# FINE STRUCTURE OF THE EYE OF A CHAETOGNATH

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ABSTRACT

Electron microscopy reveals a star-like pigment cell at the center of the eye of the arrowworm, *Sagitta scrippsae*. Between the arms of the pigment cell are clusters of photoreceptor cell processes, each process consisting of: (1) a tubular segment containing longitudinally arranged microtubules about 500 A in diameter and 20 $\mu$ in length; (2) a remarkable conical body, composed of cords and large granules, situated at the base of the tubular segment; and (3) a connecting piece which, like that of rods and cones, connects the process with the sensory cell proper and through which runs a fibrillar apparatus consisting of nine peripheral double tubules. Beneath the connecting piece lies a typical centriole with a striated rootlet. The receptor cell process is deeply recessed into the sensory cell which may possess a corona of microvilli at its inner surface. A nerve fiber arises from the outer end of the cell and passes into the optic nerve. Additional features are some supporting cells, an external layer of flattened epithelial cells, and an over-all investment of basement membrane and thick fibrous capsule. The fine structure and function of these elements of the eye are discussed in relation to earlier studies with the light microscope. The ciliary nature of the photoreceptor cell process in *S. scrippsae* points to a probable evolutionary relationship of chaetognaths to echinoderms and chordates.

The arrowworms (Phylum Chaetognatha) have long held much interest for biologists because of their unique morphology and development, ecological importance, and uncertain phylogenetic relationships. Their paired eyes, situated on the dorsum of the head, have been studied by several workers, including some eminent zoologists. The most significant investigations are those of Hesse (1) and Burfield (2), upon which are based modern descriptions of the chaetognath eye, such as that recorded by Hyman (3). This paper on the ultrastructure of the eye of an arrowworm will, we hope, considerably extend our knowledge of that organ and cast some light on the phylogenetic relationships of the phylum.

## MATERIALS AND METHODS

Arrowworms, *Sagitta scrippsae* Alvariño (4), were collected on July 10, 1962 from the Monterey Canyon in Monterey Bay, California. Living animals, undamaged by the tow and approximately 2 inches in length, were decapitated, and their heads were fixed, some in 2 per cent osmium tetroxide and some in 2 per cent osmium tetroxide–1 per cent potassium dichromate in sea water. Both fixatives had been diluted to give a final tonicity approximately that of sea water and adjusted to pH 7.2. The vials were packed in ice for transportation to Berkeley where, 6 hours later, the specimens were rapidly dehydrated in either ethanol or acetone and embedded in the epoxy resin, Epon (5). Acetone-treated specimens were stained for 15 minutes with 1 per cent potassium permanganate in acetone (6). While in the uncured Epon the eyes were dissected from the heads and oriented for sectioning in transverse, frontal and parasagittal planes. Ultrathin sections were cut on a Porter-Blum microtome with a diamond knife, according to the method of Westfall and Healy (7), mounted on parlodion-covered grids coated with carbon on their under surfaces, stained with lead

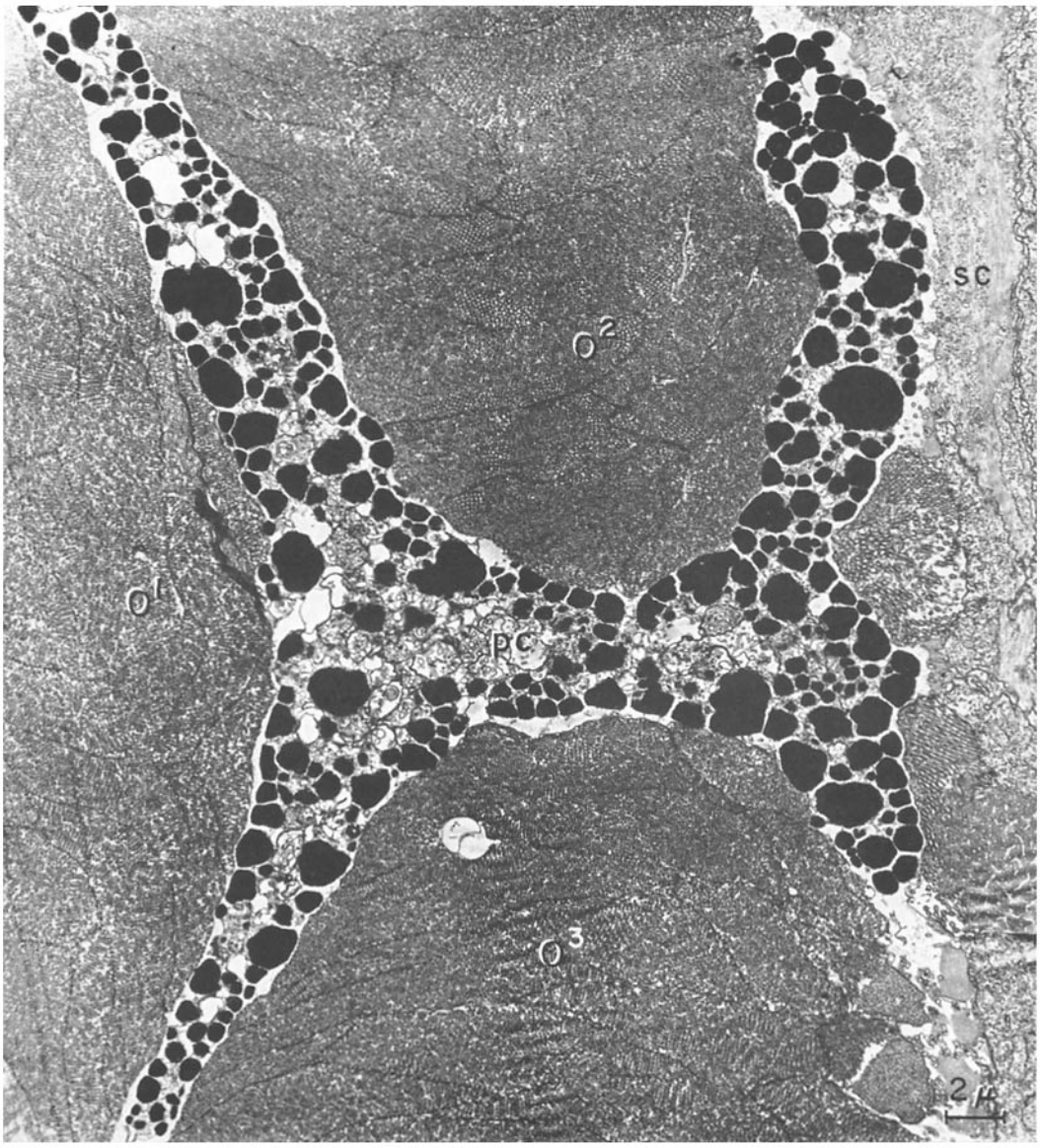



FIGURE 1 Central part of an eye frontally sectioned. $o^1$, lateral ocellus; $o^2$, anterior dorsomedial ocellus; $o^3$, posterior dorsomedial ocellus; *pc*, pigment cell; *sc*, supporting cell. × 4,000.

hydroxide (8) or lead citrate (9), and examined with an RCA-EMU-3-F. The variations in technique did not give appreciable differences in the results of electron microscopy.

## OBSERVATIONS

PIGMENT CELL: The eyes of *Sagitta scrippsae* are oval organs, flattened dorso-ventrally, and measure approximately 0.15 mm in longest axis. At the center of each eye is a mass of pigment (*pc*, Fig. 1) with concavities containing photoreceptors which Hesse (1) called eyecups or ocelli ($o^1$ to $o^3$). Although we have not attempted to confirm the pentapartite nature of the eye described by earlier workers, the organ in *S. scrippsae* appears to consist of five eyecups: one large lateral ocellus ($o^1$) and four smaller ones—two dorsomedial ($o^2$, $o^3$) and two ventro-medial (not shown).

The number of "arms" of pigment seen in a micrograph (four in Fig. 1) depends upon the plane of section, as demonstrated by Burfield (2).

Hesse and Burfield simply referred to the center of the chaetognath eye as a pigmented area or mass of pigment. We believe this mass to be a single cell because the pigmented arms are united and show no internal subdivision by cell membranes. Although we have not observed the nucleus of the pigment cell, despite examinations of hundreds of sections through at least ten eyes, we are convinced that the pigmented area is a cell, because it is surrounded by a continuous plasma membrane and contains masses of mitochondria, an endoplasmic reticulum, and cytoplasmic granules interspersed between the pigment granules. The last are highly variable in shape and size and appear to arise by the fusion of smaller, less dense, and less sharply defined bodies, as shown by serial sections of a given granule (see the two views of the same granule, *pg*, in Figs. 2 and 3). The subunits of the granule seem to be formed, in turn, by aggregations of fine particles (note arrow, Fig. 2). Fig. 2 presents the chief feature of a typical mitochondrion in *S. scrippsae*, namely, few short stubby, transversely arranged cristae projecting into a relatively spacious internal cavity. Additionally, the pigment cell contains numerous vesicles some of which are quite large (Figs. 1, 4) and filled with material which appears floccular in our preparations.

