THE EYE STRUCTURE AND OPTICAL SYSTEM
OF THE CRUSTACEAN COPEPOD, *COPILIA*

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Among the curious eyes found in nature is that of a little known marine crustacean, *Copilia*, a copepod, which is about 1 × 1 × 3 mm. This copepod is found in the Mediterranean and Caribbean Seas. In 1963, while we were working on crustacean eyes at the Zoological Research Station, Naples, Italy, Professor J. Z. Young brought to our attention this animal and its unusual eyes. Only the female of the species possesses these remarkable eyes which make up more than half of its transparent body. The eye of *Copilia* (Mediterranean) was described in 1879 by Grenacher (6) and in 1891 by Exner (1) who made measurements of its optical system. Recently Vaissière (8, 9) and Gregory (3, 4) and his collaborators (5) have made studies of the eye and the behavior of *Copilia*. Each eye appears as the single ommatidium of a compound eye (sometimes referred to as ocelli in copepods) with a corneal lens, crystalline cone, and retinula cells that form its rhabdom (Figs. 1 and 4). The rhabdom lies in a pigmented stem that oscillates back and forth (Fig. 4). It

seemed important to learn more about the optics and imaging properties of such scanning eyes and also to see by electron microscopy how the rhabdom and its rhabdomeres are structured in comparison to other arthropod visual systems (11, 12).

MATERIALS AND METHODS

Copilia quadrata and *Copilia denticulata* were collected in the Bay of Naples during summer visits to the Zoological Research Station, Naples, Italy, in 1963 and 1965. They were found in the planktonic layer at a depth of 150–200 m. Dr. Neville Moray, then working at the Station, identified these two species. The animals were immediately placed in a cold room, 12°C, and dark adapted for 1 hr before fixation. They were then fixed with 1% osmium tetroxide (OsO₄) in seawater for 1 hr at 4°C and for another hour at room temperature. After fixation, the animals were washed with distilled water, then dehydrated through a series of graded acetone solutions of 30–100%, infiltrated with Araldite monomer, and polymerized until hard at 60°C. To obtain preferred

orientation for sectioning, the eye areas were cut from the embedded animals and remounted. Sections for electron microscopy were cut with a glass knife mounted on a Porter-Blum ultramicrotome. Some sections were stained with lead hydroxide. All sections were examined with a Philips 200 EM.

In the winter of 1966, *Copilia mirabilis* was obtained at 60 m off the Florida coast, through courtesy of Dr. H. B. Owre, Institute of Marine Sciences, University of Miami, Miami, Florida, who had emphasized that these copepods were exceedingly rare in their collections. These animals were photographed and then fixed in formaldehyde, postfixed with OsO_4, embedded in Vestopal W, and sectioned for structural measurement and for comparison with the optical system of the Mediterranean species.

OBSERVATIONS

The *Copilia* eye resembles an ommatidial facet of the compound eye with a corneal biconvex lens (anterior lens) and, at some distance away from this lens, the crystalline cone (posterior lens). Attached to the crystalline cone are the retinula cells which give rise to the rhabdomeres that form the rhabdom. The rhabdom lies in the L-shaped, orange-colored stem (Figs. 1 and 4 *a*). This stem is the only pigmented part of the body. The stem is located at about the midpoint of the body and is attached to a point near the "brain" (Fig. 4). The stem oscillates back and forth in a saw-toothed pattern, varying from about 1 scan/2 sec to 5 scans/sec. The stems from both eyes move in synchronism rapidly toward each other, then separate more slowly (5). Gregory (3) has likened such scanning to a television camera. "It seems that the pattern of dark and light of the image is not given simultaneously by many receptors, as in other eyes, but in a time-series down the optic nerve, as in the single channel of a television camera."

