MORPHOLOGY OF THE OMMATIDIA OF THE COMPOUND EYE OF LIMULUS*

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PLATES 126 TO 129

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INTRODUCTION

Much of our recent knowledge of visual physiology has been derived from study of the compound eye of the horseshoe crab, *Limulus polyphemus*. These physiological studies were initiated by Hartline and Graham (1) who recorded the action potentials of single fibers of the optic nerve of this animal. Subsequent studies by Hartline and his collaborators established that the activity of any given single optic nerve fiber originates in a particular ommatidium, so that each ommatidium, though it is composed of several cells, acts as a functional unit. By analyzing the discharge in an optic nerve fiber in response to illumination under controlled circumstances, many of the basic properties of visual receptors have been explored.

Along with our increased knowledge of the physiological properties of the ommatidium of the *Limulus* eye has come the need for a better understanding of its anatomy. The early investigations, particularly those of Watase (2) and Demoll (3) accurately described the structure of the ommatidium, and recent light microscope observations reported by Hartline, Wagner, and MacNichol (4) and by Waterman and Wiersma (5) have confirmed the findings of these earlier workers. The present study is an investigation of the sensory portion of the ommatidium, principally by the use of electron microscopy. The nerve plexus of this eye will be the subject of a later paper.

Methods

The compound eyes of adult *Limulus* were excised and, without removing the cornea, were cut into pieces approximately a cubic millimeter in volume. These pieces were fixed by a modification of methods developed by Palade, Porter, and Caulfield (personal communication), and Dalton (6) for 10 to 75 minutes in 1 per cent OsO_4 in 810 mM sucrose buffered to pH 7.6 with a KOH-$K_2Cr_2O_7$ mixture such that the $K_2Cr_2O_7$ concentration was 0.5 per cent. The tissue was dehydrated in ethyl alcohol and embedded in methyl methacrylate by the usual procedure. Sections were cut with a Porter-Blum microtome and examined

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with a Phillips EM-100A electron microscope at magnifications ranging from 1,000 to 15,000 diameters at the photographic film.

For purposes of comparison a few spider and centipede eyes were excised and fixed for 30 to 75 minutes in 1 per cent OsO_4 in 200 mM sucrose buffered to pH 7.0 with a KOH-$K_2Cr_2O_7$ mixture such that the $K_2Cr_2O_7$ concentration was 0.5 per cent. Thereafter, for electron microscopy, the eyes were treated as the *Limulus* eyes described above.

OBSERVATIONS

The Ommatidium Examined with the Light Microscope.—Fig. 1 is a photomicrograph of an ommatidium in transverse section (parallel to the cornea) close to the crystalline cone. The section shows 11 wedge-shaped retinula cells arranged radially about the optical axis of the ommatidium like, as Grenecher (7) has said, the segments of an orange. In the very center of the ommatidium is the axial canal which is occupied by a dendritic "distal process" (4) of a bipolar nerve cell, called the "eccentric cell" by Hartline, Wagner, and MacNichol (4), because of the location of its cell body. The densely stained spoke-like rays (fins in three dimensions) which appear to separate the more central parts of the retinula cells constitute the rhabdom. The boundaries of adjacent retinula cells, which are not resolved in this photograph, would appear as radial lines in this slice, dividing the dark rays of the rhabdom in two. Thus the axial portion of each retinula cell contributes half of two of the darkly stained rays that make up the rhabdom. This contribution of each retinula cell is called its rhabdomere. Although the rhabdomeres are very closely applied to one another, the division between them can often be seen in both light and electron microscope sections. Grenecher (7), Watase (2), and Demoll (3) depict this division in their drawings.

The retinula cells contain pigment granules except in that part associated with the rhabdom.[1] The dense pigment in the zone around the clear central part which includes the rhabdom is, for the most part, in small pigment cells that are sandwiched between the retinula cells.

Fig. 2 is a photomicrograph of a transverse section taken near the proximal end of an ommatidium. In it is seen the cell body of the eccentric cell with its nucleus, nucleolus, and perinuclear Nissl substance. The distal process is seen entering the axial canal at the place where the rhabdom is incomplete.