PHOTORECEPTORS: Each ocellus of *S. scrippsae* is composed of about 100 narrow, but very long and closely packed sensory cells (Fig. 5), each of which terminates in a photoreceptor cell process consisting of three parts: a tubular segment (*ts*) at the distal end of the process (adjacent to pigment cell), next a conical body ($cb^1$-$cb^2$), and finally a short connecting piece (*cp*) which joins the process to the cell proper. Thus the eye of the chaetognath, like that of the vertebrate, is inverted, with the processes directed primarily away from the source of light. The receptors are frequently not straight, the tubular segments being gently undulating and sharply bent to one side at their junctions with the conical bodies.

The *tubular segment* of the process is made up of a phalanx of longitudinally arranged microtubules (*mt*, Fig. 5). In most instances, the tubules are wavy so that when the process is cut lengthwise they may appear as rows of short segments (Fig. 6), giving the distal segment a cross-banded appearance. In rare instances, however, a section may pass precisely through the long axis of a group of tubules for a short distance (Fig. 7). A tubule is circular in cross-sectional outline (Fig. 8). Its average diameter is approximately 500 A and its wall is about 100 A thick. We assume that the tubules extend the full length of the tubular segment which we have found in favorable sections to be as much as 20 $\mu$. A typical process with a diameter of 1.6 $\mu$ may contain about 800 microtubules. This estimate was obtained by dividing the cross-sectional area of a process by the cross-sectional area of a tubule and making a rough allowance for intertubular spaces. An occasional process, like the one on the right side of Fig. 6, may have tubules which appear swollen and disorganized, and in some specimens they may be greatly reduced in number. This picture is interpreted as degeneration. At the base of the tubular segment the microtubules are normally less ordered; consequently, they may be cut transversely, longitudinally, or obliquely (see top of Fig. 9). The entire array of tubules is enclosed by a membrane which is continuous with the plasma membrane of the cell proper.

The *conical body* of the process is unique among photoreceptors. It is roughly an inverted cone with its base next to the tubular segment and its apex adjacent to the connecting piece (Figs. 5, 9 to 11), and it is usually subdivided into a distal part composed of irregular cords ($cb^1$) and a basal one of large, loosely packed, irregular, and moderately dense granules ($cb^2$). The cords appear to anastomose in the distal half of the body, and there is some evidence (see arrow, Fig. 12) that they may connect here and there with the microtubules. The conical body is bounded by a membrane which is continuous with that of the tubular segment above and that of the connecting piece (*cp*, Figs. 5, 11) below. Extending along the sides of the body and continuing into the connecting piece are tubular fibrils, two of which (*f*) are seen in Fig. 11, the one on the right being cut lengthwise for a long distance. In cross-section the conical body may appear polygonal (Fig. 13). Fig. 14 shows a photoreceptor process which is unusual in that it possesses a very few granules in the region of the conical body. Perhaps this process is in a stage of development or regeneration. The base of the conical body shown in Fig. 15 contains some small vesicles (*v*) in addition to the granules.

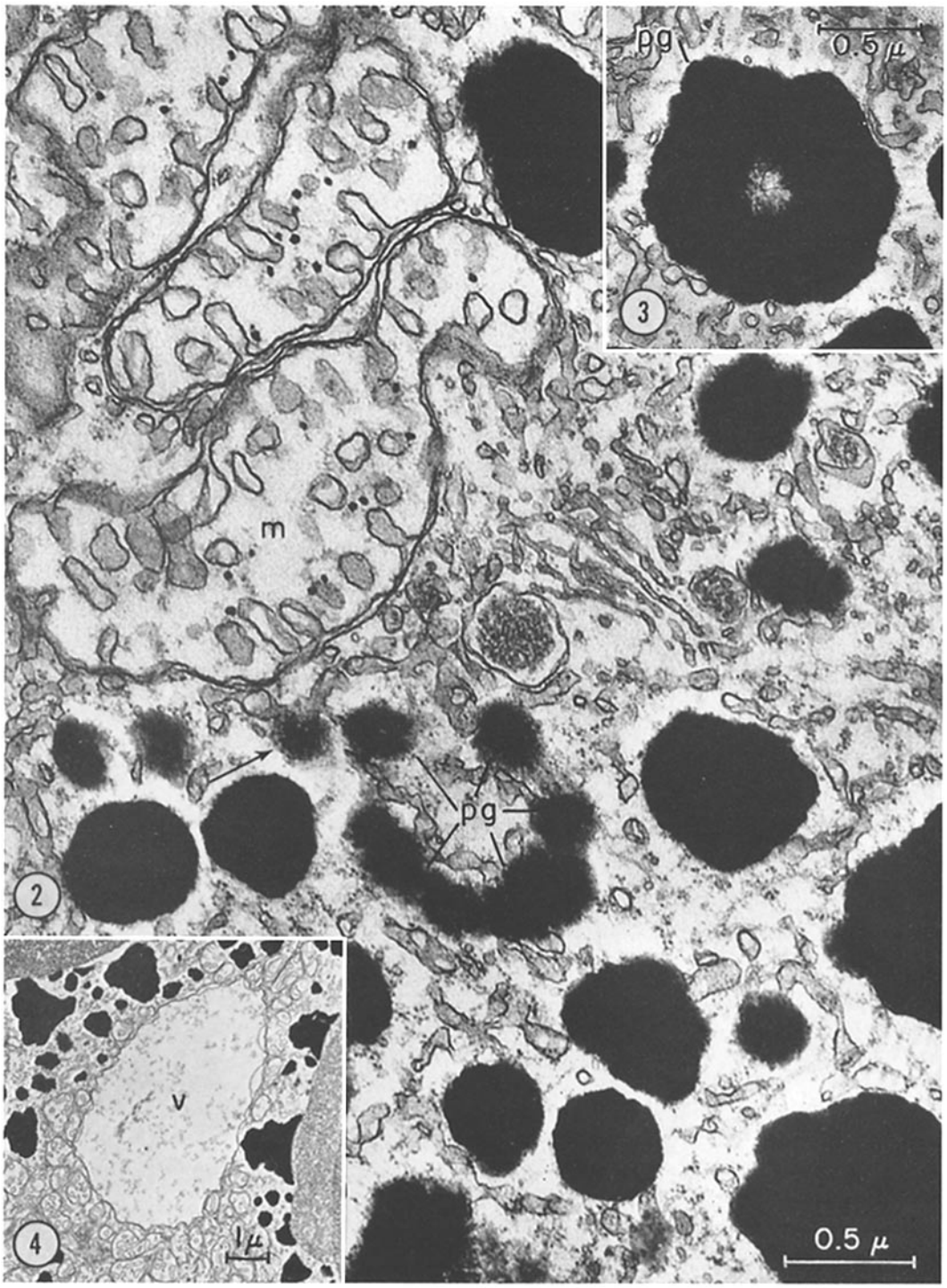
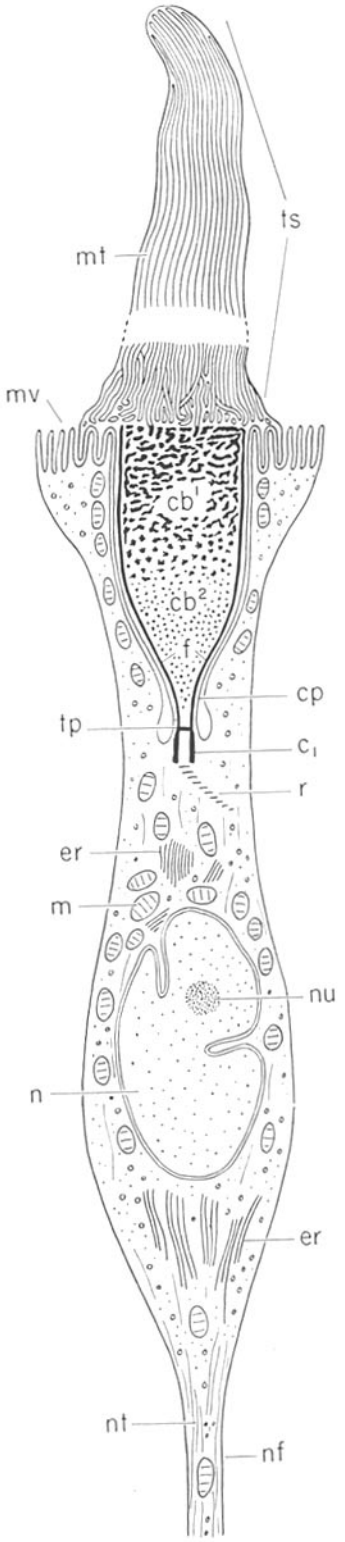



FIGURE 2 Selected part of a pigment cell showing mitochondria (*m*) and a surface section of a pigment granule (*pg*) demonstrating its subunits. Arrow indicates particles which may form the subunits. × 38,000.

