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ULTRASTRUCTURE OF A CEPHALOPOD PHOTOPHORE. I. STRUCTURE OF THE PHOTOGENIC TISSUE

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Of the many animal taxa which are capable of light production, the Cephalopoda present some of the most varied and elaborate photophores known. Photogenic organs are found within the mantle (Clarke 1965) in or by internal organs (*e.g.*, ink sac; Boletzky, 1970) or on the siphon (Hoyle, 1904), on the skin of the mantle, head and/or tentacles, and on the surface of the eye (Berry, 1920a, 1920b). There are many different types of photogenic organs and several dif-

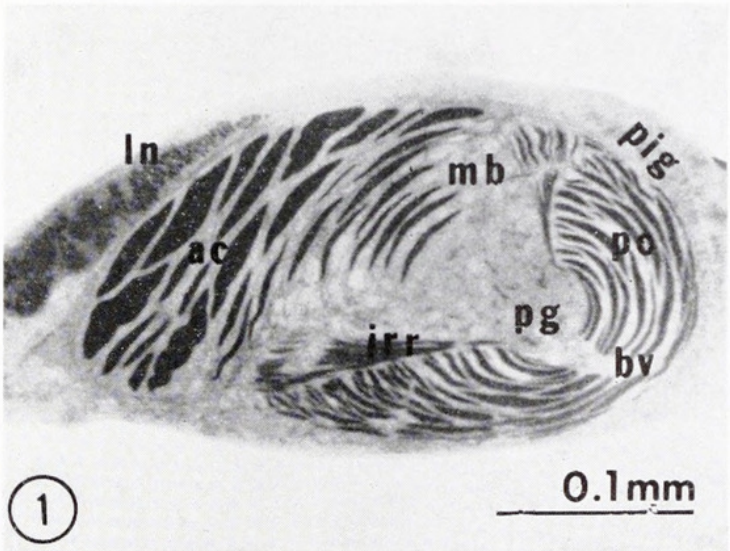
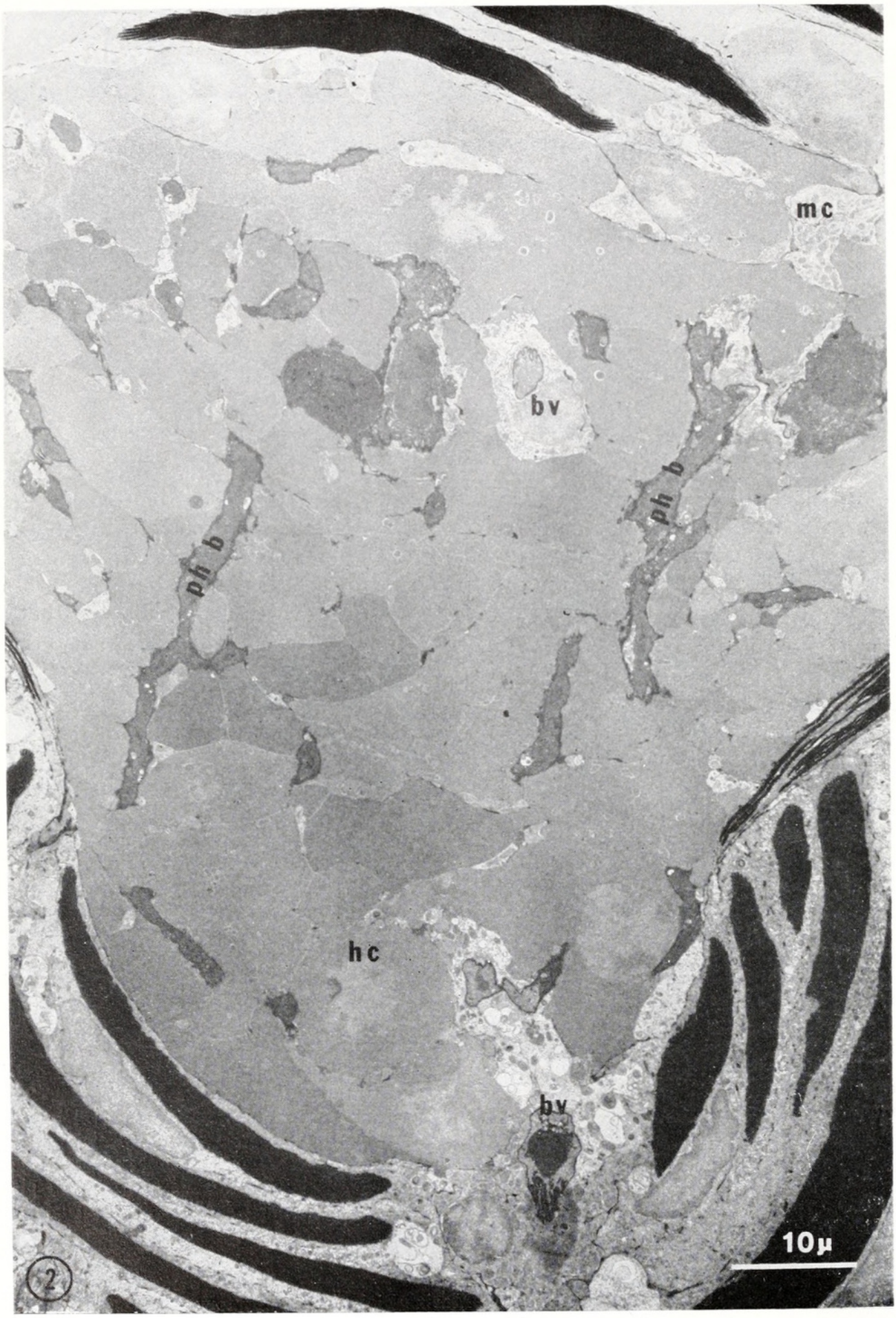


