PHOTORECEPTOR STRUCTURES. THE RETINAL CELLS OF THE COCKROACH EYE

IV. *Periplaneta americana* and *Blaberus giganteus*

J. J. WOLKEN and P. D. GUPTA. From the Biophysical Research Laboratory, Eye and Ear Hospital and University of Pittsburgh School of Medicine, Pittsburgh. Dr. Gupta is a Visiting Research Fellow; his present address is the Department of Zoology, University of Lucknow, Lucknow, India

The retinal structures of the compound eyes of insects are being investigated as part of a comparative study of the structure and biochemistry of visual organs (1–5). The compound eye of the insect is composed of numerous ommatidia, each of which consists of from four to eight retinula cells. Each retinula cell contains a differentiated structure, the rhabdomere, a photoreceptor. The rhabdomeres are analogous in function to the retinal rods of the vertebrate eyes.

One of the more primitive of the unspecialized insects is the cockroach. It has been suggested from electrophysiological studies that the cockroach eyes contain two types of visual photoreceptor systems (6, 7). It was, therefore, of interest to us to determine the *fine structure* of their rhabdomeres and to isolate their photosensitive pigments in order to make a comparison with the photoreceptors of other insects and invertebrate compound eyes. Two common, large species of cockroaches, *Periplaneta americana* and *Blaberus giganteus*, made available to us from the Gulf Research Laboratory, were used in this study.[1]

MATERIALS AND METHODS

The cockroaches, *Periplaneta americana* and *Blaberus giganteus*, were dark-adapted and their eyes excised in dim red light. The eyes were immediately dropped into a solution of 1 per cent osmium tetroxide, buffered with veronal-acetate pH 7.0 (8). Under a binocular microscope each eye was dissected into a lower half and an upper half and returned to the fixative for 4 hours at 5°C. After fixation, the eyes were washed with buffer and distilled water, and then dehydrated by a graded water-ethanol series. While in 75 per cent alcohol, muscle, tracheae, fat bodies and other extraneous eye tissues were cut away. The completely dehydrated eye parts were then carried through ethanol-*n*-butyl methacrylate mixtures (9 parts *n*-butyl methacrylate to 1 part methyl methacrylate) to a pure methacrylate monomer, embedded

in gelatin capsules, and polymerized by heat. Using a Servall (Porter-Blum) microtome with a glass knife, sections less than 0.05 μ thick were cut from the polymerized blocks. The sections were mounted on formvar or carbon-coated grids and examined with a Philips EM-100A electron microscope. Transverse and longitudinal sections, 2 to 5 μ in thickness, were also cut from the tissue blocks for light microscopy.

OBSERVATIONS

The compound eye of the cockroach is composed of approximately 2,000 ommatidia that are separated from one another by a pigment sheath extending to the basement membrane of the eye, where nerves and tracheae are clearly seen. A cross-section through the cockroach eye shows many ommatidia with their rhabdomeres surrounded by pigment granules. The proximal portion of each ommatidium appears to be made up of seven retinula cells radially arranged in the form of a cylinder. Each retinula cell is 7 to 9 μ in diameter with a large elliptical nucleus. The inner side of the retinula cell is differentiated to form the rhabdomere, its photoreceptor. All seven rhabdomeres lie in close proximity with one another, and form the rhabdome. The aggregates of intracellular pigment granules which surround the rhabdomes extend the whole length of the retinula cells and do not seem to be affected by dark-adaptation. Numerous mitochondria are dispersed throughout the retinula cell and are not necessarily associated with the rhabdomeres. Depending upon the location and angle of cut of the eye tissue slice, the rhabdome appears either as a rhomboid or as a triangle (with sides measuring from 5 to 12 μ in length). Each rhabdome is made up of seven rhabdomeres which exhibit a regular pattern of organization: one of the rhabdomeres is asymmetrical, while the others are arranged in pairs (Figs. 1 and 2). In all rhabdomes, the main axes of the paired rhabdomeres have the same orientation with respect to the asymmetrical rhabdomere. The rhabdomeres are

tightly packed around the stellate axial cavity, which is quite prominent in the distal region and becomes narrow toward the basal region.

