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The Morphology of the Eyes of *Limulus*

I. Cornea and Epidermis of the Compound Eye*

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Summary. The dioptric apparatus of the *Limulus* compound eye is composed of the corneal cuticle with its internally projecting cuticular cones and the specialized underlying epidermis. The latter is composed of three distinct cell types. The guanophores, located between cuticular cones, contain guanine as a reflecting pigment. The distal pigment cells, which clothe the sides of the cuticular cones and form a sheath around the underlying ommatidium, contain massive bundles of microtubules, abundant pigment droplets and a large Golgi system. The cone cells are positioned between the flattened tip of the cuticular cone and the apex of the ommatidium. They serve to anchor the retinula cells to the cuticle and, by virtue of long processes along the periphery of the rhabdome, perform a glial function with respect to the interaction of adjacent retinula cells. The geometry and fine structure of the dioptric apparatus provide supporting evidence for the wide angle of acceptance and lack of polarized light perception by the ommatidia.

Introduction

The eyes of *Limulus polyphemus*, particularly the compound eyes, have proved rewarding objects of study to numerous morphologists, embryologists, physiologists, and biomathematicians for well over a century. The efforts of neurophysiologists, in particular, have yielded results that in their general significance far exceed the taxonomic confines of this experimental animal. The increasing detail and precision of neurophysiological investigations as well as a tendency to extrapolate morphological inferences from physiological data suggest the need for a thorough morphological description of the eyes. Furthermore, it should be possible to bring structural features into congruence with known functional characteristics.

This paper, the first of a series designed to fulfill this need, deals primarily with the dioptric apparatus. Of the earlier authors EXNER (1891) gives a detailed and accurate description of the *Limulus* compound eye in his classical study of facetted eyes. Similar detail is evident in the study of WATÁSE (1890), whereas the often-mentioned later work by DEMOLL (1914) simplifies the morphology beyond the point of current utility. No electron microscopic studies have been published on the dioptric apparatus, but WATERMAN (1950, 1954a—c) has scrutinized this region of the eye from a variety of functional viewpoints, specifically the relative growth of the eye, directional sensitivity of ommatidia, and sensitivity to polarized light. Gross views of the eye, showing the distribution of

the interommatidial reflecting pigment, have been published by WATERMAN (1954a) and VON CAMPENHAUSEN (1967).

Materials and Methods

Animals measuring 5, 10, and 20 cm in prosomal width were obtained from the Woods Hole Biological Supply Company. Best fixation has been accomplished by slicing the eye into large cubes in a solution of 1% glutaraldehyde, 5% formalin, 3% NaCl and 3% sucrose in 0.1 M phosphate buffer. (This fixative, one of approximately 40 mixtures tried over a period of several years, is still not reliable and is being subjected to further modification, but it will on occasion give good preservation of all cells and subcellular components of the eye. However, it is not unusual to have crenated epidermal cells and frothy retinula cells along with well-preserved eccentric cells, neural elements of the plexus, and connective tissue cells.) After 6 hours of fixation, the tissue was washed briefly in buffer with 7% sucrose, postfixed for 2 hours in osmium tetroxide, and embedded in Araldite. Thin sections on Formvar-coated or uncoated grids were stained with uranyl acetate, lead citrate, potassium permanganate or a combination of these. Thicker sections were stained with 1% toluidine blue in 1% borax for light microscopy. A Philips EM-200 yielded the electron micrographs.

Results

The peculiar growth characteristics of the *Limulus* compound eye have largely been detailed by WATERMAN (1954), but some further description appears desirable with reference to the three sizes of *Limulus* studied. The length of the ellipsoidal cornea increases from 3 to 13 mm with an approximately 10-fold gain in area, while the number of ommatidia increases only from 450—850. Accordingly, the ommatidia grow in all their dimensions including cell size rather than in number only. The intraocular haemocoelic sinusoids are poorly developed in the smallest *Limulus*. In the largest animal, with its considerably greater corneal curvature, a trabeculated plate intervenes between the eye and the body cavity, making the usual dissection approaches difficult. Hence, the medium-sized animal is best suited for anatomical studies.

The corneal cuticle, 140—440 μ in thickness (the quoted range refers to the 5 and 20 cm *Limulus*, respectively), bears inward projections, the cuticular cones (Figs. 1, 3), which are 80—300 μ in length and have an inclusive angle of 20—30°, depending on the location of the cone in the eye and the age of the animal. The cornea, though transparent in young animals, often becomes heavily abraded in older, presumably less frequently molting, animals and is occasionally invaded down to the epidermis by fungal hyphae. Crescentic pore canals penetrate the cuticle between the areas occupied by cuticular cones. The lamellae of the cuticle, composed of fibrils about 150 Å in diameter in swirling, alternating layers, project concentrically from the basal surface of the cuticle to form the cones. As a result, fibrils are visible in every orientation when the cone is viewed along its long axis (Fig. 2). Only a small central area of the cornea contains a few cones normal to the surface; the most peripheral cones are inclined as much as 55° toward the center of the eye in a large animal.