FIGURE 1. Light micrograph of a whole photophogenic tissue (pg) composed of an axial cone and spheroidal knob enclosed in a posterior cup (pc) made of regular iridophores. The anterior cap (ac) grades into a conical "plug" of iridophores in the center of the axial cone. Distal to the anterior cap is the clear lens (ln) composed of iridophores with many iridosomes in each cell. Surrounding the axial cone is a conical layer of irregular iridophores (irr). The posterior cup and proximal side of the whole organ is covered with a layer of brownish pigment (pig). Muscle bands (mb) and blood vessels (bv) occasionally penetrate the posterior cup or irregular iridophores.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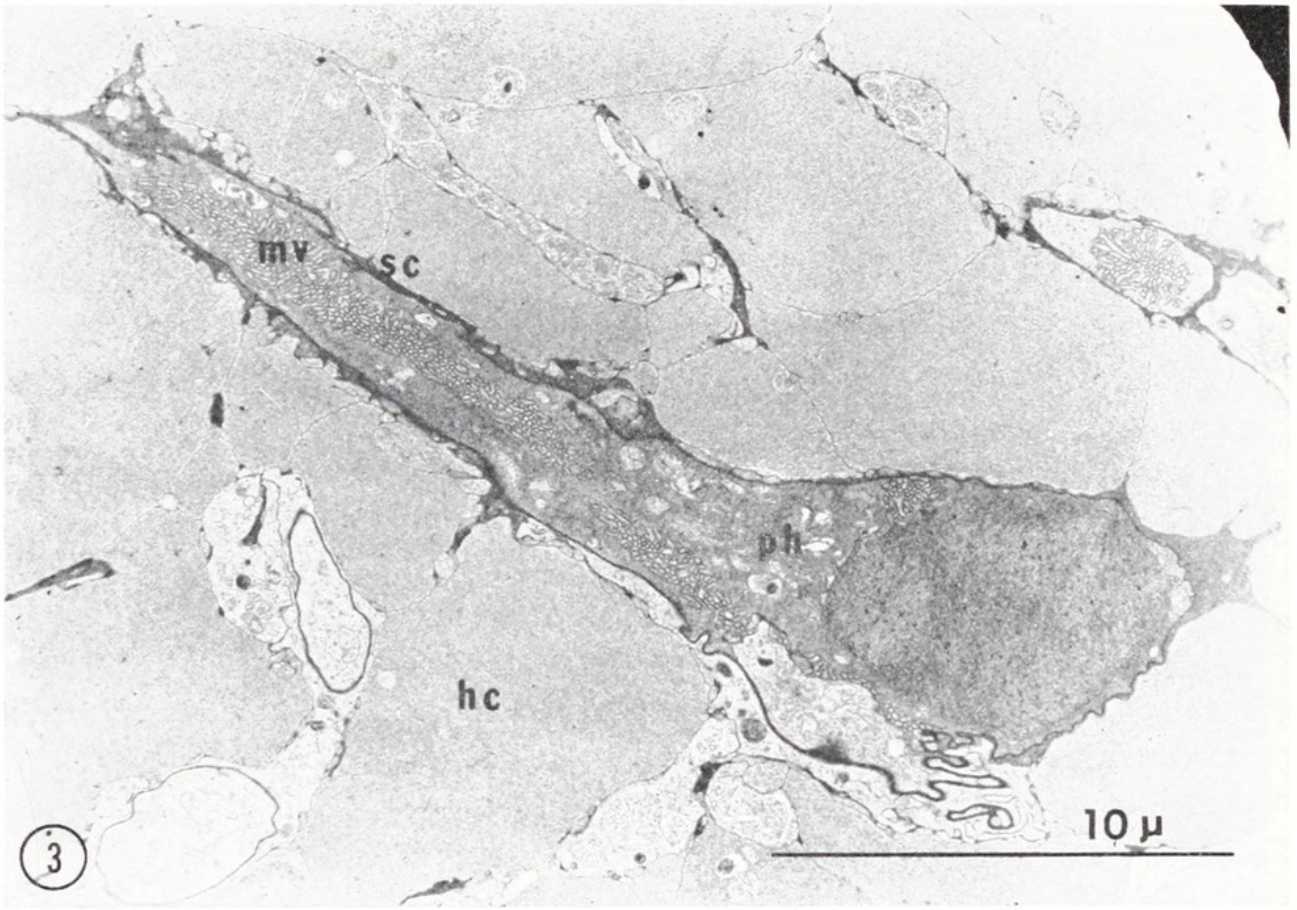
2

