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The Morphology of the Eyes of *Limulus*

II. Ommatidia of the Compound Eye*

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Summary. The *Limulus* ommatidium consists of 4 to 20 retinula cells surrounding the dendrite of the eccentric cell. Adjoining membranes are differentiated into the microvillous rhabdome in the central area of the ommatidium. Three types of pigment cells envelop the sensory cells. The distal pigment cells cover the periphery of the distal half of the ommatidium; proximal pigment cells (beneath the base of the ommatidium) and intraommatidial pigment cells provide glial wrapping for the sensory cells, the partitions between them, and the peripheral loose framework. Processes of the overlying cone cells penetrate into the ommatidium and lie at the edges of the rhabdomal fins. Numerous neurosecretory axons terminate at all levels of the ommatidium on pigment cells, conveyed there either by enveloping pigment cells or by separate neuroglial cells. Tight junctions in the ommatidium are confined to the contacts between rhabdomal microvilli. The periphery of the rhabdome is surrounded by continuous adhering junctions except at the tip and exit of the eccentric cell dendrite. The discussion centers on possible correlations between known neurophysiological characteristics of ommatidial cells and significant morphological aspects of the ommatidium, such as distribution of supporting cells, extracellular space, and junctional specializations.

Introduction

The ommatidia of the *Limulus* compound eye have served as exceptionally rewarding objects of study for neurophysiologists. Favorable characteristics include the large size and accessibility of primary and secondary sensory cells, resistance to the deleterious effects of experimental manipulation, the relative simplicity of the adjacent synaptic areas, and a long optic nerve. WOLBARSHT and YEANDLE (1967) have reviewed the extensive physiological literature as well as the few morphological studies. Four papers by MILLER (1952, 1957a, 1957b, 1958) cover a number of fine structural aspects of the *Limulus* ommatidia. A more recent study (LASANSKY, 1967) deals specifically with the junctional specializations between several cell types of the ommatidium. The present study concerns the ommatidium exclusive of the overlying dioptric apparatus (see FAHRENBACH, 1968) and the subjacent axons and plexus.

Materials and Methods

Animals of 5, 10 and 20 cm prosomal width were obtained from the Woods Hole Biological Supply Company and stored in illuminated aquaria to preclude possible degenerative changes of the eyes after prolonged darkness. Eyes that were dark-adapted for 12 hours were cubed

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in various fixatives, the most successful of which consisted of 0.75% glutaraldehyde, 5% formalin, 3% NaCl and 4.5% sucrose in 0.1 M phosphate buffer. The tissue was briefly washed in buffer with 8% sucrose, postfixed in OsO_4, and embedded in Araldite. For purposes of comparison, some eyes were fixed in LASANSKY's (1967) mixture. Thin sections were stained with uranyl acetate, lead citrate, potassium permanganate, or a combination of these. One half to 1 micron sections were stained with 1% toluidine blue in 1% borax for light microscopy. Electron micrographs were obtained with a Philips EM-200.

Results

1. Gross Ommatidial Morphology

The basic structure of the ommatidium has been outlined and illustrated several times (MILLER, 1957; MILLER *et al.*, 1961; LASANSKY, 1967); hence only a brief coverage will be included with emphasis on new observation and on those cell types not previously described. Measurements apply to the largest animals (20 cm); cellular dimensions are scaled down by about 30% in the 10 cm specimens.

Each ommatidium (Fig. 1) consists of an ovoid cellular complex, about 350 μ long und 180 μ in diameter, its narrow end abutting against the proximal surface of the cone cells. Its long axis coincides with that of the overlying, more or less inclined, cuticular cone. The body of the ommatidium is formed by the primary receptor, or retinula, cells, 70 μ in diameter, grouped like orange slices about the central tapering dendrite of the secondary receptor, or eccentric, cell. The number of retinula cells per ommatidium averages between 10 and 13 for different individuals, the observed range covering 4 to 20 cells. The retinula cells surround the dendrite with the elaborate complex of photoreceptor membranes called the rhabdome, which has the cross-sectional appearance of a starfish (Figs. 2—4). It measures about 60 μ in diameter and 180 μ in vertical extent. In many instances, an unsymmetrical retinula cell can be distinguished by the shape of its rhabdomere, i.e. that portion of the rhabdome contributed by one cell, and by some cytological peculiarities. However, these asymmetrical elements occur in random radial orientation and are not related to the position of the eccentric cell.

The eccentric cell soma has a diameter of about 70 μ and lies level with the proximal borders of the retinula cells. Its dendrite has a maximal proximal diameter of about 25 μ tapering gradually to 4 μ and terminating in a slightly expanded knob that abuts against the proximal surface of the cone cells. The frequency with which two eccentric cells per ommatidium occur varies from one in 5 to one in 30 in different individuals. Ommatidia without eccentric cells have not been seen; a single ommatidium with 3 dendrites was found, though the number of eccentric cells was not verified.

The apical half of the ommatidium is covered by a peripheral sleeve of distal pigment cells, which are anchored to the sides of the cuticular cone. Processes of the cone cells, which are situated at the tip of the cone, follow the rhabdome almost to its basal terminus, each process lying at the edge of one of the rhabdomal radii.

The proximal pigment cells form a cup-shaped mass underneath the ommatidium. The extensive, attenuated processes of these cells and of similar intraommatidial pigment cells envelop the eccentric and retinula cells, form partitions between adjacent retinula cells, and constitute the peripheral framework of the

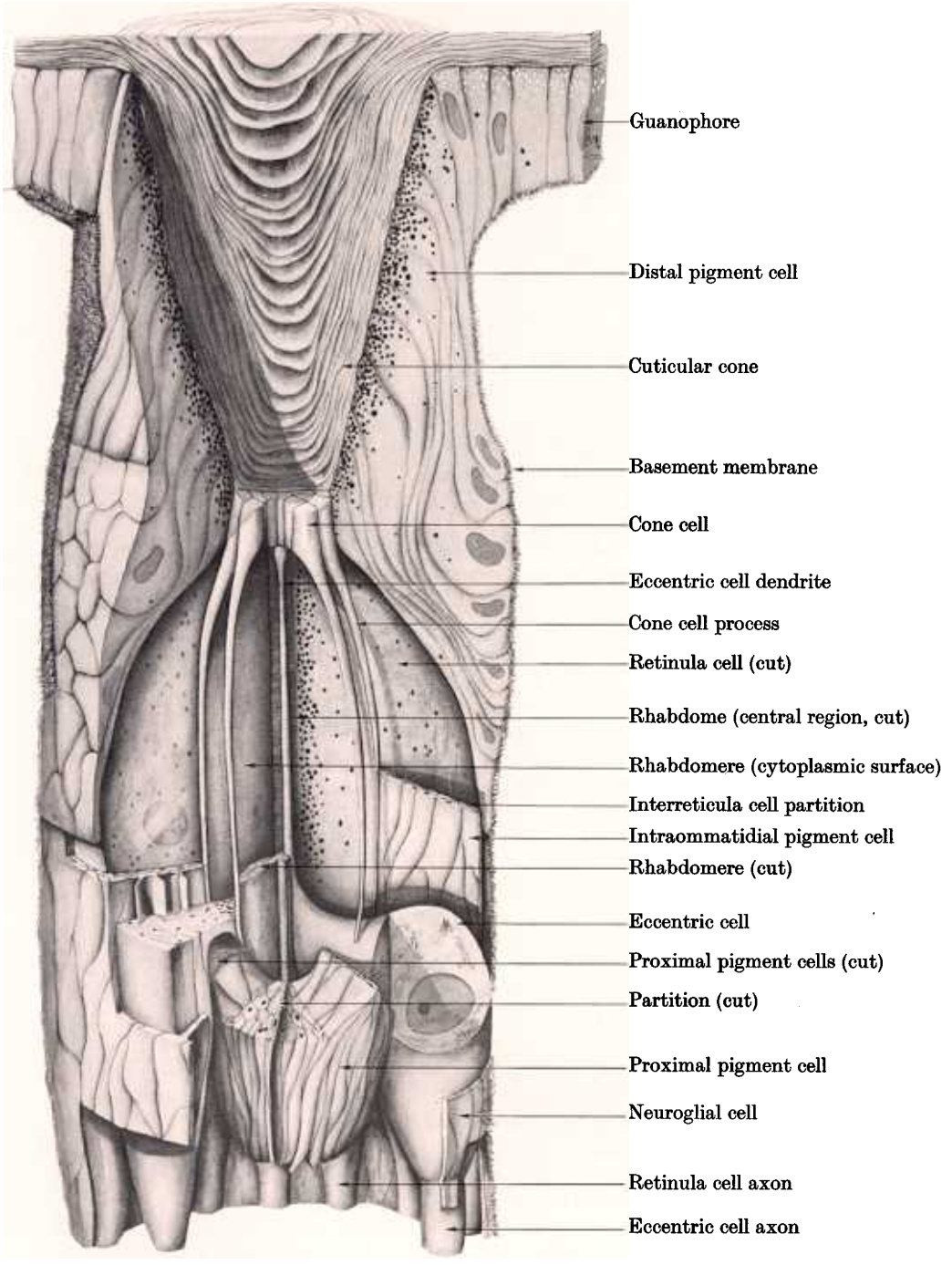


Fig. 1. Semidiagrammatic, longitudinal, cutaway view of a *Limulus* ommatidium

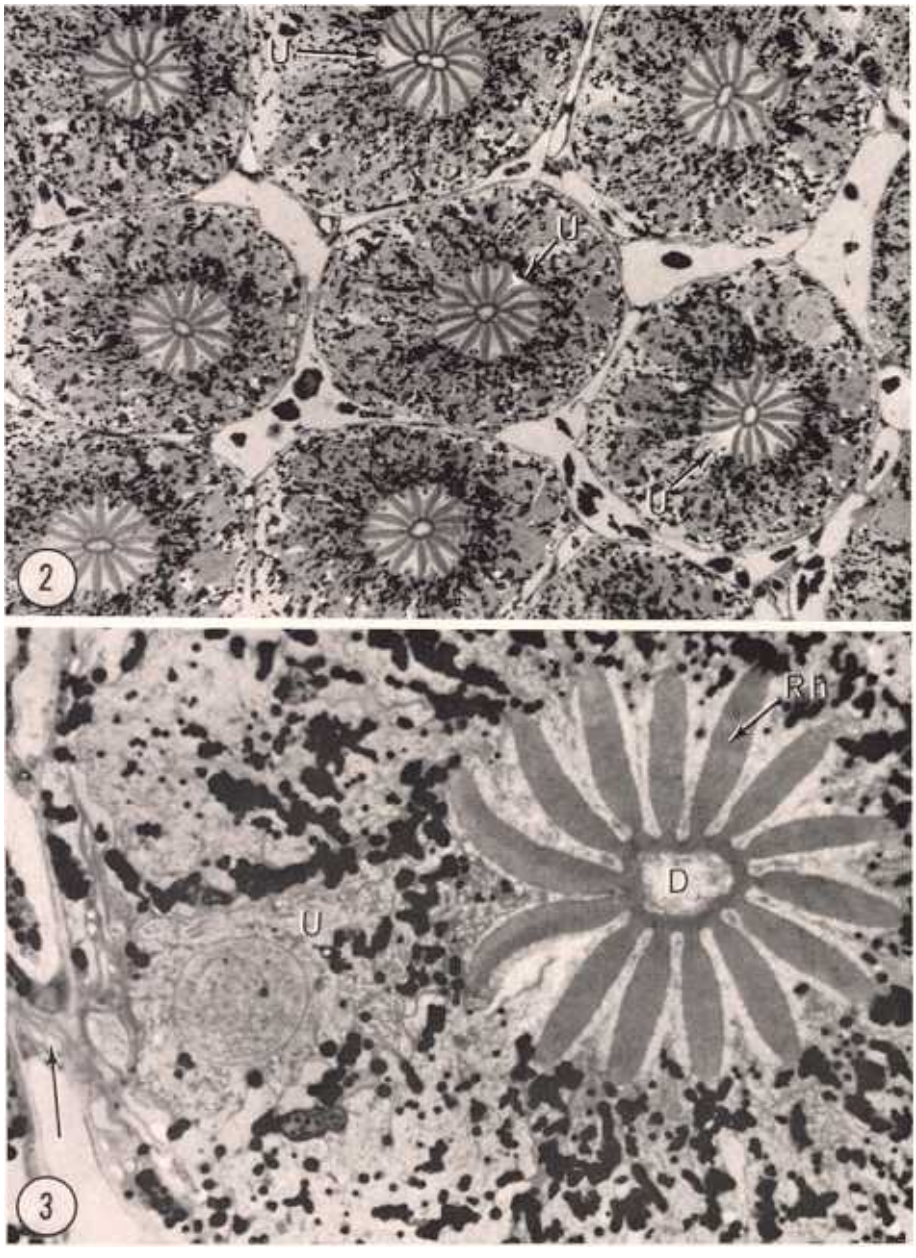


Fig. 2. Low power light micrograph of several ommatidia in cross-section, showing the stellate rhabdomes, some with bizarre fins (upper right), double eccentric cell dendrite (upper center) and several unsymmetrical retinula cells (*U*). × 280

Fig. 3. Higher power light micrograph of an ommatidium in cross-section. The eccentric cell dendrite (*D*) shows peripheral concentrations of organelles. Fine central lines in the radii of the rhabdome (*Rh*) are indicative of the zone of apposition of adjacent retinula cell microvilli. A typical unsymmetrical retinula cell (*U*), its nucleus, and its flared rhabdomere is visible. The bridge (arrow), across the interommatidial sinusoid carries neurosecretory axons. × 1,300

