FINE STRUCTURE OF THE RETINULAE IN THE COMPOUND EYE OF THE HONEY-BEE

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ABSTRACT

The retinula of the compound eye of the worker honey-bee has been examined with the electron microscope. The rhabdom lies on the ommatidial axis; it is usually cylindrical in shape, about 3 to 4 μ in diameter, and lacks an axial cavity. Cross-sections show it to be four parted, although it is formed from eight retinular cells (Figs. 2, 3). Each quadrant of the rhabdom consists of a closely packed parallel array of tubules with long axes perpendicular to the axis of the rhabdom. The tubules in adjacent quadrants of the rhabdom are mutually perpendicular. At the distal end of the ommatidium these tubules are seen to be microvilli of the retinular cells. Immediately surrounding the rhabdom, the cytoplasm of the retinular cells contains a membranous endoplasmic reticulum which is oriented approximately radially with respect to the axis of the ommatidium. Farther away from the rhabdom the cytoplasm contains numerous mitochondria.

The eyes of arthropods contain rhabdoms, elongate structures composed of closely packed, hexagonal tubules (1, 3, 4, 6, 10–12). The rhabdom is thought, for good but nevertheless indirect reasons, to be the site of photoreception in these eyes (*cf.* 4, 9). The following is a preliminary description of the microstructure of the rhabdom of the compound eye of the worker honey-bee and its relation to the retinular (sense) cells from which it arises.

The compound eye of the worker honey-bee consists of several thousand *ommatidia.* Fig. 1 is a diagrammatic representation of a single ommatidium as seen in longitudinal section. Light enters from the top and passes through the "*lens*" and *crystalline cone.* Lateral scattering to adjacent ommatidia is diminished by the sleeve of *pigment cells.* Each retinular cell is a primary sensory neuron with a short axon on its proximal end. The *rhabdom* occupies the axis of the ommatidium and is formed by the eight retinular cells. That part of the rhabdom associated with a single retinular cell is known as a *rhabdomere.* A rhabdom and its complement of retinular cells are called collectively a *retinula.*

METHODS

Worker bees (*Apis mellifera*) were taken from a small laboratory hive, decapitated, and the head halved or quartered with sharp scissors in chilled fixative. The fixative had the following composition: 0.14 M veronal acetate, 10 ml; 0.1 N HCl, 6 ml; 1 per cent $CaCl_2$, 1 ml; sucrose, 1.5 gm; 4 per cent OsO_4, 25 ml; distilled water, 8 ml. The pH was 7.8–7.9. Fixation proceeded for 30 minutes in a refrigerator (4°C) followed by 30 minutes at room temperature. The tissue was dehydrated in acetone and embedded in Araldite epoxy-resin. Sections were cut on a Porter-Blum ultramicrotome with a glass knife, flattened with xylene vapor, and picked up on carbon-filmed grids. Prior to examination the sections were stained by floating the grids section-side-down on a saturated solution of uranyl acetate in 50 per cent ethanol in the dark for 1 to 2 hours. Excess uranyl acetate was removed by thorough rinsing in 50 per cent ethanol. For purposes of orientation, thick sections were periodically cut with the microtome and examined with a phase contrast microscope. The electron microscope was an RCA EMU-3D.

RESULTS

Fig. 2 is a cross-section of a single retinula. The rhabdom (*r*), a four-parted structure, is surrounded by eight retinular cells (numbered 1 to 8) containing mitochondria (*m*) and a few scattered pigment granules (*p*). (According to Phillips (8), ommatidia are occasionally found with nine retinular cells. None were encountered in the course of this work.) The larger pigment granules (*p'*) are associated with the pigment cells. The cytoplasm of the retinular cells shows clearly two regions of differentiation. The peripheral areas contain most of the mitochondria and appear more granular; the cytoplasm immediately surrounding the rhabdom, on the other hand, is almost devoid of mitochondria and contains a membranous endoplasmic reticulum (*er*) oriented approximately radially with respect to the ommatidial axis.

