Retinal Structure. Mollusc Cephalopods: *Octopus, Sepia*.* BY JEROME J. WOLKEN. (*From the Biophysical Research Laboratory, Eye and Ear Hospital, and University of Pittsburgh School of Medicine, Pittsburgh.*)‡

The retinal structures of the eyes of the mollusc cephalopods *Octopus* and *Sepia* were investigated as part of a comparative developmental and biochemical study of photoreceptors in our laboratory (1–5). The invertebrate photoreceptors have recently been reviewed by the Milnes (6). The visual structures in this phylum range from the so called simple light-detecting cells, eyespots, to complex eyes capable of forming a visual image.

The *Octopus* and *Sepia* eyes are single lens eyes provided with a mechanism for accommodation and resembling, in these respects and in their general

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physical organization, the vertebrate eye. The lens, however, is formed out of two halves joined together; the retina is not inverted as in the vertebrate eye, and the photoreceptors are directly exposed to the incident light (7, 8). Histological studies indicate that the retina is made up of rhabdomes analogous to those of the arthropod compound eyes (9, 10). The visual pigment in molluscs is proposed to be a rhodopsin (11, 12). The *Octopus* has been used by J. Z. Young and his collaborators to study visual acuity and learning (13). Because of these interests and its phylogenetic position in the scheme of visual development, it was desirable to obtain at higher resolution additional structural information on the visual elements within the retina. Fixed, thin sectioned retinas were examined in the electron microscope, and the resulting electron micrographs studied to ascertain the structural pattern of the visual elements within the retina.

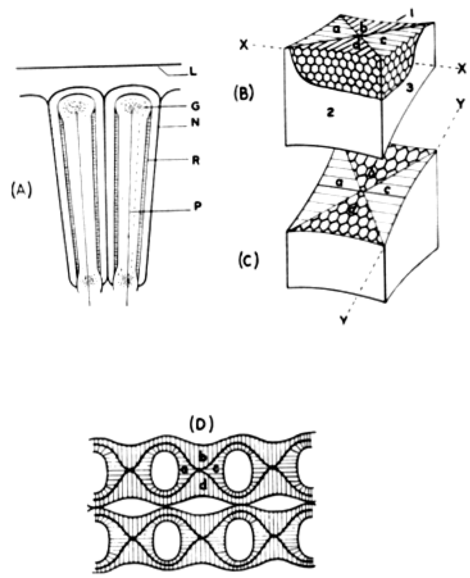
Methods

Octopus vulgaris and *Sepia officinalis* were obtained from the bay of Naples in the summer of 1957. The eyes were excised in dim red light and their retinas were dissected out and immediately fixed in 1 per cent osmium tetroxide (dissolved in sea water) for 1 to 4 hours at 5°C. After fixation the retinas were washed with distilled water, dehydrated in the usual manner by a series of ethanols, embedded in the monomer *n*-butyl methacrylate, and the monomer polymerized by heat. These procedures were carried out at the Zoological Research Station, Naples, Italy. Thin sections of the retinas were subsequently cut from the polymerized blocks using a glass knife attached to a Porter-Blum microtome. The sections were mounted on formvar and carbon-coated grids; these sections were examined with a Phillips EM 100 A electron microscope.

OBSERVATIONS AND DISCUSSION

The thin sections of fixed retinas show clearly that the *Octopus* and *Sepia* retinas are made up of rhabdomes. Each rhabdome consists of four visual retinal units (rhabdomeres or retinal rods) radially arranged (Figs. 1 to 3).