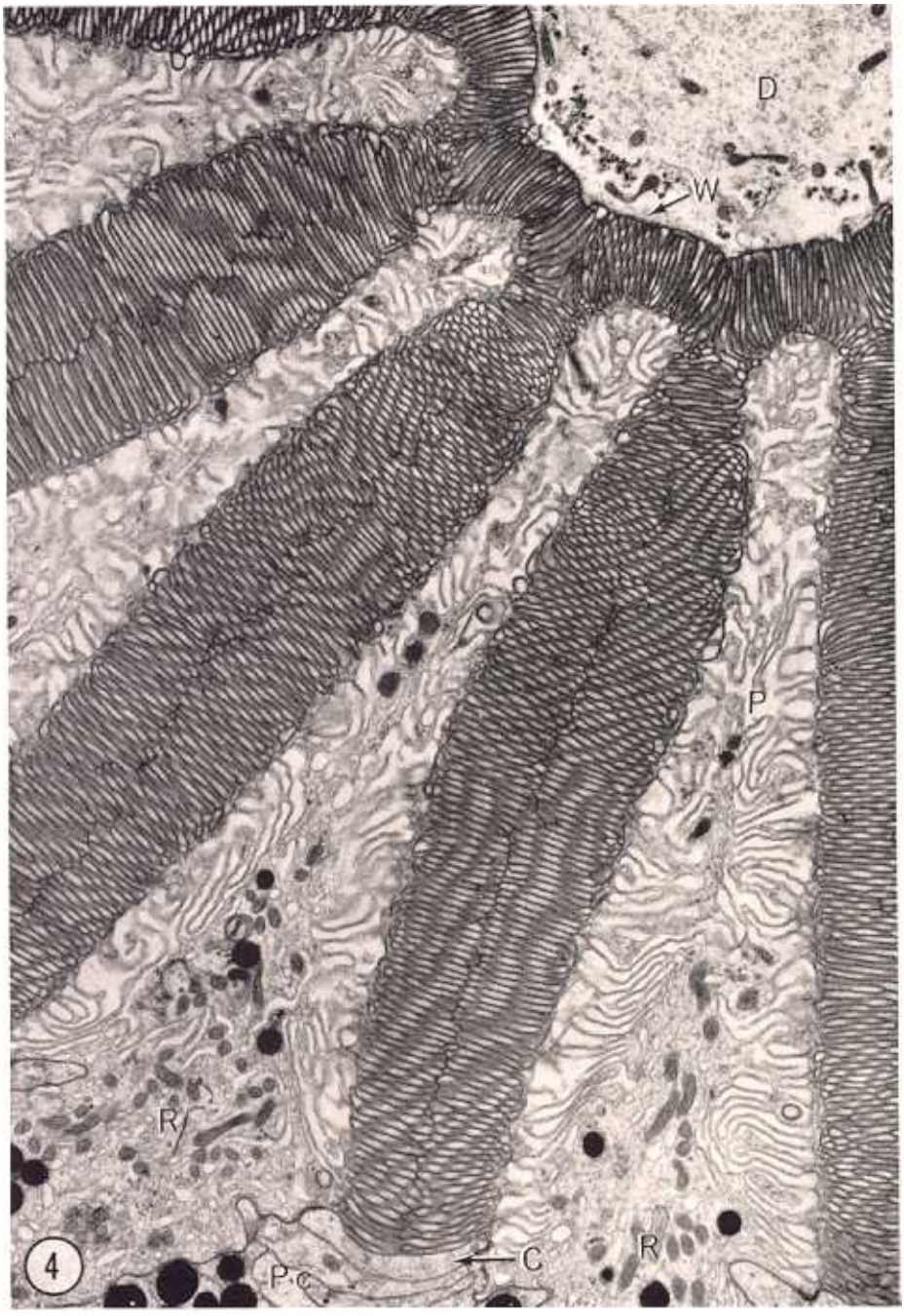


Fig. 4. Electron micrograph of a sector of Fig. 3. The eccentric cell dendrite (*D*) has a peripheral terminal web (*W*) though few microvilli. The palisade (*P*) is fully formed in this dark-adapted specimen. The broad partition of pigment cells (*Pc*) between retinula cells (*R*) starts with the cone cell process (*C*). × 7,000

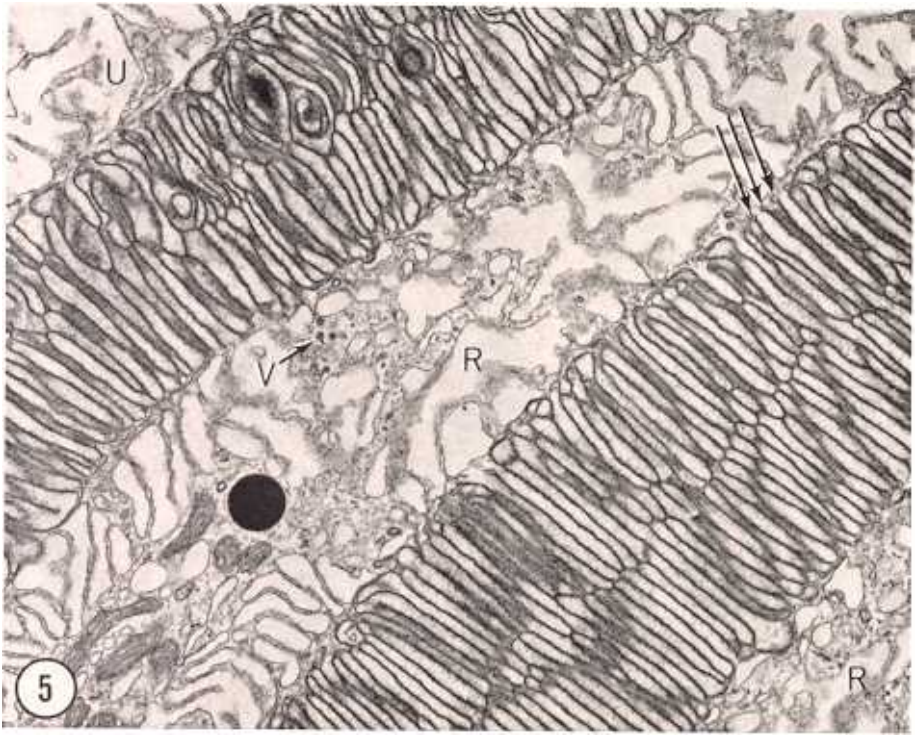


Fig. 5. Rhabdomeres of an unsymmetrical (*U*) and a regular (*R*) retinula cell. The difference in membrane thickness between cisternae of the palisade and microvilli is perceptible. Basal dilatations between microvilli are common (arrows). *V* Coated vesicles. × 15,000

ommatidium. The pigment of these cells, like that in retinula cells and in the distal pigment cells, migrates in response to changing illumination.

Approximately 100 neurosecretory axons (Figs. 6, 25—27) enter each ommatidium, terminating in about equal proportions between the proximal pigment cells, within the body of the ommatidium, and in the epidermal zone. They are frequently invested by neuroglial cells. This efferent innervation of the ommatidia will be dealt with in a future communication. The ommatidium is covered by a thick though loosely structured basement membrane backed by thin extensions of the cells lining the surrounding circulatory sinusoids. Hemocyanin easily passes through the basement membrane and penetrates deeply into the ommatidium. No hemocytes ever invade the ommatidium.

2. Retinula Cells

The retinula cell is the primary visual neuron of the ommatidium. On its medial and lateral surfaces it bears a massive array of microvilli, the rhabdomere (Figs. 2—5). The microvilli average 2 μ in length and 1000 Å in diameter. From accurately oriented sections through the length of a rhabdomere, the total number of microvilli for one retinula cell can be estimated at $4.5—5 \times 11^5$. Expressed in terms of rhabdomal (i.e. photoreceptor) membrane, it amounts to 3.6 mm^2 per ommatidium

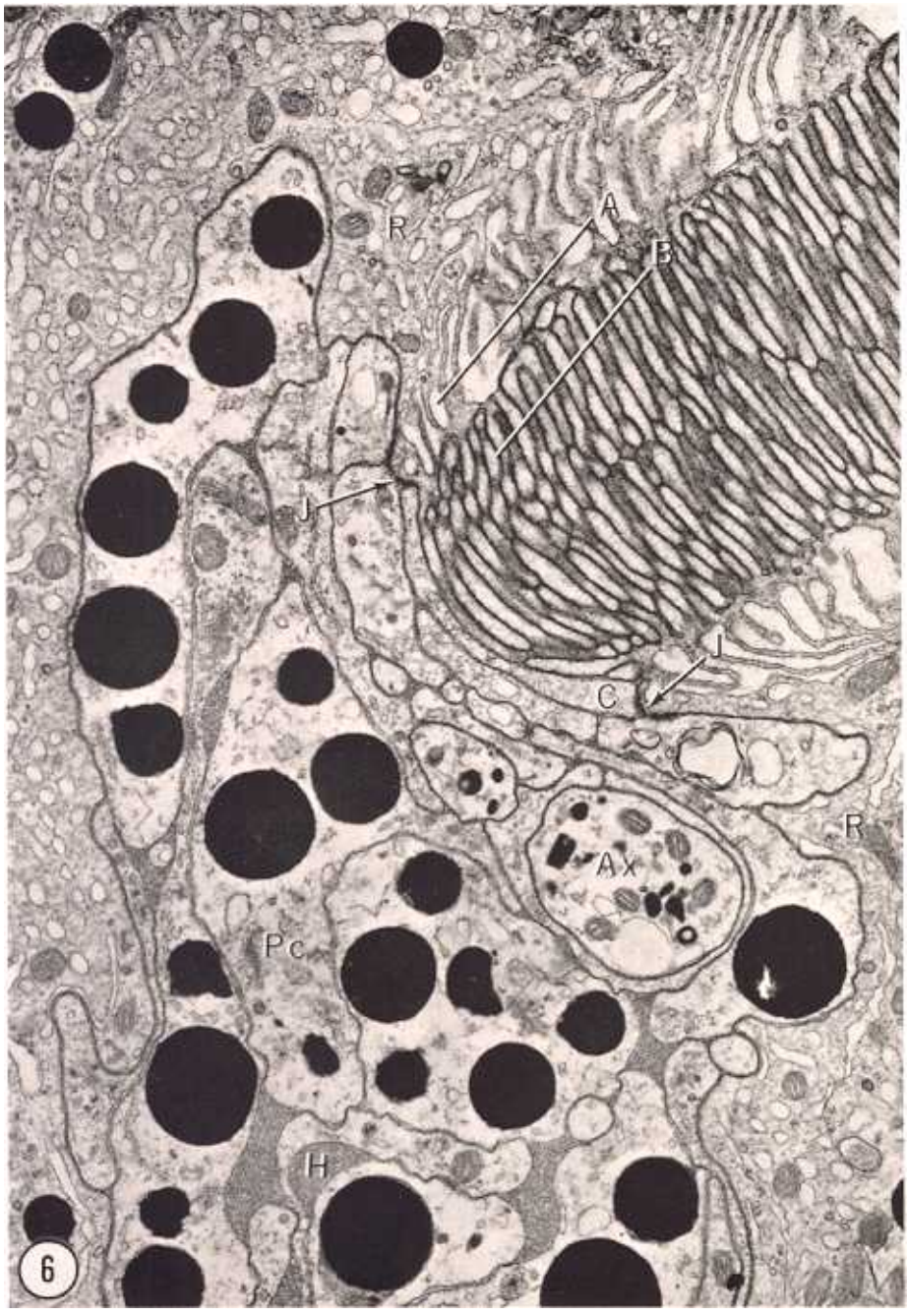
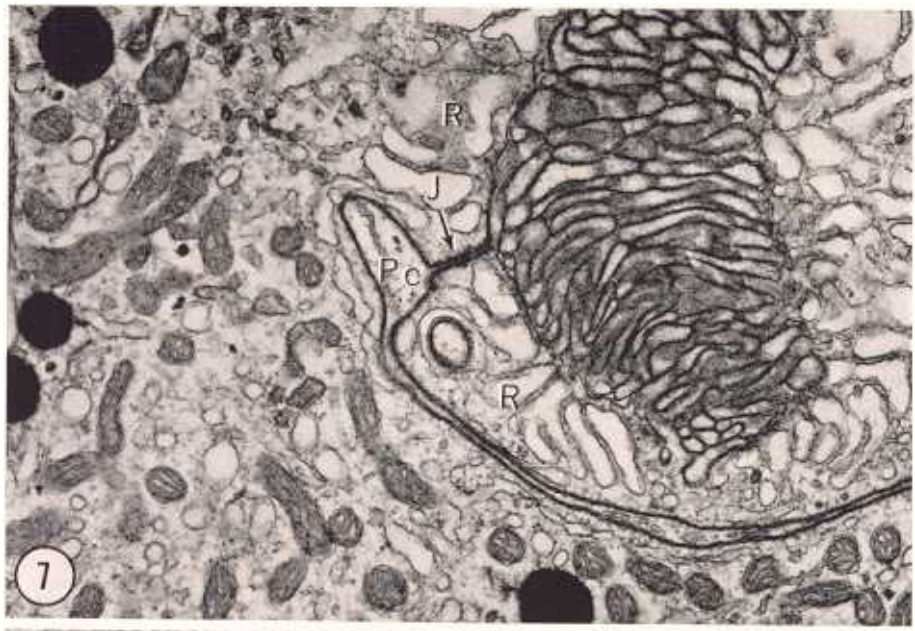


Fig. 6. The edge of a rhabdomal radius and part of the compact partition between retinula cells (*R*) in cross-section. *C* Cone cell process; *Pc* Intraommatidial pigment cells; *J* Adhering junctions; *Ax* Neurosecretory axons; *H* Hemocyanin. The two lines (*A* and *B*) indicate the planes of section of Figs. 19 and 20, respectively. × 18,000