Fig. 3 is a photomicrograph of a longitudinal section of an ommatidium taken in an axial plane. The eccentric cell body is on the lower right side of the ommatidium. The axon of this cell can be seen, although its origin is obscured by pigment. The distal process is seen as it comes out of the eccentric cell and is visible throughout its entire length in the axial canal. It ends at the most distal part of the sensory portion of the ommatidium where the crystalline

[1] This is true only of the dark-adapted eye. Preliminary experiments indicate that in the light-adapted eye pigment granules are present in the part of the retinula cell associated with the rhabdom.

cone, which has been removed in this section, would normally be. The retinula cells, which have shrunk away from the pigmented epithelial envelope of the ommatidium, are heavily pigmented except for the area of the rhabdom which is seen as a less dark rectangular space around the distal process. The retinula cells also have axons, but they are not seen in this section.

The Ommatidium Examined with the Electron Microscope.—Fig. 4 is an electron micrograph of a transverse section of that part of an ommatidium which contains its rhabdom. It shows an area corresponding to the clear central part of Fig. 1 with its axial canal and darkly stained rays of the rhabdom. In the center of Fig. 4, completely filling the axial canal, is the distal process of the eccentric cell within which may be discerned scattered dark bodies that examination at higher magnification has shown to be mitochondria. Also within the distal process are irregularly shaped closed outlines which are most numerous in a zone at the periphery. The border of the distal process is irregular and makes sporadic indentations into the rhabdom.

Both Grenecher (7) and Watase (2), among others, observed that the rhabdom has a faintly striated appearance when viewed under the light microscope, and striations are a prominent feature of electron micrographs of the rhabdom in transverse section. Most of the rays of the rhabdom in Fig. 4 have this characteristic banded appearance. However, the ray of the rhabdom enclosed by the black line, which is broader than most of the other rays because it is twisted and has been cut obliquely rather than transversely, appears to be made up of small polygons. This is better observed in Fig. 5 which shows a rhabdom in axial longitudinal section at higher magnification. Thus, in three dimensions the rhabdom resembles a honeycomb and may be thought of as composed of tubes with their long axes in transverse planes. These tubes are oriented with their long axes normal to the contours of the retinula cells. Therefore, in the relatively large regions where the retinula cells are contiguous, the long axes are perpendicular to the axial planes that bisect the rays, while in the region around the distal process the orientation of the tubes follows the sharply curving contour of the retinula cell.

Fig. 6 is an electron micrograph of parts of two rays (*Rb*) of the rhabdom in transverse section at higher magnification than Fig. 4. The rhabdom appears as a banded structure of dark lines of various thicknesses, because the thickness of the walls of the tubular units depends on the angle that the plane of the section makes with these walls. Because the plane of this section has passed through different levels of the tubular units of the rhabdom, the spacing of the dark lines varies. In some places, however, the lines are regularly spaced at the widest observed interval, which is about 140 mμ. This interval represents the diameter of the tubular units. As has been mentioned, the boundaries of the retinula cells (*Rc*) divide the rays down the center. These divisions are not well resolved in Fig. 6, but one such boundary dividing a

ray is illustrated in Fig. 7 (arrows). This boundary is interpreted as indicating the plane along which the tube-like structures end. The other ends of the tubes are often observed to be open and continuous with the cytoplasm of the retinula cell. In other words, the tube-like structures of the rhabdomere may be interpreted as being microvilli of the retinula cell's border. The walls of the tubular microvilli appear in many places to be continuous with fine linear structures (*C*) in the retinula cell cytoplasm. These structures, which are interpreted as membranes, have a thickness of about 6 mμ and a spacing of about 45 mμ, and are oriented for most of their length in a direction perpendicular to the long axes of the microvilli.

Electron micrographs of the ommatidium in transverse section have shown dark-bordered oval bodies 2 to 10 μ in diameter in the annular zone at the tips of the rays of the rhabdom. In the dark-adapted eye this zone divides the pigmented from the unpigmented parts of the retinula cells. Fig. 8 shows one of these bodies, within which may be seen dark-bordered, irregular closed outlines about 100 mμ in diameter. The oval bodies, five or more of which have often been seen in a transverse section at any level, have not been examined in longitudinal section.

Fine Structure of the Spider Eye.—The eyes of a few other arthropods were examined in an attempt to determine whether the structure of the *Limulus* rhabdom is unique. It is not. The rhabdoms of the centipede, the spider, and *Limulus* are morphologically similar.