ferent types are often present simultaneously in the same individual and even on the same organ. The function of the photogenic organs is largely unknown, but it has been suggested that they are important in countershading (Young, 1972, 1973; Clarke, 1963), predation (Nicol, 1960), and possibly sexual interaction (Clarke, 1963, Young, 1974) or schooling behavior. Both bacterial and autophotogenic organs have been reported in cephalopods (Nicol, 1960). The autophotogenic photophore presents an interesting opportunity to study the production of light by a tissue whose function is the opposite of the retina but which still has many basic similarities to photoreceptive organs. By a careful fine structural analysis of these photogenic organs, insight might be gained into the basic structural mechanism of interconversion of chemical and electrical energy into light which, in turn, might be compared in interesting ways to photoreception. The purpose of these two papers is to report the results of ultrastructual studies on one common type of photogenic organ in the squid *Pterygioteuthis microlampas,* a midwater, Pacific species which probably only encounters low levels of light, and to speculate on the possible function of these photophores.

Although Hoyle (1904) has described the histological structure of a photophore of a closely related squid (*Pterygioteuthis giardi*), for purposes of convenience to the reader and because there are some slight differences in our observations, the following brief description of the whole photophore is provided. There are several photophores located on the ventral surface of each eye, and these are of at least three types. Eleven of these photophores have similar structure and have been the subject of this study. These photophores vary in size, depending on the individual squid, but typical ones measure about 0.4 mm × 0.3 mm, are lentoid in shape, and are roughly radially symmetrical. Hoyle (1904) described a connective tissue cap which was reddish-brown; a "posterior cup" of oval or circular fibrous "scales" (=iridophores) in concentric layers; an "anterior cap" of "scales"; an "inner funnel" of rather coarse fibers (=irregular iridophores); and a central mass filled with almost structureless parenchymous tissue which is composed of a "spheroidal knob" and an "axial cone" (Fig. 1). In this paper this tissue will be referred to as the photogenic tissue and the presumed light producing cell will be called the photocyte. In addition there is also a clear layer of modified iridophores (=lens) distal to the anterior cap, which Hoyle did not include in his description. Nerves and blood vessels occasionally penetrate the sides or the base of the bulb region of the posterior capsule. Bands of muscle traverse the "inner funnel" region and attach to those iridophores which make a conical proximal "plug" as a central continuation of the anterior capsule. Apparently, each of the three types of iridophores making up the posterior and anterior capsules, the "inner funnel" and clear "lens" have different functions, and this paper will be devoted to a description of their ultrastructure and speculation as to their functions.