FIGURE 3 Another section through the same granule (*pg*) shown in Fig. 2 demonstrating the subunits fused together. × 30,000.

FIGURE 4 An example of a large vesicle (*v*) commonly found in the pigment cell. × 6,000.




The *connecting piece* of the process is the short basal segment which is bounded by a membrane and surrounded by an external space (*sp*, Fig. 11, 15) homologous with the circumciliary space in a protist. The fibrils emerging from the conical body traverse the connecting piece, passing through the terminal plate (*tp*, Figs. 5, 11) en route, and end in the axial centriole ($c_1$) or kinetosome of the photoreceptor cell proper (10, 11). The cross-sectional view of the connecting piece in Fig. 16 shows the fibrillar apparatus to consist of nine peripheral double tubules, but no central ones. In this figure can be seen the nine ridges in the surface membrane of the connecting piece corresponding in position to the nine fibrils, a feature of other ciliary-type photoreceptors (12).

The *photoreceptor cell proper* contains the centriolar apparatus. Extending down the cell from the base of the axial centriole or kinetosome is a broad striated rootlet (*r*, Figs. 5, 15), which appears to be relatively short, in comparison with that of other photoreceptors (12), and often is bent at an angle at the point of junction with the kinetosome. A cross-sectional view (Fig. 17) of the axial centriole shows it to be a cylinder composed of nine triplets of tubules oriented obliquely in a ring as in other centrioles (13). The centriole is enclosed by an irregular ill defined cytoplasmic area, which is denser than the neighboring cytoplasm and appears to consist of radiating tubular filaments. This feature we have observed in other photoreceptors (see Fig. 11 in reference 14). A typical second or oblique centriole has not been observed despite a search of sixty or more photoreceptors favorably sectioned, some of them serially. At most, we find a vague aggregation of dense material (*x*, Fig. 14) at one side of the kinetosome and near the position normally occupied by the oblique centriole in other photoreceptors (12). In one specimen a subdivision of the striated rootlet led to this spot.

FIGURE 5 Schematic representation of a sensory cell. $c_1$, axial centriole or kinetosome; $cb^1$, distal part of conical body composed of cords; $cb^2$, basal part of conical body composed of granules; *cp*, connecting piece; *er*, endoplasmic reticulum; *f*, two of the nine fibrils; *m*, mitochondria; *mt*, microtubules; *mv*, microvilli; *n*, nucleus; *nf*, nerve fiber or axon; *nt*, neurotubule or neurofibril; *nu*, nucleolus; *r*, striated rootlet; *tp*, terminal plate; *ts*, tubular segment of photoreceptor cell process (from which a long section has been deleted). Receptor cell process shown as straight and not bent at junction of tubular segment and conical body.

The photoreceptor cell is deeply recessed at the point of connection of the receptor cell process so that the cell actually encloses the basal segments of the process. The membranes of the cell proper and the connecting piece are separated by a space, as noted above, but those of cell proper and the conical body lie in close apposition. Some photoreceptor cells bear a crown of microvilli (*mv*, Fig. 5) which encircle the upper part of the conical body and, in some instances, the base of the tubular segment. Fig. 18 presents a longitudinal view of such a cell showing the conical body (*cb*) recessed into the receptor cell (*rc*) which bears the corona of microvilli (*mv*) at its inner surface. The tubular segment of the process is not shown, except for the basal ends of some of the microtubules (*mt*), because it is bent out of the plane of sectioning. Fig. 19 presents a cross-sectional view of microvilli (*mv*) encircling the conical body (*cb*) in another specimen. Each villus is enclosed in a double envelope, owing to the fact that the microvilli project *into* the receptor cell process (Fig. 5). As a consequence, the villi are clothed with the membrane of the cell proper (inner line of a profile) and the membrane of the process (the outer line of a profile). Careful inspection of Fig. 19 shows the substance of the conical body, extending between the double circles, and a limiting membrane around the entire complex. High magnification of longitudinal sections (not figured) confirm this interpretation.

The nucleus of the photoreceptor cell is situated at a variable distance from the centriolar region. It is often seen to be indented (*n*, Figs. 5, 20) and its double-membrane envelope possesses pores (see arrows, Fig. 20). Mitochondria (*m*) are abundant about the nucleus, particularly in the supranuclear area. The infranuclear zone is rich in smooth-surfaced endoplasmic reticulum (*er*). The cell terminates in a nerve fiber (*nf*) which contains mitochondria, fine neurofibrils or tubules, granules, and vesicles of various sizes, the large ones being filled with a floccular material.

The nerve fibers leaving the photoreceptor cells of a given ocellus are bundled together into a tract, and the several tracts merge at the anteromedial margin of the eye to form the optic nerve which passes to the brain. Fig. 21 shows the nerve at the point of exit from the eye. Nerve fibers (*nf*), cut longitudinally, are seen passing through a wide basement membrane (*bm*), seemingly homogeneous in composition, and a very thick capsule (*cs*) made up of strata of striated (collagen?) fibers. The optic nerve (*on*) is cut transversely as are most of the nerve fibers contained therein. The fibers range in size from 0.2 to 1.0 $\mu$. A few of the fibers, such as those indicated by arrows, are ensheathed in membranes (myelin?) but the majority appear to be non-medullated. Several nuclei (*n*) are seen that presumably belong to sheath cells, within or about which are undulating membranes. Fig. 22 presents a higher magnification of parts of several nerve fibers showing the longitudinally oriented neurofibrillae (tubules) and clusters of vesicles. Mitochondria also occur in the nerve fibers, but they are not shown in Fig. 22 and are difficult to see in Fig. 21. Since we can count about 500 to 600 nerve fibers in the optic nerve, we conclude that an eye in *S. scrippsae* has the same number of photoreceptors. There appear to be no ganglion cells, and we have observed no synapses.

OTHER STRUCTURES: Epithelial cells and possibly supportive or glia-like elements are present. The eye is bounded by a layer of flattened cells (not figured), the nuclei of which are smaller than those of the sensory cells and much elongated. Irregular septal strands radiate from this layer to the pigmented cell in the center of the eye. Whether these are extensions of the peripheral epithelial cells or are formed by separate and different cells was not determined. The septa contain bundles of fine filaments, many vesicles, mitochondria, and occasionally layers of membranes. Segments of these septal cells (*sc*) may be seen in Figs. 1, 18, and 19.

FIGURE 6 Oblique section through the tips of the tubular segments of several receptor cell processes. *pc*, pigment cell; *pg*, pigment granule; *mt*, microtubule; *ts*, tubular segment of a receptor cell process. The process at the right margin appears to be degenerating. × 34,000.

FIGURE 7 Precisely longitudinal section through a number of microtubules. × 38,000.

FIGURE 8 Microtubules near the base of the tubular segment of a photoreceptor cell process where they are irregularly arranged so that they are sectioned in various planes. Note those cut transversely. × 66,000.


7
0.5 μ
pg
pc
is
mt
0.5 μ
6
8
0.1 μ

External to the superficial layer of epithelial cells lies a basement membrane (*bm*, Fig. 21) which varies in thickness from 0.25 to 1.5 $\mu$. Outside of the basement membrane is a capsule (*cs*, Fig. 21), several microns thick, composed of layers of undulating (artifact?) fibers oriented concentrically around the eye. Because the fibers are regularly striated, they are assumed to be collagenous. Dorsally the capsule is fused with the basement membrane of the integument. The skin consists of a single layer of cuboidal cells, at least in the region of the eye, that are remarkably rich in granular endoplasmic reticulum.