Fig. 9 is an electron micrograph of a transverse section (parallel to the cornea) of a principal eye of a spider, species undetermined. In this eye, which is not a compound eye, the receptor cells (*Rc*) are not so closely applied to one another as are the retinula cells in *Limulus*, and there is a distinct separation between the rhabdomeres. An arrow marks one of these planes of separation in Fig. 9. It can be seen that the rhabdomeres have a banded appearance in transverse section. The rhabdomere at the top of the section as well as parts of the other rhabdomeres which have been sectioned obliquely have a cross-hatched appearance. Thus this rhabdomere is composed of tubular units and resembles a honeycomb. As in *Limulus*, the spider rhabdomeres are made up of specialized microvilli of the receptor cell's border, and the walls of the rhabdomere's villous units have continuity with a system of linear structures (*C*), which are also in the cytoplasm. These structures, interpreted as membranes, are regularly spaced about 35 mμ apart and tend to be concentrically coiled.

The rhabdom of the centipede, *Scutigera*, (not figured), also has a honeycomb-like structure. In both the spider and *Scutigera* the diameter of the tubular units is about 70 mμ.

DISCUSSION

In the vertebrate eye the outer segments of the rods and cones are the sites of the initial photochemical reactions of vision. The most direct evidence for this is that in the frog, the outer segments of the rods have been found by Wald and Hubbard (8) to contain all of the rhodopsin that is present in that animal's retina. We infer that the homologous structures of all vertebrate retinas are the loci of the initial photochemical reactions.

In the compound eye of *Limulus* the location of the light-sensitive pigments is unknown, and the site of the initial process of vision is less certain because there is no structure in this invertebrate eye that is strictly homologous to the vertebrate outer segments. Nevertheless, there are many good reasons for the usual assumption that the cells bearing rhabdomeres in the arthropod eye are the photoreceptors. Thus, in the compound eye of *Limulus* and other arthropods the rhabdomeres are located favorably with respect to the dioptric component of the ommatidium, they appear highly refractile under the light microscope as do the outer segments of the rods and cones, and anatomically the rhabdomere-bearing cells appear to be the first elements in the neural paths of the visual system. However, electron microscope studies have revealed important differences. Sjöstrand (9) has shown that the structural units of the vertebrate outer segments are plate-like elements which, as De Robertis (10) has demonstrated, are derived from fibrillar remnants of a cilium. In contradistinction, the rhabdomeres of *Limulus* and the spider are composed of very much larger units on the receptor cell's border, which consist of closely packed microvilli continuous with what appear to be specialized cytoplasmic cisternae. Fernández-Morán (11) in a recent note, described the insect rhabdom as composed of "fenestrated disks." Although there are considerable differences, the rhabdomeres of the arthropod eye and the outer segments of the vertebrate rods and cones have certain similarities of ultrastructure. In both cases closely packed, uniformly oriented, repeated structures present a succession of many membranes to any incoming light wave. It seems reasonable, therefore, to assume that the rhabdomere in the arthropod ommatidium is the locus of the initial photochemical events of vision.

The *Limulus* ommatidium acts as a functional unit. Hartline, Wagner, and MacNichol (4) have shown that the optic nerve impulses originate in the eccentric cell. They discussed (4) the hypothesis that electric potentials originating in the receptor generate the nerve impulses. The anatomy of the ommatidium is not inconsistent with such an hypothesis: the comparatively slow, sustained change in the ommatidial polarization that takes place in response to light could be the result of processes originating in the retinula cells' rhabdomeres that influence the eccentric cell through its distal process with which the rhabdomeres are in intimate contact.

The function of the oval bodies located in the annular zone at the tips of the rays is unknown. Their resemblance to mitochondria found in sites of steroid secretion by Belt and Pease (12) suggests that they may be mitochondria. That the oval bodies are found only at the edge of the pigmented zone in the retinula cells suggests the possibility of an association of these structures with pigment migration, which is known from preliminary experiments to occur.

SUMMARY

The sensory portion of the ommatidium of the compound eye of *Limulus* has been studied with the electron microscope. In axial longitudinal section the rhabdom appears to be made up of small polygons, and in transverse section the rhabdom appears as a banded structure of dark lines. Thus in three dimensions the rhabdom resembles a honeycomb composed of tubular units, the long axes of which lie in transverse planes and are oriented perpendicular to the retinula cell's contours. The tubular units, which are about 140 mμ in diameter in *Limulus* (70 mμ in diameter in the spider and *Scutigera*), are microvilli of the borders of the retinula cells. The walls of these microvilli are continuous with fine linear structures (membranes) in the cytoplasm of the retinula cells.

