THE MICROSTRUCTURE OF THE COMPOUND EYES OF INSECTS*

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The arthropod compound eye differs fundamentally from the vertebrate eye, both morphologically and functionally. In insects it is constructed of a number of units called ommatidia, each consisting of about eight elongate retinula cells arranged around a central axis, the rhabdom (Text-fig. 1). Surrounding the retinula cells is a sleeve of pigment cells which probably serves to isolate, structurally and optically, the different ommatidia. The proximal ends of the ommatidia rest on a basement membrane, below which lies the optic ganglion and through which penetrate nerve fibers and branches of the tracheal system. At the distal end of each ommatidium is a transparent conical structure, the crystalline cone, the consistency of which varies considerably among the different groups of insects. Overlying the crystalline cones and directly in contact with the external world is the cornea, a transparent layer whose surface is sculptured into numerous tiny facets, one for each ommatidium. Incident light penetrates a corneal facet and crystalline cone, undergoing some refraction, and then passes through the length of the rhabdom.

It is now a well established fact that in a number of species the compound eye can analyze plane-polarized light (cf. 8, 1, 26). The means by which this is accomplished, however, is not yet understood. Likewise, the degree to which each ommatidium, though a composite structure, may function as a single physiological unit, and the location and nature of the visual pigment are questions which have not yet been answered. More detailed morphological information is expected to help in understanding these problems.

Materials and Methods

The animals studied were the “flesh-fly,” Sarcophaga bullata, and the large, fast flying dragonfly, Anax junius. Both are diurnal insects with “apposition” eyes (cf. Exner, 6).

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Whole eyes and pieces of eyes were fixed for varying lengths of time in 1 per cent solutions of osmium tetroxide. The osmium was dissolved in several buffers, the most successful of which was a veronal-acetate buffer (pH 7.3, approximately the same ionic concentration as insect Ringer) similar to that recommended by Palade (17). The tissue was dehydrated in a series of alcohols and imbedded in n-butyl methacrylate to which small amounts of methyl methacrylate had previously been added to increase the stiffness of the final block. After heat polymerization sections were cut on a microtome which has been described previously (18). The microscope used was an RCA, model EMU-2B.

In all cases the heads were removed from living insects just prior to fixation, and all the animals were more or less light adapted.

In general, treatment which adequately fixes the retinula cells and rhabdom often leaves the pigment cells in poor condition.
The Rhabdom.—Under these conditions of fixation the central matrix of the rhabdom does not have any highly organized structure; its substance appears as a loose precipitate (Fig. 2).

The nature of the rhabdom and its relation to the rest of the visual cell can be seen in Figs. 2 to 4 and Fig. 6. In cross-section (Fig. 2) each rhabdomerere appears as a round structure from roughly $\frac{1}{2}$ to $\frac{3}{4}$ in diameter. As can be seen in Fig. 4, it is surrounded by a membrane which bears an intimate relation to the membrane of the retinula cell. Each rhabdomerere is crossed by a series of light and dark bands. The electron-dense bands are about 370 A apart; they are about 120 A thick and are separated by spaces of about 250 A.

The seven rhabdomeres of a single rhabdom exhibit still another pattern of organization. Viewed in cross-sections of ommatidia, the dark bands of any rhabdomere are oriented approximately parallel to an ommatidial radius drawn through that rhabdomere. In other words, the orientation of the bands of any rhabdomere is approximately matched by that of the rhabdomere on the opposite side of the central matrix. This symmetry has also been observed by Fernández-Morán (7).

In longitudinal section the rhabdomeres may display a similar ladder-like series of dark and light bands spaced about 370 A apart (Figs. 3, 5) or a reticulum of roughly hexagonal spaces separated by electron-dense substance of approximately the same spacing (Fig. 5).

What tridimensional arrangement of structures in the rhabdomere could explain these findings?