The Rhabdom

In *Copilia quadrata*, the retinula cells lie directly behind the crystalline cone and are followed by the rhabdomeres which comprise the rhabdom. The rhabdom is completely surrounded by pigment granules and measures $11 \times 17 \mu$. It extends about 60μ in length from the retinula cells to the bend of the stem (Fig. 1). Only five rhabdomeres (R_1-R_5) can be identified in the rhabdom (Fig. 1 *d*). One of them (R_1) is an asymmetric rhabdomere which is located in a nodule on the side of the stem facing the brain and lying at the base of the crystalline cone (Fig. 1 *c* and *d*). The asymmetric rhabdomere (about $1.7 \times 1.7 \times 7 \mu$) appears to be near 45° with respect to the stem. Rhabdomeres R_2-R_5 measure about $1.7 \times 0.9 \mu$ and are about 58μ in length. These lie with their longest dimension parallel to the stem. The rhabdomeres are associated with mitochondria. Structures resembling nerve vesicles are also found in this region (Figs. 1 *c*, *d*, and 2 *a*). Rhabdomeres R_1-R_3 are separated by screening pigment granules, whereas rhabdomeres R_4 and R_5 are not. The rhabdomere microstructure is that of packed tubules (microvilli) about 500 A in diameter (Fig. 2) and is similar to that found in all arthropods.

Optical System

The *Copilia* eye can be considered analogous to the superposition-type ommatidium of compound eyes in which the crystalline cone lies at some distance from the corneal lens. In addition, the crystalline cone forms a convex interface with a fluid of lower refractive index (see Fig. 1 *a*). The structure of the crystalline cone resembles that of a cornea (Fig. 1 *b*), and the structure of the material within it resembles that of glycogen (Fig.

FIGURE 1 *a* Schematic, longitudinal view of the crystalline cone and rhabdom of *Copilia* eye. *cc* (L_2), crystalline cone; *rc*, retinula cells; *pg*, pigment granules; R_1-R_5, rhabdomeres that form the rhabdom. Rectangles *a*, *b*, and *c* show the approximate areas of the electron micrograph sections.

FIGURE 1 *b* Transverse section of the crystalline cone (note the change in density toward the center). $\times$ 2000.

FIGURE 1 *c* Oblique section through the nodule showing the asymmetric rhabdomere, R_1. $\times$ 14,000.