In transverse sections of the ommatidium oval bodies interpreted as mitochondria are observed in an annular zone at the tips of the rhabdom's rays. These mitochondria, which are 2 to 10 μ in diameter, are crowded with irregular closed outlines about 100 mμ in diameter.

Possible functions of components of the ommatidium are discussed.

I am particularly indebted to Dr. Keith R. Porter and Dr. George E. Palade of the Rockefeller Institute for guidance in techniques of electron microscopy which they developed, for making available to me facilities in their laboratory, and for their helpful advice and criticism of this study. I am most grateful to Dr. James B. Caulfield of the University of Kansas Medical School for his advice on fixation. This study is an outgrowth of investigations begun with Dr. Henry G. Wagner of the Naval Medical Research Institute, Bethesda, Maryland, and I am greatly indebted to him for his continued interest. It is a pleasure to acknowledge the technical assistance of Miss Joan White. I am deeply grateful to Dr. H. K Hartline, in whose laboratory this investigation has been carried out, for his encouragement and guidance in all phases of the work.

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EXPLANATION OF PLATES

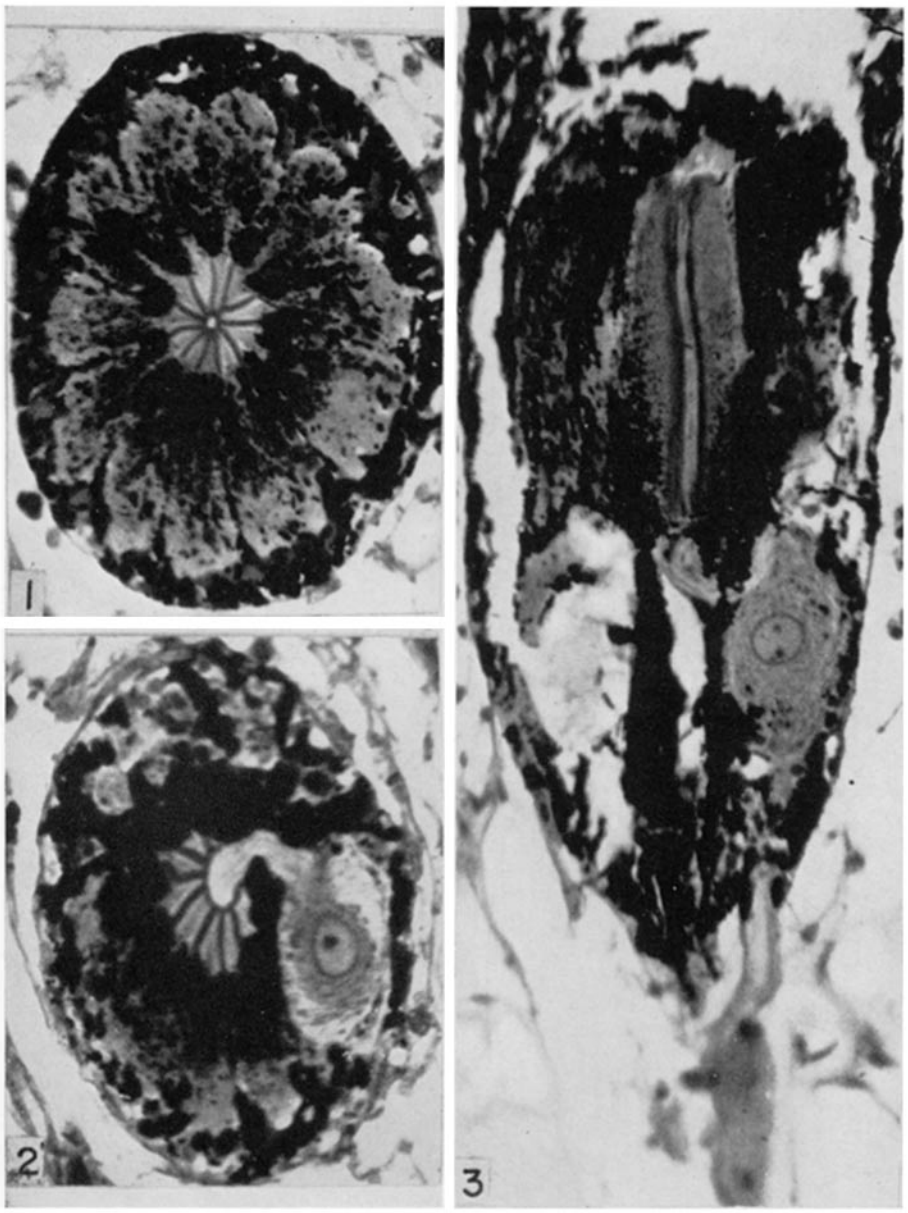
PLATE 126

FIGS. 1 to 3. Photomicrographs depicting the sensory portion of an ommatidium of the compound eye of *Limulus*. The material was fixed in 2 per cent OsO_4 in sea water, imbedded in paraffin, sectioned at 6 μ, and stained by Mallory's aniline blue method. These eyes came from animals measuring about 20 cm. across the carapace. All three figures × 500.