The corneal cuticle lies on a continuous layer of epidermis that is differentiated into three different cell types (Fig. 3). Underlying the cuticle between the cuticular cones are cells containing a reflecting pigment. The sides of the cones are covered by cells with dark purple pigment droplets; these cells form a peripheral sleeve around the distal half or two-thirds of the underlying ommatidium. The tip of

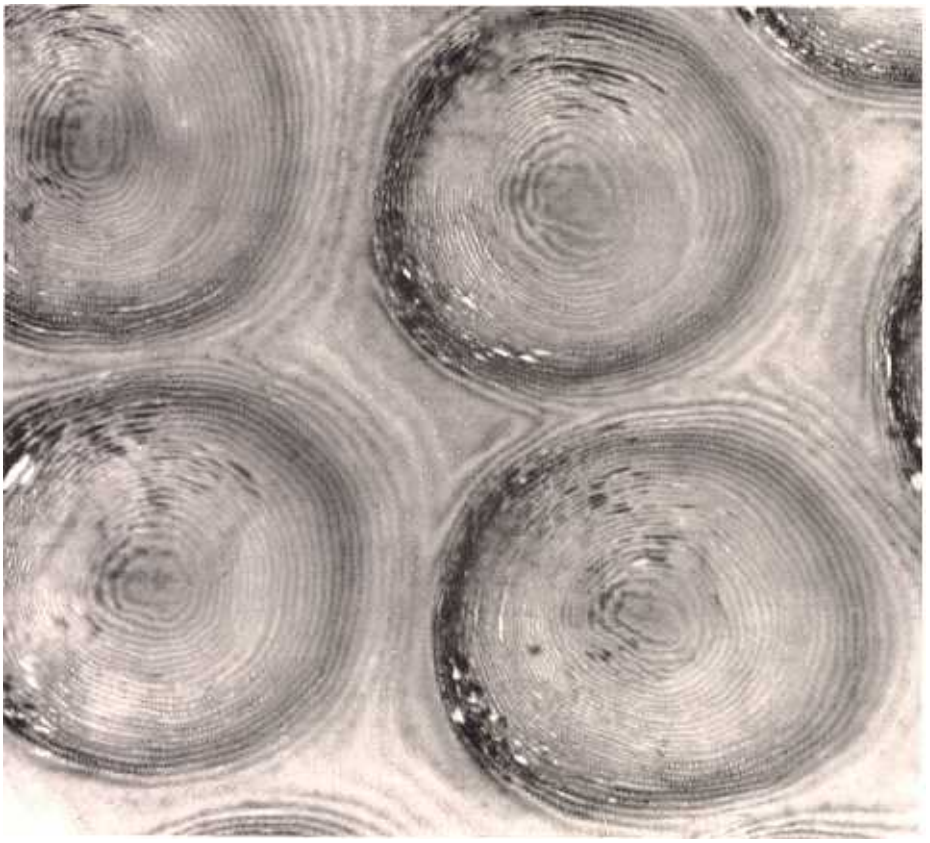

Fig. 1. Photomicrograph of a horizontal section through the corneal cuticle. Concentric rings and "contour lines" are indicative of numerous depressed lamellae above each cuticular cone. Pore canals penetrate the unmodified cuticle. × 380

the cone, which is flattened and has a diameter of 20—50 μ, abuts against a third cell type, to be called the cone cells by analogy to cells in the insect eye located in a similar place. These cells overlie the retinular part of the ommatidium.

The first cell type contains guanine as its reflecting pigment (KLEINHOLZ, 1959); hence, these cells will be referred to as guanophores (Fig. 4). They have a vertical extent of up to 80 μ, with firm attachment to the cuticle at their apical surfaces and a very delicate basal lamina separating them from the underlying vascular spaces. Laterally the cells are attached to each other by a short desmosome (Fig. 5), several tight junctions alternating with intermediate junctions, and extensive septate desmosomes, about 10 μ long. The more basal regions of the guanophores have no junctional specializations. The nuclei are large (6—10 μ) and have conspicuously clumped chromatin, at least during the intermolt period. The cytoplasm is densely packed with a variety of organelles and inclusions. Numerous mitochondria, up to 2.5 μ long, display a dense, granular matrix, presumably as a result of the fixative employed. Endoplasmic reticulum and abundant free ribosomes account primarily for the electron-opacity of the cytoplasm. Occasional glycogen rosettes are present. Golgi bodies are similar

Fig. 2. Electron micrograph of the periphery of a cuticular cone in cross-section. Directions of cuticular filaments within two lamellae are indicated by curved arrows. From the epidermis (lower left) anchoring filaments enter the cuticle (black and white arrow). × 12,500

to those in the pigmented cells, though smaller and less abundant (vide infra). Microtubules, about 220 Å in diameter, occur in bundles of several dozen to several hundred, coursing in a vertical direction (Fig. 6). As the bundles approach the cuticle, they splay out and insert in small groups on differentiated regions resembling hemidesmosomes at the apical membrane (Fig. 5). The extracellular side of each patch gives rise to a filament, about 300 Å thick, which runs deeply into the cuticle and presumably serves as anchoring filament. The apical surface of the guanophores is rather complex and convoluted, partly because of the frequently inturned attachment sites of microtubules and partly because of some degree of lateral overlap and interdigitation of neighboring cells. Infrequently one encounters a centriole or a stubby sensory cilium at the apical surface of the cell.

The most conspicuous inclusions of the guanophores are the variously shaped, angular guanine crystals. These evidently fall out of the section the moment it is cut, but the precise shape of the hole can be preserved if the section is supported on a Formvar film. Each crystal, measuring 0.5—1.5 μ in diameter, is membrane-enclosed together with a small amount of cytoplasm. The crystals appear to