FIGURE 2. Photogenic tissue of the spheroidal knob and part of the axial cone. A blood vessel (bv) enters through the posterior cup. The photocytes (ph b) branch among the homogeneous packing cells (hc). Branches of mitochondrial cells (mc) are also evident.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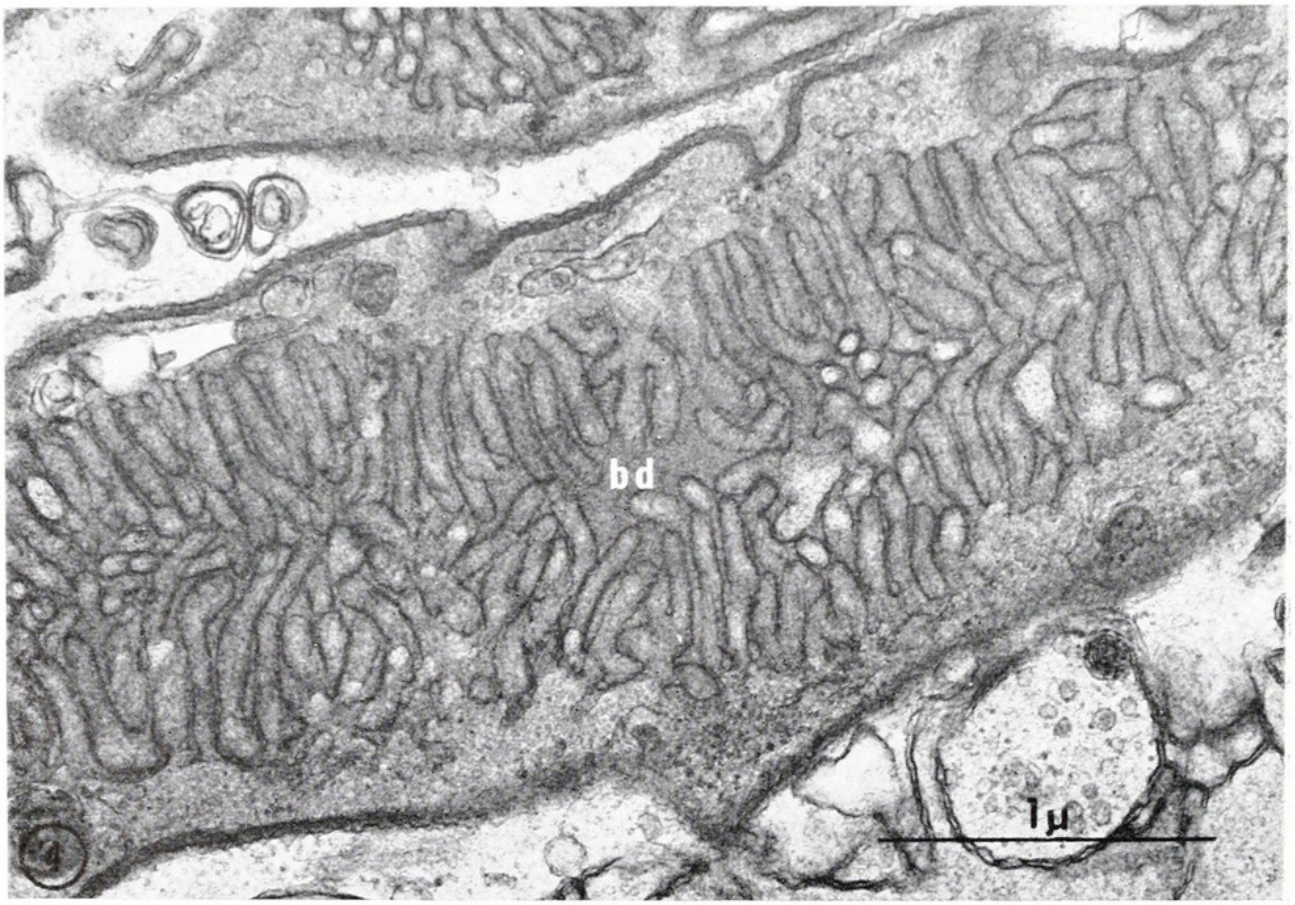
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Materials and Methods