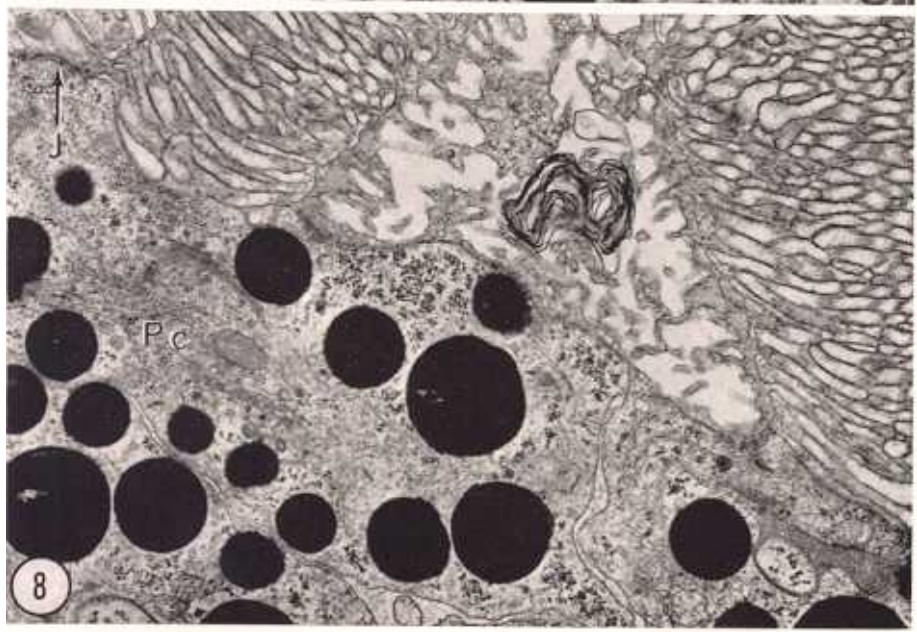


Fig. 7. Cross-section of the periphery of the rhabdome proximal to the termination of the cone cell process. An adhering junction (*J*) is established between two retinula cells (*R*). *Pc* Intraommatidial pigment cell. × 16,000

Fig. 8. A section slightly more basal in the eye than that shown in Fig. 7. The proximal tip of the rhabdome is surrounded by proximal pigment cells (*Pc*). The somata of the retinula cells lie peripheral to the pigment cells as a result of the cup-shaped disposition of this region (see Figs. 1 and 24). *J* Intermediate junction. × 16,000

(about 6×10^6 microvilli) or 25—30 cm^2 per compound eye. As previously reported by LASANSKY (1967), the microvilli are bonded to one another by tight junctions (Fig. 13, inset) as well as by similar contacts to the tips of microvilli from adjacent retinula cells. This specialized feature is evident with any of the numerous fixatives that have been explored in this study to date and under various degrees of light adaptation. Hence, the extracellular space in the rhabdome is largely limited to narrow prismatic spaces, about 250 Å on a side, between contiguous microvilli. Calculation of the approximate extracellular space within one rhabdome yields a minimum value of 7,000—10,000 μ^3. This volume might be considerably larger by virtue of the usual dilatation of the space at the base of adjacent microvilli (Fig. 5) and the loose construction of the apical region of the rhabdomeres. Basal continuation of the membrane between microvilli is relatively common, but these diverticula are never extensive or voluminous.

Each rhabdomere is backed by a zone of agranular cisternae (Figs. 4, 5, 19), 1—2 μ wide, which in various arthropods have been termed perirhabdomal vacuoles (EGUCHI and WATERMAN, 1966, 1967), subrhabdomere cisternae (LASANSKY, 1967), the palisade (HORRIDGE and BARNARD, 1965) or the „Schaltzone" (HESSE, 1901). The cisternae of this palisade are disposed irregularly but show a tendency to stack radially and longitudinally with respect to the rhabdome (Fig. 24). Continuity with the rhabdomeral membrane is precluded by the fact that the membranes of the microvilli are about 75 Å thick, whereas those of the palisade measure close to 45 Å. Coated vesicles between the palisade and the rhabdomere are not uncommon and are occasionally attached to the cell membrane between microvilli. They are not considered to be pinocytotic vesicles, as they were in the crab *Libinia* by EGUCHI and WATERMAN (1967) or in the mosquito eye by WHITE (1967), but as structures associated with a secretory process (vide infra). The palisade is most conspicuous in dark-adapted animals and may become obliterated upon thorough light adaptation.

The cytoplasm of the retinula cell has all the characteristics of a cell in intense metabolic and synthetic activity (Fig. 9). Endoplasmic reticulum fills most of the cytoplasm with concentric arrays of cisternae. These are extremely labile and account for the usual disturbingly frothy aspect of the cytoplasm after most modes of fixation. The cell contains large numbers of free ribosomes, but rarely polyribosomes. Subsurface cisternae (ROSENBLUTH, 1962; LASANSKY, 1967) are continuous with the endoplasmic reticulum, but agranular in nature. Mitochondria with predominantly longitudinal cristae are concentrated in the proximity of the rhabdomere, with a liberal sprinkling throughout the remaining cytoplasm. Small Golgi systems of 3 or 4 cisternae are common, often elaborating coated vesicles of 600—800 Å diameter that frequently accumulate into compact 1—2 μ masses, simulating glycogen to the unwary observer.

In addition to these structures, the retinula cells contain an astonishing diversity of inclusions (Figs. 10—12). Pigment droplets, probably ommochrome by virtue of their solubility characteristics (FAHRENBACH, 1968), are evenly distributed during the dark-adapted phase but concentrate in a subrhabdomeral position with protracted illumination. Also present are large masses of α-glycogen, multivesicular bodies up to 1 μ in diameter, lipid droplets, and a variety of dense bodies of presumptive lysosomal nature. These include small and large vesicles of evenly

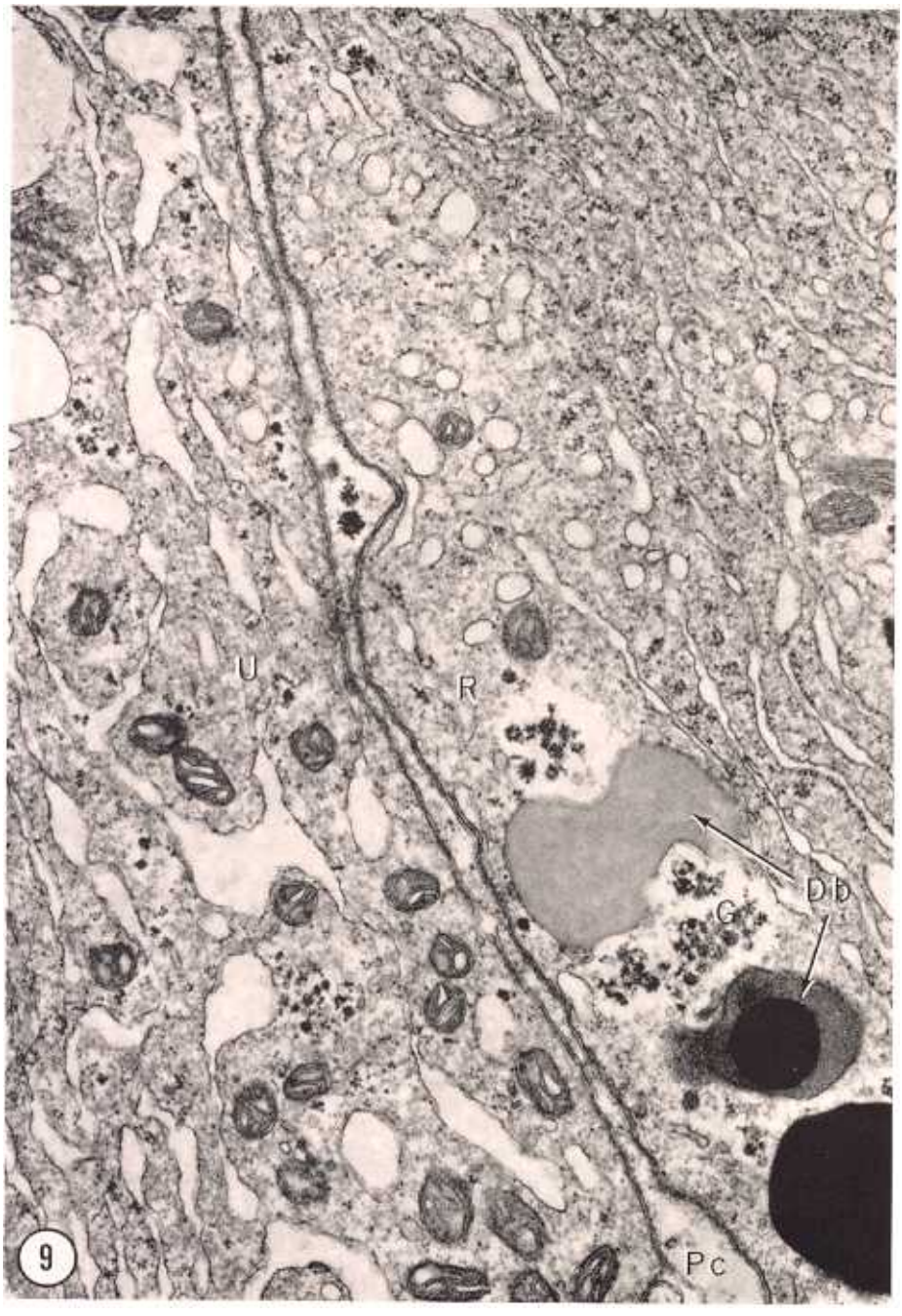


Fig. 9. Cytoplasm of an unsymmetrical (*U*) and normal retinula cell (*R*) with an intervening pigment cell partition (*Pc*). Note differences in abundance of endoplasmic reticulum and mitochondria. *G* Glycogen; *Db* Dense bodies or autophagic vacuoles. × 29,000

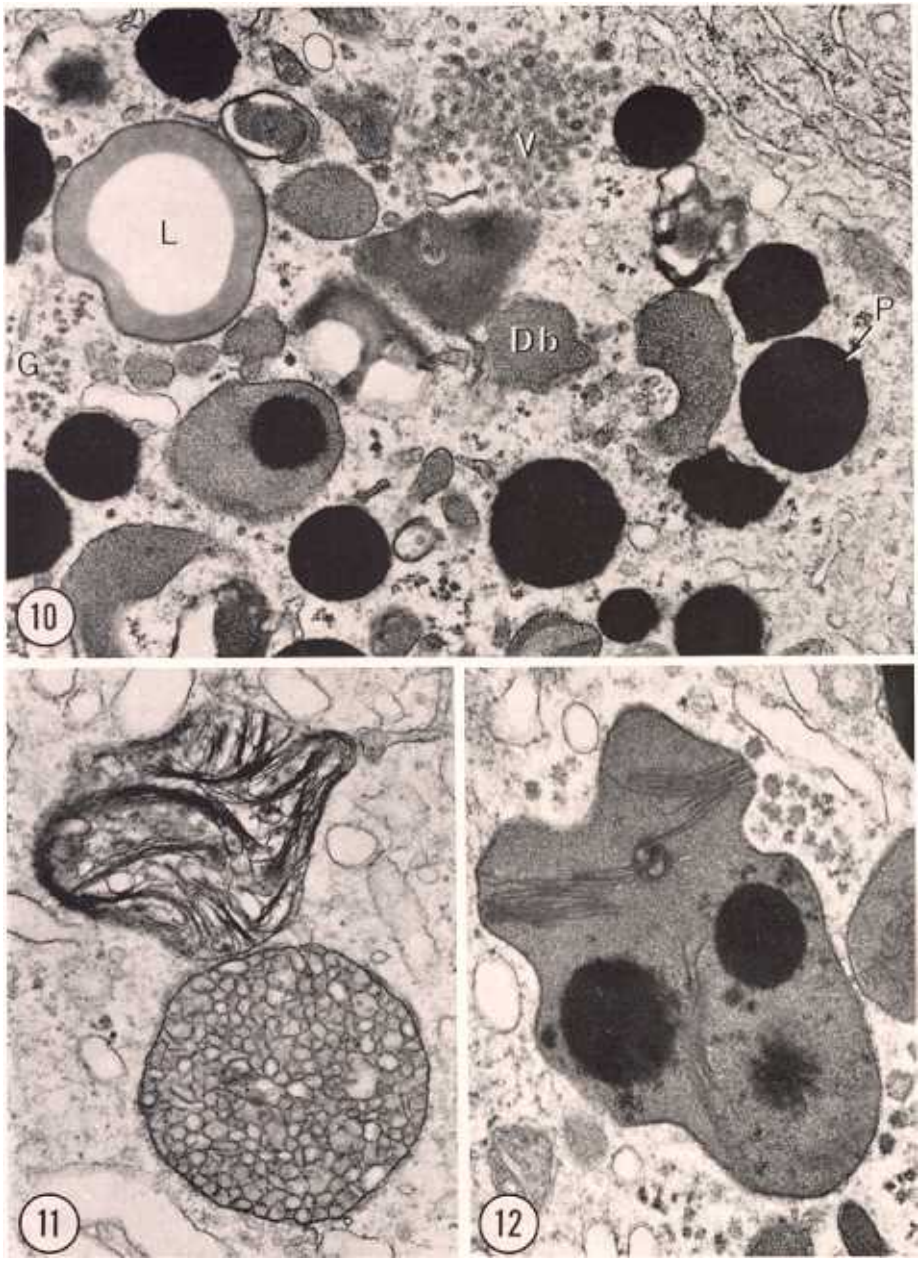


Fig. 10. A representative complement of retinula cell inclusions. *L* Lipid droplet; *DB* Dense bodies; *P* Pigment; *V* Coated vesicles; *G* Glycogen. × 28,000

Fig. 11. A multivesicular body and a presumptive residual body, containing stacks of contorted membranes. × 34,000

Fig. 12. A large autophagic vacuole, containing pigment and myeloid stacks of membranes. × 45,000

granular contents, probably regular lysosomes, similar larger bodies (autophagic vacuoles) containing pigment and myelin figures and, finally, large residual bodies filled with stacks and whorls of membranes.