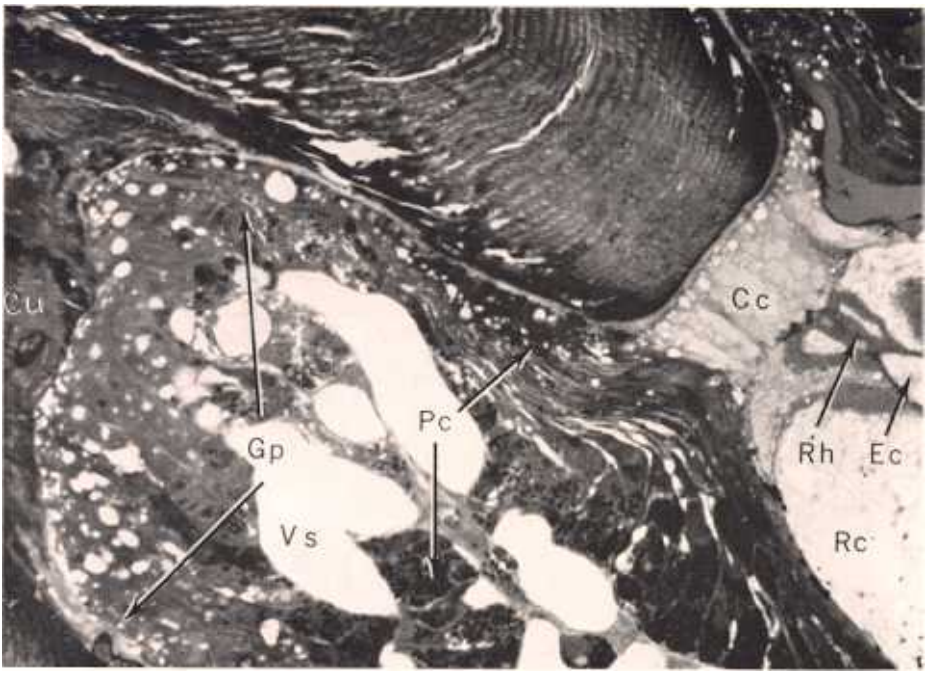

Fig. 3. Photomicrograph of a vertical section through a cuticular cone and underlying tissues. Guanophores (*Gp*) lie adjacent to the unmodified cuticle (*Cu*). Distal pigment cells (*Pc*) attach to the periphery of the cone and form a sleeve around the upper part of the ommatidium. Cone cells (*Cc*) are interposed between the tip of the cone and the ommatidium. (*Rc*) Retinula cell; (*Rh*) Rhabdome; (*Ec*) Eccentric cell dendrite; (*Vs*) Vascular sinusoids. × 570

change their position in the cells in response to changes in illumination. Other vesicles contain extremely electron-opaque spicules, possibly calcium, which occur very rarely in association with small guanine crystals. Pigment granules, autophagic vacuoles, and assorted other vesicles are identical, though less frequent, to these structures in the pigmented cell, to be described presently.

The pigmented cells surrounding the cuticular cone will be referred to as the distal pigment cells, since a second and separate group of pigment cells is located within and at the basal end of the ommatidia. The distal pigment cells (Fig. 5) are attached to the sides of the cuticular cone and to each other in a manner similar to that found in the guanophores. The cells are highly attenuated and usually have a complex shape in cross-section at a level some distance from the cuticular junction. Those cells attaching near the tip of the cone may be as long as 300 μ, forming the innermost layer of the sheath around the ommatidium, whereas the cells attaching more distally (adjacent to the guanophores) terminate at the level of the cone tip as the outermost layer of the sheath. These cells measure about 100 μ in length in a large animal. The appearance of the cytoplasm is comparable to that of the guanophores with the exception of the absence of guanine and the greater abundance of pigment droplets, microtubules, and