FIGURE 1 *d* Transverse section of the rhabdom showing the five rhabdomeres (R_1-R_5). Rhabdomeres R_4 and R_5 are interconnected (see Fig. 2 *a*) and do not appear to be isolated by pigment granules at any level. $\times$ 9450.
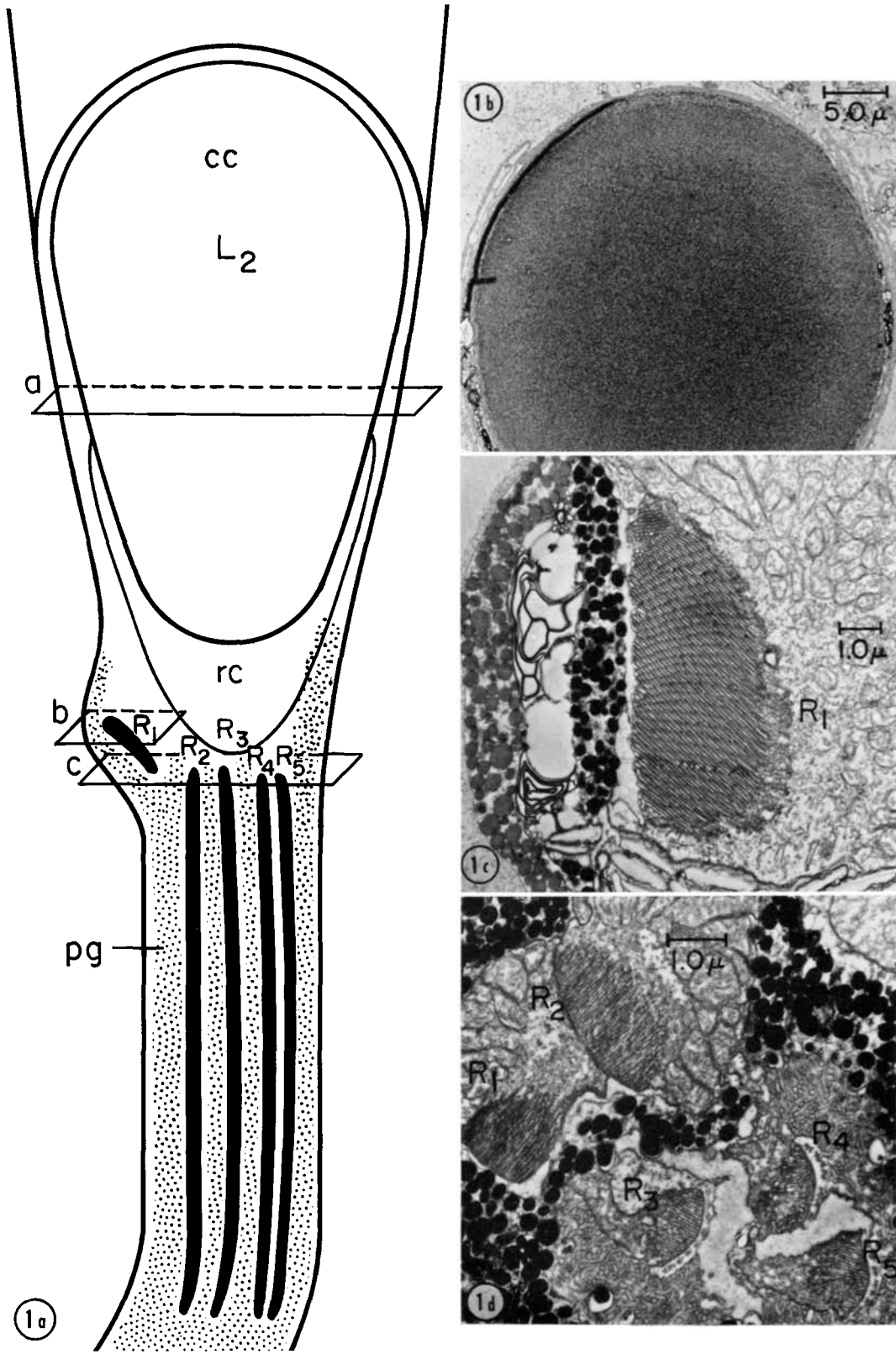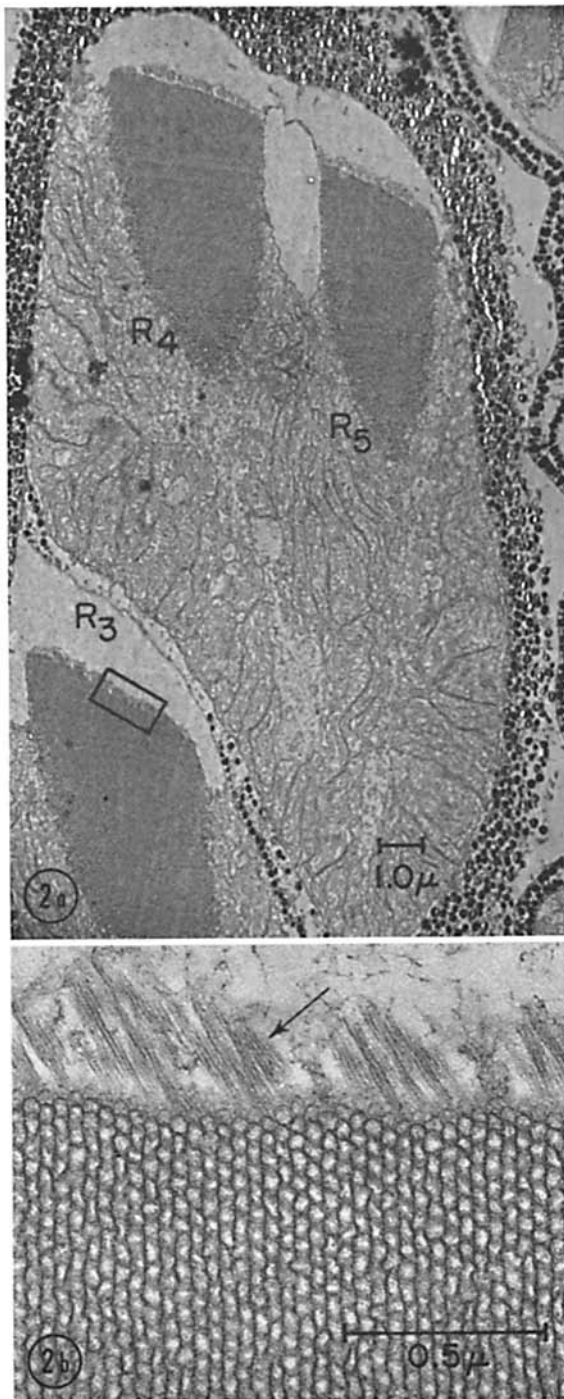
FIGURE 2 a　Oblique section through the rhabdom showing three rhabdomeres (R_3-R_5) that have crystalline-like structures which are not found on rhabdomeres R_1 and R_2. $\times$ 6200.