Grenacher recognized a long time ago that the retina consists of rhabdomes and suggested that four retinal units make up a rhabdome (9). He was unable with the limited powers of resolution then available to see the structure within an individual rhabdome. We shall refer to these retinal units as rhabdomeres, which is analogous to the terminology used for the visual elements within the insect ommatidia, although it would be just as easy to think of them as retinal rods. In a cross-section through the rhabdomes there are four sides of the rhabdomeres which are isolated by pigment cells containing screening pigment granules that probably can migrate depending on the light intensity (Figs. 1 to 3). In longitudinal serial sections the rhabdomeres measured from 50 to 60 μ in length and in numerous cross-sections and longitudinal sections from 1.0 to 1.5 μ in diameter. Depending on the angle of cutting the rhabdomeres appear as packed plates or packed hexagons (Figs. 1 *a* and *b*, and Figs. 3 *a* and *b*). In all cross-sections the lamellar structure is observed whereas in all oblique and longitudinal sections the reticular (or hexagonal) packing appears. The dense bands that form the lamellar structures are ~200 A in thickness and the less dense interspaces are ~350 A in thickness. The lamellar and reticular fine structures within the retinal units of the rhabdome, regardless of the orientation of the rhabdomeres with respect to the plane of cutting, indicate a single geometrical structure which produces two general patterns in thin sections depending entirely upon the orientation of the rhabdome with respect to the plane of cutting. The fact that the dense bands and the less dense interspaces are of the same order of magnitude in both the lamellar and the reticular design suggests a single structural packing of a rod or a tube. The measurements mentioned indicate that there are ~20 rods or tubes per micron. The tight packing of these tubes or rods produces a roughly hexagonal structure as seen at a higher magnification in Fig. 1 *a* and Fig. 3 *a*. In each rhabdome regardless of the degree of obliqueness of the sections cut, there is a reticular pattern as well as a laminated pattern as illustrated in Text Fig. 1 *c* and as shown in Fig. 2. Only in cross-sections do the four rhabdomeres of the rhabdome reveal a uniform lamellar structure. The rhabdomeres are most likely closely packed tubules that extend to the center of the rhabdome. The orientation of the rhabdomeres in the rhabdome and the internal structure are schematically illustrated in Text-fig. 1, where (A) is a longitudinal cut through several rhabdomes. In (B) and (C) the arrangement of each rhabdomere that makes up the rhabdome is depicted depending on whether the cut is through the *X-X* axis or the *Y-Y* axis. (D) illustrates how the rhabdomes appear with their four rhabdomeres as viewed in



TEXT-FIG. 1. (A), a schematic longitudinal section through two rhabdomes. *L*, limiting membrane, *G*, pigment granules, *N*, limiting fiber or membrane, *R*, rhabdomere, and *P*, nerve fiber. (B), a cross-section *X-X* of a three dimensional view of a single rhabdome made up of four retinal elements *a*, *b*, *c*, and *d*. (C), an oblique section *Y-Y* through the rhabdome showing a lamellar pattern on sides *a* and *c*, packed tubes on the opposite sides *b* and *d*. (D), a cross-section of several rhabdomes illustrating the pattern of the four retinal elements *a*, *b*, *c*, and *d* and how they may be interlocked.

cross-section on the surface of the retina. It is interesting to note in comparison the similarity of the *Octopus* and *Sepia* rhabdomeres to the rhabdomeres of the arthropods, particularly *Drosophila* and *Limulus*, as well as to those of several other insects recently observed in the electron microscope (3–5, 14–16).

The *Octopus* and *Sepia* retinas are at least $\sim$1 cm.^{2} in area; this means that they would contain about 2000 rhabdomes or about 8000 rhabdomeres per retina per eye. The number of visual elements is equivalent to the compound eye of the insect *Drosophila melanogaster*. The surface area available per rhabdome, assuming that the carotenoid pigment molecules would be spread on a monolayer over the entire surface, would permit $\sim 1 \times 10^9$ rhodopsin molecules per rhabdomere. How the rhodopsin molecules themselves would be oriented (with respect to each other) within the tubules of the rhabdomeres is at present unknown, but we have previously suggested that in all photoreceptors the pigment molecules would be oriented as monolayers between lipides and aqueous protein complexes (1, 2, 4).

SUMMARY

The retinas of the eyes of *Octopus* and *Sepia* were fixed in 1 per cent osmium tetroxide, dehydrated, and embedded in *n*-butyl methacrylate. Thin sections of the retina, cut from the polymerized blocks, were studied by the electron microscope. Each retina was found to be made up of rhabdomes analogous to those of the arthropod compound eyes. There are four retinal rhabdomeres radially arranged to form a rhabdome. A central space containing pigment cells with screening pigment granules appears to separate the rhabdomes. Each retinal rhabdomere, measures $\sim$1.0 μ in diameter and $\sim$60 μ in length, and is constructed of packed tubules. There are $\sim$20 tubules per micron whose dense bands are $\sim$200 A and whose interspaces $\sim$350 A, as measured from the electron micrographs. The rhabdomere structure of packed tubes, in preference to packed plates, is similar to that found for the insect *Drosophila* and for the visual cells of other arthropods. Electron micrographs together with a schematic model illustrate the structural packing of the rhabdomes and rhabdomeres.

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Explanation of Plates

Plate 420

Fig. 1. *Sepia.* An oblique section to show the general patterns of the rhabdomes and structure within the rhabdomere. × 7,000.

(*a*) The hexagonal structure at higher magnification. × 35,000.

(*b*) The lamellar structure at higher magnification. × 35,000.