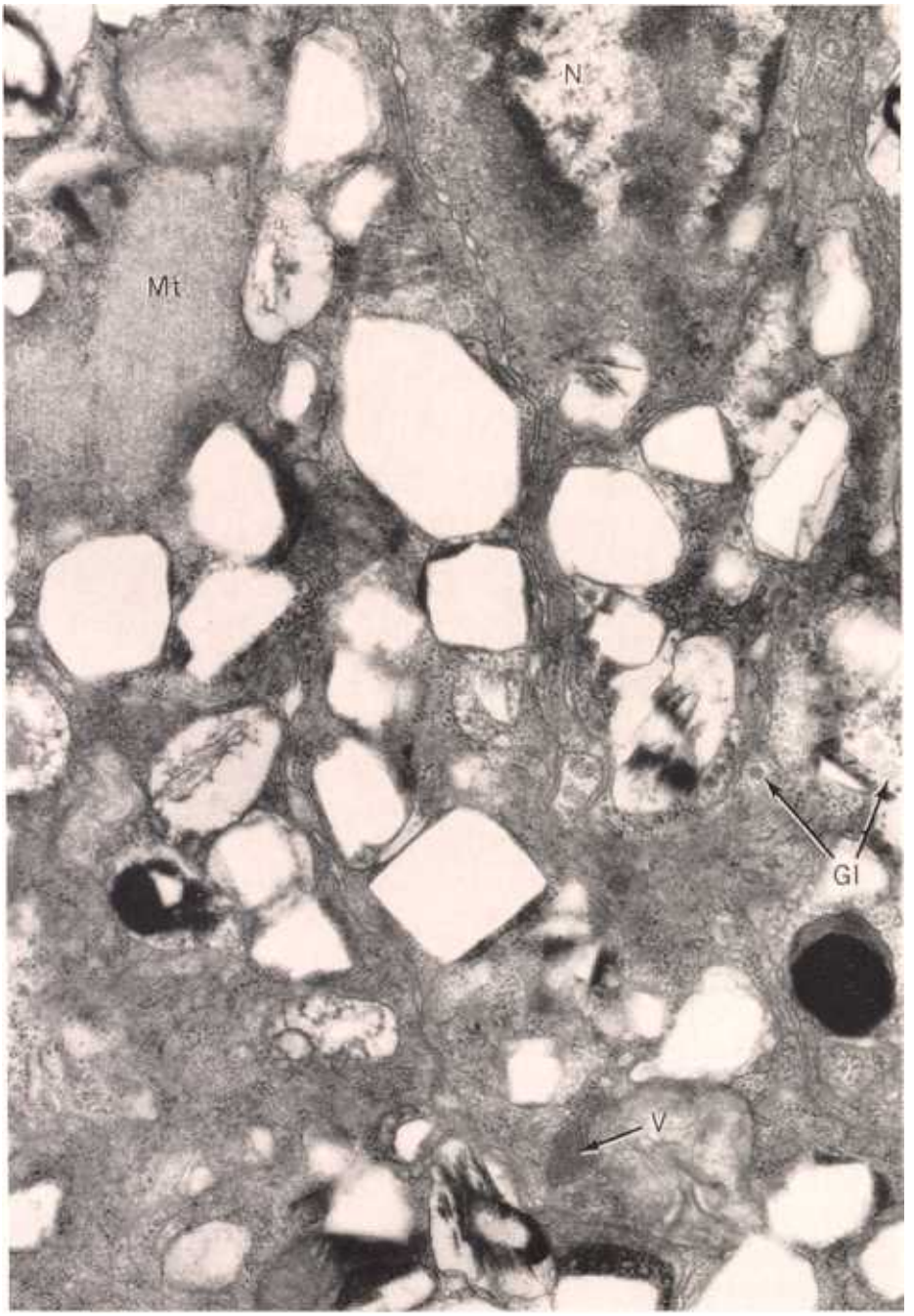

Fig. 4. Electron micrograph of a guanophore. Guanine crystals of various angular shapes have fallen out of the section. A number of vesicles contain spicules or opaque granular matter (*V*). (*Gl*) Glycogen; (*Mt*) Microtubules; (*N*) Nucleus. ×27,000

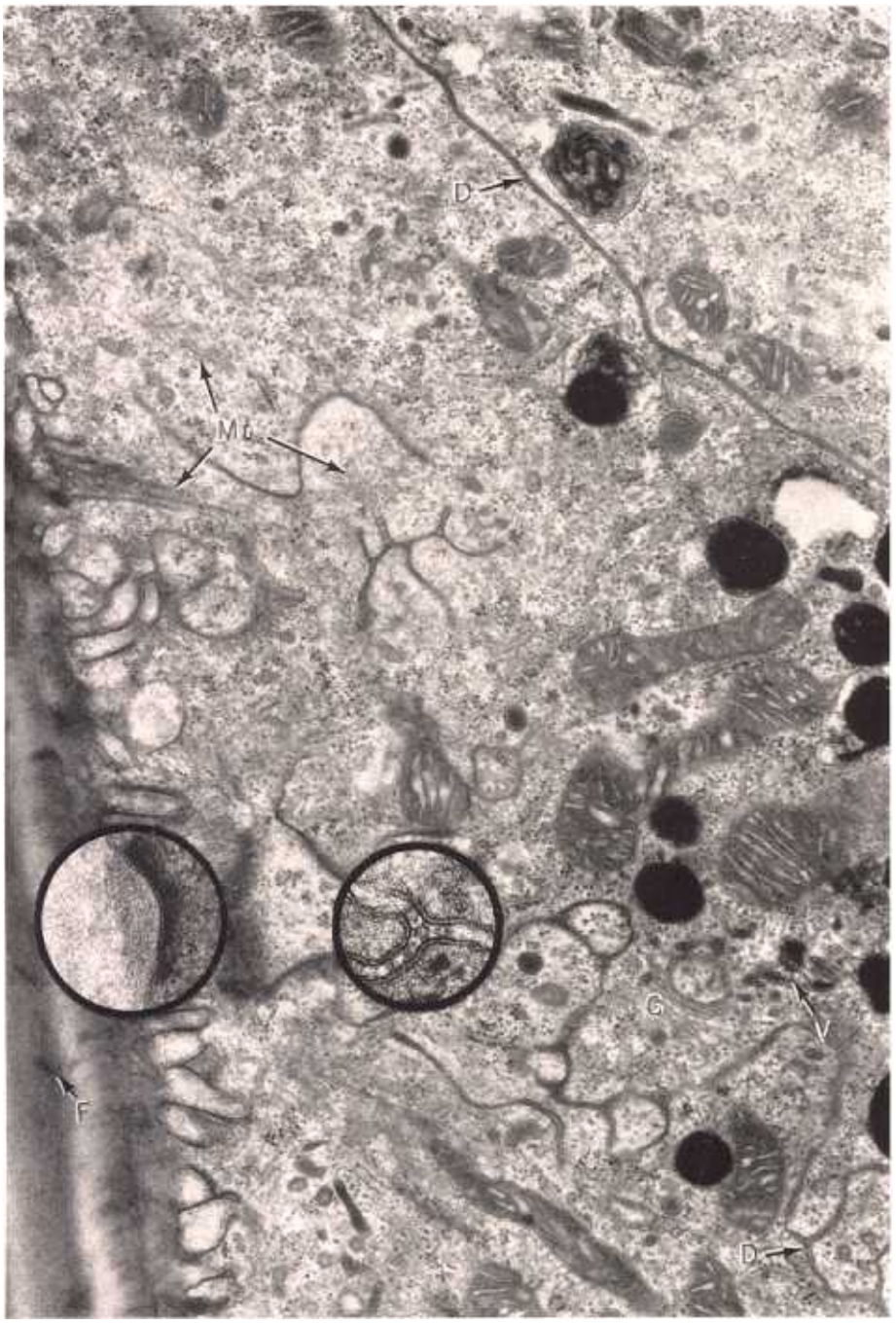

Fig. 5. Electron micrograph of several distal pigment cells adjacent to the cuticular cone. Few pigment granules are present in this region due to the dark-adapted state of the eye. The cuticle contains anchoring filaments (*F*), which originate from dense areas (left inset) at the convoluted apical surface of the pigment; microtubules (*Mt*) insert on these. Desmosomes and septate desmosomes (*D*; right inset) join the cells. A small Golgi complex (*G*) is in immediate proximity to vesicles (*V*) with very opaque content. × 26,000; left inset × 70,000; right inset × 130,000

