellae in the cytoplasm of the rod spherule. She observes further that the lamellae are never found in continuity with the spherule plasma membrane.

[4] Carasso (footnote 3) also noted, that in her material synaptic vesicles are not found in the bipolar dendrite comparable to those observed in the spherule cytoplasm.

cates that acetylcholine released at nerve terminals occurs in multimolecular units. The units appear to be held in a bound state intracellularly. Although the occurrence of these units can be varied widely by experimental means, the size of each parcel remains relatively constant (3). The synaptic vesicles are the structural elements that have been suggested as the possible repositories of acetylcholine at nerve terminals (6, 22, 5). They occur abundantly at nerve endings; and frequently it can be demonstrated that their limiting membranes are in continuity with the synaptic membranes, suggesting the release of the vesicle contents into the synaptic space. The evidence from this study supports the hypothesis that the synaptic vesicles may be the structural means of achieving acetylcholine transport at the rod-bipolar synapse.

The predominant localization of the synaptic vesicles along the lamella raises the possibility that the lamella may be actually concerned with impulse transmission rather than being an accessory structure of this complex synapse. It is possible that the lamella influences the intracellular potential within the spherule and thereby causes the seemingly preferential aggregation of vesicles at this site.

In support of findings by De Robertis and Franchi (7) in the rabbit, synapses have also been observed in the rat retina in which the dendrite of the bipolar cell penetrates directly into an expanded portion of the rod cell body. All the synaptic components found in the rod spherule are also contained in this paranuclear synapse. It appears likely that irregularities in the differentiation of the retinal layers may preclude the formation of a spherule and result in a synaptic connection closer to the rod nucleus. In this respect, it is of interest to recall that Ramón y Cajal (21) illustrated an occasional intimate connection of the spherule with the rod cell body at the outermost extent of the outer plexiform layer (Plate V, Fig. 2, by Ramón y Cajal (21); and also reproduced as Fig. 30 by Polyak (20)).

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Explanation of Plates

BCN, Bipolar cell nucleus
BDP, Bipolar dendritic process
ER, Endoplasmic reticulum
M, Mitochondrion
OPL, Outer plexiform layer
PM, Plasma membrane
RAD, Rod arciform density
RCN, Rod cell nucleus
RSL, Rod synaptic lamella
SYN, Rod-bipolar cell synapse
SV, Synaptic vesicles

Unless otherwise indicated, the solid bar on each figure represents 1 micron.