FIGURE 2 b　Enlarged area (indicated by rectangle in Fig. 2 a) of rhabdomere R_3, showing the microtubules and crystalline-like structure (arrow). $\times$ 53,000.

3). The concentration of this material varies across the diameter, the greatest concentration being in the center (Fig. 1 b). Therefore, the crystalline cone would have the properties of a lens. The *Copilia* eye with its corneal (anterior) lens, L_1, and its crystalline cone (posterior lens), L_2, may then be considered to be a two-lens optical system in which the posterior lens is
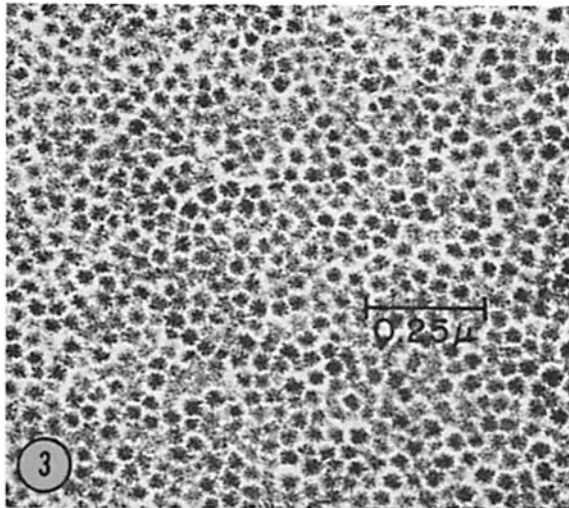
positioned a short distance in front of the rhabdom (Fig. 4).

Eyes of both *Copilia quadrata* and *Copilia mirabilis* were measured to see if there were differences in the dimensions of their optical systems. These measurements indicated that the diameters and shapes of the anterior lens (L_1) and the posterior lens (L_2) were approximately similar for both species. Also the distances between lenses L_1 and L_2 were of the same order of magnitude.

In order to determine the imaging and optics of the *Copilia* eye, the lenses were oriented as accurately as possible for measurements of their radii of curvature, and sections were then cut for light microscopy. Measurements of the spatial relationships of the parts of the eye were taken from photographs of *Copilia*. The following measurements were obtained: the anterior lens, diameter 0.172 mm; radius of curvature of the front surface, 0.0994 mm; radius of curvature of the rear surface, 0.202 mm; thickness, 0.070 mm. The posterior lens diameter was 0.039 mm; radius of curvature of the front surface, 0.019 mm; radius of curvature of the rear surface, 0.0102 mm; thickness, 0.0553 mm. The distance between the adjacent surfaces of the two lenses was 0.61 mm, and the distance from the rear surface of the anterior lens to the asymmetric rhabdomere was 0.67 mm.

DISCUSSION

The rhabdom is the open-type in which the rhabdomeres are separated (Fig. 1 *d*). A similar rhabdom structure is found in the insects *Musca domestica* and *Drosophila melanogaster* (11, 13). This structure differs from that of the freshwater crustaceans *Daphnia pulex* and *Leptodora kindtii* that have a closed-type rhabdom in which the rhabdomeres are fused (11, 14). The open-type rhabdom is common to most diptera that navigate at high light levels. Most arthropods that navigate at low light levels have a closed-type rhabdom with a significantly higher effective cross-section necessary for light gathering efficiency (11, 15). However, *Copilia* lives at a depth where the light level is near that of moonlight (5) and, therefore, requires a more efficient light collecting system. Another factor related to navigation at low light levels is the relative speed of the anterior lens. If this lens had a high relative speed, it could compensate for a less efficient rhabdom.