The nucleus of the retinula cell is 10—12 μ in diameter (Fig. 21). Its chromatin is finely dispersed and the nucleoli (Fig. 23) are usually attached to the inner membrane of the nuclear envelope by a small heterochromatic mass, suggestive of a nucleolar organizer. All the retinula cell nuclei of an ommatidium tend to lie in one transverse plane except for the unsymmetrical retinula cell, where the nucleus lies more distal (Fig. 3).

The unsymmetrical retinula cell is distinguishable by its flared rhabdomere (Fig. 3), the microvilli of which have irregular shapes and arrangement contrasting with the very orderly structure of adjacent rhabdomeres (Fig. 5). The cytoplasm of this cell is markedly more vesicular (Fig. 9), a feature evident in the light microscope as a less opaque appearance. In addition, the cell appears to be relatively deficient in ribosomes but with some concentrations of typical tubular agranular reticulum and the normal complement of other organelles and inclusions with a possible preponderance of glycogen.

The gradually tapering axons of the retinula cells are for some distance along their lengths indistinguishable in cytoplasmic constitution from their respective somata (Fig. 24).

3. Eccentric Cells

The cytoplasm of the eccentric cell (Fig. 18) differs conspicuously from that of the retinula cell. In the soma the endoplasmic reticulum consists of scattered branched cisternae continuous with a network of tubules of the agranular reticulum that permeates the cell to the tip of its dendrite. Subsurface cisternae are common in the soma. Most remarkable is the extraordinary abundance of small and large Golgi complexes, the largest measuring 10 μ in length. Both smooth and coated vesicles are elaborated by these. Long mitochondria (up to 10 μ), accumulations of glycogen, lipid droplets, various dense and residual bodies, and ubiquitous microtubules complete the inventory of cellular contents. The nucleus, about 15 μ in diameter, has the vesicular appearance typical of a neuronal nucleus.

The dendrite measures about 200 μ in length and exhibits several distinctive features (Figs. 13, 15). It is filled with neurotubules, about 200 Å in diameter, for the most part oriented longitudinally though somewhat irregularly. The agranular reticulum seems to form a three-dimensional open meshwork throughout the dendrite, condensing here and there into tight tangles of tubules or whorls of cisternae (Fig. 16). Subsurface cisternae are quite rare. Glycogen is distributed in the dendrite in small groups of rosettes, but massive amounts appear with numerous mitochondria, often centered on a few lipid droplets (Fig. 17). Two dendrites in one ommatidium are usually separated by rhabdomal microvilli.

As LASANSKY (1967) previously observed, the surface of the dendrite has a covering of microvilli backed by a fibrous terminal web (Fig. 15). From longitudinal sections through the full length of the dendrite, the number of microvilli per unit length can be ascertained. With section thickness and variable dendrite diameter included, an approximate value of 30,000—50,000 microvilli per dendrite can be calculated. Inasmuch as many of these widely spaced microvilli appear to

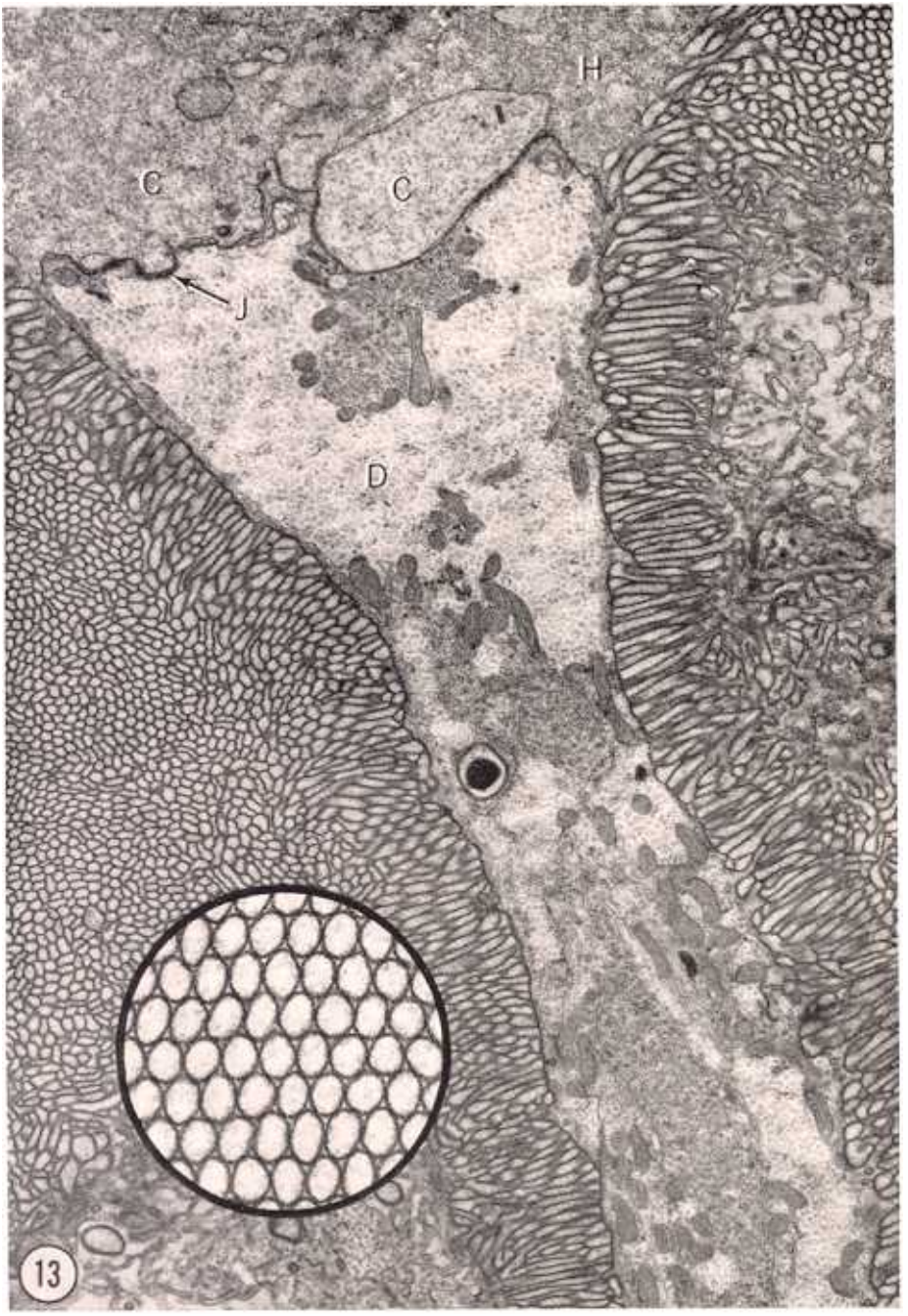


Fig. 13. Longitudinal section of the tip of the eccentric cell dendrite (*D*). Cone cells (*C*) are attached to the dendrite by adhering junctions (*J*). A small hemocyanin filled space (*H*) provides direct access for the dendrite to hemocoel. In the dendrite note the terminal web (absent at the apical surface) and abundance of organelles. The inset illustrates the tight junctions between contiguous microvilli. × 15,000; inset × 60,000

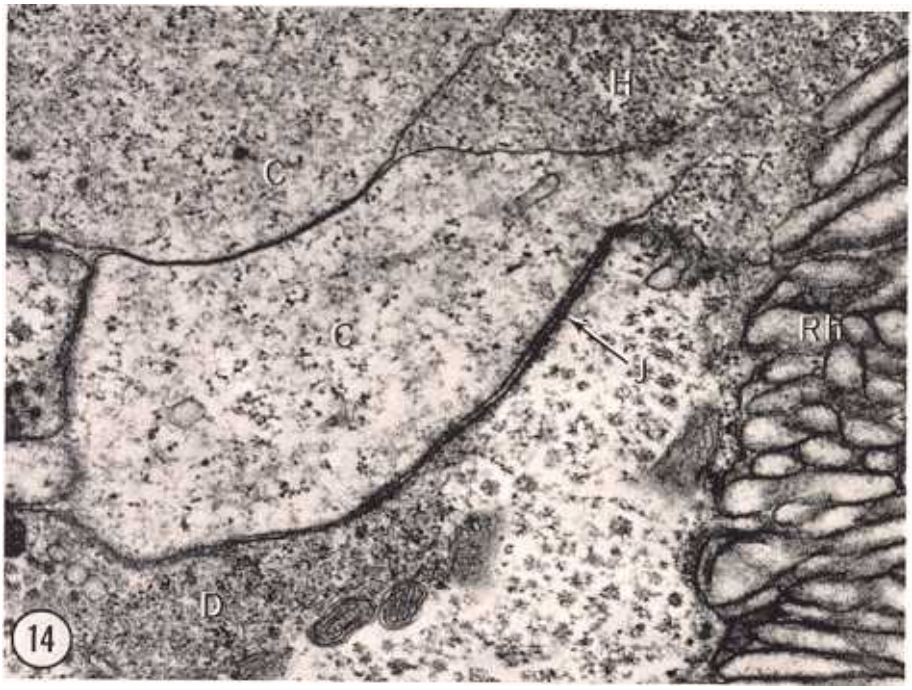


Fig. 14. The apical region of the dendrite (*D*) from a section adjacent that shown in Fig. 13. Cone cells (*C*), the dendrite and the rhabdome (*Rh*) abut against a hemocyanin filled, extracellular space (*H*). *J* Adhering junction. × 34,000

be mere stubby projections (even allowing for section thickness), it is likely that the contribution of the dendrite to total rhabdome surface is considerably less than 1%. Tight junctions are the rule between retinula cell microvilli and the surface of the dendrite.

The tip of the dendrite (Fig. 13) expands to a 7—8 μ knob from a 4 μ neck. Its apical surface is devoid of microvilli and terminal web and abuts against the cone cells or spaces between them. The tip is sometimes smoothly rounded, though more commonly indented by adjacent cone cells. The rhabdome has not been observed to cover the tip of the dendrite. The terminal knob is usually rich in cytoplasmic contents, particularly glycogen and mitochondria. The zones of contact between the knob and cone cells are focally differentiated into adhering junctions, that is 150—200 Å intercellular clefts flanked by conspicuous condensations of intracellular filamentous material (Fig. 14). Blood, devoid of hemocytes but identifiable by the characteristic hemocyanin molecules, quite commonly diffuses into the ommatidium along the cone cell-retinula cell boundaries from the peripheral vascular spaces. This blood becomes more concentrated, as seen in the tighter packing of the hemocyanin, while it stagnates in the ommatidium. It thereby serves as a marker for tissue spaces in continuity with the hemocoel. The possibility of artifactual introduction of the blood is ruled out by the changed density. Thus, the tip of the dendrite and, as a consequence, the extracellular space of the rhabdome have apparently free access to the hemocoel (Fig. 14).

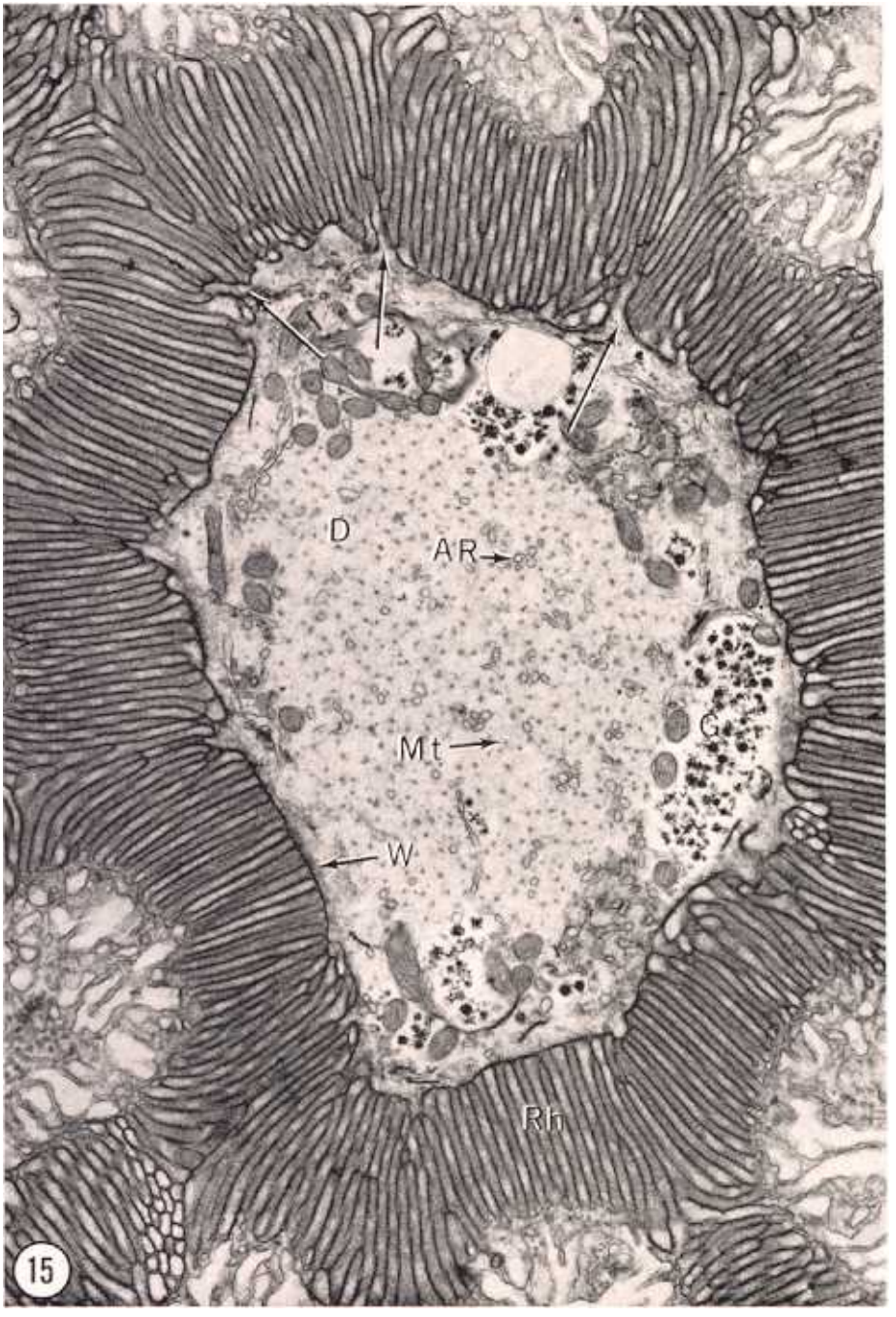


Fig. 15. Cross-section of the eccentric cell dendrite (*D*) and part of the surrounding rhabdome (*Rh*). Mitochondria and glycogen (*G*) are located adjacent to the fibrous terminal web (*W*). The center of the dendrite contains evenly dispersed profiles of the agranular reticulum (*AR*) and microtubules (*Mt*). The dendrite shows few microvillous projections (arrows). × 18,000

