THE COMPOUND EYE OF A CRUSTACEAN, *LEPTODORA KINDTII*

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A comparative study of the structure and chemistry of the retinal photoreceptors of the eye is under continuous investigation in our laboratory. A part of this study is focused on the image-forming compound eyes of the arthropods, which include the arachnids, insects, and Crustacea (13–18). The present report is a study of the eye structure of *Leptodora kindtii*, a planktonic crustacean belonging to the order Cladocera which is found in many fresh-water lakes of North America, Europe, and Asia. The general morphology and behavior of *Leptodora kindtii* were described more than a half-century ago by Gerschler (5). *Leptodora* is a relatively large organism (up to 18 mm in length), nearly all transparent, with one median, spherical eye. Because of its eye structure and various types of neurons (9), as well as its general behavior to light stimuli, *Leptodora* is of interest to us in studies of visual physiology.

MATERIALS AND METHODS

Leptodora kindtii were collected from a depth of 1 meter at the University of Pittsburgh Field Biology Laboratory, Lake Pymatuning, Pennsylvania. For electron microscopy, the organisms were immediately dark-adapted for 1 hour and then fixed for 30 minutes at room temperature with 1 per cent osmium tetroxide (OsO_4) in lake water, which included 45 mg/ml of sucrose and was buffered with Veronal-acetate to pH 7.5. After fixation, the organisms were washed with distilled water, then dehydrated by a series of graded alcohols, infiltrated with Vestopal W, flat-embedded, and polmerized. To obtain a preferred orientation for sectioning, areas were cut from the embedded material with a jeweler's coping saw and remounted. Sections were cut through the crystalline cone and photoreceptor regions of the compound eye, using a glass knife on a Porter-Blum ultramicrotome. All sections were examined with an RCA-EMU-3F microscope.

To determine the reactions of *Leptodora* to polarized light, an experimental method similar to that used by Waterman (10) for *Daphnia* was adopted, in which a low voltage, 25 watt, tungsten filament bulb provided a vertical beam of white light which passed successively through a heat filter, a depolarizer, and adjustable polarizer, (Polaroid), linearly polarizing the light almost completely. The experimental vessel was a Petri dish 10 cm in diameter and 1 cm deep, painted black to eliminate any reflections from the glass. The light intensity on the surface of the dish was about 20 foot-candles. Active, swimming *Leptodora* (50 per dish) were focused on a photographic easel and irradiated for 5 seconds with polarized light, then photographed on a plate, 3¼ x 4 inches. This operation was followed by a dark period of 15 to 20 seconds, and then another exposure on a photographic plate was made. 95 such plates which had been exposed in this manner were developed. The negatives were enlarged by printing, and the plane of polarization was indicated on each print. To obtain the angle of polarization, a line was drawn through the long axis of each *Leptodora* with respect to the plane of polarization and the angle was measured. The frequency for each angle from 3000 such measurements was tabulated and the data statistically evaluated.

OBSERVATIONS

The entire eye of *Leptodora kindtii* is contained within the transparent exoskeleton at the anterior end of the organism (Fig. 1). The eye is free to

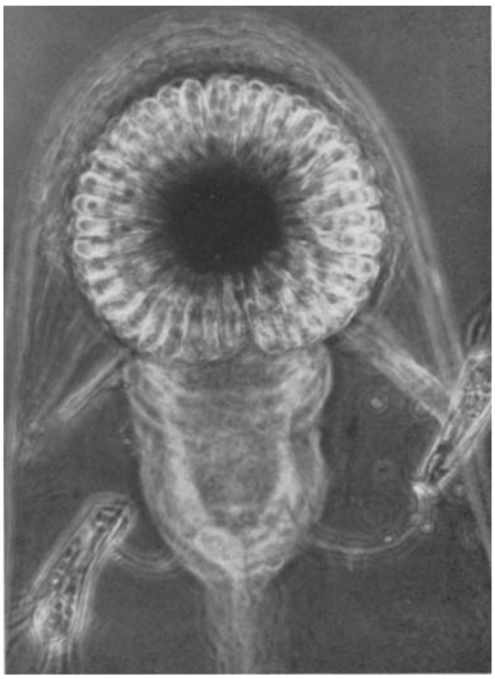

FIGURE 1 The compound eye of *Leptodora kindtii*, showing radial arrangement of ommatidia. × 100.