Since this work was started, Fernández-Morán (7) has reported a similar
structure in the eye of the house fly, *Musca domestica*. He believes that the cross-sections indicate a series of parallel membranes which run the length of the rhabdomere. The ladder-like appearance of longitudinal sections he ascribes to another series of membranes, at right angles to the first, and arranged like a stack of coins within the rhabdomere. He believes both sets of membranes to be fenestrated, and that this explains the hexagonal, honeycombed appearance of many sections. That is, a section which appears reticulated is, in his interpretation, precisely in the plane of a single fenestrated membrane.

We doubt this interpretation for several reasons:

1. It is exceedingly unlikely that longitudinal sections could continue for several micra without passing out of the plane of a single membrane (ca. 400 A thick in *Sarcophaga*). It is hardly possible for this to occur as frequently as the reticulated pattern is observed. Furthermore, in many cases (Fig. 5, for example) the section appears slightly oblique to the long axis of the rhabdomere.

2. In certain longitudinal sections, when the plane of the section should be nearly perpendicular to the planes of both series of membranes postulated by Fernández-Morán, a cross-hatched pattern of squares or rectangles should be evident. This we have never seen.

3. In cross-sections and longitudinal sections which show the ladder-like pattern, we have never seen perforations through the membranes.

We believe the following interpretation provides a better explanation.

A rhabdomere is composed of long, slender, hexagonal compartments whose walls are dense membranes. The hexagonal compartments are arranged with their long axes all parallel to one another, at right angles to the long axis of the rhabdomere, and parallel to an ommatidial radius. Text-fig. 3 is a diagram of such a structure. We have constructed a model by embedding hexagonal pencils in a matrix of paraffin, the pencils all parallel to one another, and oriented transversely to the long axis of the model. The paraffin is painted black and represents the electron-dense substance seen in the sections of the eye. Cutting it in the three major planes gives rise to rows of dark and light bands as well as the reticulated pattern. Oblique sections can produce such distortions of the reticulum as are often observed in sections of the eye.

The Retinula Cells.—The relationship of the retinula cells to the rhabdomeres has been referred to above and is illustrated in Fig. 4. The most striking feature of the retinula cells is the large number of mitochondria they contain (Fig. 7). These vary in size (0.2 to 2 μ) and shape and are abundantly supplied with an internal structure of double membranes. They appear similar to the insect mitochondria described by Chapman (4), Kisch and Philpott (11), and the
vertebrate mitochondria described by Sjöstrand (21) in that their internal membranes seem to form complete septa, unlike those described by Palade (16). As far as can be determined, their internal membranes are continuous with the inner layer of their limiting membrane. A few measurements of the thickness of the internal membranes give a value of about 150 ± 30 Å, in adequate agreement with those of other workers.

The cytoplasm of the retinula cells often appears vacuolated, perhaps because its fixation has not been optimum. Each retinula cell is thought to be associated with a single nerve fiber, but we have no evidence on this point. The pigment granules of the retinula cells are considered in the next paragraph.

**Distribution of Pigment.**—The pigment cells contain numerous, often somewhat hexagonal granules of pigment (Fig. 2) about 0.35 μ in diameter. The retinula cells contain comparatively fewer granules, distinguished by their smaller size, 0.15 ± 0.03 μ in diameter (Figs. 2 and 3). These have an interesting distribution within the retinula cells, clustering close to the membrane which separates the retinula cell from its rhabdomere (Figs. 2 and 3).

**Tracheae.**—Tracheae and their smaller endings, the tracheoles, are easily
identified in these sections (Figs. 1, 3, 8). The spiral, filamentous thickening known as taenidia can be seen projecting into the lumen of the trachea in Fig. 3. Fig. 8 shows that the surface of the trachea is marked by vertical lines which run between taenidia. Whether these represent small ridges separated by depressions in the cuticular substance of the tracheal wall or actual thickenings is not certain, though the former seems more likely.