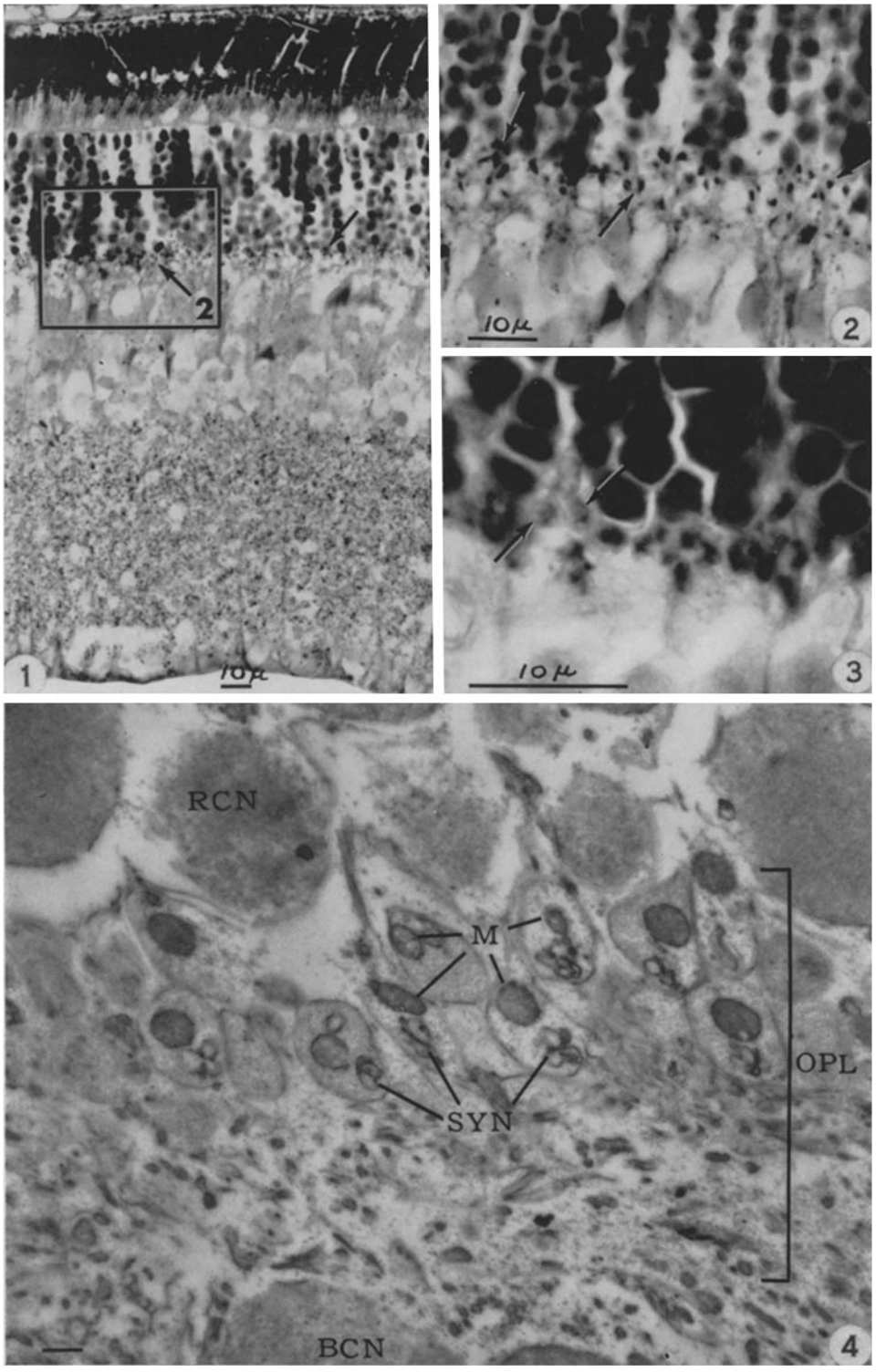
Plate 217

Fig. 1. A section of an albino rat's retina fixed in acetic-osmic-bichromate and stained with Bodian's protargol method, to show the appearance of the various layers. Darkly stained bodies located in the cuboidal epithelium ("pigment" layer) and in the outer plexiform region (arrows) are mitochondria. × 450.

Fig. 2. An enlargement of the delimited rectangular area in Fig. 1, showing the mitochondria in the outer plexiform layer (arrows) to better advantage. × 1000.

Fig. 3. A thick (*ca.* 1μ) section of rat's retina, fixed in buffered osmium tetroxide, embedded in methacrylate, and stained lightly with hematoxylin