## DISCUSSION

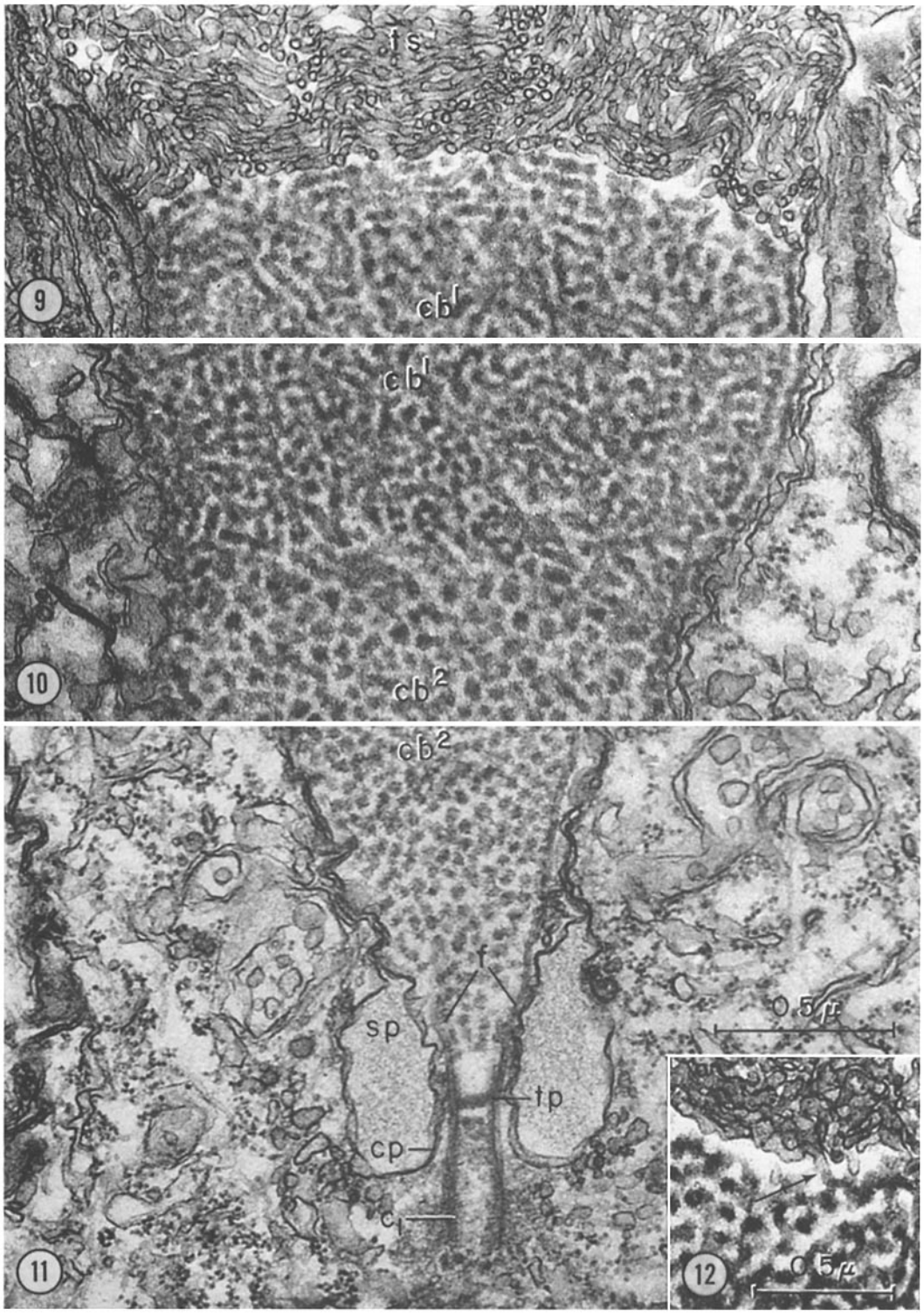
The investigations of Hesse (1) and Burfield (2), the latter largely confirming the observations of the former, laid the foundation for our present knowledge of the structure of the chaetognath eye. Both workers studied arrowworms in the genus *Sagitta* to which also belongs the form we examined. These workers corrected certain errors made by the earlier zoologists, *i.e.*, Hertwig (15) and Grassi (16), who misinterpreted the organization of the receptor cells and the compartmentalization of the eye. For example, Hertwig thought that there were biconvex lenses lying within the concavities of the central mass of pigment. Hesse clearly showed, however, that these regions represent the closely packed photoreceptor cell processes. This observation was confirmed by Burfield. Another error was the description by the nineteenth-century workers of a tripartite eye. Hesse and Burfield demonstrated, however, that the eye is composed of five subdivisions: one large lateral, two small mediodorsal, and two small medioventral ocelli. But the limitations of the light microscope, even in the hands of Hesse and Burfield, led to other misinterpretations which may now be corrected, if our observations are valid.

RODS: First, the nature of the distal part of the photoreceptor cell process, called a rod by Hesse and Burfield, was not fully understood. They illustrated the rods as being cross-striated. The electron microscope reveals, however, that this segment of the process consists of an array of narrow tubules *longitudinally* arranged. Sections in which the tubules are cut obliquely would give a false impression of cross-banding (see Fig. 6). Both workers, however, assigned the function of photoreception, correctly, in our opinion, to this segment of the process. The tubules, like the discs in the rods and cones of a vertebrate eye or the microvilli in the rhabdomeres of an arthropod eye, probably contain a photopigment. In this connection, it may be significant that Burfield observed a faint pink coloration in the rods of the living animal (*Sagitta bipunctata*).

Whereas the vertebrate discs and the microvilli in arthropod and molluscan rhabdomeres are transversely arranged with respect to the long axis of the receptor cell, the tubules in the eye of the arrowworm are longitudinally disposed. The orientation of the photoreceptor cell organelles probably bears a functional relationship not to the axes of the receptor cell but to the direction of incident light, such that the surfaces of the organelles—discs, microvilli, or tubules—are at right angles to the light, the most efficient arrangement for the trapping of photons by the photopigment (17). A lengthwise organization of the tubules within the rods of the chaetognath eye appears to be the most favorable one for photoreception, considering the dorsoventral compression of the eye and the relatively short arms of the pigmented cups. There appear to be exceptions, however, to the above principle. We found, for example, that most of the tubules in the ocelli of sea stars are oriented more or less parallel to the long axis of the pigmented eyecup and to the direction of

FIGURES 9 TO 11 Segments from a photoreceptor cell process. Fig. 9: the boundary between the tubular segment (*ts*) and the top of the conical body ($cb^1$). Fig. 10: transition between the upper part of the conical body composed of cords ($cb^1$) and the lower half ($cb^2$) containing irregular granules. Fig. 11: the base of the conical body ($cb^2$) and the connecting piece (*cp*) of the receptor cell process. $c_1$, axial centriole or kinetosome; *f*, two of the nine fibrils, the one to the right being sectioned longitudinally for more than 1 $\mu$; *sp*, space between the connecting piece and the distal part of the receptor cell proper; *tp*, terminal plate. × 54,000.

FIGURE 12 An example of an apparent connection between a microtubule and a cord in the distal end of the conical body (arrow). × 42,000.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9
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10
cb2
cb2
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cp
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11
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incoming light (12). On the other hand, in the ocelli of a hydromedusan the tubules, although poorly ordered into arrays, tend to be perpendicular to the chief axis of the eyecup (18).

The usage of the term rod for the part of the receptor cell process containing the tubules is open to question. The rod (or cone) of a vertebrate eye is the outer segment of the photoreceptor cell process, that is, the part distal to the connecting piece. In the chaetognath eye, however, the outer segment of the process is subdivided into two very different regions: the array of tubules distally and the conical body basally. Accordingly, the vertebrate rod is *homologous* with the entire photoreceptor cell process of the arrow-worm and *analogous* with the tubule-containing segment only.

CONICAL BODY: Second, Hesse and Burfield mistook the cone-shaped body in the photoreceptor cell process for a clear refractive region of the cell proper. We have shown, however, that the conical body lies within the process between the tubules and the connecting piece. Because the process is deeply recessed into the cell, the conical body appears to lie within the cytosome. Without the greater magnification of the electron microscope these workers could not resolve the double set of membranes and the narrow space separating the conical body from the cell proper, although Hesse was remarkably perceptive in noting that the conical body (*Knauf*) was bounded by a narrow, unstained zone.

This body is unique. We know of nothing like it in any other photoreceptor. Although it may appear clear when viewed with the light microscope, it is actually quite dense, being composed of irregular osmiophilic granules and cords. It has a superficial resemblance to the paraboloid in the vertebrate cone (19, 20). The two structures are not homologous, however, because the paraboloid lies not within the process but deep in the cone-cell, just above its nucleus. Moreover, the paraboloid of the cone-cell is predominantly glycogen in content, judging from its positive periodic acid–Schiff (PAS) reaction (21) and the star-like pattern of the granules of which it is composed (19). Although Hesse reports that the basal end of the conical body is very chromophilic, no critical histochemical study has yet been made of this body. However, our electron micrographs reveal irregular granules which appear to be fused distally into cords. The units are much larger than most glycogen granules and do not show the usual astral clusters of subparticles.