These observations have their counterpart in classical light microscopy. The careful and incisive description of the honey-bee's retinula which Phillips published in 1905 (8) has this to say:

> The adult retinular cells are extremely complicated structures, due to the fact that each cell has so many differentiations internally. The central part of each cell is differentiated into a sector of the rhabdome. . . . Outside the rhabdome is an area of clear protoplasm in which the nervous elements of the cell are found, and still outside of this is the granular portion of the cell in which pigment granules are found. Each of these cells then secretes part of the rhabdome, acts as a pigment cell by the accumulation of pigment on its outer surface, and is, in addition, a nerve-ending cell.

The "area of clear protoplasm" which Phillips describes around the rhabdom corresponds to the region of cytoplasm which contains few mitochondria. The "fibrils" or "nervous elements" which "run into the rhabdome where they all end" very likely reflect the presence of the oriented cytoplasmic membranes evident in the electron microscope. Whether these are properly called "nervous elements" is perhaps a moot point, for essentially nothing is known of the means by which the absorption of light in the rhabdom leads to depolarization of the retinular cell.

The rhabdom of another ommatidium is seen at higher magnification in Fig. 3. The retinular cells (numbered *1* to *8*) are seen to cooperate in pairs in the formation of the rhabdom; thus, the rhabdom is divided roughly into quadrants, each sector of which represents two completely fused rhabdomeres. Typically, the division into quadrants is not quite equal. Two of the quarters (*1* to *2*, *5* to *6*) are wedge-shaped, but their points fail to meet at the center of the rhabdom. The other two (*3* to *4*,

FIGURE 1

Longitudinal view of a single ommatidium of *Apis mellifera*, redrawn from Phillips (8). An ommatidium contains 26 to 27 cells: *L*, lens (chitinous secretion of the corneal pigment cells); *CC*, crystalline cone (formed by the vacuolation of four cells); *O. P. C.*, outer pigment cell (12 cells); *C. P. C.*, corneal pigment cell (2 cells); *R.*, rhabdom; *RET. C.*, retinular cell (8 or 9 cells); *RET. N.*, retinular cell nucleus; *B. M.*, basement membrane; *A.*, axonal process.

7 to *8*) meet in such fashion that together they have the appearance of an hourglass. The rhabdom is divided in this fashion over its entire length.

Each sector of the rhabdom is traversed by 20 to 25 dense lines oriented radially with respect to the ommatidium; *i.e.* perpendicular to the border of their associated retinular cell. Each dark line is about 150 A wide and often appears double. These lines are sometimes contiguous with the cytoplasmic membranes (*e.g.* Fig. 3, cell *3*). Between the dark lines in the rhabdom are less electron-opaque regions about 500 A wide containing diffuse lines of intermediate density at the center.

Similar patterns of radially oriented dense lines are seen in the cross-sections of rhabdoms from other arthropods (1, 3, 4, 6, 10–12). Likewise, the three-dimensional structure of the honey-bee's rhabdom is also similar to that described for other arthropods. Each rhabdomere consists of closely packed microtubules whose long axes are perpendicular to the retinular cell surface. A cross-section of the rhabdom thus cuts the tubules in longitudinal section. Fig. 4 is a view of the tubules cut in cross-section. The thin strand of material which occupies the center of each tubule—and which probably corresponds to the line of intermediate density seen in longitudinal sections of tubules (Fig. 3)—does not appear in micrographs of rhabdoms from other arthropods. Favorable sections (Fig. 5) show that these tubules are microvilli of the cytoplasm, as found by Miller (6) in *Limulus* and by Fernández-Morán (3) in certain insects.

Two additional structures are revealed in Fig. 3. Close to the point where the membranes of neighboring retinular cells join the rhabdom are accumulations of electron opaque material, both in the substance of and immediately adjacent to the membranes (*d*). Eight of these dense areas are regularly observed in each ommatidium. They seem to run the length of the retinula. They are possibly desmosomes, sites of adhesion between cells (*cf.* 2), and it is intriguing to speculate that they form a permeability barrier, isolating the rhabdom from the intercellular fluid bathing the retinular cells much as the terminal bars of kidney tubule cells of the mouse seal the intercellular spaces between microvilli (5).