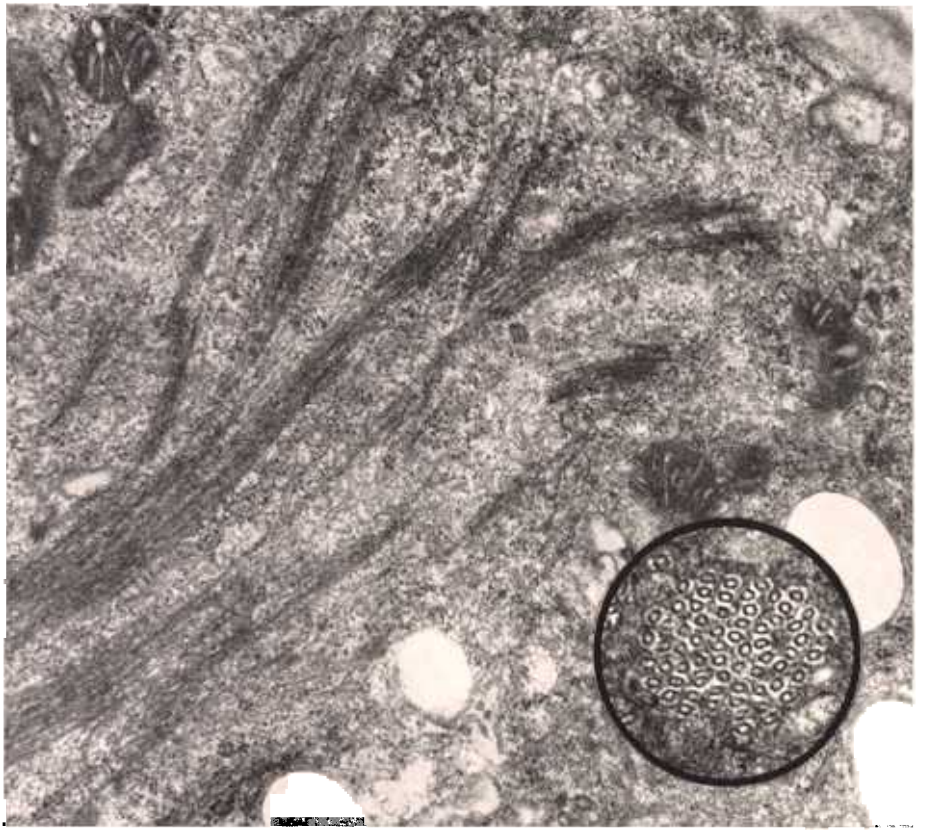

Fig. 6. A large bundle of microtubules in a pigment cell, splaying out on approach to the cuticle (upper right). Each microtubule is usually surrounded by a halo of more translucent cytoplasm (inset). × 31,000; inset × 78,000

Golgi bodies. The large systems of Golgi membranes (Fig. 7) are peculiar in that numbers of coated and smooth vesicles, filled with an opaque material, are liberated from the *concave* side of the crescentic stacks of cisternae rather than from the more usual convex region. The fact that the electron opacity of some of these vesicles approaches that of the pigment droplets leads one to suspect that the larger pigment droplets are formed by coalescence and condensation of detached Golgi vesicles. The pigment droplets remain membrane bounded and measure between 0.2 and 0.8 μ in diameter. They do not at any stage display the substructure peculiar to melanin and commonly fall out of the section with permanganate staining. The pigment is readily extractable by acidified methanol or formic acid, characteristics which indicate that it is an ommochrome (BUTENANDT et al., 1958). A number of other inclusions are commonly found in these cells. Among these are coated vesicles, membrane-bounded dense bodies with the appearance of lysosomes, as well as large autophagic vacuoles of diverse contents. Microtubules are attached to the apical surface in a manner identical to that in the guanophores. Bundles of 10—30 microtubules appear to run the full length of the cell to its proximal termination. The distal pigment cells are

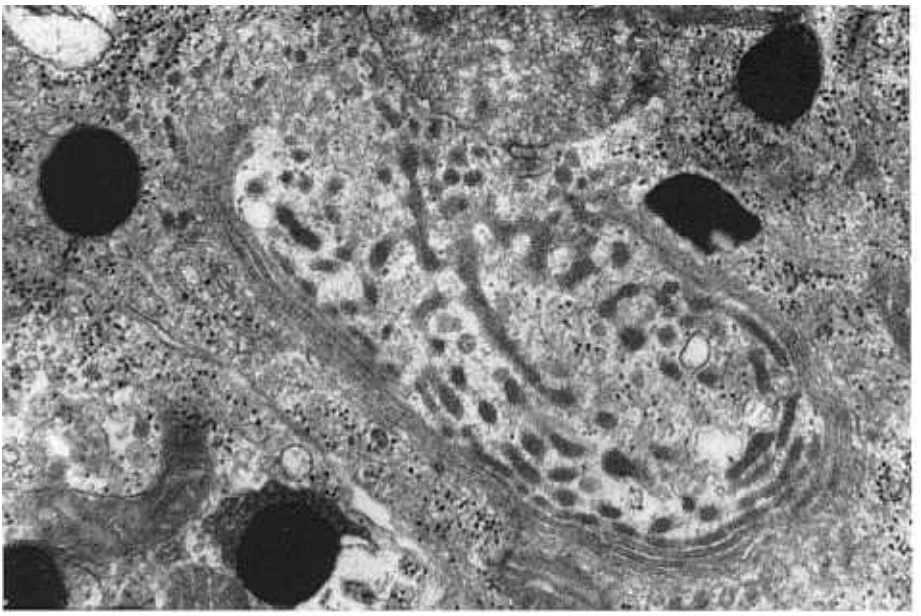

Fig. 7. A large Golgi complex (in a pigment cell) liberating numerous coated and smooth vesicles on its concave side. × 35,000