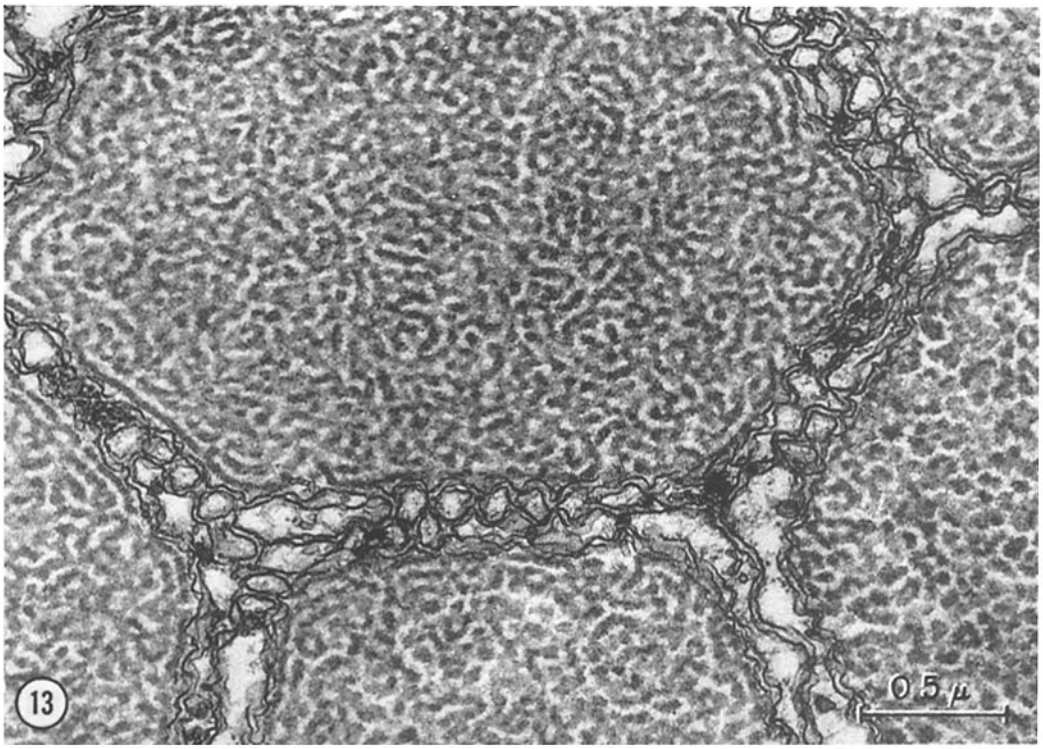
If the conical body of the chaetognath eye is stored nutrient, such as glycogen, one would expect mitochondria in the immediate vicinity to effect energy transfers. Mitochondria situated below the centriole and separated from the conical body by the narrow connecting piece would seem to be too distant to function in this instance. The reader will recall, however, that the photoreceptor cell process is deeply recessed into the receptor cell. The distal end of the cell that encircles the conical body contains mitochondria, many just inside the plasma membrane. Consequently, mitochondria actually lie very near the granules under discussion, although physically separated from them by two membranes.

Serving as an optical system is another possible function of the conical body. Both Hesse and Burfield attributed refractive properties to it. Much of the light entering the eye would pass through the conical bodies before striking the tubules and becoming absorbed by the photopigment. Light unabsorbed by the tubules would be captured by the pigment cell and thereby prevented from stimulating receptors in other

FIGURE 13 Cross-sectional view of parts of several conical bodies, showing their polygonal shape. × 38,000.

FIGURE 14 Longitudinal section through the proximal part of a photoreceptor cell process which may be in the process of development or regeneration. $c_1$, axial centriole; *cp*, connecting piece; *f*, two of the fibrils; *g*, a few granules in the region of the process usually occupied by the conical body; *x*, condensation which might represent the remnant of the oblique centriole. × 38,000.

FIGURE 15 Longitudinal section through the proximal part of a photoreceptor cell process and its insertion into the receptor cell proper. $c_1$, axial centriole; *cb*, conical body; *cp*, connecting piece; *f*, fibrils; *r*, striated rootlet; *sp*, space between connecting piece and receptor cell proper; *v*, small vesicles in the conical body. × 38,000.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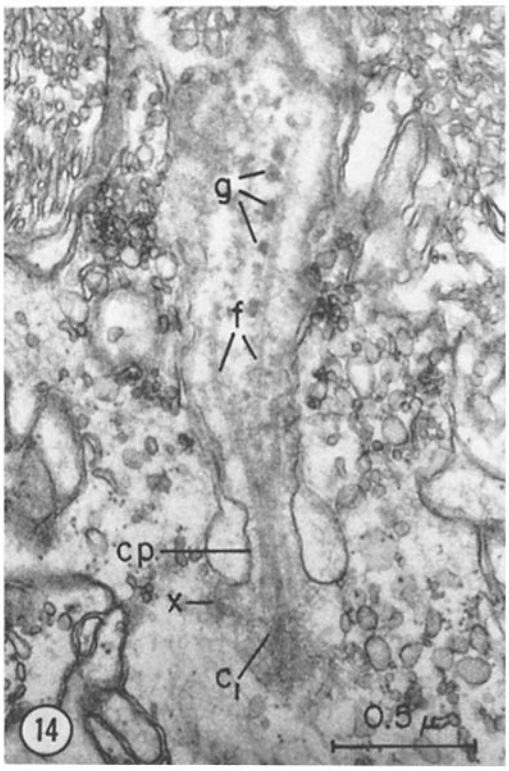
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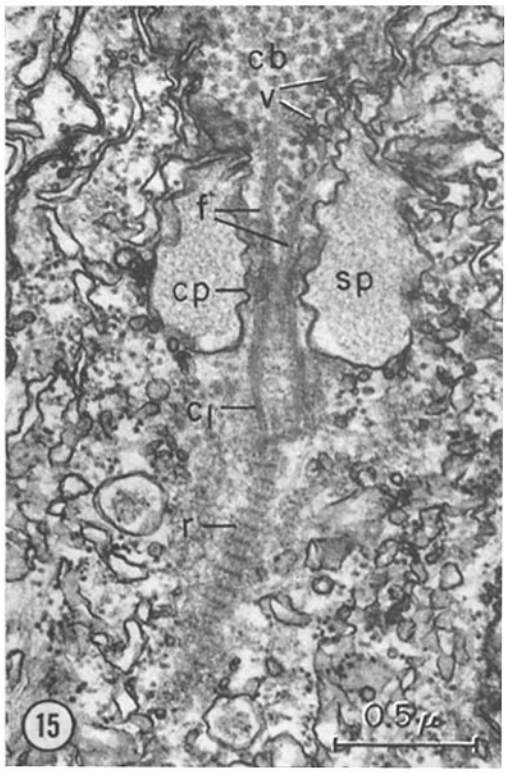
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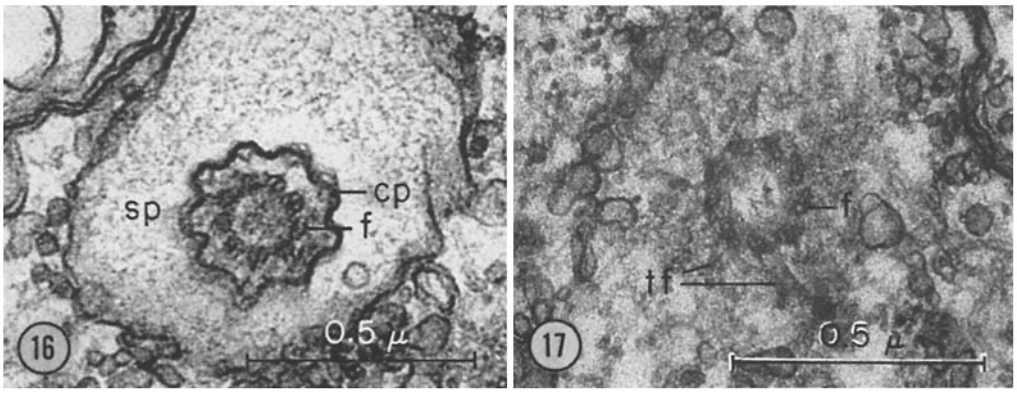



FIGURE 16 Cross-section of the connecting piece (*cp*) of a photoreceptor cell process. *f*, one of the nine peripheral fibrils; *sp*, space between connecting piece and receptor cell proper. Note double tubular nature of the fibrils, absence of central fibrils, and nine ridges on surface of connecting piece. × 60,000.