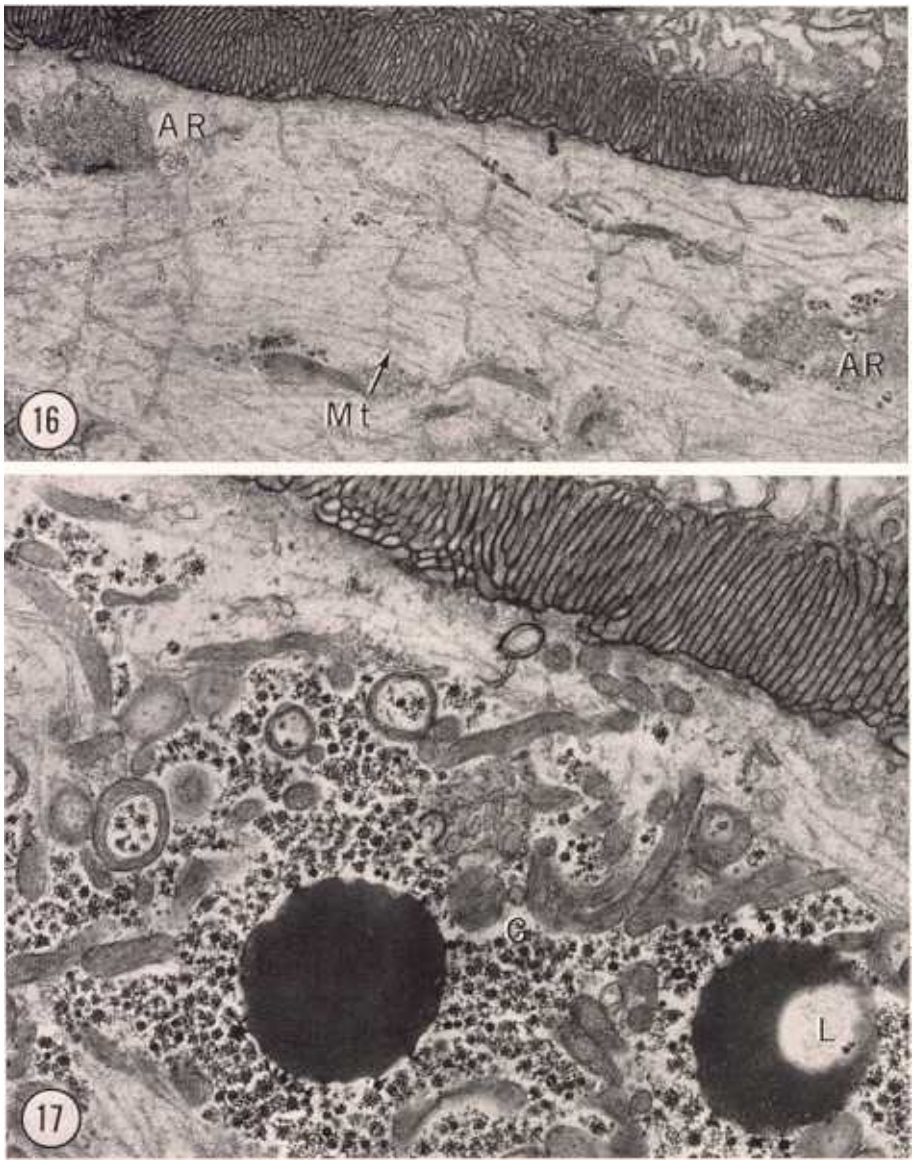


Fig. 16. Longitudinal section of the eccentric cell dendrite, illustrating the disposition of the agranular reticulum, including two clusters of tubules (*AR*), and longitudinal microtubules (*Mt*). × 9,000

Fig. 17. A characteristic accumulation in the dendrite, consisting of glycogen (*G*) and mitochondria, many of these cup-shaped, grouped around lipid droplets (*L.*). × 16,000

Occasionally, analogous conditions prevail in the proximal region of the rhabdome. The dendrite exits usually in a lateral direction through a breach in the rhabdome or the rhabdome terminates at the same level. Since the rhabdome is entirely surrounded by proximal pigment cells (vide infra) at its basal terminus

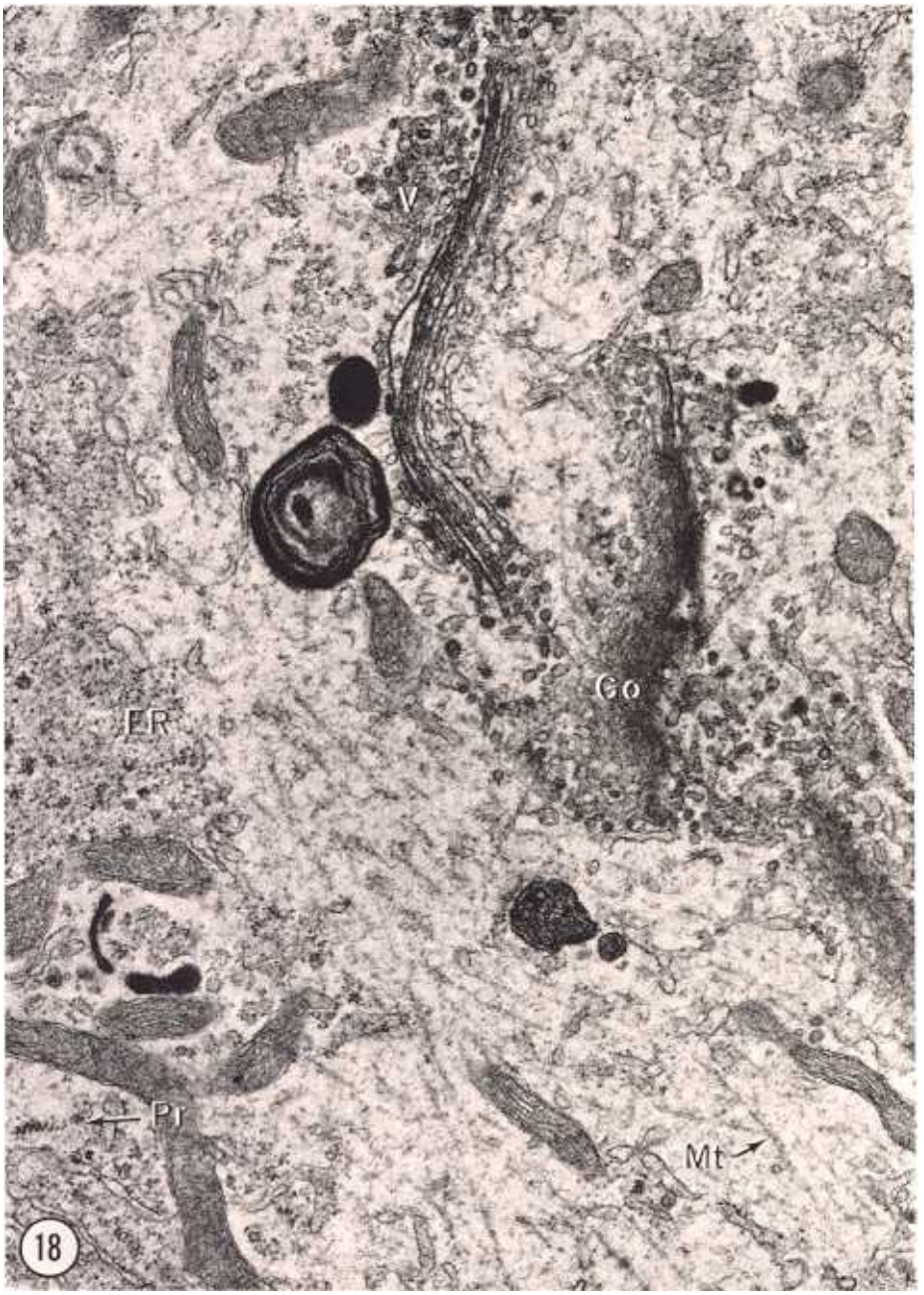


Fig. 18. Cytoplasm of the euentric cell, showing large Golgi systems (*Go*) [elaborating masses of coated vesicles (*V*)], endoplasmic reticulum (*ER*), polyribosomes (*Pr*), microtubules (*Mt*), mitochondria and assorted dense bodies. × 26,000

(Fig. 24), the dendrite acquires a sheathing of these immediately upon leaving the rhabdome. The junctional zone between the three involved cell types is not sealed tightly enough to exclude occasional infiltration of hemocyanin into the

terminal region of the rhabdome. Most likely the passage way is formed by pigment cell to pigment cell clefts, since these spaces contain hemocyanin in greater or lesser amounts throughout the eye, whereas the pigment cells doubling in function as glial cells adhere more tenaciously to their respective neurons.

The eccentric cell axon is usually slightly constricted at its origin from the soma, i.e. at the axon hillock. Beyond this point the diversity of cytoplasmic contents decreases abruptly.

4. Cone Cells

The fine structure and disposition of these epidermal cells as well as their intraommatidial processes have been described in the first paper of this series (FAHRENBACH, 1968). GRENACHER (1879) has a noteworthy illustration that suggests cone cell processes. Closer investigation of the proximal part of the ommatidium discloses that the cone cell processes terminate short of the end of the rhabdome, their position being supplanted in an identical manner (with respect to shape and junctions) by distally directed processes of the proximal pigment cells (Fig. 8). This change-over accounts for the appearance of pigment in what, judged by their position, look like cone cell processes. Actually, pigment droplets are extraordinarily rare in the cone cells.

Since the two facing cell projections, i.e. of the cone and proximal pigment cells, do not necessarily meet end-to-end, they provide one of the rare occasions of retinula-retinula cell contact. Such a zone of apposition is differentiated into an adhering junction (Fig. 7).

5. Pigment Cells

The distal pigment cells, which form a peripheral sleeve over the distal half of the ommatidium, have been previously described (FAHRENBACH, 1968).

Pigment cells in the body of the ommatidium can be designated as proximal and intraommatidial cells that cannot be differentiated by cytoplasmic criteria, although several nuclear types can be distinguished. About 100 proximal pigment cells are grouped in a mass under the ommatidium (Fig. 24). Their nuclei are elongated like the attenuated shape of the cells themselves and measure 10—12 μ in length and 2—5 μ in diameter. Heterochromatin is distributed in coarse clumps. The pigment is concentrated in a subommatidial position in a dark-adapted animal. Long distal processes of complex cross-sectional shape project between and around eccentric and retinula cells. Since any one cell can be followed only for a limited, even though considerable, longitudinal extent, it is uncertain where the proximal pigment cells terminate in the ommatidium and to what degree they overlap with intraommatidial pigment cells. Binucleate cells have only rarely been seen in the present study, an observation supported by the work of WATÁSE (1890) on dissociated cells of the ommatidium.

At medial levels in the ommatidium, the two types of pigment cells contribute to the ommatidial architecture in several ways. They form a tight glial covering over the visual cells and, thereby, partitions between them. These consist of up to 6 more or less loosely packed sheets of visual cells, although on occasion a 300—400 Å thick cell constitutes the sole barrier (Fig. 9). Generally, the partitions are quite compact near the rhabdome (Fig. 6) but loosen in a radial direction. Retinula cells, and to a lesser degree, the eccentric cell are invaded by deep infoldings of