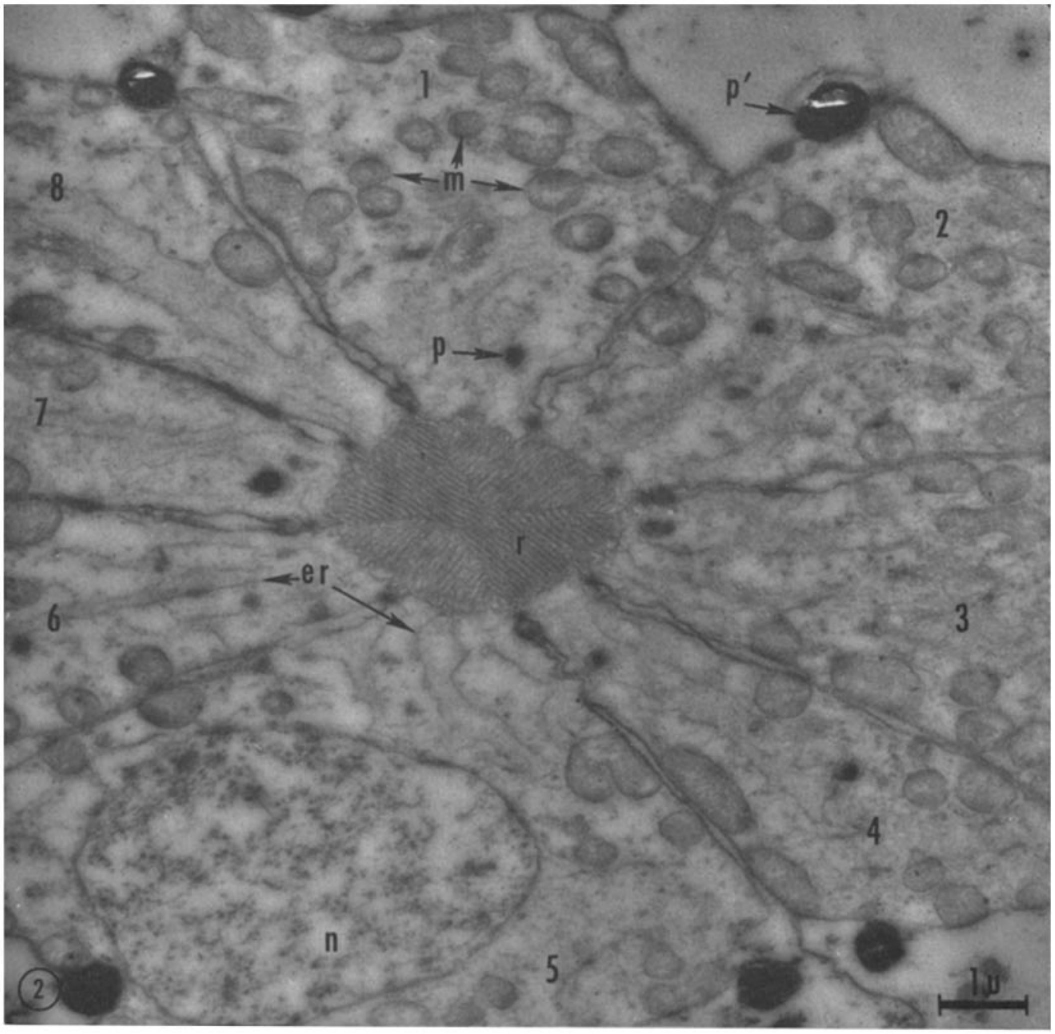
Four roughly circular membrane-limited bodies (*f*) 0.155 μ ($\pm$ 0.011 μ S.E.) in diameter are present in cross-sections of ommatidia. They are found on radial extensions of the sutures which divide the rhabdom into four parts. Because they are always present in cross-sections made at any level, they must be fibers with long axes parallel to the rhabdom. When viewed in cross-section they appear to contain dense granules 100 to 250 A across. These granules may be cross-sections of fibrils, but the evidence is not sufficient on this point. It is not known whether the fibers arise from the basal ends of the retinular cells, from pigment cells, or from cells below the basement membrane. If they are centrifugal fibers from the *lamina ganglionaris* or optic lobe they are of obvious importance.

Although the rhabdoms in Figs. 2 and 3 are typical, ommatidia have been observed in which the rhabdom is greatly flattened, split, or has otherwise departed from a cylindrical shape. In the retinula of Fig. 6 the rhabdom is double over at least part of its length. Using as reference points the intercellular fibers which were described in the preceding paragraph, one can number and pair the retinular cells as in Fig. 3. This has been done in Fig. 6, showing how this rhabdom is derived from the usual form.