to show some of the constituents of the rod terminal spherule. Osmiophilic bodies in the center of two spherules (arrows) are mitochondria. Below each mitochondrion a density corresponding to the rod spherule–bipolar cell synaptic connection can be identified. × 2300.

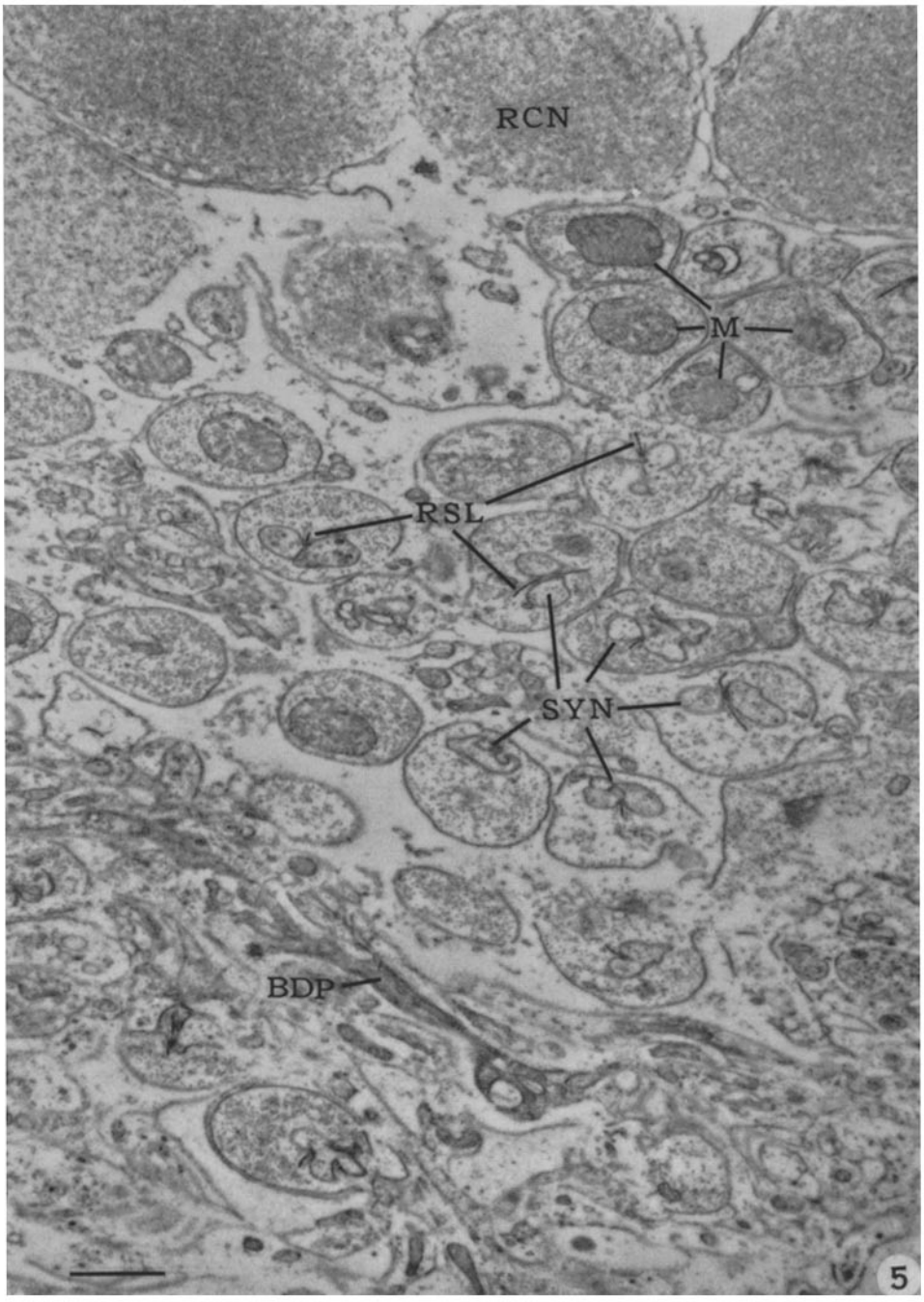
Fig. 4. An electron micrograph of a section of the block from which Fig. 3 was taken shows further the contents of the rod terminal spherule. These are: mitochondria (*M*), the rod-bipolar cell synapse (*SYN*), rod cell nuclei (*RCN*), and bipolar cell nuclei (*BCN*). The approximate thickness of the outer plexiform layer (*OPL*) is indicated. × 6000.