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PLATE 420
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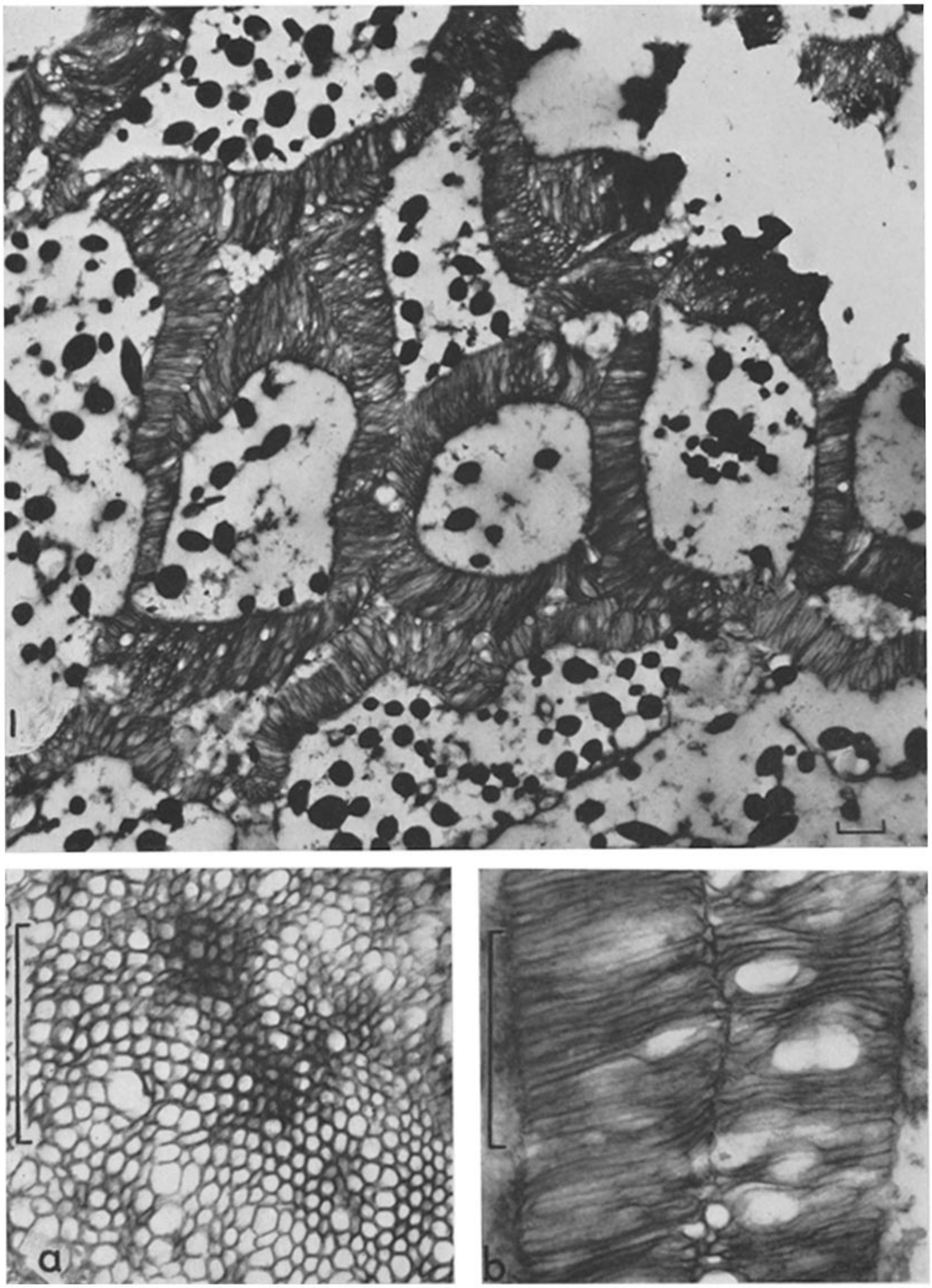


(Wolken: Retinal structure of mollusc cephalopods)