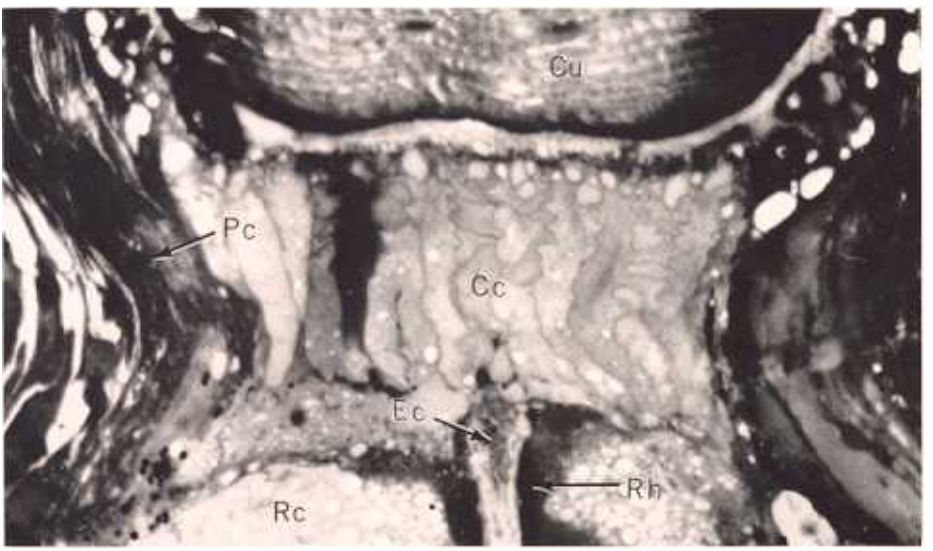

Fig. 8. Photomicrograph of the flattened tip of the cuticular cone (*Cu*), cone cells (*Cc*) and underlying retinula cells (*Rc*). The layer of distal pigment cells (*Pc*) is more loosely structured near the ommatidium than at the periphery of the sheath. The light zone between the cone and cone cells, occupied by fungal cells at the far left and right, may be a developing molting space. (*Rh*) Rhabdome; (*Ec*) Eccentric cell dendrite. × 1,300

reactive to changing levels of illumination. During the dark-adapted state the pigment is located basally around the periphery of the ommatidium, whereas

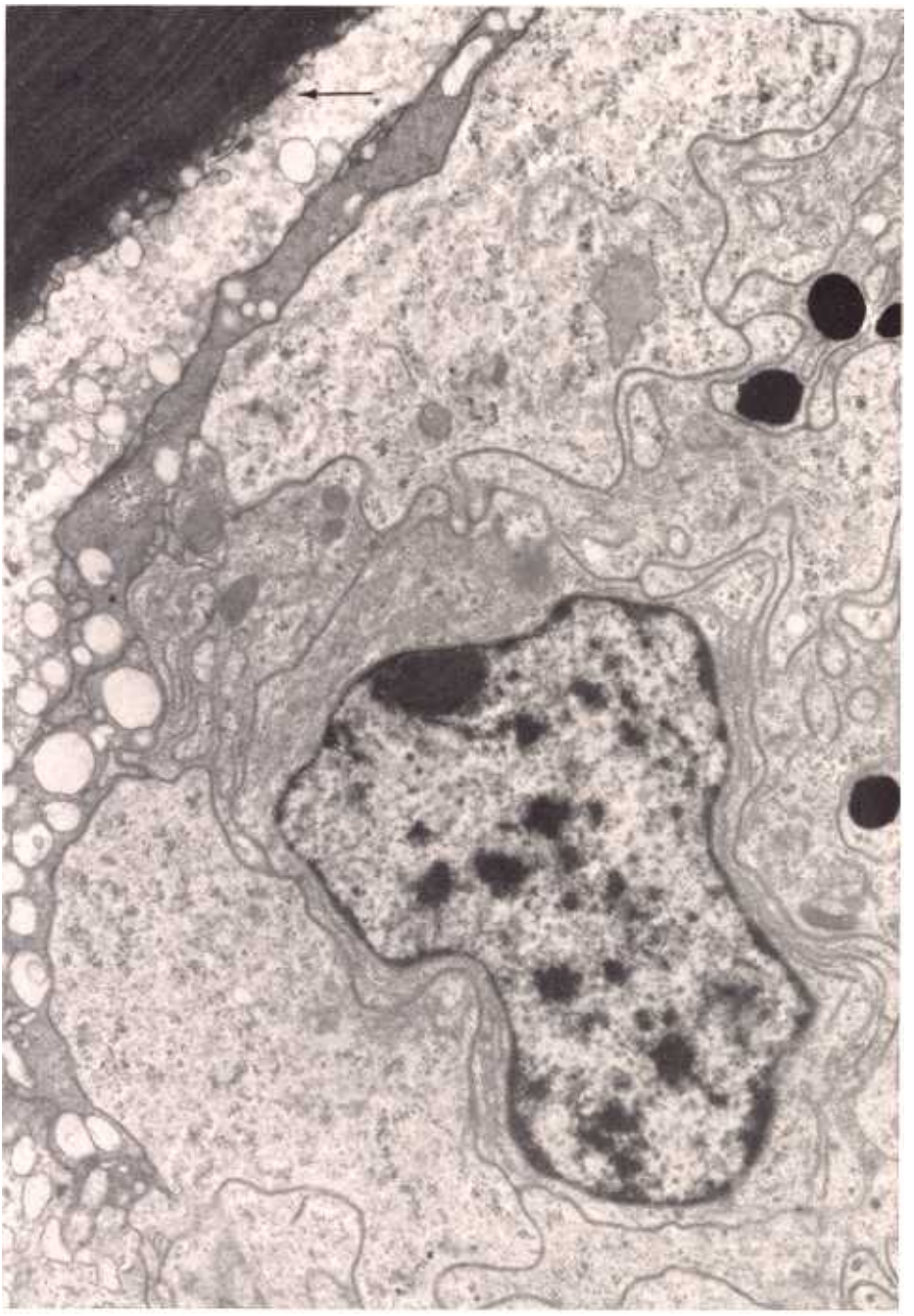

Fig. 9. Electron micrograph of cone cells, illustrating various degrees of vesiculation and electron opacity in adjacent cells. No microtubules are visible in the cells or near epithelio-cuticular junctions (arrow). Pigment at right side belongs to bordering distal pigment cells. × 13,300