DISCUSSION

This description extends our knowledge of the fine structure of retinulae to another order of insects, the Hymenoptera. In all cases studied to date, the rhabdomeres have a similar ultrastructure of small tubules, extending in a closely packed array from one or two surfaces of the retinular cell. The way in which the several rhabdomeres of an ommatidium are arranged to form the rhabdom, however, varies greatly in different groups.

Fernández-Morán (3, p. 608) has stated that the organization of the rhabdom of the honey-bee is is essentially similar to that of Diptera. The present work and a recent report by Naka and Eguchi (7) show this statement to be in error. The point would not be worth laboring except that Wolken and Gupta (11) have recently put forward the generalization that the rhabdoms of arthropods fall into two groups: "open" and "closed." They suggest that the open type, characterized by separate and distinct rhabdomeres projecting into a large axial space, is found in apposition eyes. The closed type, on the other hand, possessing wedge-shaped rhabdomeres in close proximity to one another around a narrow axial cavity, is said to be typical of superposition eyes. This generalization is not justified. The open type of rhabdom has, in fact, been observed only in some Hemiptera and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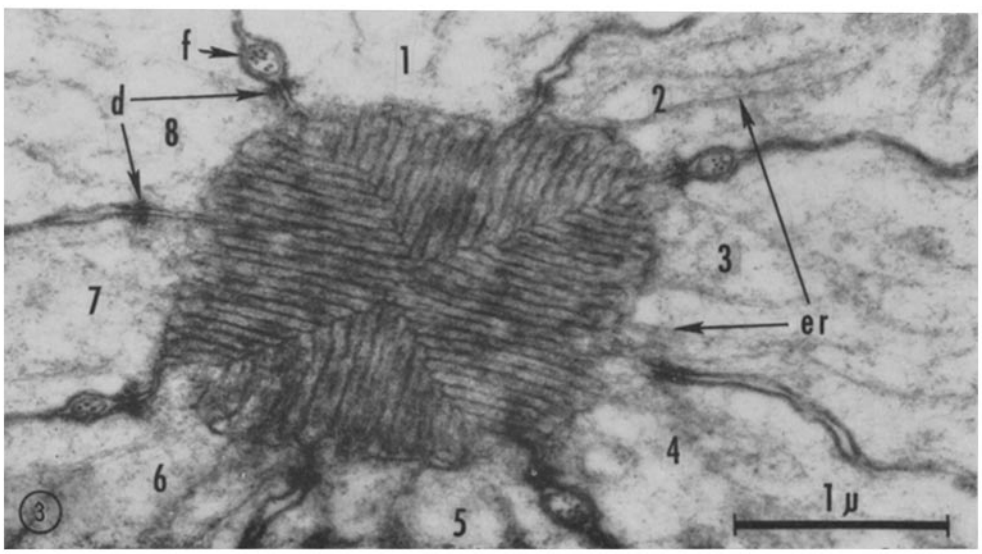
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Diptera. In all other insects that have been examined the rhabdomeres are fused to varying extents, and there may or may not be an axial "cavity" in the rhabdom. The honey-bee, for example, is a diurnal insect with an apposition eye; yet it has a fused (closed) rhabdom with no trace, incidentally, of a cavity or tube in the center.

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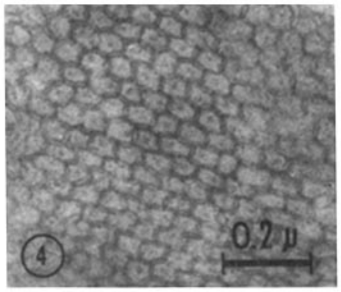
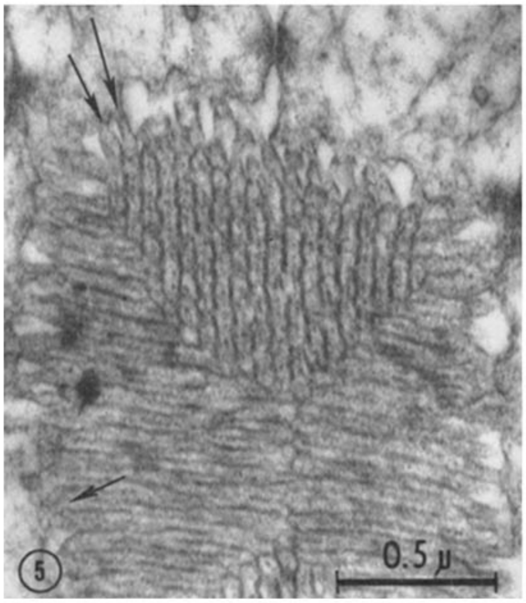
Figure 2