To determine if the image is formed at the rhabdom, it is necessary to know the focal lengths of the anterior and posterior lenses. The focal lengths could be calculated by using our measurements for the radii of curvature of the lenses if we knew their respective refractive indices. Exner (1) found that the focal length of the anterior lens in water was 0.93 mm. Grenacher (6) had previously measured the distance from the corneal lens to the stem and found it to be 0.9–1.0 mm. These values are greater than those we obtained for our specimens. For *Copilia quadrata* and *Copilia mirabilis* our measurement of the separation of the lenses was 0.65 mm (Fig. 4), which is in agreement with Gregory et al. (5). Therefore, we cannot assume
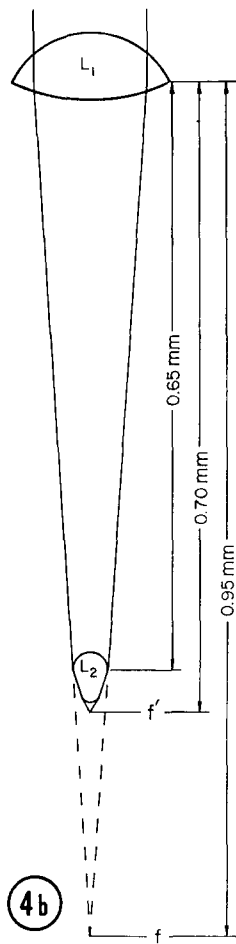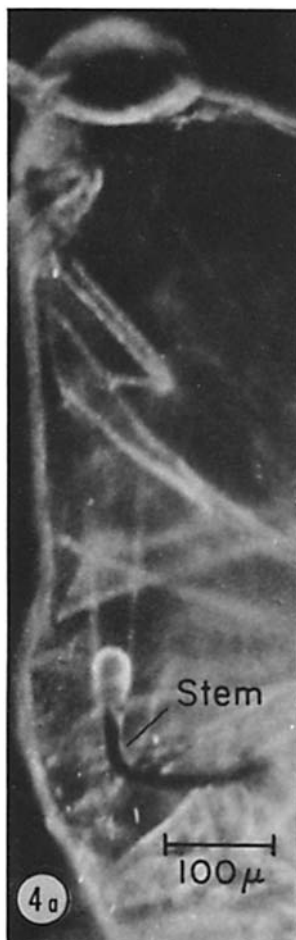
FIGURE 4 a   Dark-field light micrograph of a live *Copilia quadrata*. (Courtesy of Dr. Neville Moray). X 120.

FIGURE 4 b   Schematic of the optical system, showing positions of the anterior lens (L_1) and the crystalline cone or posterior lens (L_2) f, focal point of the corneal lens; f′, focal point of the total optical system.

that the anterior lenses in our specimens have the same focal length as that found by Exner (1). Since we were not able to measure the focal length of the lenses in our specimens, we had to search for data that would permit its computation. We found that effective values of refractive index ranged from 1.42 as measured by Walls (10) to 1.5 as measured by Kuiper (7). Walls (10) also measured a value as high as 1.72^+ in a silurid (fish) lens. If we take a value of 1.42 for the anterior lens, this lens would have a focal length of 0.98 mm; if we take a value of 1.50, the focal length would be 0.52 mm. Since the distance to the rhabdom is 0.69 mm, a refractive index of 1.46 for the corneal lens would place the image directly on the rhabdom. However, this does not take into account any refractive power of the posterior lens.

A function of this posterior lens would be to

shorten the focus of the anterior lens, which leads us to believe that the refractive index for the anterior lens is closer to 1.42. If so, then we have a unique type of optical system, an optical "light amplifier," that is, a system that increases the light collecting efficiency of the anterior lens. Since *Copilia* lives at depths where the light levels are low, such an optical system would be most useful.

To see how the optical system would work, we took a value of 1.425 for the refractive index of the anterior lens, a value which gave for this lens a focal length of 0.93 mm (i.e. the focal length measured by Exner). Using this focal length and the known position of the rhabdom, we found that the strength of the posterior lens which would be necessary to place the image at the rhabdom level was 0.128 mm. The anterior lens alone has a relative

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speed or focal ratio of 5.5:1; but, when the posterior lens is taken into consideration, the focal ratio changes to 2.5:1, or an increase in light collecting efficiency of five times.