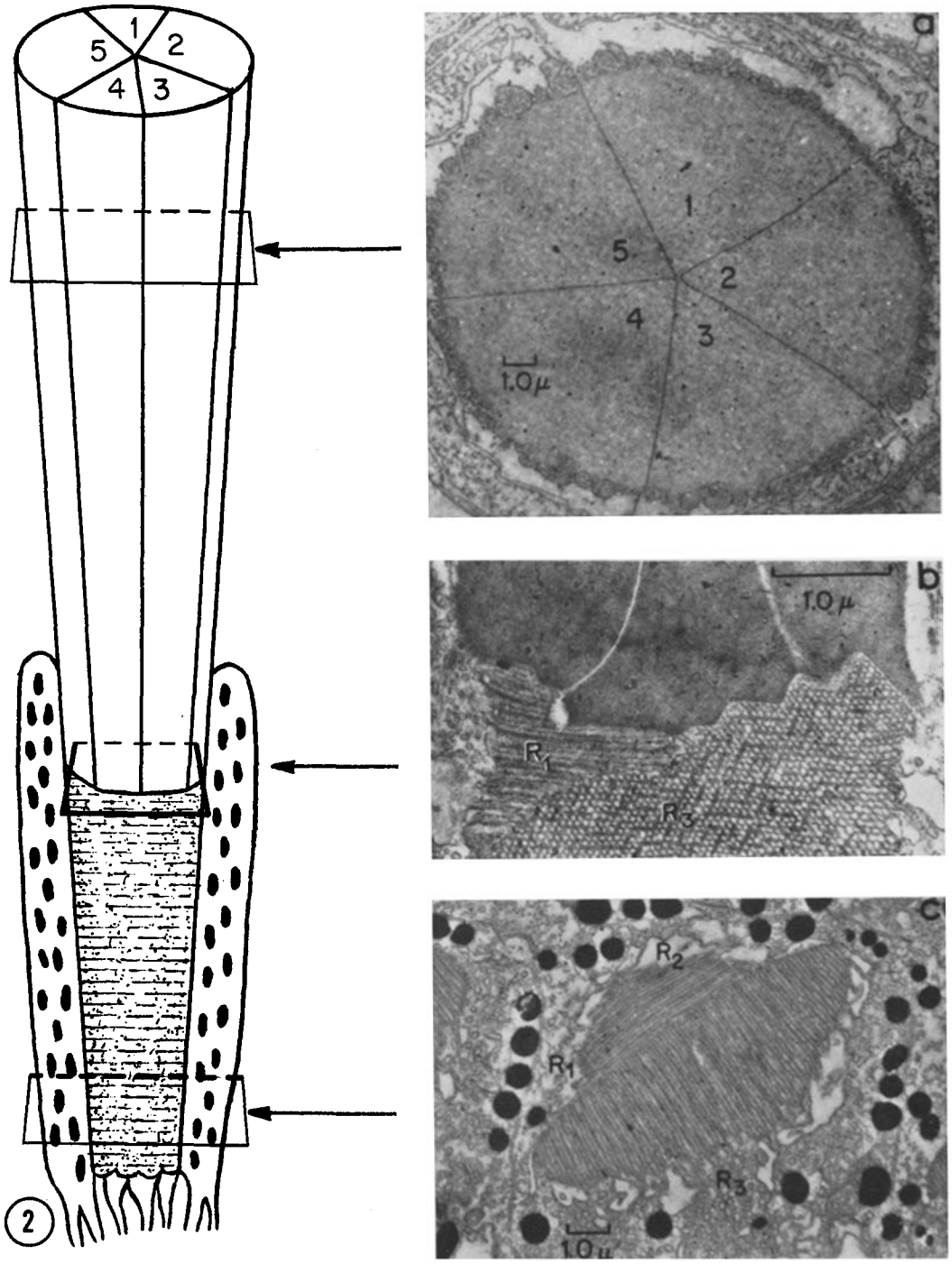

FIGURE 2 Schematic diagram of ommatidium with electron micrographs which indicate the structure at various levels. (a) Cross-section of crystalline cone. (b) Longitudinal section, crystalline cone and rhabdome connection. (c) Cross-section, through a single rhabdome showing rhabdomeres R_1, R_2, R_3. Fig. (a), × 4800; Fig. (b), × 17,400; Fig. (c), × 6200.

move and can rotate 10° in either direction. A small area in back of the eye is for accommodation of the optic processes. The brain lies in close proximity to the eye elements, with no optic nerve chord, as such, between the eye and the brain.

The compound eye of *Leptodora* consists of approximately 500 ommatidia that are radially arranged 360° around it as shown in Fig. 1. The interstitial material between the surface of the eye sphere and the curved external chitinous wall probably serves as a common lens for all the ommatidia.