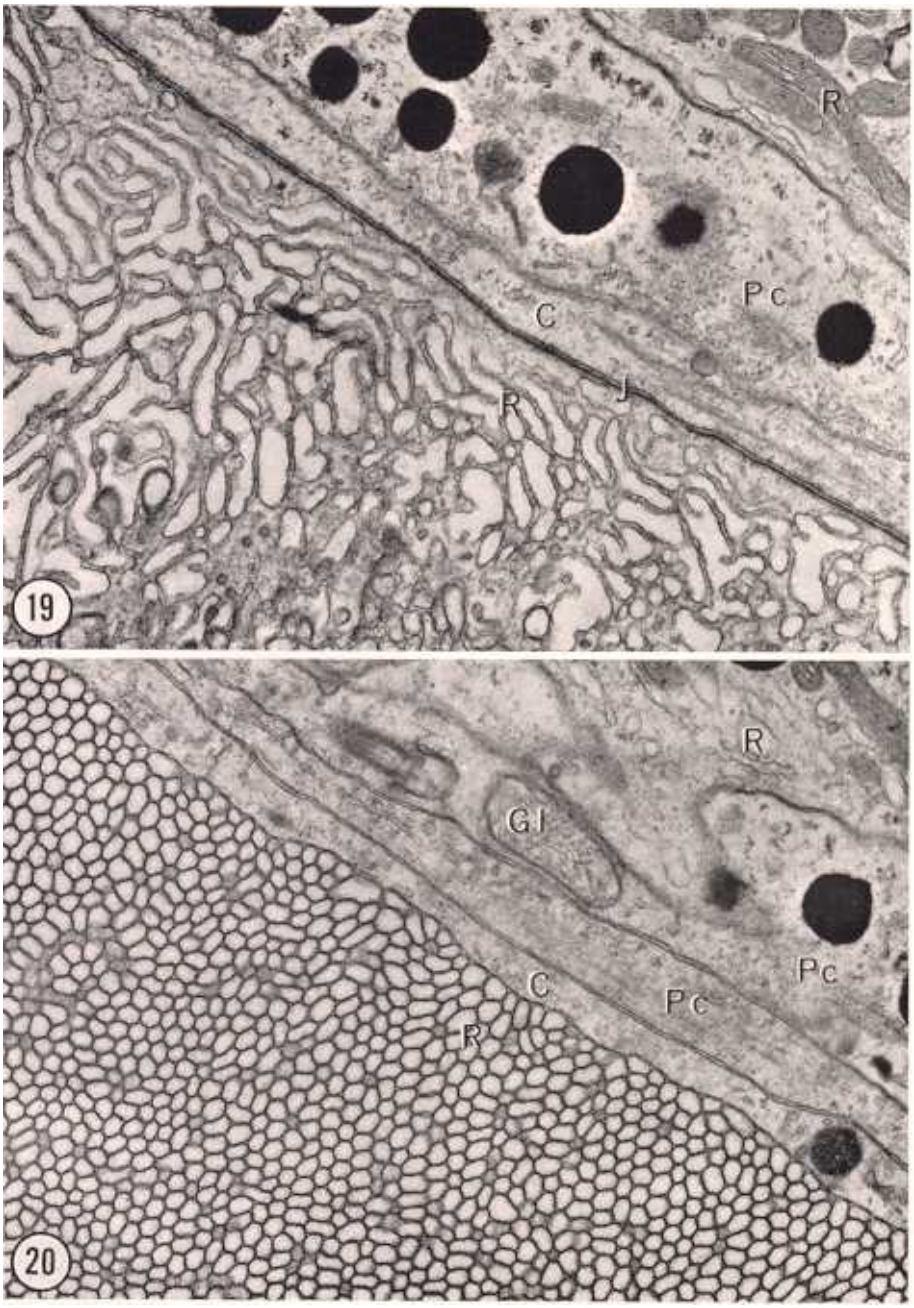


Fig. 19. Longitudinal section of the edge of the rhabdome in a plane indicated by line *A* in Fig. 6. The cone cell process (*C*) is bonded to the palisade zone of the retinula cell (*R*) by a continuous adhering junction (*J*). *Pc* Pigment cell. × 20,000

Fig. 20. Longitudinal section of the edge of the rhabdome in a plane indicated by line *B* in Fig. 6. No junctions are in evidence between the retinula cell (*R*), cone cell processes (*C*), pigment cell (*Pc*) or glial cell process (*Gl*). The rudimentary cilium is located in a pigment cell. × 20,000

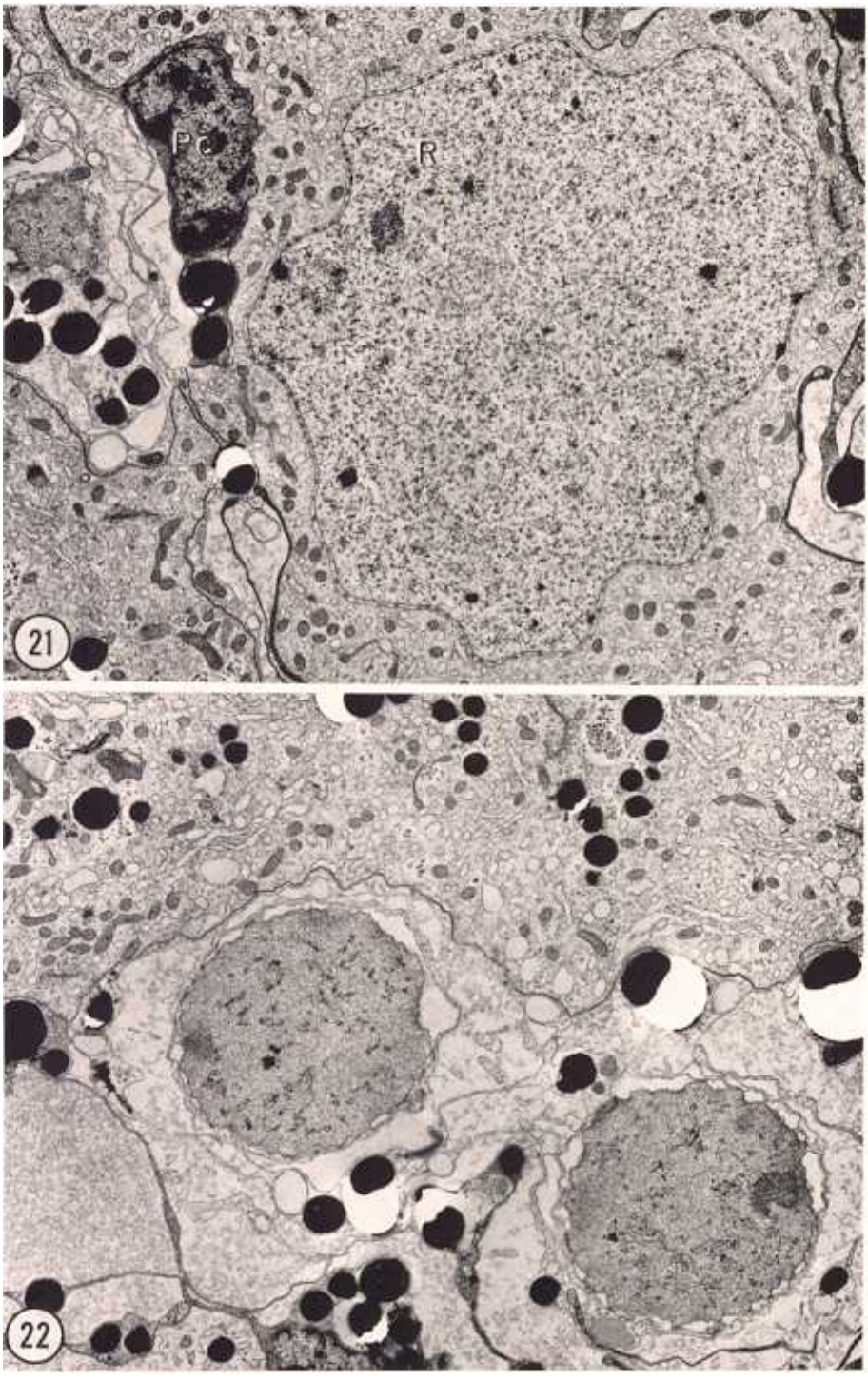


Fig. 21. A comparative illustration of the nucleus of a retinula cell (*R*) and an intraommatidial pigment cell (*Pc*). The retinula cell nucleus has an abundance of nuclear pores. × 7,500

Fig. 22. Two nuclei, presumed to belong to a second type of intraommatidial pigment cell. × 7,500

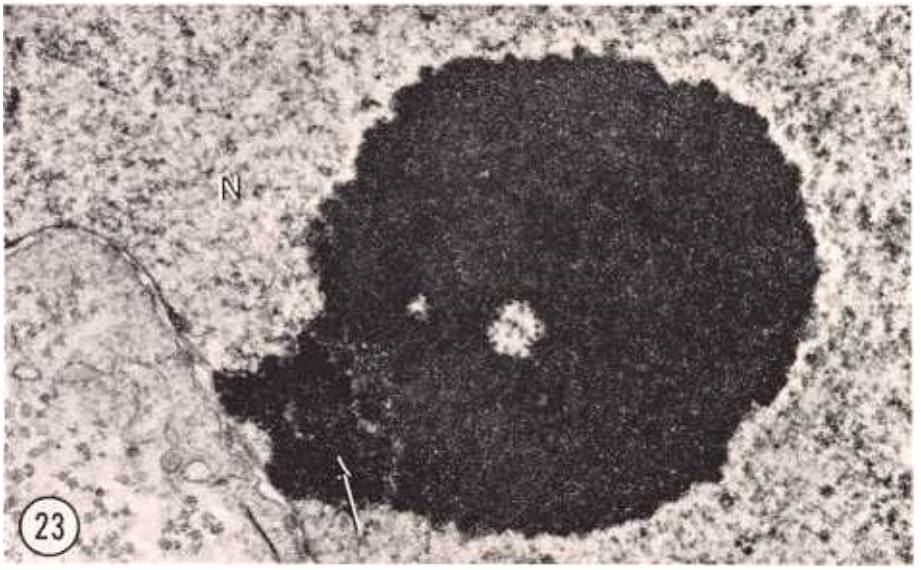


Fig. 23. The nucleolus of a retinula cell nucleus (*N*), attached to the nuclear envelope by a heterochromatic mass (arrow). × 24,000

pigment cells. The periphery of the ommatidium is composed of the peculiar cancellate framework of pigment cell sheets with hemocyanin filling the interstices (Fig. 25). Similar infiltration occurs along the very narrow clefts between pigment cells; here hemocyanin is found as small masses of characteristic molecules, particularly in the compact parts of the partitions (Fig. 9). LASANSKY (1967) referred to these accumulations as granular ground substance.

In addition to quantities of pigment, both cell types contain abundant mitochondria, α-glycogen, small amounts of granular and agranular reticulum, occasional subsurface cisternae and conspicuous bundles of microtubules. The latter comprise 10—100 or more tubules, in strict longitudinal orientation, generally situated in the edges of the fluted projections of the pigment cells (Figs. 25, 26). During light adaptation the proximally located pigment migrates distad along with some accompanying cytoplasm. During this process, rows of pigment droplets are often seen aligned in pearl-necklace regularity alongside a bundle of microtubules. Occasionally centrioles and associated rudimentary cilia are seen consisting primarily of a 9 + 0 basal body and a well-developed ciliary vesicle (SOROKIN, 1962) (Fig. 20).

Two nuclear types are found among the intraommatidial pigment cells. The more common is relatively pyknotic in appearance, elongated in outline with a smaller cross-section of 1—2 μ (Fig. 21). The second type is spherical with a diameter of 4—5 μ and finely dispersed chromatin (Fig. 22). No clear difference could be established between the two types of cells.

6. Neuroglia Cells

All pigment cells of the ommatidium act in a supportive or quasi-glial role for the sensory cells as well as for the neurosecretory axons (Figs. 6, 26). In addition, however, regular unpigmented neuroglial cells are found in the ommatidium with

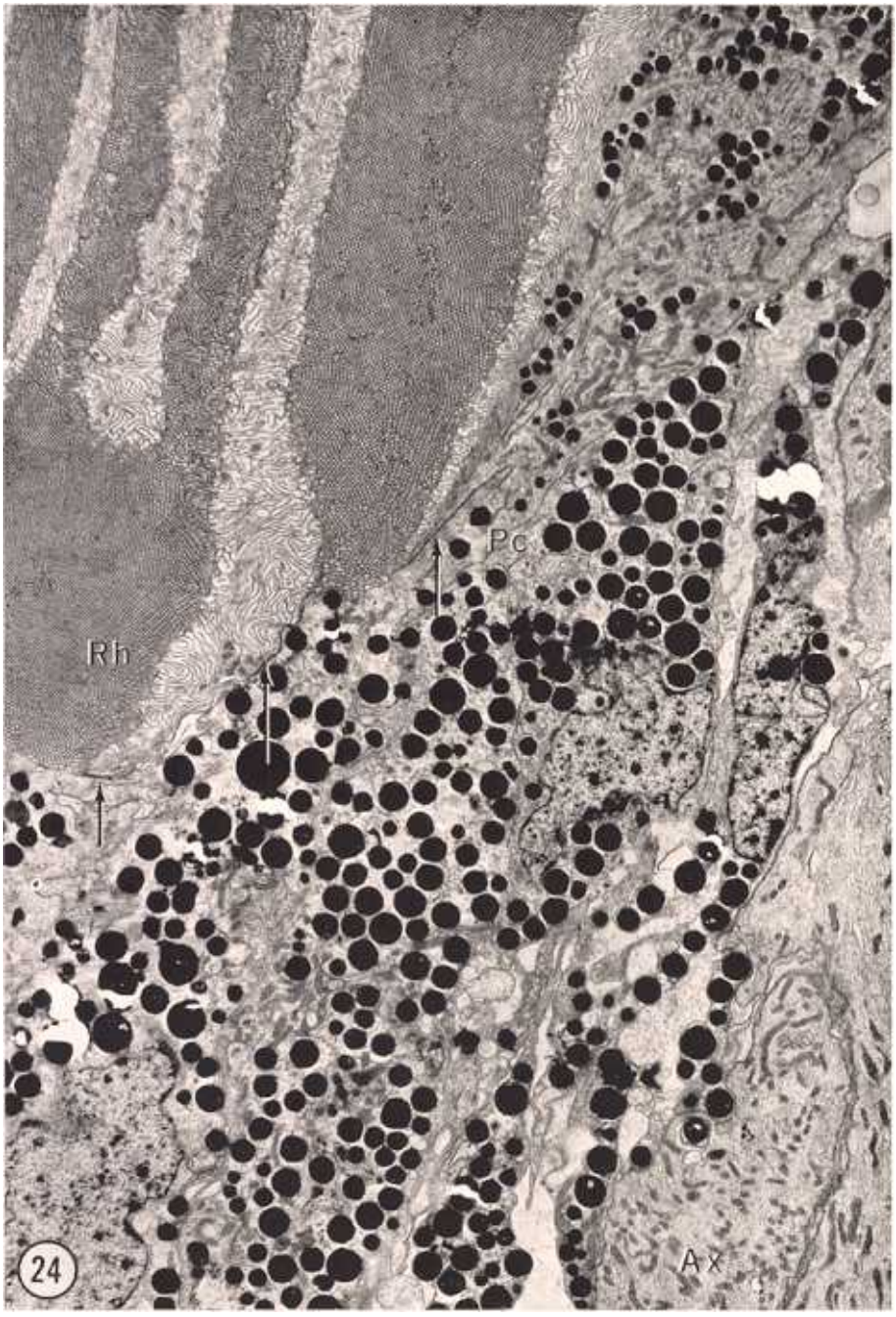


Fig. 24. Longitudinal section of the base of the rhabdome (*Rh*) and the surrounding cup of proximal pigment cells (*Pc*). Note the relatively large nuclei as compared to those of intraommatidial pigment cells (Fig. 21). Adhering junctions (arrows) are present between pigment cells and non-rhabdomeral surfaces of the retinula cells. Two retinula cell axons (*Ax*) leave the picture at the lower right corner. × 4,500