Each rhabdomere averages 2 μ in diameter and 100 μ in length. The lateral portions of contiguous rhabdomeres are pressed against each other and, therefore, appear to be narrow. In transverse or slightly oblique sections, a rhabdomere shows either a lamellar or hexagonal pattern, while in oblique longitudinal sections, both patterns are observed (Figs. 1 to 4). In many sections the hexagonal structure predominates over the lamellar structure. The rhabdomeres of the cockroach are of a single geometrical structure of tightly packed tubules with the long axis lying at right angles to the longitudinal axis of the rhabdome. The walls of the tubules are osmiophilic and appear as electron-dense bands in comparison with the less dense interspaces. The lamellar appearance of a rhabdomere, with alternating dense and less dense bands in perfect transverse or vertical longitudinal sections, is due primarily to the arrangement of the tubules and to the differential staining properties of its wall and cavity (Fig. 3.) However, in oblique and horizontal longitudinal sections, the tubules are tightly packed and appear as hexagons (Figs. 4 and 4 a). Since most of the thin-sections are cut at an oblique angle, the hexagonal pattern predominates, especially at higher magnifications. In the lamellar structure, the electron-dense bands are of the order of 200 A in thickness, while their interspaces vary from 350 A to 400 A wide; each tubule is about 500 A in diameter and its wall is of the order of 50 A in thickness. There are approximately 400 tubules in 1 μ^2 of surface, or about 80,000 such tubules in a single rhabdomere.

Except for minor variations in the relative size and angle of location, the number of retinula cells in the upper and lower halves of a compound eye of *Periplaneta americana* do not differ from one another. *Blaberus giganteus* has a similar arrangement of its seven retinula cells and its rhabdomeres that form the rhabdome. The rhabdomeres are also tightly packed tubules of the order 500 A in diameter.

DISCUSSION

The fine structure of the cockroach rhabdomere is similar to that described in a variety of arthropods (9–11) and molluscs (12). No histological differences were found between the upper and lower

halves of the compound eye of the cockroaches which is in agreement with previous studies of Walther (7). We have observed that there are seven retinula cells which form the rhabdome, although eight such visual cells were found in the cockroaches, *Blatta* (*Stylopyga*) *orientalis* and *Blatella germanica* (13, 14). The eighth cell, as found in some insects, is probably a rudimentary structure located close to the basal membrane (15). It does not extend the entire length of the rhabdome and no rhabdomere is differentiated from it. The pigment granules immediately surrounding the rhabdomeres have previously been observed in histological studies of a variety of cockroach eyes (13). These pigment granules, because of their location, probably prevent light from passing transversely from each rhabdomere to its retinula cell.

Electron microscopy of the arthropod visual cells indicates that there are two arrangements for the rhabdomeres that form the rhabdome. One can be considered a "closed" type in which the greater portion of the mesial border and the entire inner margin of the retinula cells are modified to form wedge-shaped rhabdomeres that are in close proximity with one another around a narrow axial cavity. The second is then an "open" type in which the rhabdomeres project through a neck-like portion of their retinula cells, extending into a comparatively large axial cavity. The closed type rhabdome is found in the cockroach and in a number of insects generally described to possess "superposition" eyes, e.g., grasshoppers, locusts, dragonflies, moths and butterflies (9, 10), as well as in the retinas of the cephalopod molluscs, *Octopus* and *Sepia* (12). The open type, on the other hand, is characteristic of dipterous and hymenopterous insects, e.g., *Drosophila*, houseflies, flesh-flies, and honeybees (1, 9, 11, 16), all of which have "apposition" eyes. Some of these insects have been found to orient with respect to the plane of polarization when illuminated with polarized light.