The animals used in this study were taken at night by a closing trawl off the island of Oahu, Hawaiian archipelago. Once on shipboard they were fixed in a mixture of 6% glutaraldehyde buffered to pH 7.5 with 0.1 M collidine buffer and adjusted to approximately 1200 milliosmoles with sucrose as modified from Bell, Barnes and Anderson (1969). Fixation began at ambient temperature and then the vials containing the whole animal were chilled on ice and kept at 4° C until the ship returned the next morning. The photophores were then dissected off the surface of the eye, washed in cold collidine buffer for 1 hour and then post-fixed in 1% OsO_4 in veronal-acetate buffer at pH 7.6 for one-half hour. The photophores were then dehydrated through an ethanol series and then embedded in Luft Epon for thin sectioning. These sections were stained with either uranyl acetate in methanol followed by lead citrate or in lead citrate alone. This fixation procedure seemed to be adequate despite the difficulties caused by working on shipboard, and the time delays involved. However, in some areas there are membrane whorls that are probably artifact. Despite slight variations in fixation procedures these whorls occasionally remained randomly associated with membranous structures in the cells. Because of the difficulty in obtained animals (one or two per night is an average catch), the expense of ship time, and the otherwise satisfactory fixation, the results are reported with no further apology.

Observations

Figure 2 shows a low power micrograph of the "spherical knob" and most of the "axial cone" region of the photogenic tissue. The photogenic tissues are surrounded by stalks of iridophores which are occasionally penetrated by blood vessels and nerves. These iridophores will be discussed in a separate paper. In addition to elements of circulatory system and the nervous tissue, these are four cell types: the photocytes; their associated sheath cells; the packing cells which occupy most of the volume of the photogenic tissue; and the mitochondrial cells. Each of these cell types will be discussed in turn. Although it has not been possible to examine each of these cells in physiologically active state, by elimination and analogy it has been possible to assign these four cell types functions on the basis of their morphology.

Photocytes

The photocytes are branching, ramifying cells which are interspersed with no apparent pattern between the other cells of the photogenic tissues. They are characterized by an extensive body of microvillous protrusions which occupy most of the volume of the cell and are arranged about a central lumen filled with dense