(Ladman: Rod-bipolar cell synapse)

PLATE 218

FIG. 5. An electron micrograph of the outer plexiform layer. Rod cell nuclei are at the upper margin of the picture; numerous rod terminal spherules, each containing a single mitochondrion and a rather pleomorphic, although usually bifid, invaginating bipolar dendritic process, appear in various planes of section below. Associated with the synapse is a dense lamellar structure, the rod synaptic lamella, which in many planes of section gives the impression of being bar-shaped. A portion of a bipolar dendritic process courses through the field below. × 13,000.

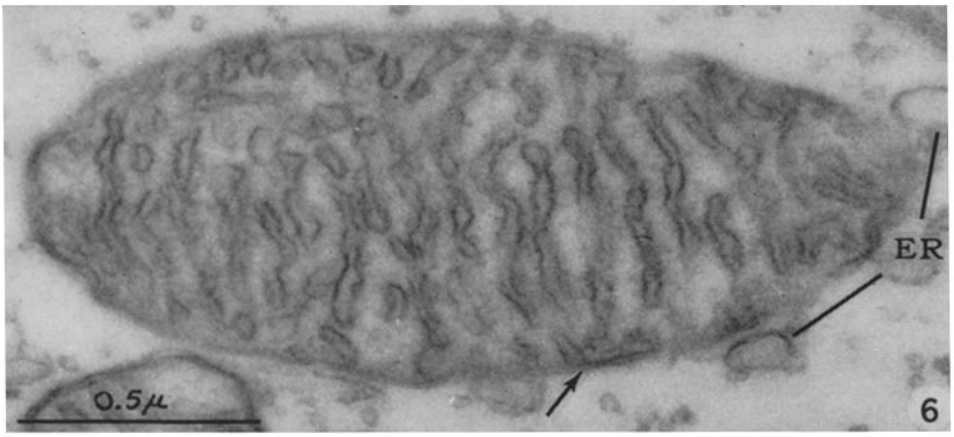


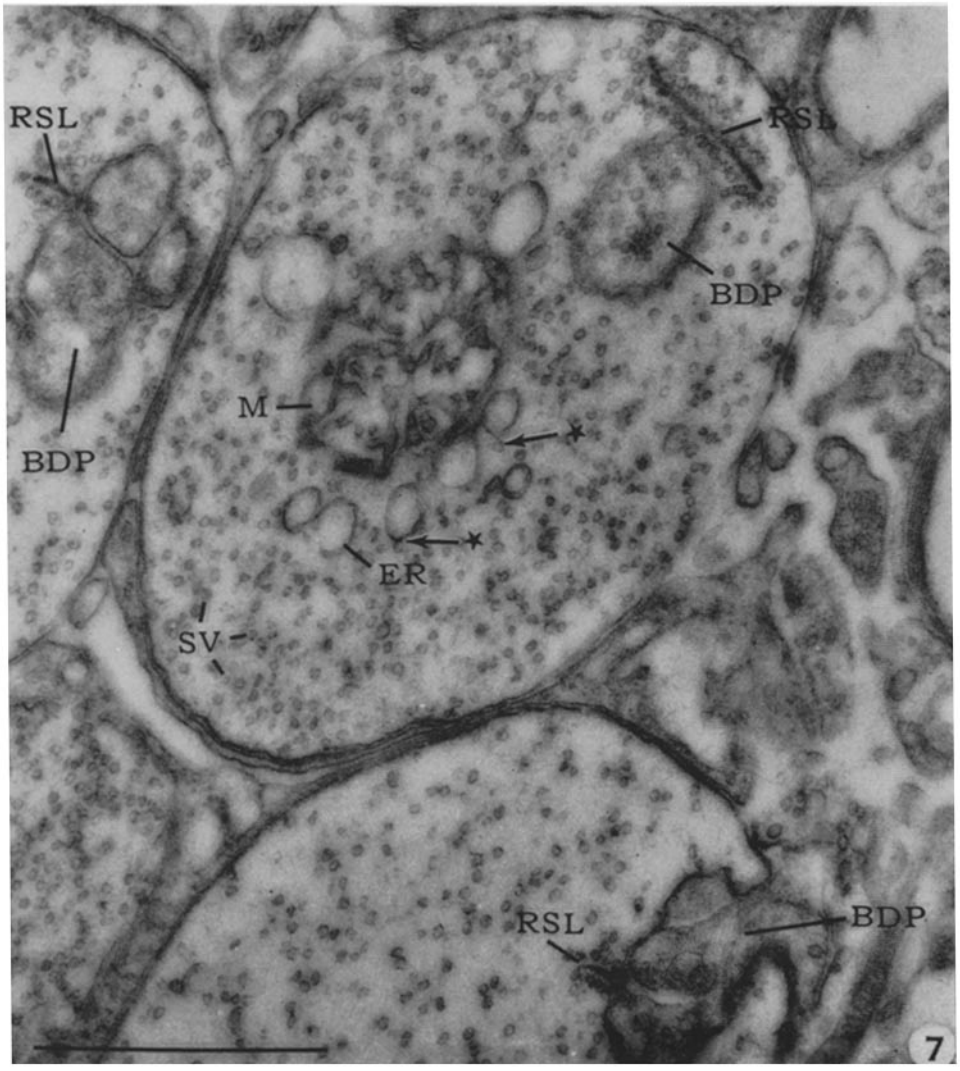
(Ladman: Rod-bipolar cell synapse)

PLATE 219

FIG. 6. A higher magnification of a rod spherule mitochondrion, showing the irregularly tortuous pairs of membranes of the cristae that appear to be interrupted in some places and that are in continuity (arrow) with the inner of the two membranes that characteristically bound this organelle. This sectioned mitochondrion measures approximately $0.7 \times 1.8\ \mu$. Around the mitochondrion, a few vesicular profiles of agranular endoplasmic reticulum are seen. $\times$ 65,000.

FIG. 7. A section through a whole rod spherule and portions of two others, showing a mitochondrion in which the plane of section coincides with that of the crista. The latter, seen in full-faced view, appears to be a tortuous reticulum. Surrounding the mitochondrion are seven large profiles of the agranular endoplasmic reticulum (*ER*). Small synaptic vesicles (*SV*) fill the cytoplasm, and occasionally there appears to be a continuity of the membrane bounding the synaptic vesicle with that of portions of the endoplasmic reticulum (*). At the upper right, the bipolar dendritic terminal has been partially sectioned; and adjacent to it is a portion of the rod synaptic lamella surrounded by aggregations of synaptic vesicles. At the left, the bipolar dendritic process has been sectioned at a right angle to the long axis of the invagination; the relationship of the dendrite to the rod synaptic lamella within the substance of the spherule is shown. At the lower right, a third bipolar process is seen penetrating the rod spherule; and a portion of the rod synaptic lamella can also be detected here. Between the spherules, the small cytoplasmic extensions of other cellular elements can be seen. These are probably either the communicating cytoplasmic strands of rod cells between the myoid region and the spherule or supporting cell expansions of a glial nature. $\times$ 39,000.