Our observations suggest a possible relationship between these two kinds of arrangements of the rhabdomeres and their visual physiology. The action potentials and electrical response of several arthropod eyes, as investigated by Autrum (17, 18), indicate that there are two physiological types: a "slow" type eye characterized by a negative monophasic potential which is dependent upon the state of dark-adaptation, and a "fast" type eye in which the electroretinogram (ERG) is diphasic,
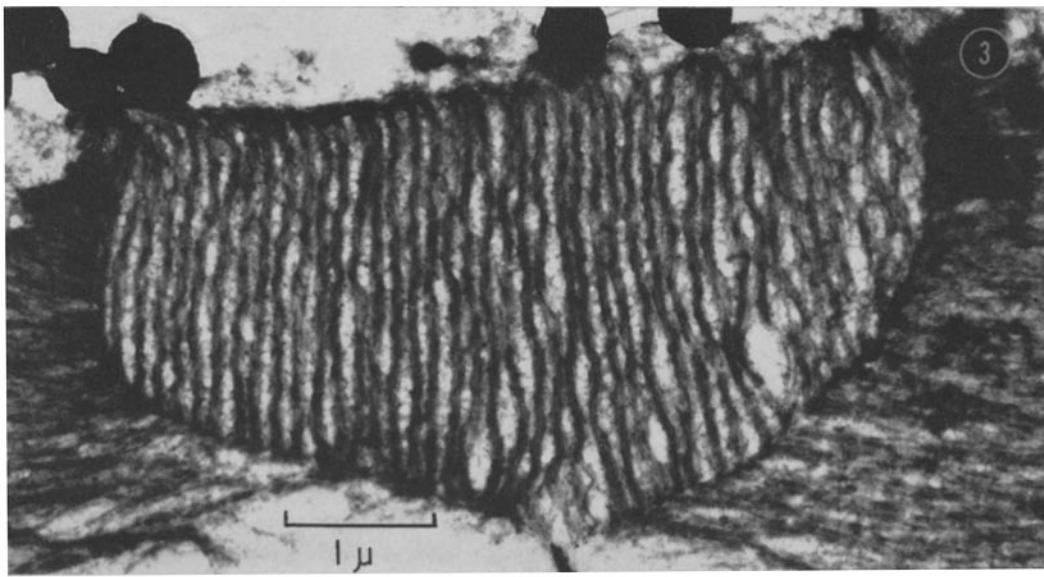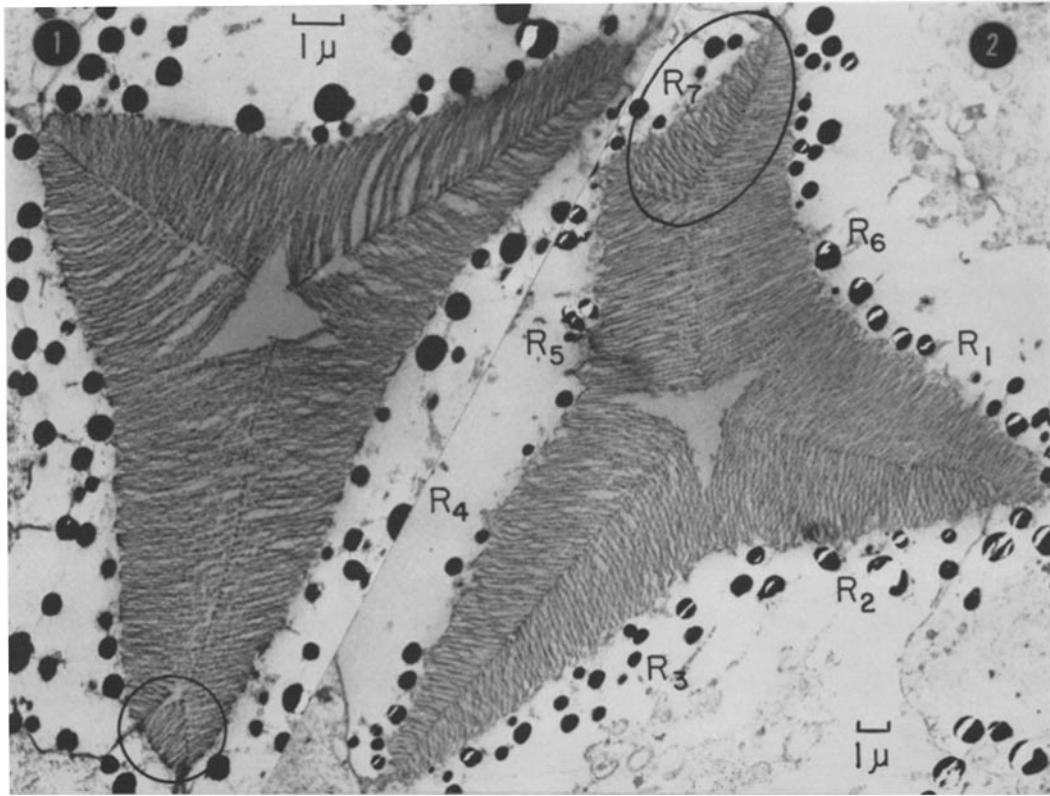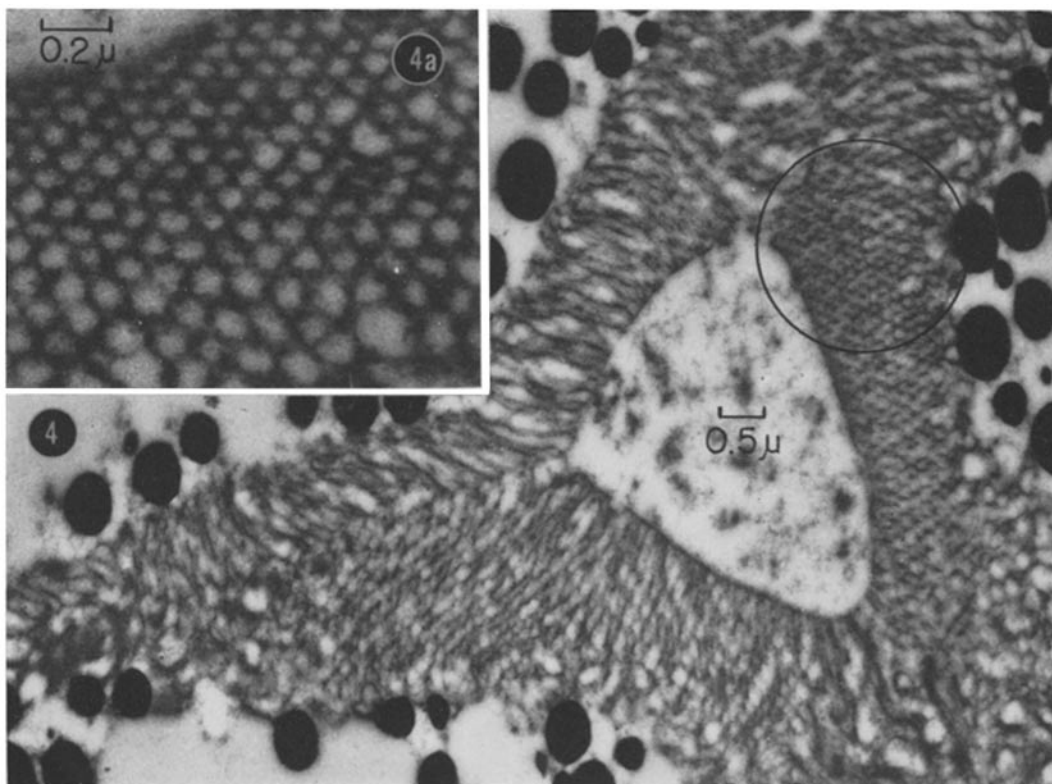
FIGURE 4