Cross-section of most of a single retinula. The retinular cells are numbered *1* to *8*; the plane of the section passes through the nucleus (*n*) of cell *5*. The rhabdom (*r*) is a four-part structure, although there are eight retinular cells. The cytoplasm adjacent to the rhabdom has prominent membranes (*er*); more peripherally it appears granular and contains numerous mitochondria (*m*). The small pigment granules (*p*) are located in the retinular cells, the larger granules (*p'*) in the outer pigment cells. × 12,000.

Figure 3

Cross-section of a typical rhabdom under higher magnification. The eight retinular cells are numbered as in Fig. 1. Characteristic fibrillar structures (*f*) and electron-opaque accumulations (*d*) are evident, as is also part of the endoplasmic reticulum (*er*). The rhabdom is composed of four wedge-shaped aggregates of microtubules which extend from the surfaces of the retinular cells. A cross-section of the rhabdom therefore cuts the tubules lengthwise. × 29,000.





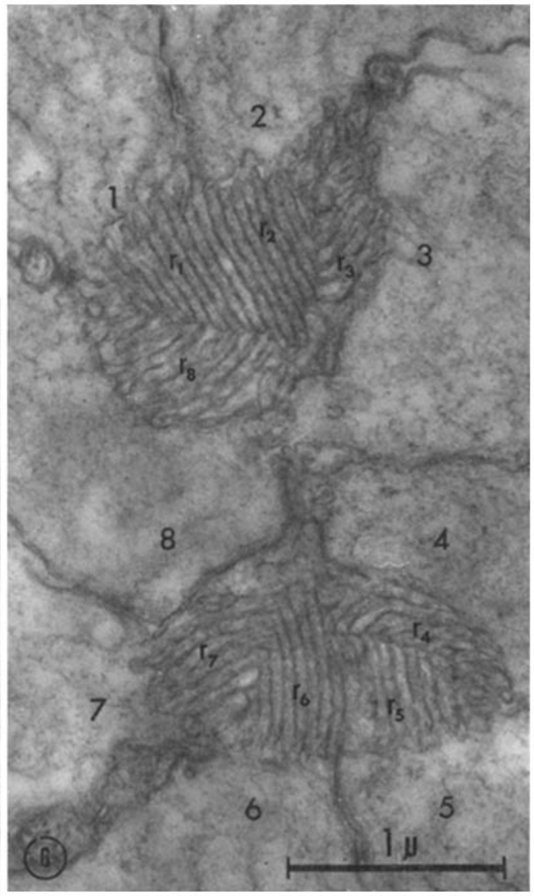


Figure 4

Part of a rhabdom cut in longitudinal section. The close packed tubules seen in Figs. 1 and 2 in longitudinal section are here cut in cross section. There is a strand of electron-opaque material in the center of each tubule. × 60,000.

Figure 5

Part of a rhabdom viewed in cross-section showing (arrows) places where the insides of the tubules are clearly continuous with the cytoplasm of the retinular cells. Fig. 4 is a cross-section near the distal end of the rhabdom, and, unlike Fig. 2, there are open spaces between the rhabdom and the retinular cell cytoplasm. Such spaces are observed regularly in sections from just under the crystalline cones. × 43,000.

Figure 6

Cross-section of a retinula in which the rhabdom is double over at least part of its length. The tubules nevertheless still take one of two orientations which are nearly perpendicular to one another. The cells are numbered *1* to *8* as in Fig. 3, and the corresponding rhabdomeres are labeled r_1 to r_8, showing how this pattern is related to the more usual cylindrical rhabdom. × 29,000.