(Ladman: Rod-bipolar cell synapse)

PLATE 220

FIG. 8. A region of the rod spherule showing a mitochondrion with its internal structure, capped at the upper left by a portion of a slightly distended cisterna of the agranular endoplasmic reticulum which has been sectioned longitudinally. At the left is a part of the bipolar dendritic process and its associated rod synaptic lamella, sectioned at a right angle to its long axis and projecting into the rod spherule cytoplasm at a right angle away from the bipolar process. Between the lamella and the bipolar process is a density, the rod arciform density (see Fig. 15). Synaptic vesicles occur in the cytoplasm of both the rod spherule and the bipolar process. × 40,000.

FIG. 9. At the base of the spherule, the invagination of the bipolar dendritic process is often represented in section by a number of membrane-bounded projections containing synaptic vesicles The spherule membrane (arrows) which surrounds the bipolar process appears to have a greater density than the non-synaptic surface. A portion of the rod synaptic lamella (*RSL*) with adjacent synaptic vesicles is at the top of the field. × 62,000.

FIGS. 10 to 14 are selected fields containing the rod synaptic lamella that illustrate the topography of the lamella and its relationship to the rod-bipolar synaptic junction, as seen in various planes of section (see Text-fig. 1).

FIG. 10. A horizontal section through the distal projection of the bifid bipolar process, showing the position of the lamella between the two bipolar projections. Numerous synaptic vesicles surround the lamella. × 36,000.

FIG. 11. At a level nearer the base of the spherule, the cytoplasm of the two bipolar projections becomes continuous. The lamella that in Fig. 10 appeared as a single band is now visualized as two separate bands on either side of the isthmus of the bipolar process. Note the increased density of the spherule cytoplasm at the isthmic region. The apparent increase in the density of the spherule cytoplasm is due to the fact that part of the arciform density is included in the thickness of the section (also see Fig. 15). × 30,000.

FIG. 12. The plane of section has passed obliquely to the long axis of the synapse, and here the rod synaptic lamella is shown in relation to one of the bipolar processes. To the left is a portion of the spherule mitochondrion. × 36,000.

FIG. 13. Occasionally, the spherule is favorably sectioned and shows the position of the lamella in relation to the stem of the bipolar dendritic process nearer to its bifid processes. The lamella appears to extend to the point where the dendrite begins to enlarge to form the bifid processes (*). × 31,000.

FIG. 14. In adjacent spherules, portions of the lamella have been cut in a plane which is rotated slightly from that represented in Fig. 13. Thus, at the right, both the thickness and extent of the lamella are shown in relation to a portion of the bipolar dendrite. The curvature of the lamella is also evident. At the top of the arch, a few vesicles of agranular endoplasmic reticulum are seen; and the membranes of some of them appear to be continuous with some of the synaptic vesicles, which are numerous in this region and surround the lamella. At the left, only a small portion of the lamella, which is thought to be similarly disposed as that shown at the right, is seen. In this plane of section, the bipolar dendrite at the left consists of a number of membrane-bounded projections, two of which appear to be oriented more longitudinally. × 31,000.