FIG. 1. Transverse section of an ommatidium near the crystalline cone. In the center of the ommatidium is the axial canal, occupied by the dendritic distal process of the eccentric cell. The wedge-shaped retinula cells are arranged radially around the axial canal; their boundaries are in axial planes which divide in two the densely stained spoke-like rays of the rhabdom. The ommatidium is enveloped by a heavily pigmented layer of epithelial cells.

FIG. 2. Transverse section of an ommatidium at the level of the entrance of the distal process into the axial canal. The eccentric cell body with its distinct nucleus, nucleolus, and Nissl substance in the perikaryon are seen on the right. The rhabdom is incomplete where the distal process enters the axial canal.

FIG. 3. Longitudinal section of an ommatidium made in an axial plane. The eccentric cell body is seen on the right. The origin of its axon is partially hidden by pigment. Its distal process is seen along the entire length of the axial canal. The rhabdom is the relatively clear rectangular space around the axial canal. The retinula cells are, for the most part, shrouded by pigment. Their axons are not seen.

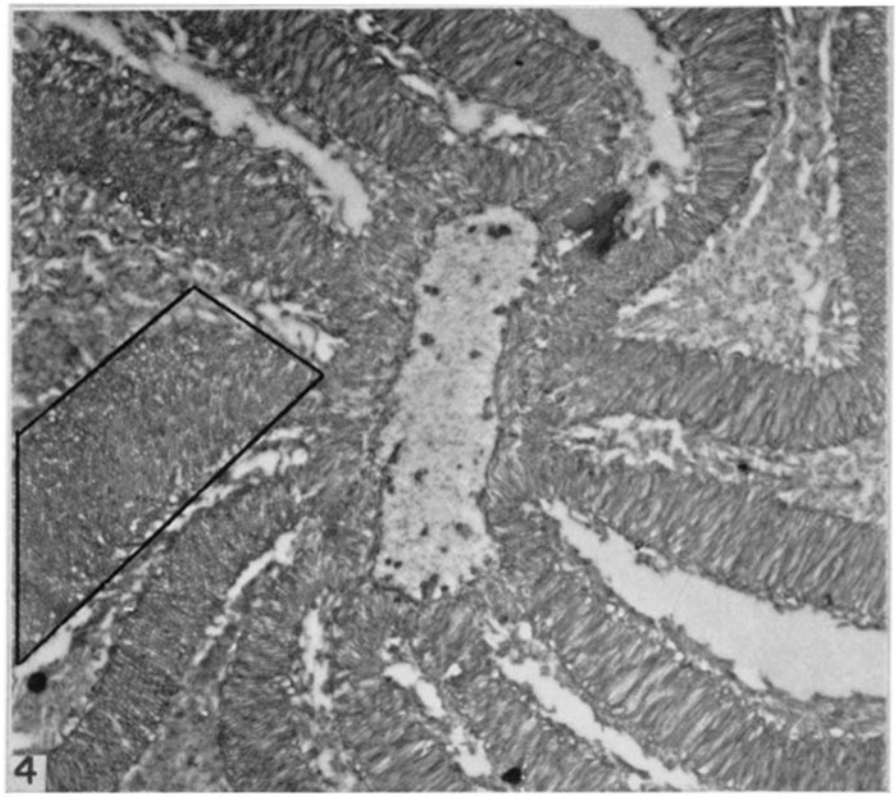