An oblique section through a rhabdome, showing hexagonal structures of packed
tubules in the rhabdomeres. × 13,000.

FIGURE 4 a

An enlarged area of Fig. 4 of the hexagonal structures of the rhabdomere tubules.
× 44,400.

the magnitude and the form of the potential being
independent of the state of dark-adaptation. It is
of interest then that, except for the adult dragon-
flies, all the arthropods having a "closed" arrange-
ment for their rhabdomes possess a "slow" type
electrical response. However, all the dipterous and

hymenopterous insects that have an "open" type
arrangement possess the "fast" type electrical re-
sponse. In the "fast" type eye characteristic of
diurnal insects, the rhabdome occupies only a small
part of the volume. In the "slow" type eye char-
acteristic of nocturnal insects which have a light-

FIGURES 1 and 2

Cross-section through two rhabdomes showing the arrangement of the seven rhabdomeres
(R_1 to R_7) which form each rhabdome. Note the encircled asymmetric rhabdomere and
the *fine structure* of the rhabdomeres. Fig. 1, × 6,500. Fig. 2, × 4,650.

FIGURE 3

A cross-section of one of the asymmetric rhabdomeres, showing its lamellar structure
of packed tubules. × 20,000.

gathering problem, the rhabdome takes up a much larger part of the volume. For example, the volume of the rhabdome of the cockroach is about five times the volume of the *Drosophila* rhabdome.

A visual pigment-complex containing retinene$_1$ has been extracted from the cockroach rhabdomeres and its concentration calculated from absorption spectra to be 1×10^8 molecules per rhabdomere, which is within the range of pigment molecules found for all photoreceptors so far studied (19). Retinene is a linear molecule of the order of 5 A in diameter which when combined with a protein, such as in rhodopsin, would have a diameter of 40 to 50 A. Each rhabdomere was observed to consist of tubules 500 A in diameter whose walls are 50 A in thickness. Each rhabdomere contains 80,000 such tightly packed tubules. If the number of retinene molecules were distributed on the surface of these tubules as a monolayer, as previously suggested in our photoreceptor model (1), there would be just enough cockroach rhodopsin molecules to cover all of the surfaces of these tubules.

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