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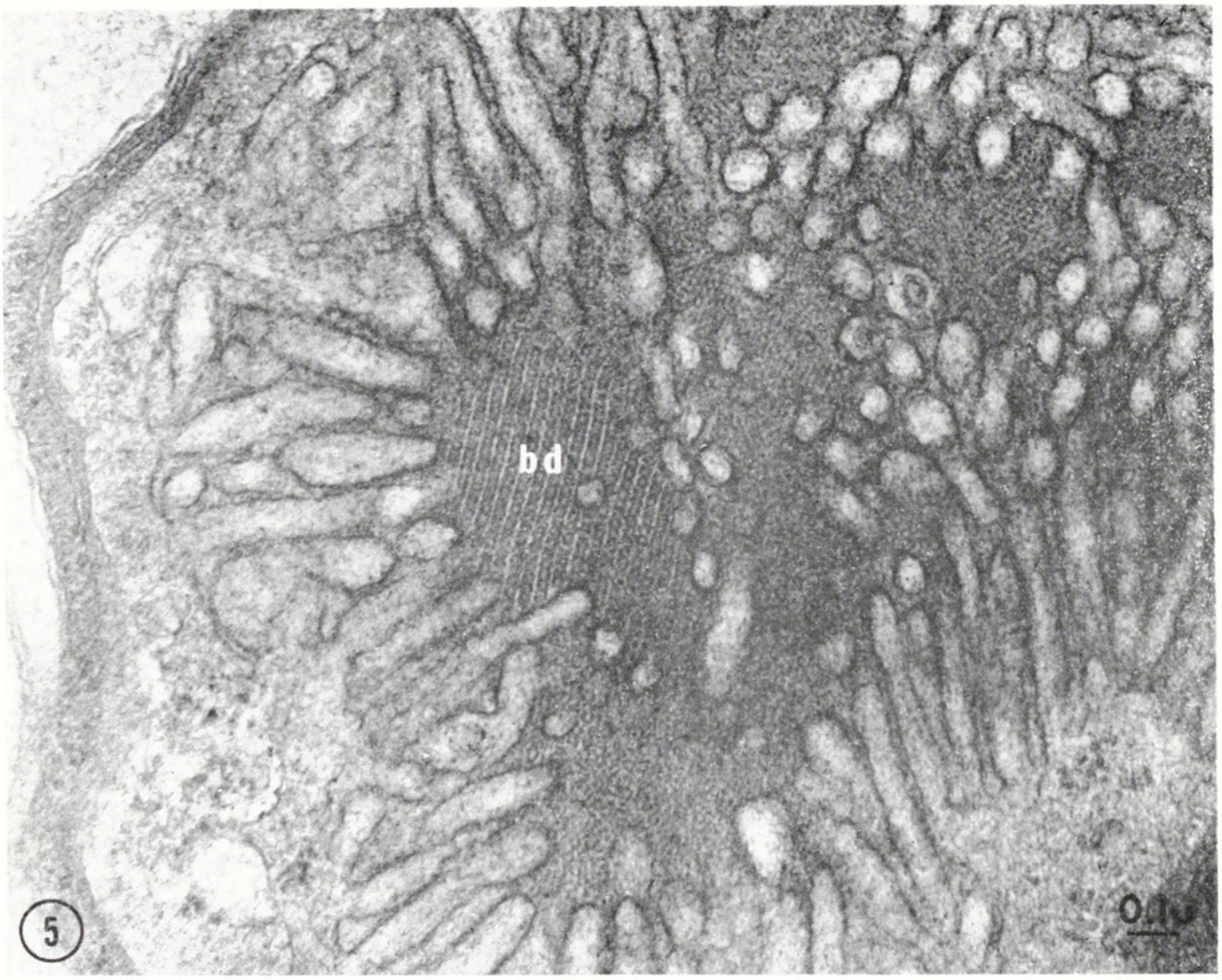
Figure 3. Mature photocyte (ph) in the nuclear region. The photocyte is enclosed in a sheath (sc) which separates it from the packing cells (hc). The microvilli (mv) are quite evident.