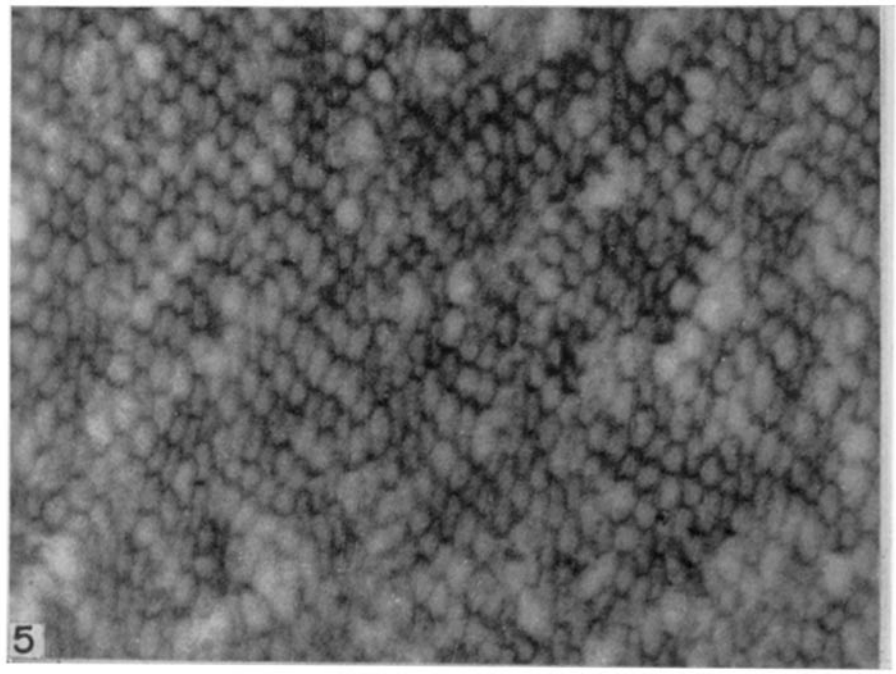
(Miller: Morphology of *limulus* ommatidia)

PLATE 127

FIG. 4. Electron micrograph of the rhabdom of *Limulus* in transverse section. In the center is the distal process of the eccentric cell, within which can be seen irregular closed outlines which are more numerous in a thin zone around the periphery, and a few dense structures which are mitochondria. Most of the rays of the rhabdom show a characteristic banded appearance. The ray enclosed by a black line is wider than the others because it has been twisted and sectioned obliquely. It appears to be made up of small polygons. Thus in three dimensions the rhabdom resembles a honeycomb the tubular units of which have their long axes in transverse planes. The orientation of the long axes is perpendicular to the contours of the retinula cells. ×3600.

FIG. 5. Electron micrograph of the rhabdom in axial longitudinal section. This figure depicts an area similar to that bounded by the black line on Fig. 4. It illustrates the appearance of the polygonal outlines of the tube-like structures of the rhabdomeres. The units, which have a diameter of approximately 140 mμ, appear somewhat elongated due to knife compression. × 20,000.