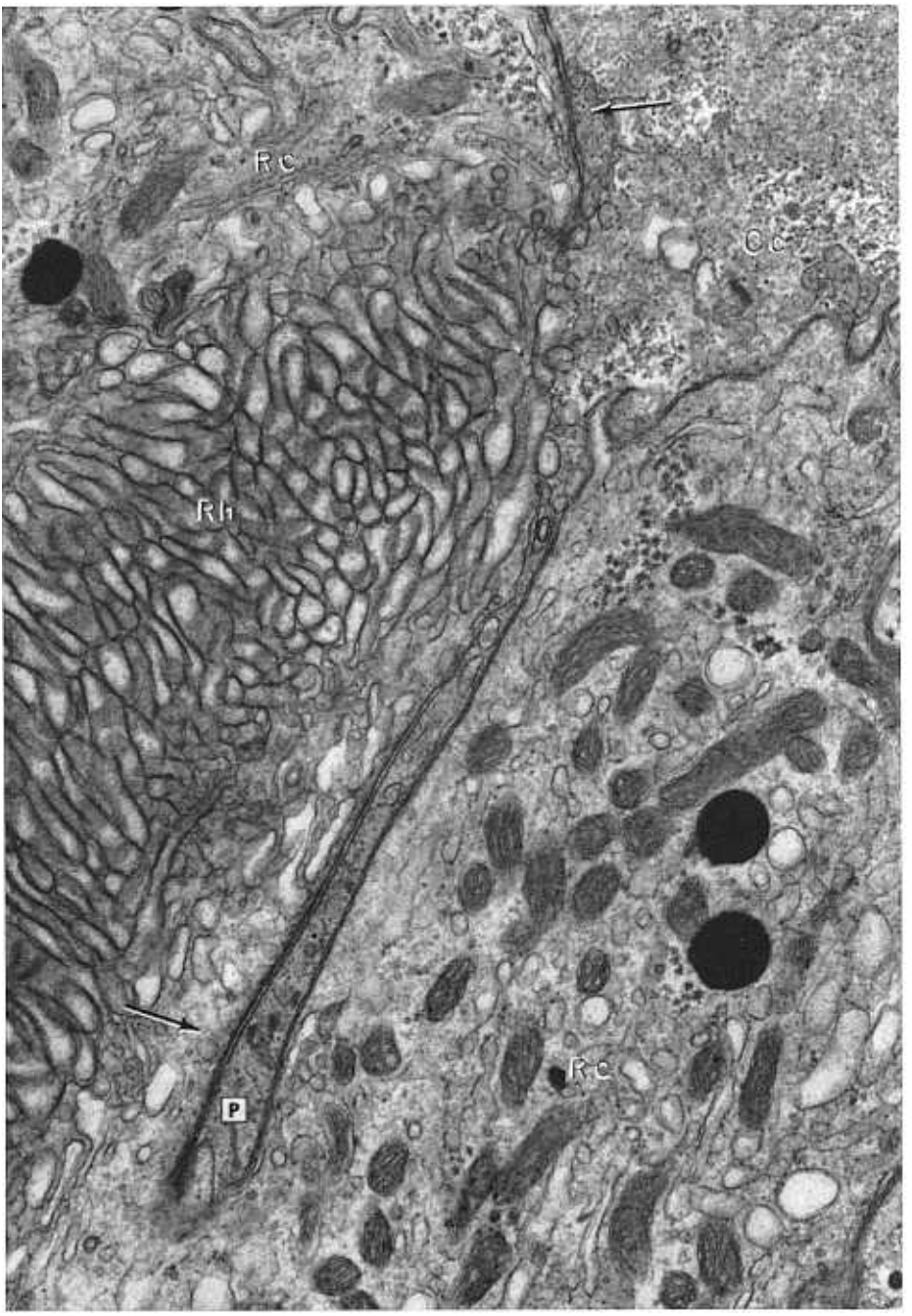

Fig. 10. Electron micrograph of the apical region of an ommatidium in vertical section. A cone cell (*Cc*) above two retinula cells (*Rc*) sends a process (*P*) toward the base of the ommatidium alongside the rhabdome (*Rh*). Junctional modifications are indicated by arrows. × 38,000

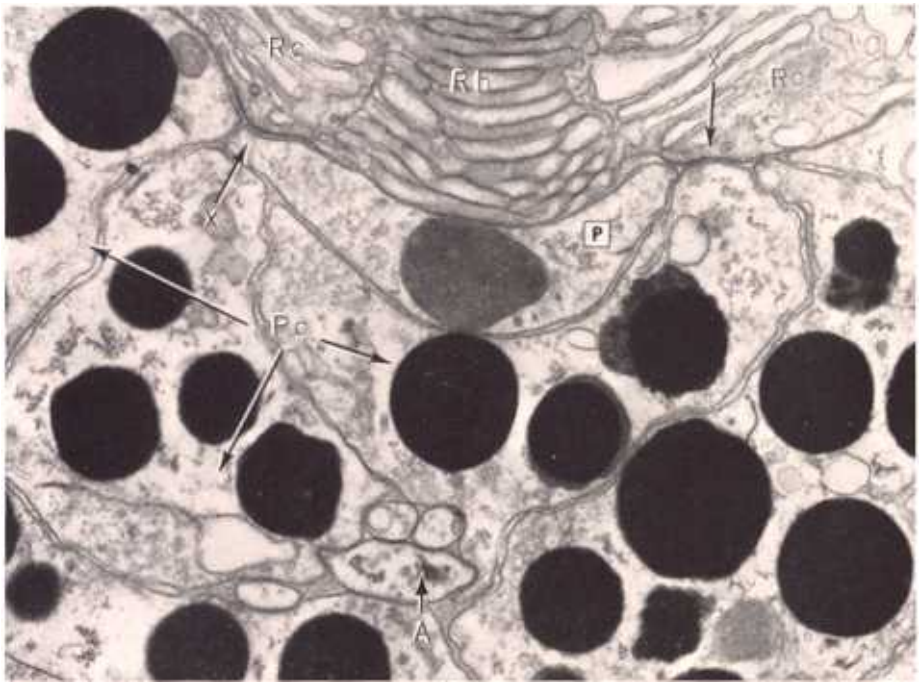

Fig. 11. Electron micrograph of the edge of one ray of the rhabdome (*Rh*), the eccentric cell process (*P*), which separates two adjacent retinula cells (*Rc*), and proximal pigment cells (*Pc*). Note junctions between retinula cells, cone cell process and proximal pigment cells (*X*). Three small axons (*A*) course between pigment cells. × 24,000

on exposure to light the pigment migrates toward the apex of the cells to form a tight cuff around the cone.