Figure 4. Higher magnification of the microvillous region of one branch of a photophore. The microvilli project into a central blood filled lumen (bd). The sheath can be seen to be of homogeneous density and lined by membranes of both sides.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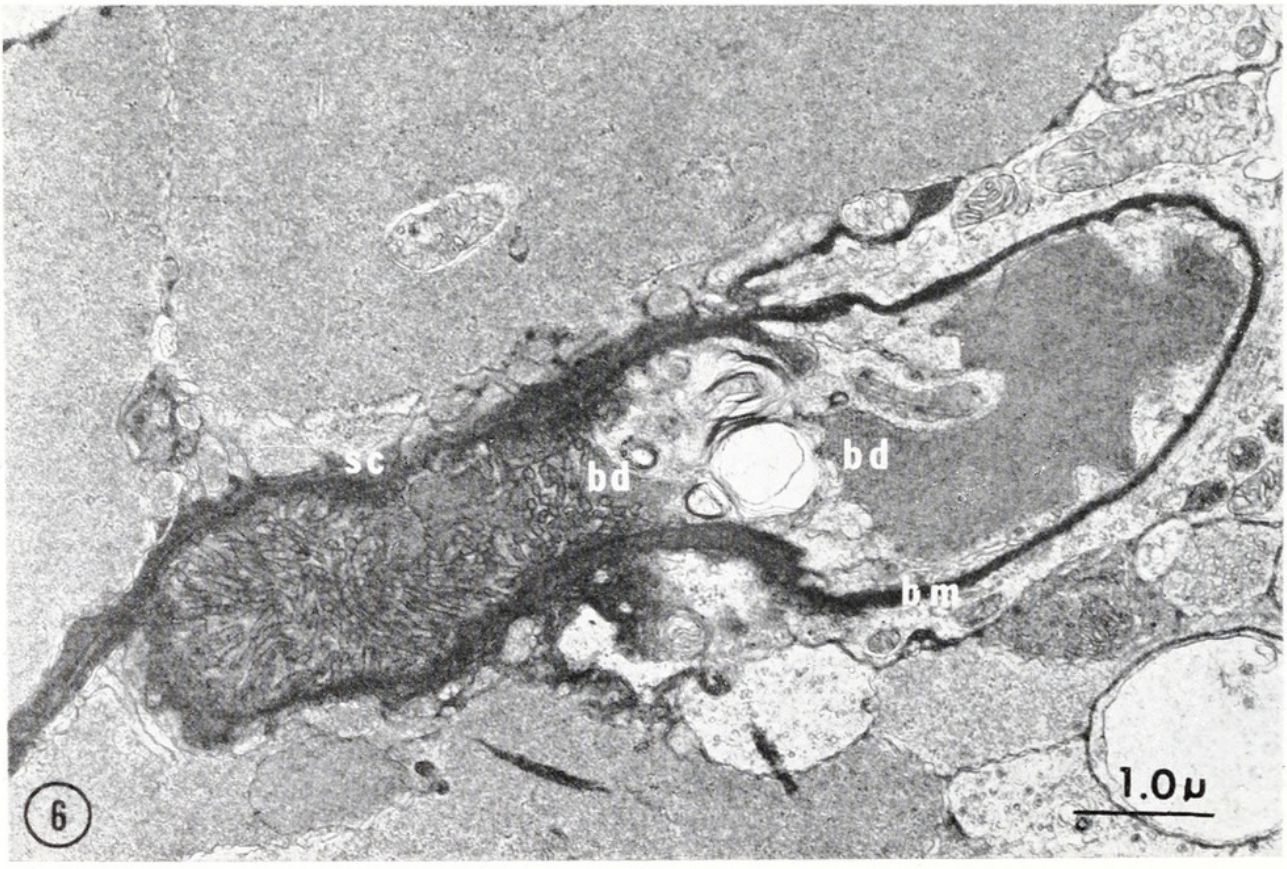
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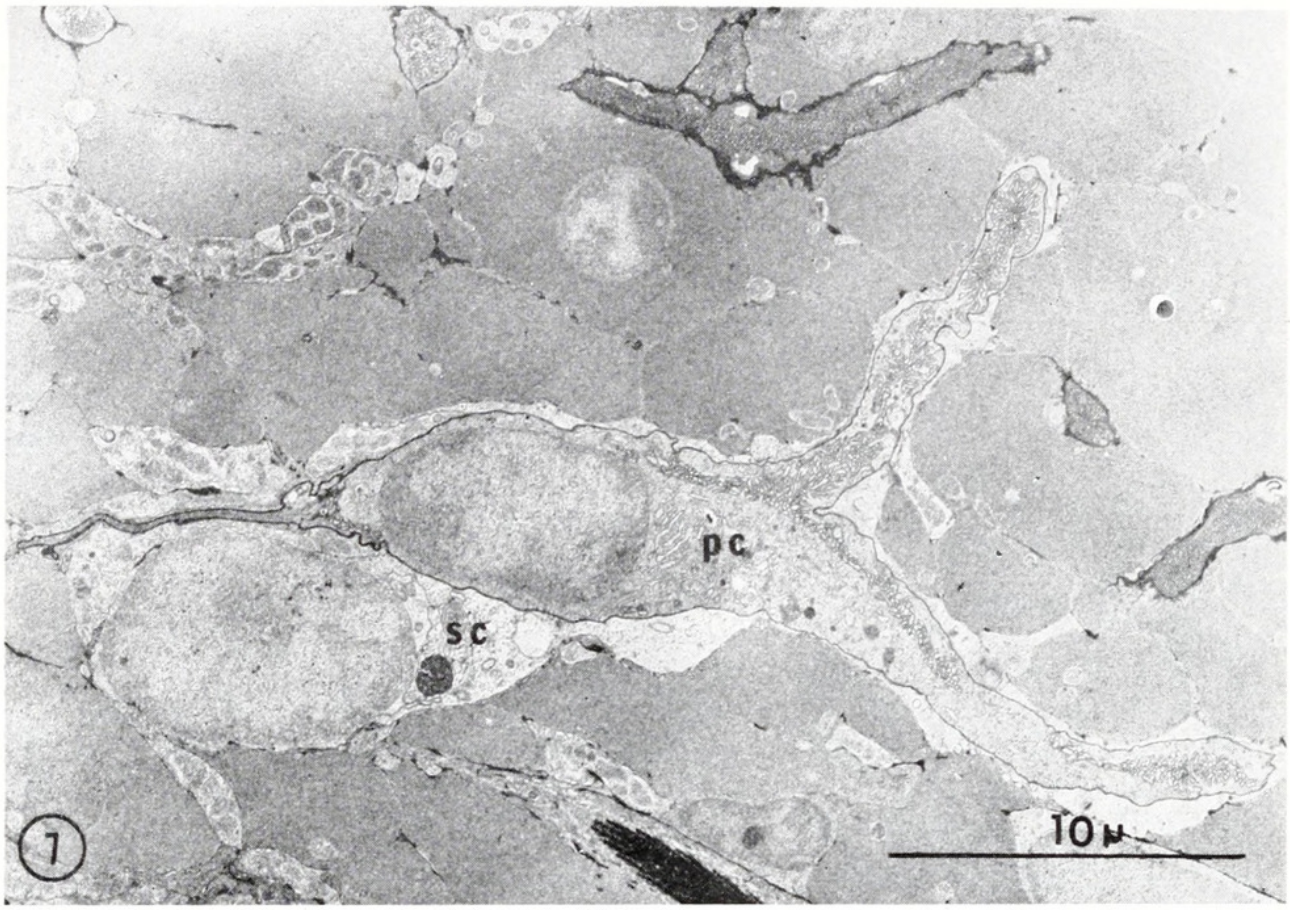
material (Fig. 3). Surrounding each photocyte is a dense sheath of acellular homogeneous material which separates the cell from either the surrounding packing cells or engulfing sheath cell (see below). In sections the nucleus of the photocyte appears more or less homogeneous when compared, for example, with the nuclei of the packing cells or the sheath cells.