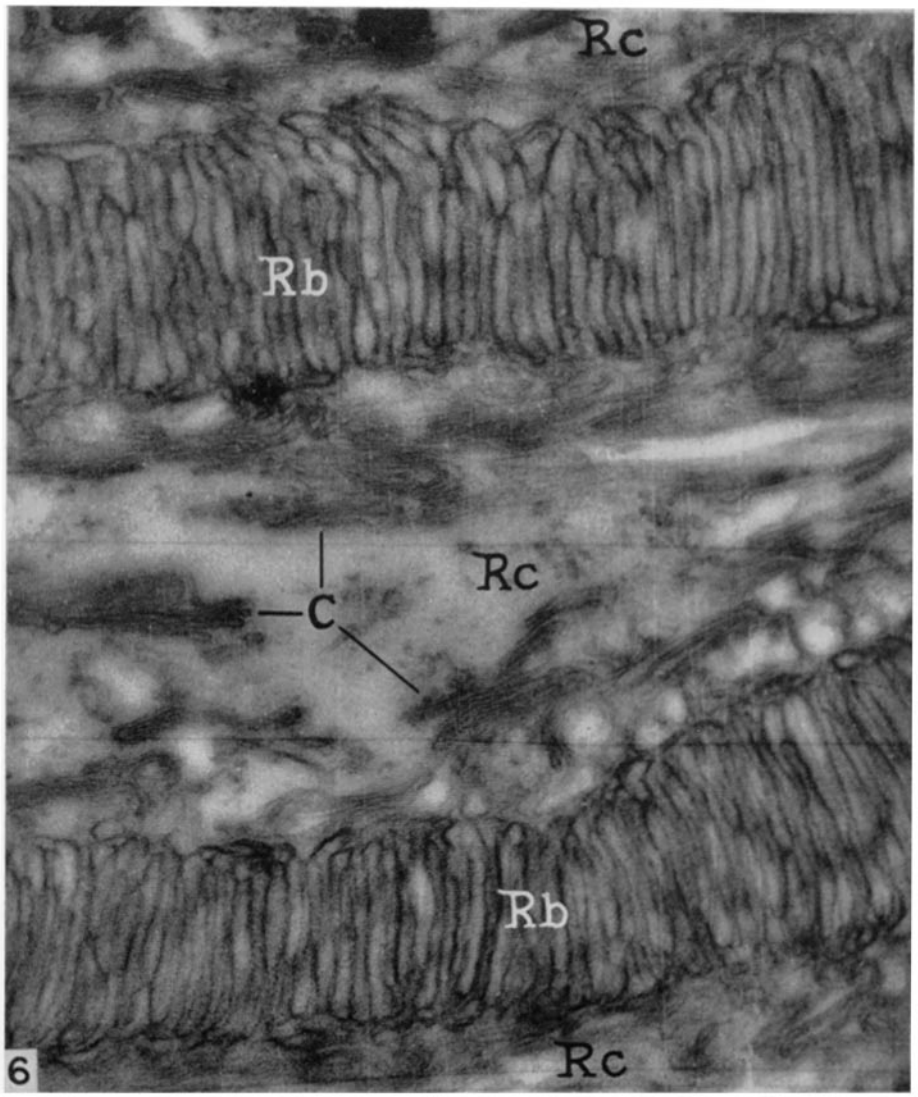


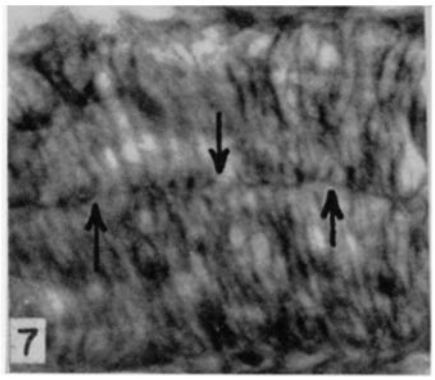
(Miller: Morphology of *limulus* ommatidia)

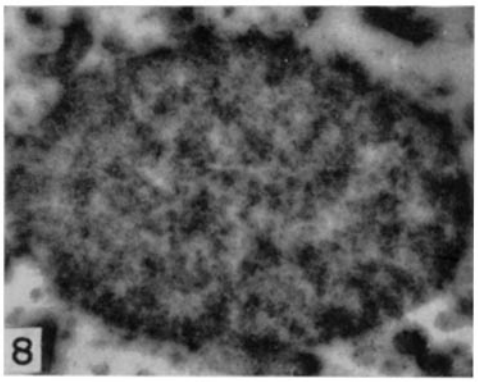
PLATE 128

FIGS. 6 to 7. Electron micrographs of rays (*Rb*) of the rhabdom of *Limulus* in transverse section. Fig. 6 shows the rhabdom's characteristic banded structure of dark lines. The division between the rhabdomeres of adjacent retinula cells (*Rc*) is not resolved on Fig. 6 but is seen on Fig. 7 (arrows). This boundary is interpreted as representing the plane of abutment of the tubular units of the rhabdomeres. The other ends of the tubular units are seen in Fig. 6 to be continuous with the retinula cell cytoplasm. Therefore, the tube-like structures may be thought of as microvilli of the retinula cell's border. The dark lines of the rhabdom, which represent the walls of the microvilli, are seen to be continuous with fine linear structures (*C*), interpreted as membranes, which, for most of their length, are oriented perpendicular to the walls of the microvilli. Fig. 6 × 17,000. Fig. 7 × 16,000.

FIG. 8. Electron micrograph of an oval body in a *Limulus* retinula cell. These oval bodies, which are interpreted as mitochondria, measure 2 to 10 μ in diameter. They are seen in transverse sections at all levels of the dark-adapted retinula cell in an annular zone at the tips of the rhabdom's rays. Within the oval bodies are many closed irregular outlines which are roughly circular and about 100 mμ in diameter. × 9000.