**Text-Fig. 4.** Schematic drawing of a portion of a rhabdom from the dragonfly, *Anax junius*. The double-headed arrow R represents the orientation of the long axis of the rhabdom; AD, BD, CD are the borders of the three rhabdomeres viewed in cross-section. Small double-headed arrows indicate the orientations of the long axes of the hexagonal compartments within the three rhabdomeres, X, Y, and Z. MNOP is the approximate plane of the section seen in Fig. 9.

*The Rhabdom of the Dragonfly, Anax junius:*

In this species the rhabdom is not composed of individual rhabdomeres separated by a central matrix. It is a rod-shaped structure which in cross-section looks like a pie that has been cut into three large pieces. Each third is distinguished by a slightly different orientation of a series of dark stripes which cross it, very much as in a single rhabdomere of the eye of *Sarcophaga.* Longitudinal
sections reveal the two distinctive patterns observed in similar sections of the eye of the fly. Thus it appears as though the rhabdom were formed by the fusion of three “rhabdomeres,” each composed of an array of long hexagonal compartments running at right angles to the axis of the rhabdom as described for Sarcophaga. The hexagonal “cells” are about 400 to 425 Å in diameter in this species (Text-fig. 4, Fig. 9).

The relation of the rhabdom to the visual cells has not yet been properly studied. Surrounding the rhabdom there is a space (corresponding to the Schaltzone revealed by histological techniques) crossed by fingers of cytoplasm in which are found mitochondria. Mitochondria also occur in the bodies of the retinula cells.

DISCUSSION

The nature and function of the rhabdom are so closely linked that it is fruitful to consider them together.

Nowikoff (14, 15), impressed with the earlier thought that the rhabdom is a rod of cuticle, developed the theory that this structure serves merely to scatter light laterally to the visual cells and so bring about a more efficient use of the available light energy. This view assumes that all of the “transducer” action of the eye, the initiation of nerve impulses by the action of light, occurs in the bodies of the retinula cells, leaving only an accessory role to the rhabdom. The hypothesis that the rhabdom is a rod of cuticle received its first experimental support from Machatschke (13) who found that, like cuticle, the rhabdom resists peptic digestion, and that it stains in much the same way. Richards (19), reviewing the identification of chitin, has questioned the specificity of the diaphanol-iodine-zinc chloride staining reaction used by Machatschke. In addition he claims the rhabdom lacks the resistance to hot, concentrated alkali which would be expected if it were composed of chitin. Nevertheless, Nowikoff’s theory, essentially unchanged, still finds adherents (2).

Another school has maintained that the rhabdom is somehow actively engaged in the excitatory process. Some early workers thought that the rhabdom is formed, at least in part, of nerve fibers (20, 10), and this view still appears (cf. Snodgrass (24): “...the striations of [the rhabdomere] appear to be rodlike thickenings of neurofibrillae traversing the cell.”).

The microstructure of the rhabdom seems incompatible with the idea that it is either nerve fiber or cuticle. Superficially its fine internal structure resembles that of the outer segments of vertebrate rod cells (22). Also the numerous mitochondria of the retinula cells remind one of the ellipsoid of the rod inner limb, a tightly packed group of mitochondria at the base of the outer segment (23). Rhodopsin, the visual pigment of the vertebrate rod, is located in the rod outer limb; it is tempting to argue by analogy that the rhabdom of the compound eye also contains the visual pigment, and hence is the site of photoreception.

Pigment migrations are a well known part of light and dark adaptation in
the eyes of certain insects. According to Lüdtke (12), the rhabdons of the eye of the backswimmer, Notonecta glauca, also display movements. During dark adaptation the rhabdom pushes peripherally, its tip intruding between the cells of the crystalline cone. Thus under low levels of light intensity it is found in the position best suited to intercept what light reaches the eye. This observation also suggests that the visual pigment is located in the rhabdom.

The chemical composition of the pigment granules seen in these sections is not known, but probably they are related to the "ommochromes" described by Butenandt and his coworkers (cf. 3). The granules of the pigment cells are thought to isolate the ommatidia from one another, but little is known about the function of the pigment granules of the retinula cells of such insects as Sarcoptes. There does not appear to be any reason for associating them with the excitatory process. Their position, forming a curtain behind the rhabdomeres, makes Nowikoff's theory that the rhabdom is concerned with distributing light to the retinula cells more unlikely. These granules seem so placed as to prevent light from passing transversely from each rhabdomere to its retinula cell.