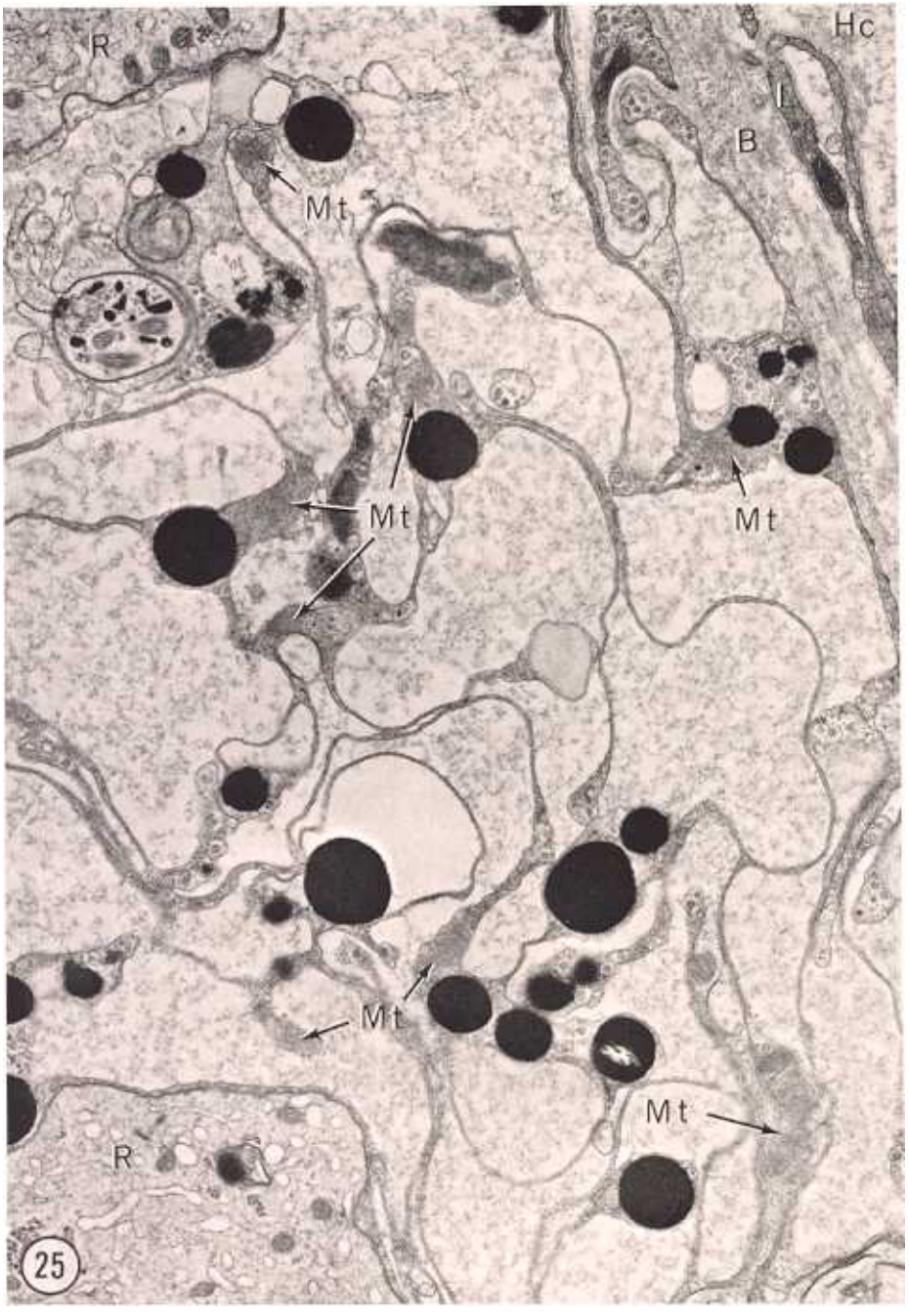


Fig. 25. Cross-section of the periphery of an ommatidium. The area shown constitutes the loose portion of the pigment cell partition between two retinula cells (*R*), both of which have a tight investment of one pigment cell. Numerous bundles of microtubules (*Mt*) are located in the pigment cells. Interstitial spaces contain granular appearing hemocyanin. The basement membrane (*B*) and vascular lining cells (*L*) separate the ommatidium from the hemocoel (*Hc*). The region at the upper left is shown in Fig. 26. × 13,000

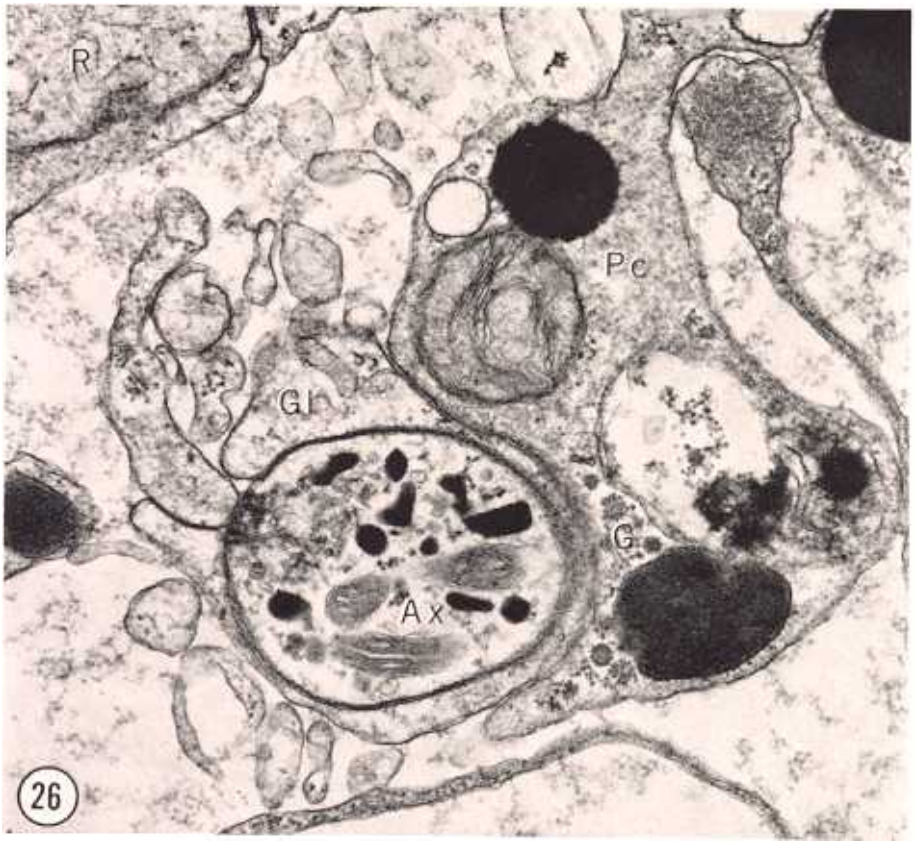


Fig. 26. An enlargement of part of Fig. 25. A typical bundle of microtubules in the edge of an intraommatidial pigment cell is shown at the upper right. The neurosecretory axon (*Ax*) is invested by a neuroglial cell (*Gl*). The intra-axonal differentiated area is indicative of a secretory terminus. *R* Retinula cell; *Pc* Pigment cell; *G* Glycogen. × 32,000

a pronounced concentration between the distal pigment cells. Their cytoplasm appears empty except for a few mitochondria, small amounts of endoplasmic reticulum and few free ribosomes. Occasionally, masses of tubular agranular reticulum are seen. The elongate nucleus, measuring about 4 by 8 μ, contains large, evenly distributed clumps of chromatin. Processes of these cells are extremely elongated in the long axis of the ommatidium (Fig. 27). They contain more or less deeply embedded neurosecretory axons and are most conspicuous between the distal pigment cells because of their abundance and their contrasting lack of cytoplasmic opacity. They do not form a glial wrapping around axons and at best show short mesaxons.

7. Junctional Specializations

The extensive tight junctions within the rhabdome have been mentioned (Fig. 13). Outside this area they are almost completely absent, being confined to spot-like welds between pigment cells. Viewed in longitudinal sections of entire

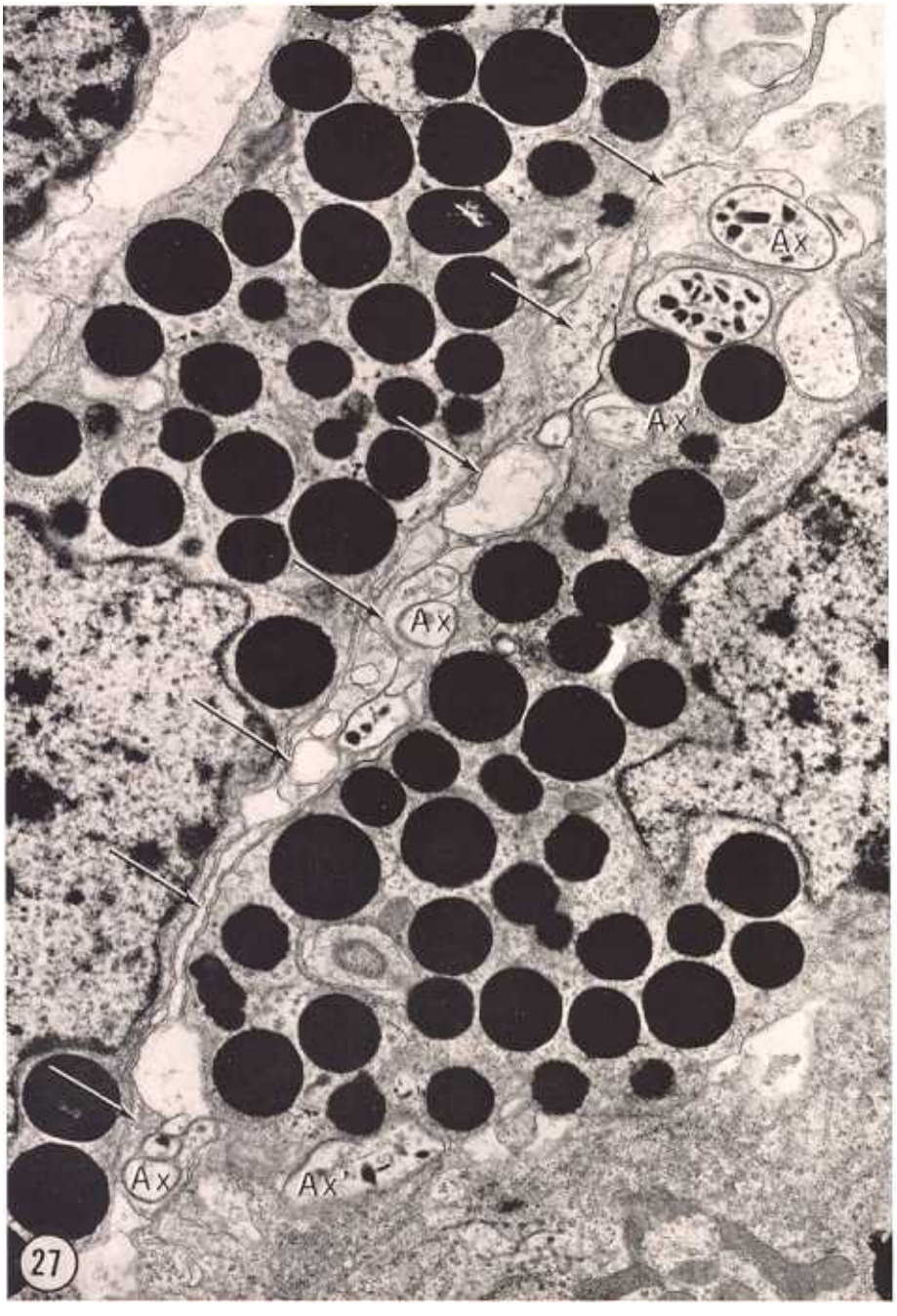


Fig. 27. Longitudinal section of proximal pigment cells at the base of the ommatidium. Numerous neurosecretory axons (*Ax*) are invested by one or two attenuated and slightly vesiculated glial cells (arrows). Two axons (*Ax*') are carried by pigment cells. × 14,000

ommatidia, an intercellular cleft of 200 Å or slightly larger is continously maintained in the compact region of the partitions between retinula cells as well as at the surface of the sensory cells over distances of as much as 30—50 μ. Eyes fixed according to the method of LASANSKY (1967) contain an abundance of tight junctions between all cell types.

A second type of junction is common in the ommatidium, namely an adhering junction, be it a *fascia, zonula* or *macula adhaerens* (FARQUHAR and PALADE, 1963). The rhabdome is sealed on all sides by adhering junctions against the extracellular space, the exceptions being the tip and the exit of the eccentric cell dendrite. The cone cell processes and a few adjacent pigment cells are bonded to both neighboring retinula cells along each radius of the rhabdome (FAHRENBACH, 1968) (Figs. 6, 19). Proximal to the termination of the cone cell process, the two adjacent retinula cells adhere by a short adhering junction (Fig. 7) or, more commonly, an extension of a proximal pigment cell supplants the position of the cone cell process with similar junctional specializations. In addition, cone cells and proximal pigment cells are extensively bonded by adhering junctions to any nonrhabdomeral surface of the retinula cells (Fig. 24).

The only consistent adhering junctions with the eccentric cell are those between the tip of the dendrite and the adjacent cone cells (Fig. 14). In very rare instances, the periphery of the rhabdome is breached to allow an unmodified surface of a retinula cell to touch the surface of the dendrite. Such contact zones consist entirely of adhering junctions.