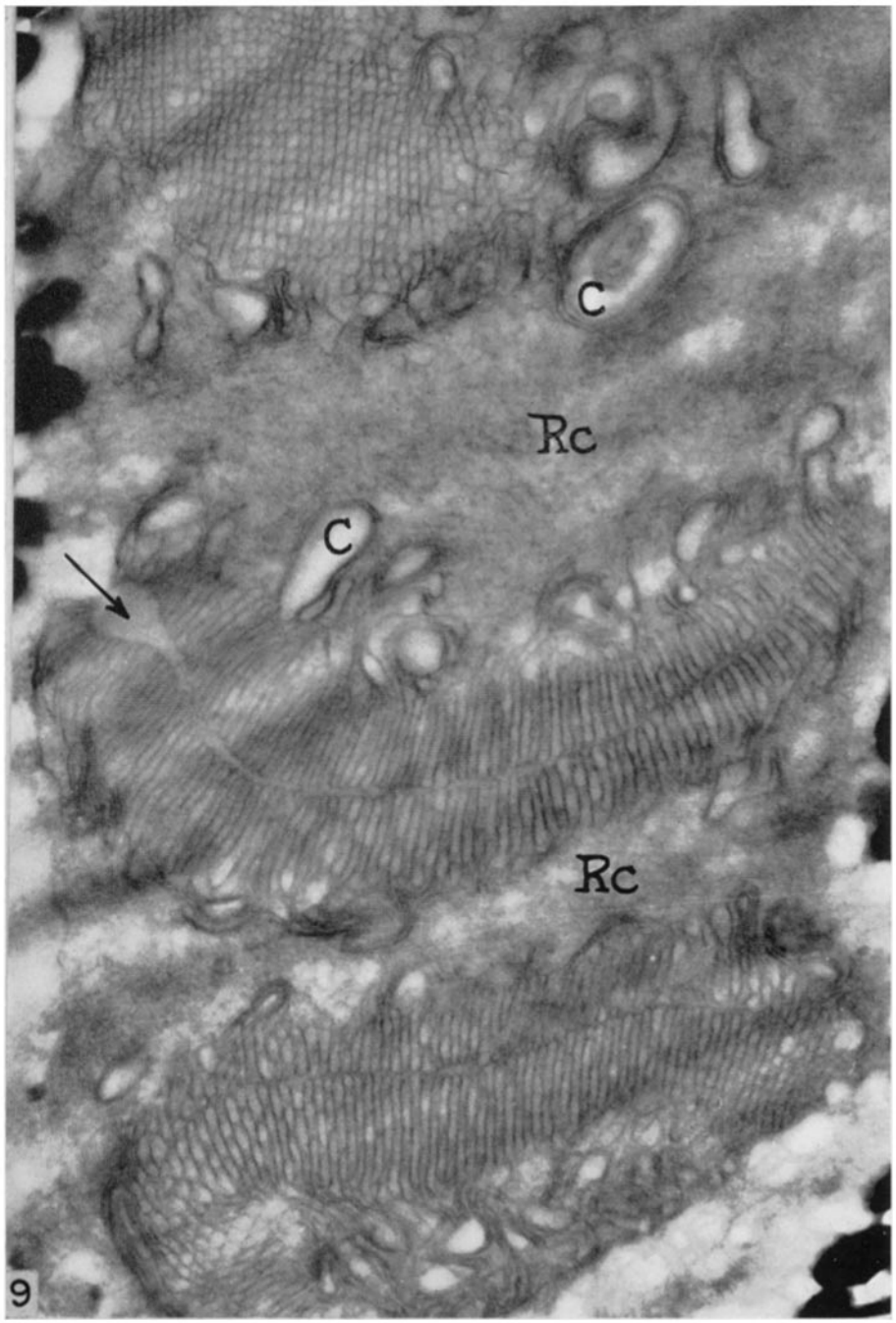




(Miller: Morphology of *limulus* ommatidia)

PLATE 129

FIG. 9. Electron micrograph of a principal eye of a spider in transverse section. The receptor cells (*Rc*) of this eye are not as closely applied to one another as are the retinula cells in *Limulus*. The plane of separation between two rhabdomeres is marked by an arrow. Most of the rhabdomeres have a predominately banded structure, but the rhabdomere at the top of the figure, because it has been sectioned obliquely, has a reticular appearance. In three dimensions the spider rhabdom resembles a honeycomb and, like that of *Limulus*, is composed of tube-like microvilli of the receptor cell's border. In this eye the tubular units have a diameter of about 70 mμ. As in *Limulus*, the dark lines of the rhabdomere (walls of the microvilli) are continuous with fine linear structures (*C*), which in this eye tend to be concentrically coiled. × 25,000.



(Miller: Morphology of *limulus* ommatidia)