The hypothesis that the rhabdomere is the site of photoreception implies that biochemical activities of the rhabdomere are linked with other metabolic processes of the retinula cell. Thus we regard a fly rhabdomere as a part of a single retinula cell specialized to perform a specific biochemical task. The morphological evidence presented in these pictures supports this view, but further information concerning the relationship of rhabdomere to retinula cell during development, particularly in the dragonfly eye, is desirable.

How are these observations related to the insect's ability to determine the plane of polarized light? Stockhammer (25) has recently studied the birefringence of thin sections of insect eyes. He finds the cornea optically anisotropic but concludes that it is not the structural element responsible for analyzing polarized light, for an analyzer must not only be able to resolve light into ordinary and extraordinary rays polarized at right angles to one another (birefringence), but must be able to reduce selectively the intensity of one of these rays. Birefringence alone is not enough.

According to Stockhammer, in the fly the rhabdomeres also are birefringent; between crossed Nicols they appear as bright spots. However, the rhabdomeres of a single ommatidium are not equally bright; a rhabdomere appears about as bright (or dim) as the rhabdomere opposite it. It should be recalled that the orientation of internal structure also is similar in opposite rhabdomeres (cf. Fernández-Morán). Stockhammer's observation means that the two planes of 

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3 The reduction in intensity of one of the rays can be achieved by diverting it by reflection as in a Nicol prism, or by absorption. If in a birefringent medium the absorption of one ray is greater than that of the other, the crystal is said to be dichroic. The amount of light absorbed by a dichroic medium therefore depends on the plane of polarization of the incident light.
vibration into which a rhabdomere resolves the light reaching it from the polarizer are parallel, or nearly so, to the planes of vibration in the rhabdomere on the opposite side of the central matrix. But just as the dioptic apparatus seems unable to account for the ability of the insect eye to analyze polarized light, the birefringence of the rhabdom cannot alone be responsible either. However, as Stockhammer points out, since his experiments were carried out with light of wave lengths longer than 480 m# while the sensitivity of the bee to polarized light is reported to be greatest in the near ultraviolet and violet (9), the matter may not yet be settled. The situation is also confused by the fact that de Vries, Spoor, and Jielof (27) found no optical anistropy in the sections of insect eyes they examined. Further, evidence presented by Waterman (28) suggests that the compound eye of Limulus detects the plane of polarized light by reflection-refraction phenomena at the cornea.

If, as has been suggested by other workers (27, 25), the basis for polarization analysis is a dichroic visual pigment, one should expect the molecules of this pigment to be definitely oriented in each rhabdomere, perhaps similarly oriented in the pairs of rhabdomeres lying across from each other in the rhabdom. It may be that the ordered internal structure of the rhabdom revealed in the present pictures is related to such an organization of the molecules of visual pigment.

SUMMARY

The apposition eyes of two diurnal insects, Sarcophaga bullata (Diptera) and Anax junius (Odonata), have been examined with the electron microscope. In the latter case only the rhabdom is described.

The rhabdom of the fly consists of a central matrix and seven rhabdomeres, one for each retinula cell. The rhabdomeres show an ordered internal structure built up of transverse tubes, hexagonal in cross-section. These slender compartments running the width of the rhabdomere are 370 A in diameter. After fixation with osmium tetroxide the walls of the compartments are more electron dense than the interiors. The retinula cells contain mitochondria, and pigment granules smaller than those found in the pigment cells. These granules tend to cluster close behind the membranes which separate the retinula cells from their rhabdomeres.

The rhabdom of the dragonfly is a single structure which appears to be composed of three fused “rhabdomeres,” each similar to a rhabdomere of Sarcophaga.