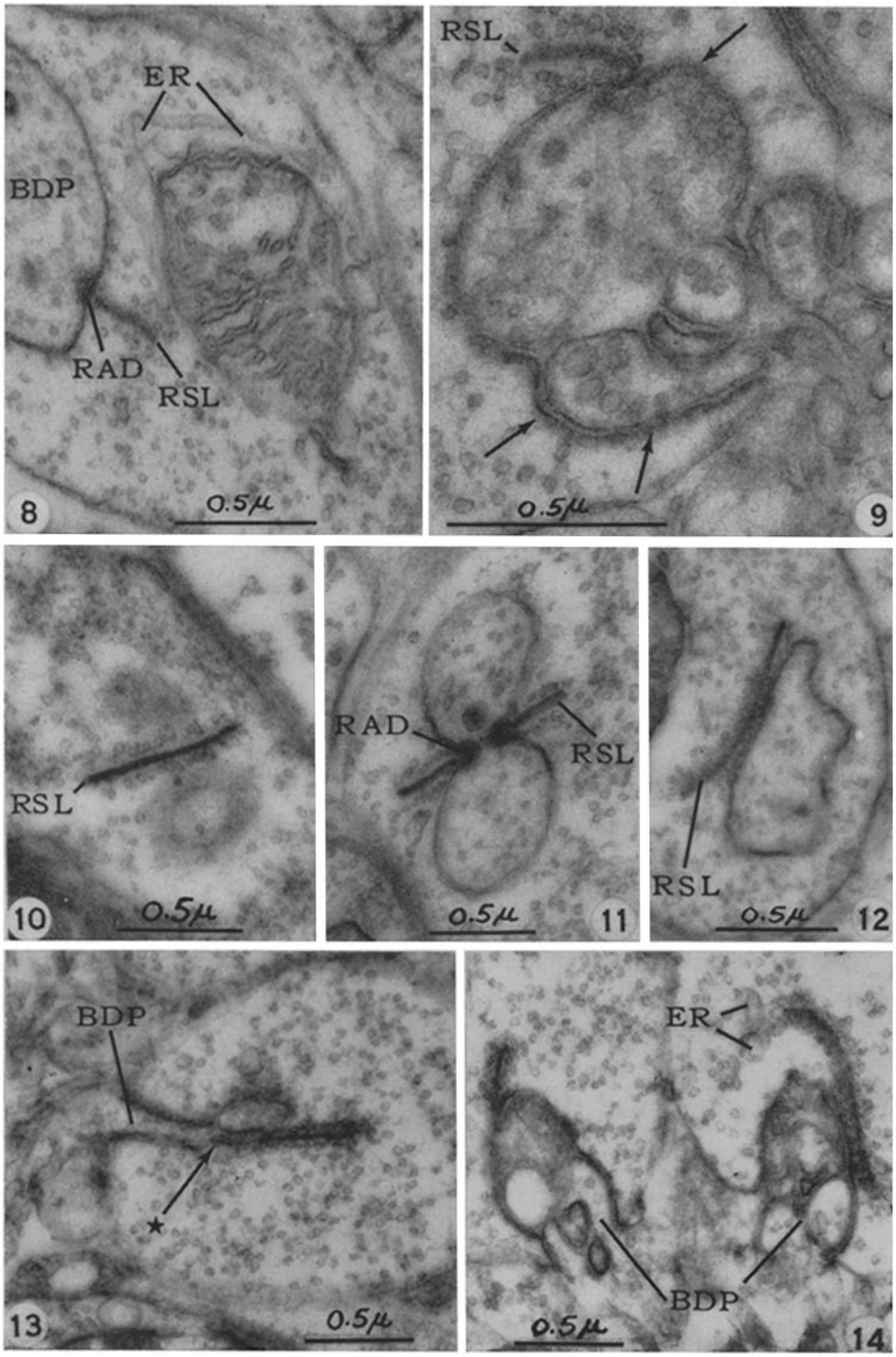


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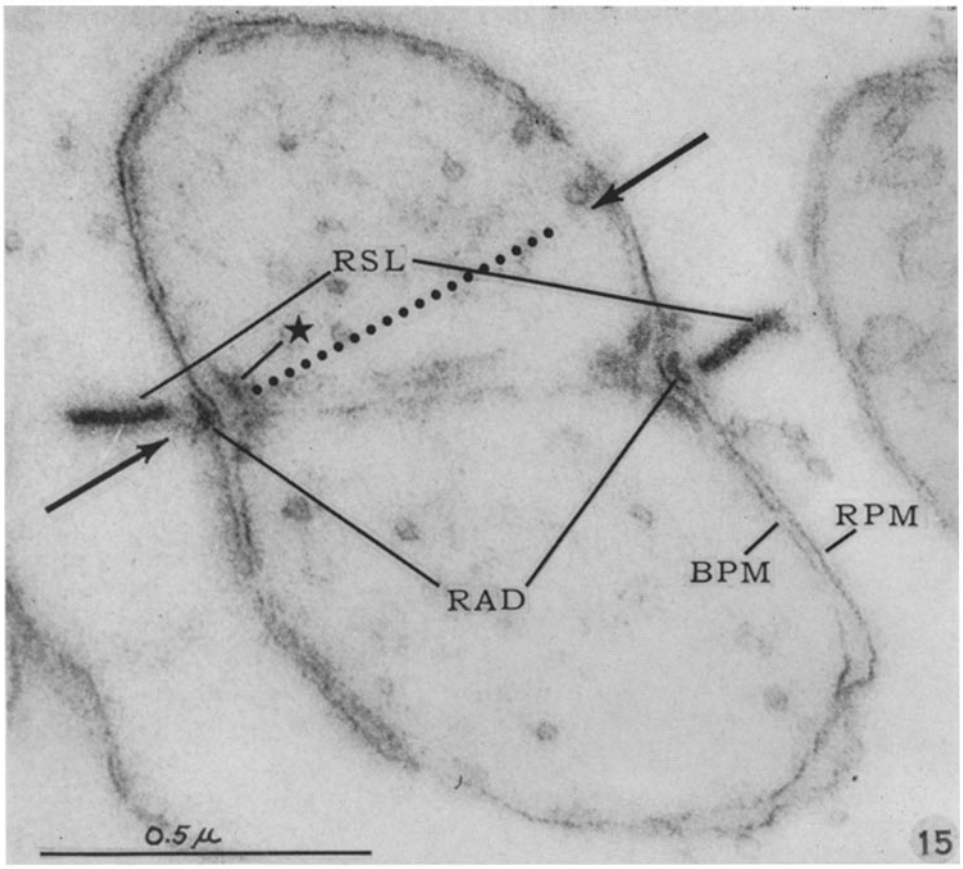
FIG. 15. A horizontal section of the synaptic junction from a retina fixed in a slightly hypotonic osmium tetroxide solution, to show the disposition of the rod synaptic lamella at right angles to the synapse, and the rod arciform density positioned between the lamella and the synaptic membranes. The lamella appears to be composed of dense rodlets or cylinders approximately 25 mμ in length and 5 mμ in diameter. The rodlets, tightly packed parallel to one another, are disposed with their long axes perpendicular to the plane of the lamella. Adjacent rodlets appear to be separated by a space of approximately 5 mμ. These relationships are also shown in Fig. 18.