FIGURE 17 Cross-section through the receptor cell at level of axial centriole or kinetosome. *f*, one of nine fibrils (note its triplet tubular nature); *tf*, tubular filaments radiating from centriole. × 66,000.

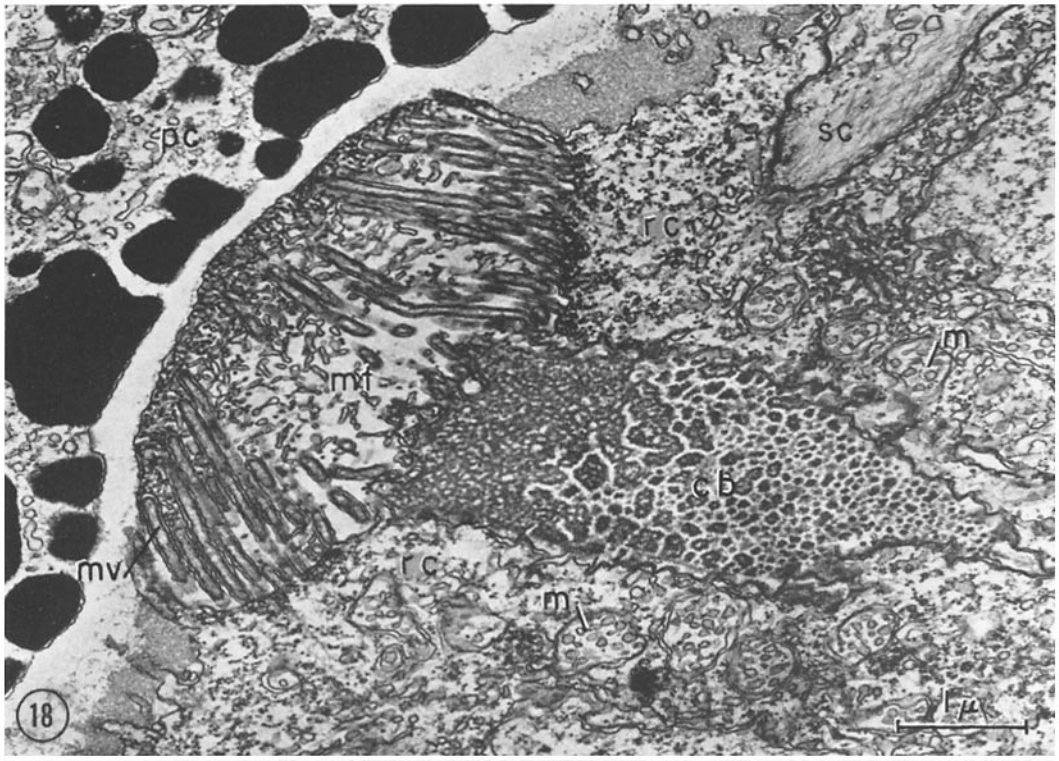
ocelli. Since chaetognaths live in subsurface marine waters where light is weak and diffuse, small lenticular bodies within the eye might be useful in trapping the light sufficiently to stimulate the photoreceptor tubules. Photons having entered the conical body might be reflected back and forth by its sides and concentrated before leaving its distal end to enter the tubules. It is even tempting to speculate that the conical body might have wave guide effects (22) or that it might act as a resonator in the manner of a laser (23).

Other possible functions of the conical body occur to us. Maybe the cords are incipient tubules. One occasionally sees an apparent connection between the base of a tubule and a cord of the conical body (Fig. 12). It would be surprising, however, if the tubules develop from the irregular cords by growth and canalization, because most organelles that are light-sensitive, whether discs, tubules, membranes, or microvilli, appear to arise by invagination or evagination of the cell membrane or derivatives of it, such as the membrane of a cilium-like process (24). The connections between tubules and cords may have physiological rather than developmental significance. Perhaps some substance utilized in the photochemical reactions moves from the cords, in which it is stored in a concentrated form, into and along the tubules. Finally, the conical body might be involved in the transmission of excitations. Since the mechanism of conduction of signals in the best known system, the vertebrate rod-cell, is not yet understood, one can do no more than speculate in this instance. Although it would be expected that electrochemical excitations would travel down the membrane of the process to its base (25), they might, however, be transmitted *via* the conical body or by the fibrils which run under its surface and through the connecting piece to the kinetosome.

FIBRILLAR APPARATUS: Third, Hesse and Burfield could not see the details of the fibrillar apparatus which the electron microscope reveals. However, both investigators described a fibril

FIGURE 18 Example of photoreceptor cell with corona of microvilli (*mv*) at its inner end which project into the tubular segment of photoreceptor cell process. *cb*, conical body of process; *m*, mitochondria; *mt*, microtubules; *pc*, pigment cell; *rc*, receptor cell; *sc*, part of a supporting cell. Tubular segment of process not shown, except for bases of a few microtubules which are sharply bent at junction with conical body. × 17,000.

FIGURE 19 Cross-sectional view of receptor cell with microvilli (*mv*). *cb*, distal end of conical body; *mt*, bases of a few microtubules; *sc*, part of a supporting cell. × 23,000.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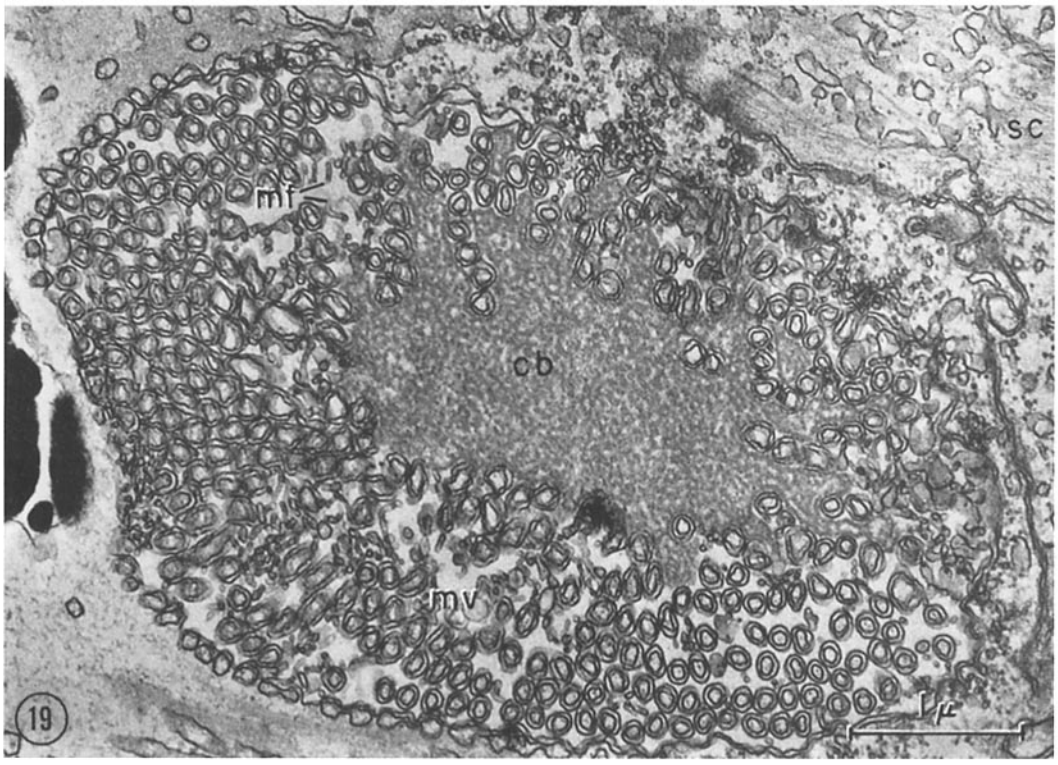
18
1 μ