The ommatidia are large conical structures 180 μ in length and of a diameter ranging from 30 μ at the outer portion to just a few microns at the base. An ommatidium (see schematic diagram in Fig. 2 and electron micrographs Fig. 2 *a*, *b*, and *c*) consists of crystalline cone cells (*a*), rhabdomes (*b* and *c*), and pigment cells. The retinula cells contain many vesicles, numerous mitochondria, and have differentiated structures, the rhabdomeres Fig. 2 *c*, R_1, R_2, and R_3). The rhabdomeres are analogous to the outer segments of the retinal rods of the vertebrate eye. The rhabdomeres of the retinula cells form the rhabdome (Figs. 2 *c* and 3), the photoreceptor area.

The crystalline cone consists of five crystalline cone cells that are arranged in five pie-shaped segments (Fig. 2 *a*). Although the crystalline cone continues proximally to the surface of the rhabdome as in the apposition-type eye, observations of pigment migration indicate that, under certain conditions of dark adaptation, "crossing" among adjacent crystalline cones could result in the formation of a superposition image. As the crystalline cones continue inward, the space between them increases and is taken up with pigment cells.

The rhabdomes are affixed directly to the ends of the crystalline cones (Fig. 2 *b*). The four radially arranged retinula cells that form the rhabdome show only three closed rhabdomeres (Figs. 2 *c* and 3). One of the rhabdomeres (R_3) is large in comparison to the other two rhabdomeres, (R_1 and R_2), and appears to be two fused rhabdomeres.

The rhabdomere *fine structure* is that of tightly packed tubules (Fig. 4 *a*); however, the tubules can appear as lamellae (Fig. 4 *b*), depending on the angle of cut. These tubules have an outside diameter of 500 A and an inside diameter of 400 A, with a total wall thickness of 100 A. Each membrane that forms the wall is of the order of 40 to 50 A. The tubules of the small rhabdomeres (Fig. 2 *c*, R_1 and R_2, and Fig. 3) are arranged 90° with respect to the large rhabdomeres (R_3). The ends of the rhabdomere tubules (referred to by some authors as microvilli) appear continuous with the cytoplasm (Fig. 3). This has also been observed for the rhabdomeres of other arthropods (2, 3, 7, 8).

The data for polarized light analysis (Fig. 5) from 3000 trials were analyzed using an IBM 7090 computer. The mean, variance, and standard deviation of the frequency with which *Leptodora* selected a given angle of polarized light was calculated. The data had a mean value of 32.68, a variance of 130.48, and a standard deviation of 11.42. Statistical methods indicate that significant points must have a value that differs from the mean by three times the standard deviation in any given situation. The significant positive level by this method is 66.88 ($3 \times 11.42 + 32.62 = 66.88$). The frequency of all angles from 0 to 90° gave only two points, at 0° and 90° with respect to the plane of polarization, which had values above 66.88. On the basis of this study, *Leptodora kindtii* does have a strong tendency to orient at 0° and 90° to plane-polarized light (Fig. 5). Sunlight passing through clear water is not likely to be polarized; however, particles, of the order of 1 μ or less, suspended in the water tend to serve as polarizers (11). *Leptodora* are found in quite turbid water, and there could be sufficient polarized light beneath the water surface to influence orientation.

DISCUSSION

It is difficult, at present, to relate the optics of the compound eye to the structure of the ommatidia and to the behavior of the organism upon light stimulation. However, some structural observations and other descriptive details can be indicated.

It is doubtful, from our present studies, that the crystalline cone has any lens cylinder properties; if so, measurable changes in indices of refraction would be needed (1, 12).

Compound eyes form two types of rhabdomes: the "open," in which the rhabdomeres are not in contact with each other (4, 6, 7, 17) as in the fruitfly, *Drosophila*, and the housefly, *Musca domestica*; and the "closed," in which the rhabdomeres are fused (18) as in the cockroach, *Periplaneta Americana*, and the honeybee, *Apis mellifera*. It was hypothesized that the open and closed eye types were peculiar to a specific kind of vision, but recent electron microscope studies indicate that such structural organization may be peculiar