The rod arciform density is a dense, concave structure, the concavity of which faces the lamella. Its course appears to follow that of the lamella (see Fig. 16), and like the lamella it is contained wholly within the rod spherule cytoplasm. Between the margins of the concavity, it measures approximately 50 mμ, and at its greatest thickness midway between the tips of the crescent it is approximately 20 mμ. The plasma membrane of the rod spherule (*RPM*) is separated from the arciform density by an interval of about 10 mμ. The bipolar plasma membrane (*BPM*) is less well preserved here; but, in the region opposite the lamella and arciform densities (*), the plasma membrane of the bipolar cell shows a corresponding increase in density. (This can also be observed in Figs. 8, 11, and the synapse at the right in Fig. 16.) The heavy interrupted line between the arrows represents the plane of section which is figured in the synapse at the left of Fig. 16. × 87,000.

FIG. 16. The plane of section of the synapse at the left is thought to be that shown by the interrupted line between the arrows in Fig. 15. In the upper part of the region there are a large number of synaptic vesicles. It can be noted in Fig. 15 that synaptic vesicles are few in number and indistinct in outline. It is assumed that most of them disintegrated during fixation because of the low tonicity of the fixative. The dark, broader band beneath the aggregation of vesicles is the rod arciform density (*RAD*). Between it and the next dark line is another space which in places lacks clear definition. This latter space is the synaptic space between respective rod and bipolar plasma membranes. Finally, a broader, somewhat less dense strip is beneath this space. The density of this strip corresponds to the density of the bipolar plasma membrane (*) at the point where the interrupted line intersects that membrane internal to the rod arciform density shown in Fig. 15 at *. The density of the bipolar plasma membrane is also shown in the synapse at the right. A similar picture would be obtained if the synapse at the right were sectioned in a plane represented by the black line. × 59,000.