vsc
mf
cb
mv
19
1 μ

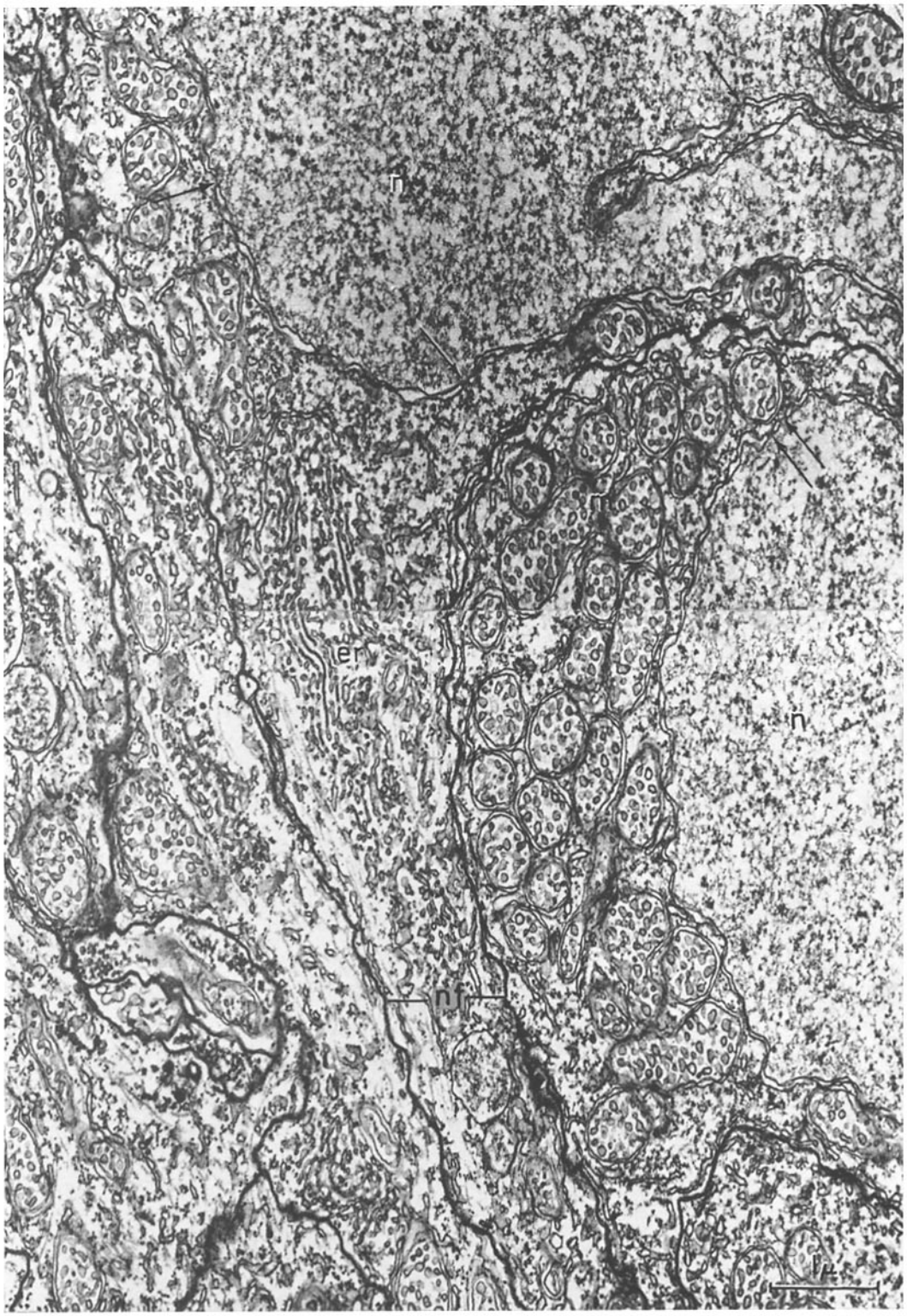



FIGURE 20 Nuclear region of two receptor cells. *er*, endoplasmic reticulum; *m*, mitochondria (particularly numerous in supranuclear region of cell to the right); *n*, nuclei; *nf*, nerve fiber or axon leaving infranuclear region of receptor cell in center of figure. Arrows indicate nuclear pores. × 17,000.

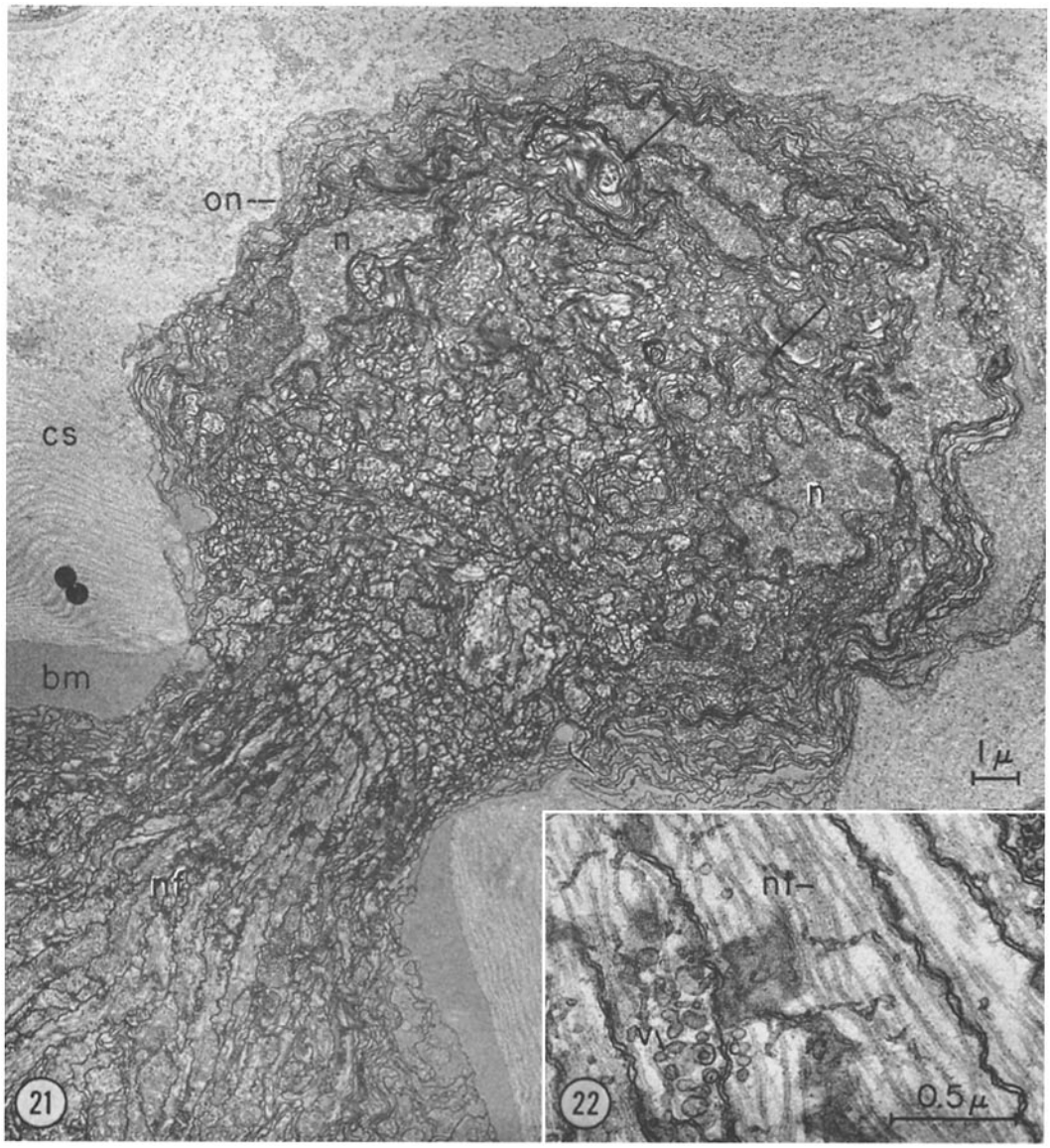



FIGURE 21 Frontal section through junction of eye (lower left corner) and its optic nerve (*on*), largely cut transversely. *bm*, basement membrane; *cs*, capsule containing layers of collagenous (?) fibers; *n*, nuclei of sheath (?) cells; *nf*, nerve fibers passing from eye to nerve. Arrows indicate membranes (myelin ?) investing some nerve fibers. Polystyrene balls, 0.5 μ. × 6,000.

FIGURE 22 High magnification of parts of several nerve fibers shown in Fig. 20. *nt*, neurotubules or neurofibrils; *v*, vesicles. × 34,000.

passing from the base of the conical body. Hesse even saw a small granule on this thread, near the distal end, and stated that it reminded him of the basal body in a rod (*Stiftchen*) in other eyes. Both he and Burfield regarded the thread as a neurofibril which traverses the sensory cell and continues from the outer end of the cell as a neurite, even though they could not follow it around the

nucleus. Moreover, Hesse thought that both the conical body and the rod were thickened specializations of this neurofibril. The granule that Hesse described was undoubtedly the axial centriole, and the fibril above and that below the granule, the connecting piece and striated rootlet, respectively, which we see. Since the fibrillar apparatus of any receptor cell has not been shown to be a conducting system, it seems inadvisable to designate a part of it a neurofibril. Burfield (2) showed some converging lines at the base of the conical body (his Plate IX, Fig. 60) in a semi-diagrammatic sketch of a single sensory cell, but he gave no explanation of them. He might have seen very vaguely some of the nine fibrils extending along the sides of the conical body, or the lines might have been added to impart a three-dimensional aspect to the figure.