8. Basement Membrane

The basement membrane, which covers the surface of each ommatidium and is continuous with that of the epidermis and of the subjacent plexus, is a complex sheath comprising cellular and fibrillar elements. It is 1—3 μ thick, usually less than that over the ommatidium proper and thicker around the ramifications of the plexus. Its composition resembles that of the enveloping sheaths of peripheral arteries of *Limulus* (DUMONT *et al.*, 1965), but it is more homogeneous. The diffusely fibrous material, presumably mucopolysaccharide, contains scattered banded filaments of collagen (HARPER *et al.*, 1967), about 100 Å in diameter and often in excess of 10 μ long as seen in thin sections. Values of the periodicity lie between 490 and 540 Å. Highly attenuated connective tissue cells form the hemocoelic boundary of the basement membrane. As mentioned previously, the basement membrane is easily permeable to hemocyanin molecules.

Discussion

The terminology used here calls for a brief explanation, especially since it deviates from that employed by LASANSKY (1967), who assigned all non-neural elements of the ommatidium to the glial category. The plan of the *Limulus* eye closely resembles that of an exocone insect eye, including the cuticular cone, cone cells, and several types of sheathing pigment cells. The three types of insect pigment cells (BULLOCK and HORRIDGE, 1965) parallel those in the *Limulus* eye. Primary pigment cells (distal p.c.) appear to function as an iris; accessory pigment cells (intraommatidial p.c.) fill the spaces between retinula cells; and basal pigment cells (proximal p.c.) lie underneath the ommatidium but extend between

retinula cells. In view of these similarities and of the existence of neuroglial cells in the *Limulus* eye, it would seem inappropriate to lump these diverse cell types into one category.

Perhaps the most vexing problem of the present investigation is that of fixation and its bearing on physiologically important aspects of the ultrastructure of the eye. Specifically, there is a discrepancy between the abundance of tight junctions described here and in the study of LASANSKY (1967). In eyes fixed with a great variety of fixatives, both hypo- and hypertonic, the only reliable tight junctions are those between the rhabdomeral microvilli and those that are part of standard junctional complexes between epidermal cells. The principal difference between the fixative of LASANSKY and those employed in the present study is the considerably higher concentration of sucrose in the former. That this high sucrose content is responsible for the artifactual formation of tight junctions is borne out both by the results of the comparative fixations used here and by studies by MAUNSBACH (1966a, b), who has treated this phenomenon of glutaraldehyde fixation in some detail. In view of the considerable hypertonicity of the fixative employed for all successful fixations of the present study, the small tight junctions between pigment cells should probably be viewed with suspicion. Conversely, the good over-all histological and cytological preservation inspires more confidence in the regularity of narrow intercellular clefts seen between visual, pigment, and glial cells as well as around neurosecretory axons.

WHITE (1967), studying the response of the larval mosquito eye to light- and dark-adaptation, observed the formation of tight junctions between microvilli as a function of increased rhabdome volume. Inasmuch as the rhabdomes of the *Limulus* eye do not appear to change volume under similar experimental conditions, the ubiquitous tight junctions are most likely not an expression of a particular functional state. WHITE's suggestion that the varying diameter of microvilli and, thereby, the abundance of tight junctions are influenced by differential ionic distribution during various physiological states can be viewed differently, namely that such ion distribution changes the response of the particular morphological component to the fixative. A pure glutaraldehyde fixative would probably accentuate such differences on account of its low rate of penetration (GRIFFIN, 1965).

Several features of morphological rather than neurophysiological importance merit discussion first. The unsymmetrical retinula cell, despite its rather constant occurrence in well-fixed ommatidia, is not oriented to relate to that in adjacent ommatidia or to any other element of the eye, and its significance remains enigmatic. Its normal complement of tight junctions in the rhabdome precludes its being easily implicated in any neurophysiological processes other than those observed in other retinula cells.

The membranes of the rhabdomal microvilli have the faintly beaded appearance common in photoreceptor membranes of both vertebrates and invertebrates (FERNÁNDEZ-MORÁN, 1962; NILSSON, 1965; WHITE, 1967), a feature that is probably expressive of the visual pigment molecules [rhodopsin in *Limulus* (HUBBARD and WALD, 1960)] incorporated in the membrane structure. In this connection, on the basis of experiments dealing with the photoelectric (early receptor) potential of the *Limulus* eye, SMITH and BROWN (1966) have suggested that the

visual pigment may be such an intimate component of the rhabdomeral membrane that it directly influences the electrical properties of the membrane. Granules in the rhabdome, repeatedly mentioned by LASANSKY (1967), have not been observed.

The peculiar prominence of coated vesicles at the base of rhabdomers in various arthropods has been commented on by a number of authors (WADDINGTON and PERRY, 1960; MELAMED and TRUJILLO-CENÓZ, 1966; EGUCHI and WATERMAN, 1967, 1968; WHITE, 1967). The interpretation of this association has uniformly been one of presumptive protein uptake in the manner illustrated by ROTH and PORTER (1964) for the mosquito ovary. In that instance, coated vesicles sequester proteins out of the easily accessible hemocoel, migrate inward and fuse to form larger yolk droplets. In the retinula cells of the *Limulus* eye, coated vesicles are virtually absent from the periphery of the cell and are common at the base of the rhabdomeres, a site that appears singularly unpromising of having free access to diffusing proteins from the hemocoel.

An alternate interpretation is suggested by this disposition, namely that of secretion of a substance into the rhabdome by way of coated vesicles. The possibility of export is supported by several observations and experimental results. FRIEND and FARQUHAR (1967) documented in a detailed study of absorptive and secretory processes in the rat vas deferens a category of coated vesicles originating from Golgi cisternae and migrating outward. Some of these transport hydrolytic enzymes to larger elements of the lysosomal series, whereas others fuse with the surface, although the nature of their contents remains unknown. For this latter type the function of mucopolysaccharide transport has been suggested by FAHRENBACH *et al.* (1966) in fibroblasts of ligamentum nuchae of the calf. Several lines of investigation provide supporting evidence for this hypothesis. The role of the Golgi complex in synthesizing complex carbohydrates has been demonstrated repeatedly by its incorporation of labeled glucose and galactose (PETERSON and LEBLOND, 1964; PETERSON-NEUTRA, 1965; NEUTRA and LEBLOND, 1966a, b), its participation in sulfation (LANE *et al.*, 1964) and its acid mucopolysaccharide content in secreting cells (BERLIN, 1967). Circumstantial evidence that points to mucopolysaccharide transport by coated vesicles in animal cells has been considered by FAHRENBACH *et al.* (1966). Coated vesicles in plant cells also merit consideration, in that protein uptake by them is an exceedingly unlikely function. Conversely, their production is clearly associated with the Golgi system (BONNETT and NEWCOMB, 1966; NEWCOMB, 1967) and is particularly prominent in differentiating cells with their presumptive larger output of structural polysaccharides.

The membrane array under the rhabdomeres, referred to as the palisade, is replaced by shielding pigment during light adaptation (MILLER, 1958) either by obliteration or by displacement. It is, therefore, unlikely that these cisternae participate in photochemical transduction as suggested by RUCK (1964) and LASANSKY (1967), but rather that they function in changing the refractive index of the near-rhabdomeral cytoplasm, as detailed by HORRIDGE and BARNARD (1965) in locusts. Similar changes occur during light and dark adaptation in the crab *Libinia* (EGUCHI and WATERMAN, 1967). It follows from these observations that a morphological or functional connection between the palisade and subsurface cisternae is unlikely. The latter are not particularly common in the retinula cells and have no relationship to tight junctions contrary to the suggestion of

LASANSKY (1967). The occasional subsurface cisternae of pigment cells generally face adjacent pigment cells rather than visual cells. In the eccentric cell soma, these cisternae have a size range and distribution similar to that described by ROSENBLUTH (1962) in vertebrate neurons.

The density of organelles and inclusions in the retinula cells point to a high rate of protein synthesis and catabolism. It is more likely that the protein involved in this turnover is the visual pigment rather than the shielding ommochrome, inasmuch as a vastly greater concentration of ommochrome in the pigment cells is not paralleled by a proportionate amount of endoplasmic reticulum. EGUCHI and WATERMAN (1967) have determined that in the crab *Libinia* the quantity of endoplasmic reticulum, multivesicular and lamellar bodies increases with light adaptation, involving these cytoplasmic structures further in an at least indirect participation in the visual process. Although multivesicular bodies have been assigned with good cause to the lysosomal sequence (GORDON *et al.*, 1965), they occur in such conspicous quantities and sizes in arthropod photoreceptor cells (FAHRENBACH, 1964; TRUJILLO-CENÓZ, 1965; MELAMED and TRUJILLO-CENÓZ, 1966; EGUCHI and WATERMAN, 1967) that it seems reasonable to ask whether these structures might not represent a membranous substratum for the storage of the visual pigment or of a part thereof. Cytochemical techniques are essential to elucidate the function of these bodies.

The neurophysiological studies of visual processes in the *Limulus* eye have achieved such a degree of complexity that they almost exceed the competence of the average morphologist. Nevertheless, it seems desirable to discuss those morphological features that are most likely to have physiological importance, particularly as they provide a substratum for further experimental investigations.

The electrical responses of retinula and eccentric cells are well known primarily as a result of numerous studies using direct intracellular recording techniques. The retinula cells display large amplitude slow potentials with small or no superimposed spikes, while the eccentric cell shows a small amplitude slow potential with large superimposed spikes (MACNICHOL, 1956; FUORTES, 1958; BEHRENS and WULFF, 1965). The large action potentials observed in the optic nerve originate exclusively in the eccentric cell, specifically in the region of the axon hillock (HARTLINE *et al.*, 1952a, b, 1956; WATERMAN and WIERSMA, 1954). The generator potential of the retinula cells in one ommatidium shows a high level of synchrony, a phenomenon congruous with the existence of electrical coupling between retinula cells (TOMITA *et al.*, 1960; SMITH *et al.*, 1965; STIEVE, 1965; BEHRENS and WULFF, 1965) and the corresponding presence of tight junctions between retinula cell microvilli demonstrated by LASANSKY (1967) as well as in the present study. Numerous correlated morphological and neurophysiological studies have convincingly demonstrated the reliable association between sites of tight junctions and the existence of electrotonic transmission (BARR *et al.*, 1965; BENNET *et al.*, 1963, 1967a—d; FURSHPAN and POTTER, 1959; PAPPAS *et al.*, 1967; ROBERTSON, 1955, 1961). Tight junctions, identical in appearance, prevail between the retinula cells and eccentric cell dendrite. However, the generator potential in the retinula cells precedes that of the eccentric cell by 6—30 msec (BEHRENS and WULFF, 1965), a synaptic delay that is not characteristic of electrotonic transmission with its more usual delay of less than 1 msec. Chemical transmission for this same junction has

been suggested (FUORTES, 1959), but the typical morphological features of a chemical synapse are not present. No attempt has been made in the present study to differentiate between tight junctions and gap junctions (REVEL and KARNOVSKY, 1967). The latter type would be similar to tight junctions in its physiological characteristics, but might require larger amounts of extracellular content.

Electrotonic synapses are frequently irreciprocal, or rectifying, in their transmission characteristics. Incomplete irreciprocity may account for the appearance of small spikes in the retinula cells synchronous with those of the eccentric cell, a phenomenon that may be due to antidromic invasion of spikes into the retinula cells through the retinula-eccentric cell dendrite junction. On the other hand, reciprocity in the retinula-retinula cell junctions would be expected. If they were irreciprocal, one might have to consider directional transmission toward one cell (the unsymmetrical retinula cell ?) and some neurophysiological nonequivalence from cell to cell. This latter possibility may account for the occasional precedence of the generator potential of the eccentric cell over that of the retinula cells (STIEVE, 1965). If one contemplates the extraordinary complexity of intra- and extracellular compartmentalization in the ommatidium, it appears likely that a wide range of subtle neurophysiological differences detected in the course of double electrode, intracellular recording experiments (DOWLING, 1968) can be explained by the nonequivalent placement of microelectrodes.

The remaining physiological considerations pertain to the ommatidium as a whole and specifically to the potential pathways of current flow as suggested, for example, by the current flow diagrams of LIPETZ (1960) and RUCK (1962). All sensory cells are surrounded by usually multiple layers of overlapping pigment cells, which have a much higher resistivity than the sensory cells (LIPETZ, 1958). Combining this observation with the results obtained on glial cells of the leech (KUFFLER and POTTER, 1964; NICHOLLS and KUFFLER, 1964), one can conclude that the ommatidium is contained within a highly insulating envelope of pigment cells, which probably maintain their level of polarization irrespective of electrical activity in their immediate vicinity. The clefts between the pigment cells would provide a patent, though tortuous and restricted, pathway of current flow, which is probably occluded to a considerable degree by the extensive adhering junctions around the rhabdome. Thus, the extracellular space of the rhabdome is continuous with the hemocoel primarily at the tip of the dendrite. The site where the dendrite exits from the rhabdome appears to be better sealed, although occasional hemocyanin infiltration can be observed. Hence, the extracellular space of the rhabdome is also in continuity with the 200 Å cleft between the soma of the eccentric cell and its surrounding glial cell. Since no special junctions exist between glial cells in this area, current could flow into the surrounding hemocoel at the level of the eccentric cell soma. In addition to the somewhat speculative functions of the perirhabdomal junctional complexes, there is the certain one of providing structural support between adjacent retinula cells. This is suggested by the common existence of adhering junctions or desmosomes at analogous sites in the compound eyes of insects (GOLDSMITH, 1962; HORRIDGE, 1966; WHITE, 1967) and crustaceans (EGUCHI and WATERMAN, 1966). GOLDSMITH (1962) originally suggested that these junctions constitute a block to a radial, low resistance pathway for ionic diffusion, although the theoretical need for this arrangement in the absence of an eccentric cell has not been explored.

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