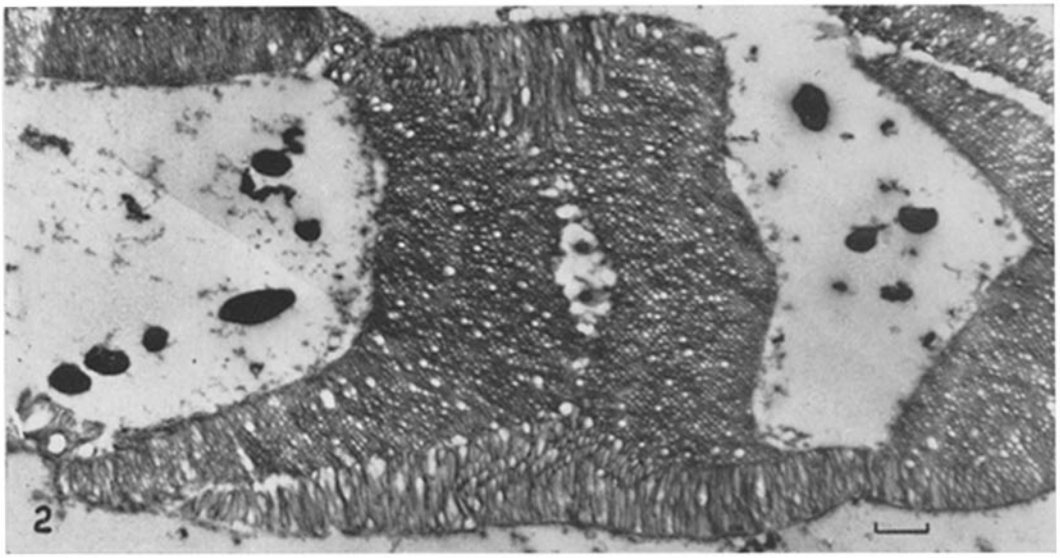
PLATE 421

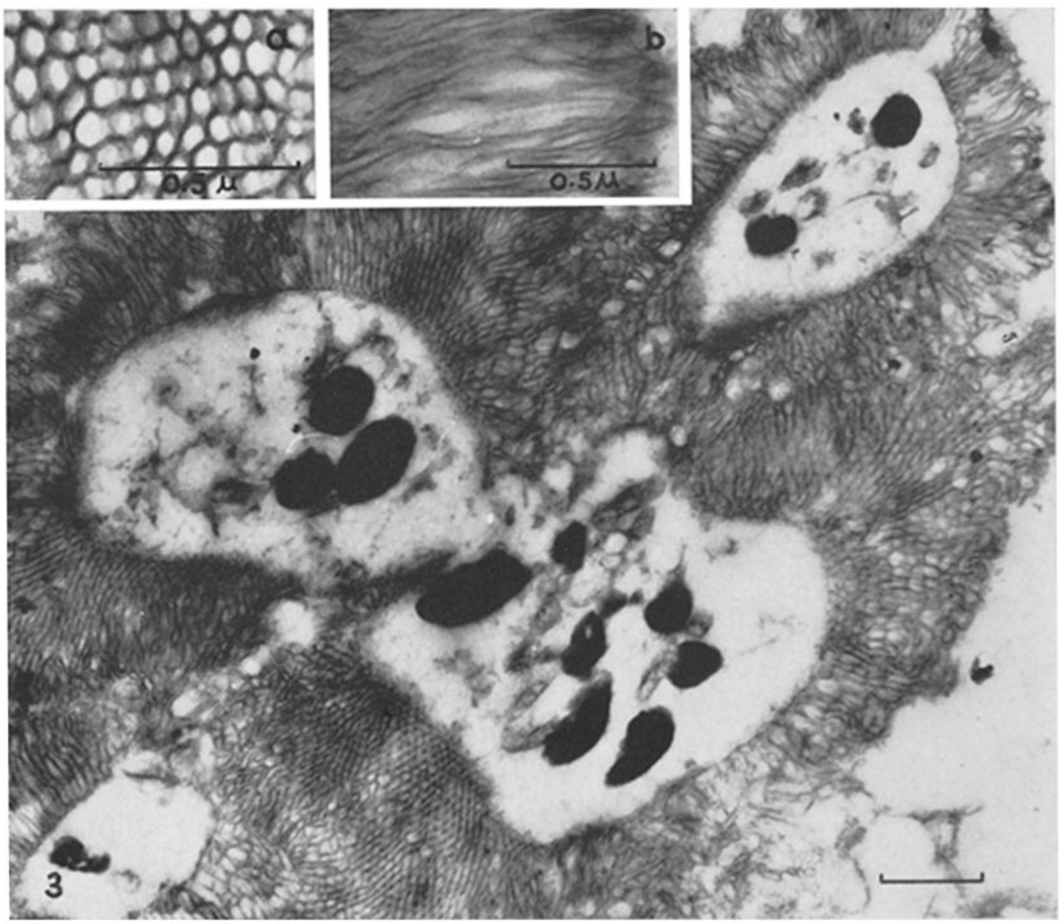
FIG. 2. *Sepia*. An oblique section of a rhabdome showing two opposite sides of the rhabdomere hexagonal structure and two sides of the lamellar structure. × 7,000.

FIG. 3. *Octopus*. A slightly oblique section showing a lamellar and hexagonal structure. × 15,000.

(*a*) A higher magnification showing the spacings of the lamellar structure. × 43,000.

(*b*) A higher magnification of the hexagonal pattern of packed tubes. × 58,000.





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