The presence of the fibrillar and centriolar apparatus clearly demonstrates that the photoreceptor cell process of *S. scrippsae* is ciliary in type (12). The similarity between the connecting piece in this arrowworm (Figs. 11, 16) and that which we described in the amphibian frontal organ (26) and in the reptilian parietal eye (27) is very striking. The nine peripheral fibrils are double tubules, and the surface membrane has nine ridges which correspond to the fibrils. Central elements have not been seen in our electron micrographs of photoreceptors of this chaetognath eye, although they were observed in photoreceptors of certain coelenterates (18), echinoderms (12), and amphioxus (28).

NEURAL STRUCTURES: Fourth, earlier workers have been uncertain of the origin of the fibers in the optic nerve. Burfield (2) states that "the nerve enters the anterior border of the eye capsule, and then divides into fibrillae which pass into the inner portion of the organ,. . . coming very close up against the outer ends of the visual cells, and it is possible, though it could not be clearly seen, that the fibrous extensions of the visual cells actually form the optic nerve fibers" (pp. 61, 66). Hesse (1) observed that fibers from the anteromedial sensory cells extended into the optic nerve in a fresh specimen of *Sagitta bipunctata*, but he was unable to trace the other fibers in this species or in a larger arrowworm, *S. hexaptera*, even in microscopic sections. By constructing montages of the entire eye of *S. scrippsae*, we have established with certainty that the axons from the photoreceptor cells do indeed enter the optic nerve. On the basis of a count of about 500 fibers in the nerve, we conclude that there are approximately 500 sensory cells.

CAPSULE: Fifth, our electron micrographs clarify earlier descriptions of the investment of the eye. Burfield (2) states that the eye is enclosed by a very thin membrane in which small nuclei can be seen. Outside this membrane is a firm capsule formed by the basal membrane of the epidermis. We assume that the membrane he saw is the superficial layer of epithelial cells which we observe. Apparently, he did not see the basement membrane, which averages about 1 $\mu$ in thickness and is non-cellular. We are in agreement with him concerning the thick capsule which we believe to be composed of collagenous fibers.

PHYLOGENY: The evolutionary relationships of the chaetognaths have been a subject of speculation since the discovery of the organisms in 1768. These worms have been considered to be related to no less than eight other invertebrate groups (2, 3). Hyman (3) notes that Darwin introduced his paper on the arrowworms by stating that they are "remarkable for obscurity of affinities." Although Hyman believes that the chaetognaths most resemble the aschelminths in adult morphology, she places them in the deuterostomia because of their equal and indeterminate cleavage and the absence of eutely. She points out, however, that the embryonic coelom, although an enterocoel, does not arise by outpouching of the archenteron and that the adult body cavity is a pseudocoel. Hyman (3) concludes her treatise on the chaetognatha with the statement: "The possibility that the chaetognaths are remotely related to the dipleurula ancestor of the Deuterostomia is the only justification for placing them, as done here, among the Deuterostomia" (p. 66).

Our studies on the fine structure of light-sensitive organs (12) suggest the possibility that the deuterostomia and protostomia may be distinguished on the basis of the nature of their photoreceptors. The deuterostomes (chordates and echinoderms) plus the coelenterates, on the one hand, appear to possess characteristically a ciliary-type photoreceptor, that is, a light-sensitive structure derived embryologically from a process with a fibrillar apparatus similar to that in a cilium. The photoreceptors of protostomes (arthropods, annelids, molluscs) plus the flatworms, on the other hand, are rhabdomeric in type and do not seem to develop typically from

cilia-like processes. We have clearly shown here that the photoreceptors in *Sagitta scrippsae* are ciliary in type. Assuming that other chaetognaths are like this species, we may say that the arrow-worms belong to the deuterostomia with respect to one point of adult anatomy: the basic organization of their photoreceptors. Before the ciliary or non-ciliary nature of a receptor cell process becomes useful in determining broad phylogenic relationships, however, many more animals need to be examined with the electron microscope, and certain exceptions (12) require confirmation.

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## REFERENCES

1. HESSE, R., Untersuchungen über die Organe der Lichtempfindung bei niederen Thieren. VIII. Weitere Thatsachen. Allegmeines, *Z. wissensch. Zool.*, 1902, **72,** 565.
2. BURFIELD, S. T., Sagitta, *Proc. Tr. Liverpool Biol. Soc.*, 1927, **41,** Mem. 28.
3. HYMAN, L. H., Smaller coelomate groups, *in* The Invertebrates, New York, McGraw-Hill, 1959, **5.**
4. ALVARIÑO, A., Two new pacific chaetognaths, *Bull. Scripps Inst. Ocean. Univ. Calif.*, 1962, **8,** 1.
5. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 409.
6. PARSONS, D. F., A simple method for obtaining increased contrast in Araldite sections by using postfixation staining of tissues with potassium permanganate, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 492.
7. WESTFALL, J. A., and HEALY, D. L., A water control device for mounting serial ultrathin sections, *Stain Technol.*, 1962, **37,** 118.
8. MILLONIG, G., A modified procedure for lead staining of thin sections, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 736.
9. REYNOLDS, E. S., The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, *J. Cell Biol.*, 1963, **17,** 208.
10. PITELKA, D. R., Electron-microscopic structure of protozoa, *in* International Series of Monograms on Pure and Applied Biology, (G. A. Kerkut, editor), New York, The Macmillan Company, 1963, **13.**
11. PITELKA, D. R., and CHILD, F. M., The locomotor apparatus of ciliates and flagellates: relations between structure and function, *in* Biochemistry and Physiology of the Protozoa, (S. H. Hutner, editor), New York, Academic Press, Inc., 1964, **3.**
12. EAKIN, R. M., Lines of evolution of photoreceptors, *in* General Physiology of Cell Specialization, (D. Mazia and A. Tyler, editors), New York, McGraw-Hill, in press.
13. GIBBONS, I. R., and GRIMSTONE, A. V., On flagellar structure in certain flagellates, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 697.
14. EAKIN, R. M., QUAY, W. B., and WESTFALL, J. A., Cytological and cytochemical studies on the frontal and pineal organs of the treefrog, *Hyla regilla*, *Z. Zellforsch.*, 1963, **59,** 663.
15. HERTWIG, O., Die Chaetognathen. Ihre Anatomie, Systematik und Entwicklungsgeschichte, Jena, Gustav Fischer, 1880.
16. GRASSI, B., I Chetognati, *in* Fauna und Flora des Golfes von Neapel, Leipzig, Wilhelm Engelmann, 1883, Monograph V.
17. WOLKEN, J. J., A structural model for a retinal rod, *in* The Structure of the Eye, (G. K. Smelser, editor), New York, Academic Press, Inc., 1961, 173.
18. EAKIN, R. M., and WESTFALL, J. A., Fine structure of photoreceptors in the hydromedusan, *Polyorchis penicillatus*, *Proc. Nat. Acad. Sc.*, 1962, **48,** 826.
19. CARASSO, N., Rôle de l'ergastoplasme dans l'élaboration du glycogène au cours de la formation du "paraboloïde" des cellules visuelles, *Compt. rend. Acad. sc.*, 1960, **250,** 600.
20. EAKIN, R. M., The effect of vitamin A deficiency on photoreceptors in the lizard, *Sceloporus occidentalis*, *Vision Research*, in press.
21. SIDMAN, R. L., and WISLOCKI, G. B., Histochemical observations on rods and cones in retinas of vertebrates, *J. Histochem. and Cytochem.*, 1954, **2,** 413.
22. ENOCH, J. A., Wave guide modes: are they present and what is their role in the visual mechanism, *J. Opt. Soc. Am.*, 1960, **50,** 1026.
23. SCHAWLOW, A. L., Optical masers, *Scient. Am.*, 1961, **204,** 52.

24. EAKIN, R. M., and WESTFALL, J. A., The development of photoreceptors in the stirnorgan of the treefrog, *Hyla regilla*, *Embryologia*, 1961, **6,** 84.

25. WALD, G., BROWN, P. K., and GIBBONS, I. R., The problem of visual excitation, *J. Opt. Soc. Am.*, 1963, **53,** 20.

26. EAKIN, R. M., Photoreceptors in the amphibian frontal organ, *Proc. Nat. Acad. Sc.*, 1961, **47,** 1084.

27. EAKIN, R. M., and WESTFALL, J. A., Further observations on the fine structure of the parietal eye of lizards, *J. Biophysic. and Biochem. Cytol.*, 1960, **8,** 483.

28. EAKIN, R. M., and WESTFALL, J. A., Fine structure of photoreceptors in amphioxus, *J. Ultrastruct. Research*, 1962, **6,** 531.