Reasons are given for believing that the rhabdom may be the site of photo-reception, as well as the organ for analyzing plane-polarized light, as suggested by other workers.

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EXPLANATION OF PLATES
Fig. 1. Low power cross-section through several ommatidia. In this section the contents of the pigment cells have largely been removed or destroyed in preparing the section, but branches of the tracheal system (T) are readily evident, as are a few remaining pigment granules (PG1). Each ommatidium (O) consists of an array of seven retinula cells (not clearly delineated from one another in this picture) arranged radially about the rhabdom. The central matrix (CM) of the rhabdom appears light; but in addition, each retinula cell donates a darker rhabdomere (R) to the formation of the rhabdom. In the two ommatidia at the left one can count all seven rhabdomeres. Compare with Text-fig. 2. X 6,700.

Fig. 2. Cross-section through a single ommatidium. The banded appearance of the individual rhabdomeres (R) is evident. Note that the dark and light bands of the several rhabdomeres are radially arranged with respect to the axis of the ommatidium. Between the rhabdomeres, at the center of the ommatidium, is the central matrix of the rhabdom (CM). The cell membranes of the retinula cells (RCMem) are preserved allowing one to count the seven cells. There are numerous mitochondria (M) present, particularly in the retinula cells. Surrounding the ommatidium are the pigment cells (PC) which contain large pigment granules (PG1). Within the retinula cells are smaller pigment granules (PG2) which are found near the rhabdomeres. X 17,000.
(Goldsmith and Philpott: Microstructure of compound eyes of insects)
Fig. 3. Longitudinal section through a single ommatidium. The ladder-like appearance of the rhabdomeres (R) is one of two distinct patterns obtained in longitudinal sections. Note that, due to the star-shaped appearance of the central matrix in cross-section (Fig. 2), this longitudinal section passes successively (from upper right to lower left) through retinula cell (RC), rhabdomere (R), central matrix of the rhabdom (CM), retinula cell, central matrix, a narrow strip of retinula cell, another rhabdomere, central matrix, and retinula cell. That the pigment granules of the retinula cell (PG2) tend to be found immediately behind the rhabdomeres is evident from this picture. The granules are not sufficiently numerous that they would appear in every cross-section, which probably explains their apparent absence from some of the visual cells in Fig. 2. Mitochondria (M) are present, and there is a large trachea (T) in the upper right. × 17,500.

Fig. 4. Cross-section of a single rhabdomere (R) showing its relation to the retinula cell (RC) and central matrix of the rhabdom (CM). × 31,000.

Fig. 5. This is a section, nearly longitudinal, through the edge of a rhabdom such that two adjacent rhabdomeres (R) are sectioned. These two rhabdomeres illustrate the two patterns seen in longitudinal section, the hexagonal reticulum, and the "ladder" of dark and light bands. Mitochondria (M) are present. × 29,500.
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Fig. 6. Cross-section of a rhabdom near the apex of the crystalline cone (which is really a watery “pseudocone” in this species). All seven rhabdomeres can be seen. × 27,500.

Fig. 7. This figure shows well the concentration of mitochondria (M) in the cells immediately surrounding the rhabdom. Sections of several somewhat twisted rhabdomeres (R) are seen at the top. × 11,500.

Fig. 8. A nearly tangential section through a trachea. L, lumen of the trachea; Tn, taenidia; VR, probably small, vertical ridges. × 24,500.
(Goldsmith and Philpott: Microstructure of compound eyes of insects)
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Fig. 9. Slightly oblique longitudinal section through a rhabdom of the dragonfly *Anax junius*. At the top the hexagonal compartments of one of the rhabdomeres are seen in cross-section. In the lower portion of the picture the knife has passed forward (or back) into the other two rhabdomeres so that the compartments are revealed almost in longitudinal section. Rhabdomeres are indicated with the letters $X$, $Y$, and $Z$ to correspond to the notation in Text-fig. 4. $\times$ 32,000.
(Goldsmith and Philpott: Microstructure of compound eyes of insects)