In order to see how efficient this optical system would be, we constructed a holder for a 15 × 25 mm focal length Hastings triplet lens, and we mounted the lens 17.5 mm in front of the film plane of a 4 × 5 inch (Burke and James) commercial view camera. The image was then focused on the film plane as formed by a combination of that lens with the regular camera lens (a 6 inch focal length lens with a focal ratio 1:6.8, manufactured by the American Optical Company, Southbridge, Mass.), and photographs were taken. With the camera lens alone, an exposure of $\frac{1}{25}$ sec at f/18 was required to record an image. However, when the second lens was introduced, the exposure had to be reduced to $\frac{1}{200}$ sec to record an image with the same density on the negative. Calculations indicated that the lens speed was increased from f/18 to f/5.6, or an increase of more than eight times in image brightness. The photographs also showed that the size of the image formed by the combination of these lenses was reduced to about one-third, but this is much less than the gain in image brightness. A similar optical system has been described for a focal reduction camera used on the large telescope at Yerkes Observatory, Williams Bay, Wisconsin (2).

Although the *Copilia* eye is considered primitive in the respect that its field scanning mode is slow, perhaps it has adapted to low light levels by having a comparatively "advanced" optical system. If our model for the *Copilia* optical system is correct, then one of the functions of this system is that of a light amplifier.

REFERENCES

1. EXNER, S. 1891. Die Physiologie der Facettirten Augen von Krebsen und Insekten. Franz Deuticke, Vienna, Austria.
2. GASCOIGNE, S. C. B. 1968. The optics of large telescopes. *Quart. J. Roy. Astr. Soc.* 9:98.
3. GREGORY, R. L. 1966. Eye and Brain. McGraw-Hill Book Company, New York.
4. GREGORY, R. L. 1967. Origin of eyes and brains. *Nature.* 213:5074.
5. GREGORY, R. L., H. E. ROSS, and N. MORAY. 1964. The curious eye of *Copilia*. *Nature.* 201: 1166.
6. GRENACHER, H. 1879. Untersuchen über das Sehorgan der Arthropoden, insbesondere der Spinnen, Insekten und Crustaceen. Vandenhoeck & Ruprecht, Göttingen, Germany. 185.
7. KUIPER, J. W. 1966. On the image formation in a single ommatidium of the compound eye in diptera. *In* The Functional Organization of the Compound Eye. C. G. Bernhard, editor. Pergamon Press, Inc., London. 35-50.
8. VAISSIÈRE, R. 1961. Morphologie et histologie comparées des yeux des Crustacés Copépodes. *Arch. Zool. Exp. Gen.* 100:126.
9. VAISSIÈRE, R. 1961. Morphologie et histologie comparées des yeux des Crustacés Copépodes. Ph.D. Thèse. Centre National de la Recherche Scientifique, Paris, France. 125.
10. WALLS, G. L. 1942. The Vertebrate Eye. Cranbrook Institute of Science, Bloomfield Hills, Mich. 29, 265.
11. WOLKEN, J. J. 1966. Vision: Biophysics and Biochemistry of the Retinal Photoreceptors. Charles C. Thomas, Publisher, Springfield, Ill. 193.
12. WOLKEN, J. J. 1967. The eye of the crustacean, *Copilia*. *J. Gen. Physiol.* 50:2481. (Abstr.)
13. WOLKEN, J. J., J. CAPENOS, and A. TURANO. 1957. Photoreceptor structures. III. *Drosophila melanogaster*. *J. Biophys. Biochem. Cytol.* 3:441.
14. WOLKEN, J. J., and G. J. GALLIK. 1965. The compound eye of a crustacean: *Leptodora kindtii*. *J. Cell Biol.* 26:968.
15. WOLKEN, J. J., and P. D. GUPTA. 1961. Photoreceptor structures. The retinal cells of the cockroach eye. IV. *Periplaneta americana* and *Blaberus giganteus*. *J. Biophys. Biochem. Cytol.* 9:720.