The third type of modified epidermal cells consists of the cone cells (Fig. 8). In a large animal, about 100 of these cells form a squat cylinder with the same diameter as the flattened tip of the cuticular cone and a depth of about 30 μ. The cell margins interdigitate deeply in all directions, but few junctional specializations are apparent. Dense patches at the epithelio-cuticular junction are associated with delicate tonofilaments but no microtubules. The cytoplasm of the cone cells is of low electron opacity, contains a small amount of endoplasmic reticulum and free ribosomes, rare mitochondria and virtually no microtubules or pigment (Fig. 9).

Several cone cells, their number probably corresponding to the number of retinula cells comprising the subjacent ommatidium, form long, flattened processes that penetrate into the ommatidium (Fig. 10). These are located at the tip of each rhabdomeral ray (as seen in cross-section, Fig. 11) and follow it to its basal termination, about 120—180 μ. Inasmuch as the central areas of the retinula cells contact each other within the rhabdome, the cone cell process provides the first quasi-glial element separating adjacent retinula cells. The process is joined over its full length to the two retinula cells by junctions resembling

fasciae adhaerentes. Proximal pigment cells, which form the remainder of the septum between any neighboring retinula cells, participate frequently in this junctional specialization.

Numerous neurosecretory axons approach the epidermis by way of the periphery of the ommatidia and terminate between pigmented cells and guanophores. Their origin, precise course, and function is undergoing further study.

Discussion

The presence of swirling filaments in the cuticular cones would lead one to expect a certain amount of form birefringence in these structures. Examination of the cornea with polarized light reveals that the cones act as very efficient depolarizers (unpublished observations by the author). Specifically, the tips of the cones appear bright when viewed through crossed polaroids, with the faintest indication of a Maltese cross, presumably a function of a slight preponderance of radially oriented filaments over circumferential ones, while the remainder of the cuticle remains relatively dark. This finding explains the lack of polarized light perception at normal incidence (WATERMAN, 1954c) and suggests, furthermore, that the apparent differential sensitivity of the ommatidia to polarized light at angles deviant from normal is a function of refraction and reflection within the cuticle and cones. In all cases a beam of depolarized light of varying intensity would arrive at the rhabdome.

The geometrical optics of the dioptric apparatus have been treated in considerable detail by EXNER (1891), who, however, arrived at an angle of acceptance of 8° for each ommatidium, a value that has been greatly revised by neurophysiological experiments (WATERMAN, 1954b). Calculations based on the measurements cited in the present paper and the refractive indices of cuticle (1.54) and tissue fluid (1.34) show that an incident cone of light of 32° in water could be reflected once in a 30° cuticular cone by internal reflection and that a 70° cone of light would be accepted by the ommatidium. These values would increase in air and would decrease in the more pointed, peripheral cones of large animals. Considering the possibility of light scatter at the surface or in the cuticle, these values, based purely on the morphology of the cornea, agree quite well with WATERMAN'S results, namely a significant light response to an incident cone of light of 80° (in air) and occasional responses to much higher angles.

The microtubules of guanophores and pigment cells seem to fulfill an anchoring function analogous to the situation encountered in subcuticular cells of insects (BASSOT and MARTOJA, 1965; NOIROT-TIMOTHÉE and NOIROT, 1966) or crustaceans (BOULIGAND, 1963). In addition, the microtubules probably play a role in pigment migration as suggested for fish melanophores by BIKLE, TILNEY and PORTER (1966). The totality of the phenomenon of dark-light adaptation, which involves retinular cells, guanophores, proximal and distal pigment cells as well as neurosecretory activity, requires further study before a detailed treatment can be presented.

The cone cells in *Limulus* were noted by EXNER (1891) and have been ignored since that time. Considering their cytological specializations, they occupy an important place in the functioning of the ommatidium. The absence of microtubules may be a consequence of the absence of pigment. Furthermore, the cytoplasm has a more optically homogeneous consistency without bundles of

microtubules, which would probably be more refractile than ordinary cytoplasm. The location of the cone cells, as well as their long processes into the underlying ommatidium, suggests that these cells may, in fact, be homologous to the Semper, or cone, cells of the insect compound eye. Processes of these cells have been described in Diptera (WADDINGTON and PERRY, 1960), Hymenoptera (GOLDSMITH, 1962) and Orthoptera (HORRIDGE, 1966). In *Limulus*, the cone cells are bonded both to the cuticle and the retinula cells by specialized junctions, thus providing support in keeping the retinula cells in register with the overlying cuticular cone. Furthermore, it is likely that the continuous junctions at the periphery of all the rhabdomeres provide an impediment to free ion diffusion along the intercellular clefts from the rhabdome to the tissue spaces surrounding the ommatidium as has been suggested by GOLDSMITH (1962) for the junctions adjacent to the rhabdome in the ommatidia of the eye of the bee. Even a partial blockage of this potential low-resistance radial shunt would produce a higher current density around the eccentric cell dendrite and thus would favor the transmission of electrical excitation from the retinula cells to the eccentric cell dendrite.

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