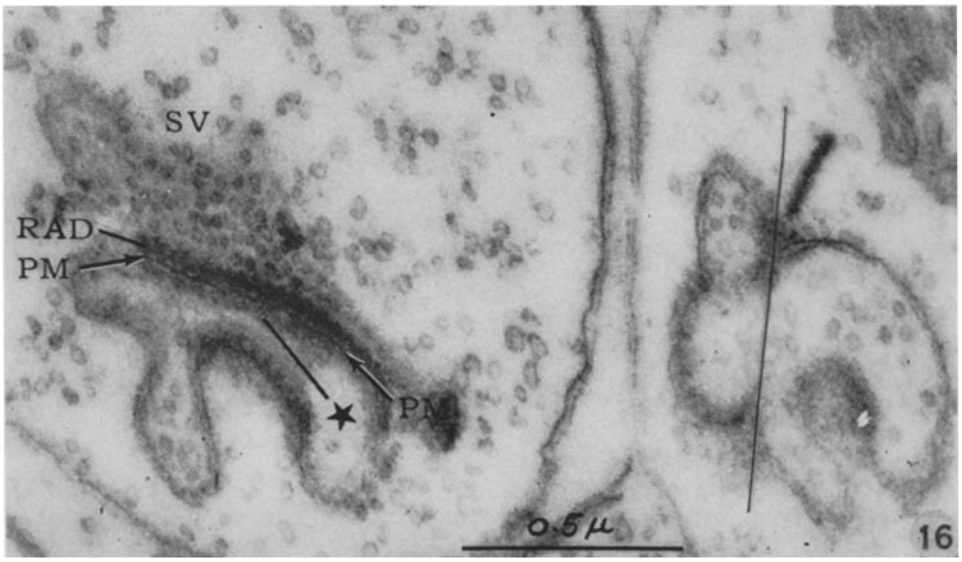


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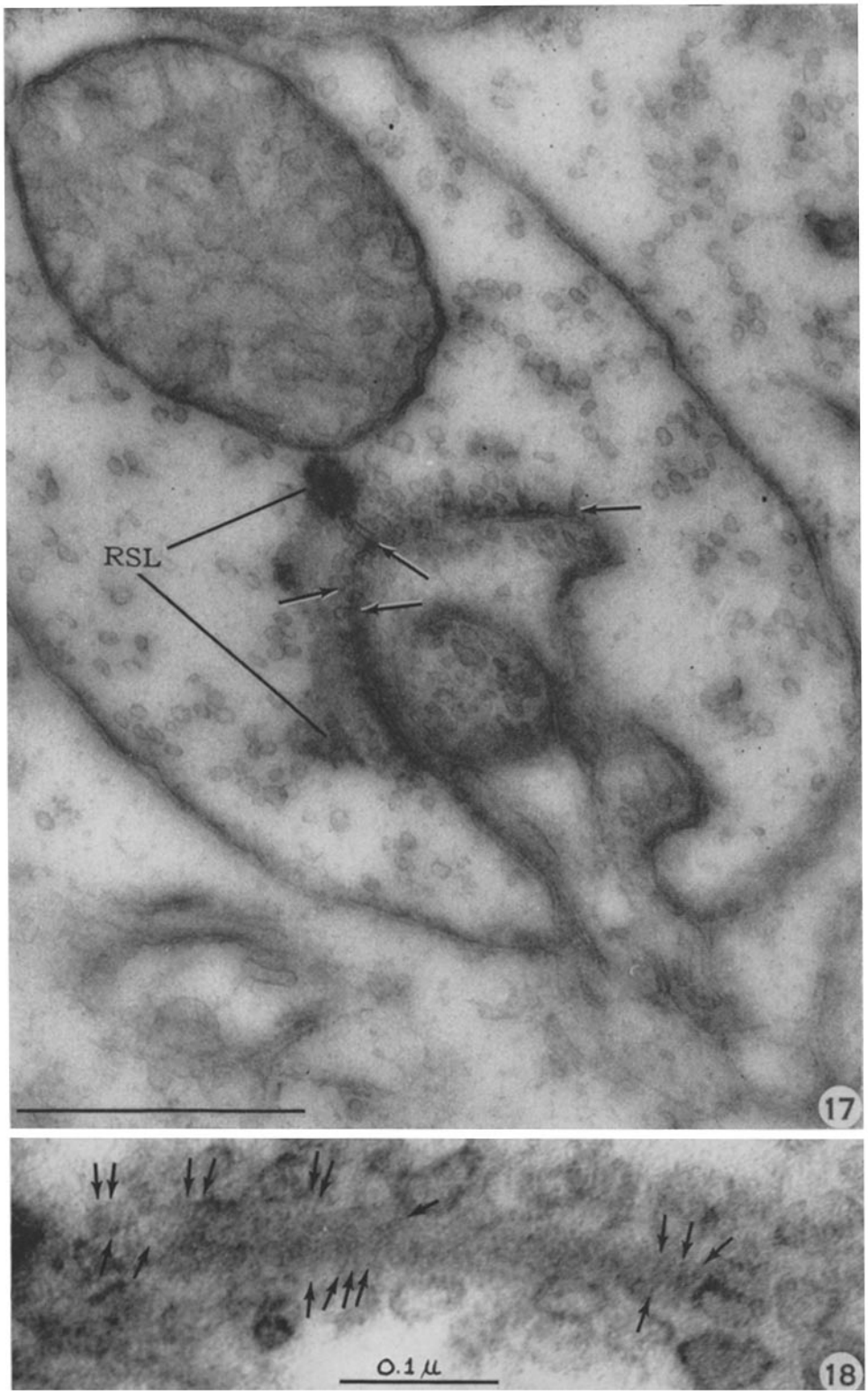
FIG. 17. A section in the long axis of the rod spherule that passes through the bipolar dendritic invagination. At the upper left is the spherule's mitochondrion sectioned in a plane perpendicular to its long axis. The double membrane surrounding the mitochondrion is quite evident. The contiguous plasma membranes of the spherule and bipolar dendrite at the synaptic region are very dense. At the upper border of the invagination, many synaptic vesicles of the rod spherule are closely grouped about the synaptic membranes; occasionally, the membranes of the vesicles appear to be in contact with the plasma membrane (arrows), providing a continuity of the interior of the vesicles with the space which separates the synaptic membranes. A very small tangential portion of the rod synaptic lamella can also be identified. × 43,000.

FIG. 18. At high magnification, this figure illustrates the banded nature of the rod synaptic lamella. The striations are oriented with their long axes perpendicular to the plane of the lamella (arrows). In regions of favorable orientation, they appear to consist of dense rodlets separated by lighter spaces. × 238,000.



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