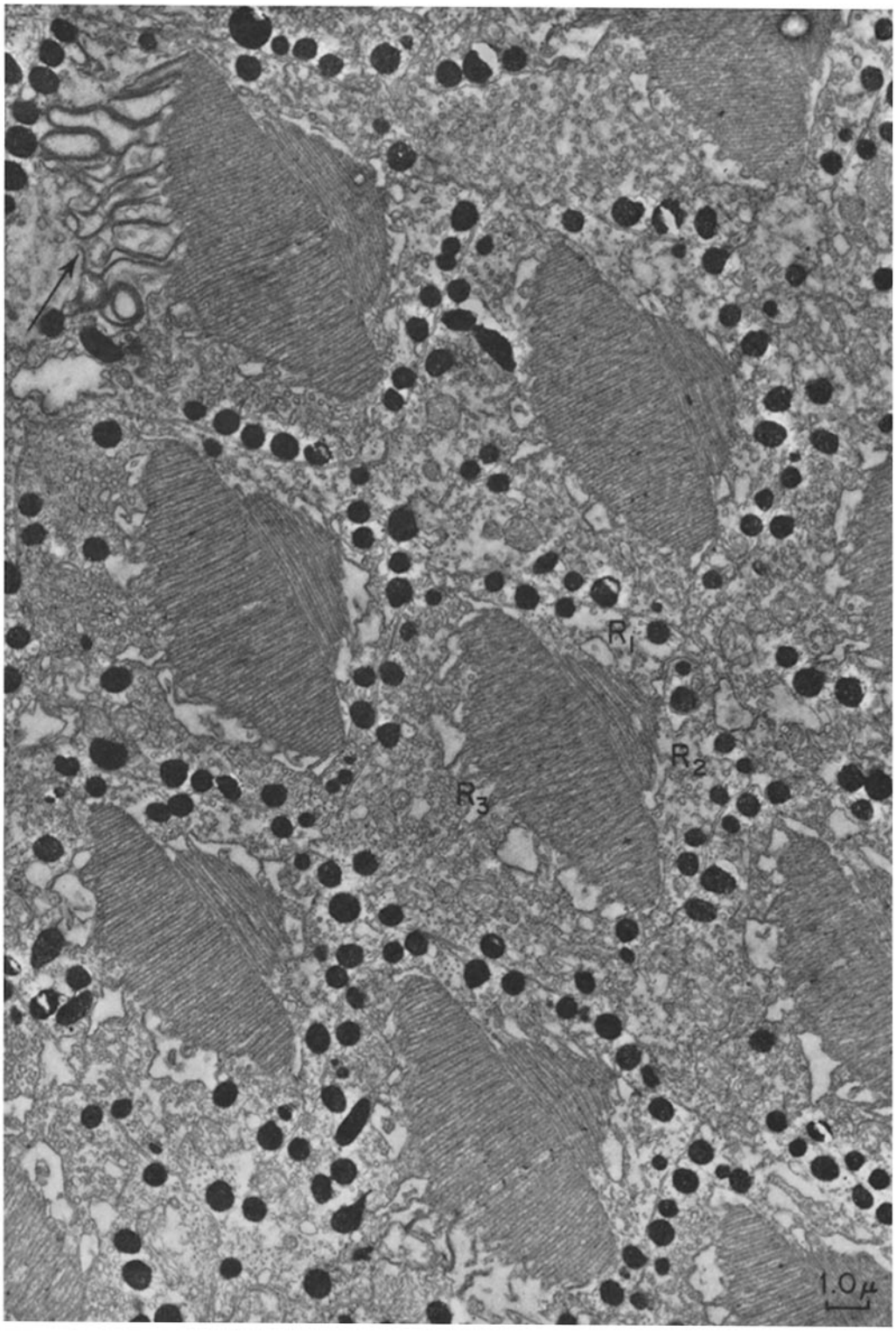

FIGURE 3 Cross-section, through rhabdome area, showing many rhabdomes. Note that the tubules (or microvilli) of the rhabdomeres are in contact with the cell cytoplasm (see arrow). × 6200.

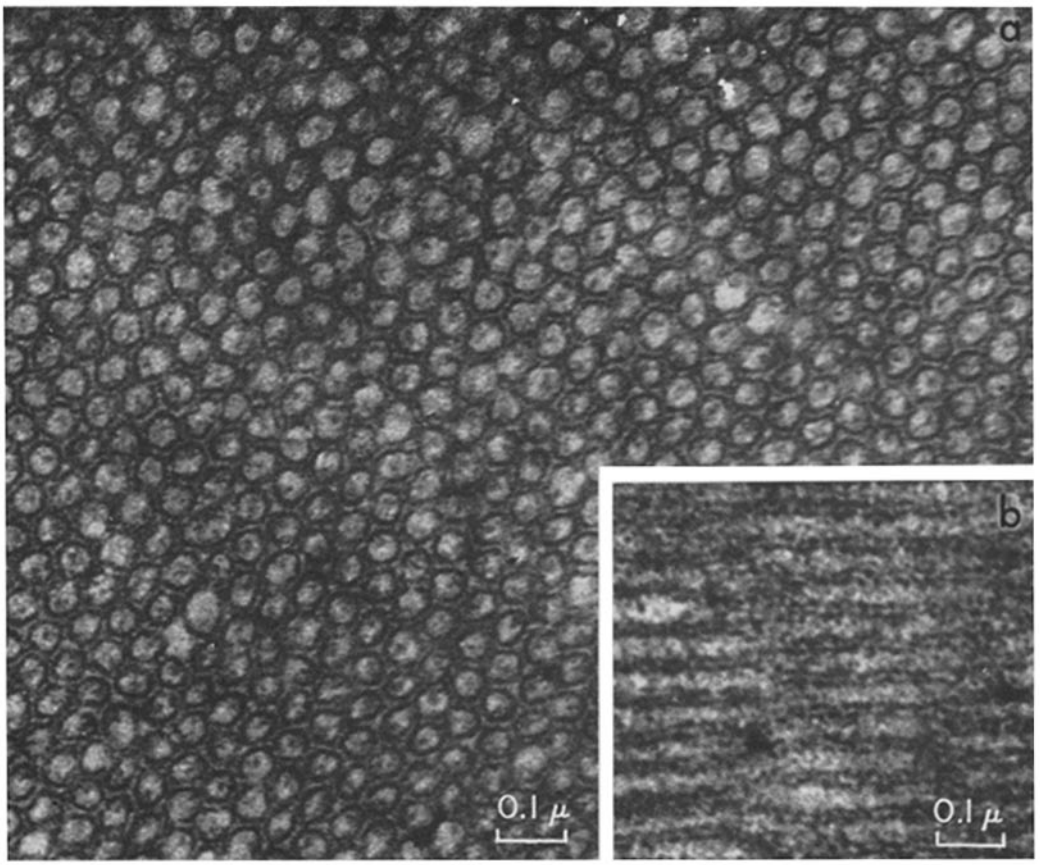

FIGURE 4 Higher magnification of a section of the rhabdomere. (a) Cross-section, showing the tubules (or microvilli). (b) Longitudinal section of the tubules which appear as lamellae. Fig. (a), × 80,000; Fig. (b), × 95,000.

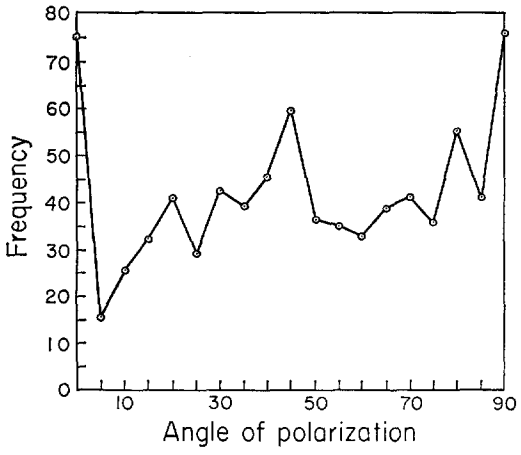

FIGURE 5 The angular frequency of orientation of *Leptodora* with respect to the angle of polarization.

to the species and not correlated with its behavior or type of vision.

The *fine structure* of the rhabdomere is similar in geometry and dimension to that described for a variety of arthropod and mollusc photoreceptors; *i.e.*, tightly packed tubules (4, 8, 13, 17). It is also similar to that found in other crustacea; *e.g.*, the fresh-water *Daphnia*, and the marine *Copilia* which is found at depths of 200 meters (16). The observation that four retinula cells form only three fused rhabdomeres has been described for the dragonfly, *Anisoptera* (6, 8). However, the large rhabdomere (R_3) could be formed by fusion of two of the rhabdomeres.

The data concerning polarized light analysis by the arthropods, whether intra- or extraocular, are, at present, incomplete. It is not possible from these

studies of *Leptodora* to draw any direct correlation between the structure of the compound eye, the structure of the rhabdome, and the structure of the rhabdomere with polarized light analysis.

The behavioral studies also indicate a spectral sensitivity, or action spectrum, for *Leptodora* near 520 mμ in its swimming response; in addition, microspectrophotometric analysis of the ommatidia of the eye showed an absorption peak near 510 mμ (16). In the absence of isolation, these data indicate that in this spectral range the organism probably possesses a "rhodopsin-like" visual pigment.

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