The microvillous bodies comprise a discrete part of each photocyte and when apparently mature, are easily distinguished by their electron density. The individual microvilli are cylindrical and have a rounded end. They average 0.1μ in diameter and vary in length from 0.4μ to 0.6μ (Fig. 4). The microvilli project into a central lumen which apparently is filled with blood (Fig. 5). Cephalopod blood frequently has a characteristic paracrystalline appearance when fixed for electron microscopy (Barber and Graziadei, 1965) (Fig. 6). The hemocyanin molecules tend to form chains, each unit of which measures approximately 24 nm in diameter. Cut at various angles other characteristic spacing patterns are evident (Fig. 5) so blood can often be distinguished from electron dense substances. However, the blood does not always fix in this paracrystalline pattern but in these instances other characteristics (basement membrane, homogeneous density) can be used to distinguish blood from other materials of similar density. The microvillous body can often be seen to be continuous with blood vessels and in a few instances it appears that the microvilli are bathed by blood directly from the capillaries (Fig. 6). The sheath which surrounds each photocyte often appears continuous with the basement membrane of the blood vessel.

In the region of the axial cone photocytes which appear to be in stages of development can frequently be found. These immature photocytes are characterized by complete enclosure in a sheath cell, few and less dense microvilli in poorly organized discontinuous microvillous bodies, and by areas of somewhat less dense cytoplasm between the microvilli and the sheath (Fig. 7). As the microvillous body develops, this cytoplasmic area is mostly replaced by microvilli and what cytoplasm remains becomes increasingly dense (Fig. 8). Concommitant with this increase in density, the surrounding sheath cell apparently is retracted leaving the branches of the photocyte surrounded by a dense homogeneous sheath of variable thickness which is continuous with the basement membrane of the circulatory system. The microvilli apparently originate in conjunction with membranous components of the cytoplasm (possibly endoplasmic reticulum) and are frequently associated with infolding of the photocyte surface (Fig. 9). In some instances blood can be found contained within membranous networks within the cytoplasm which appear to be giving rise to microvilli. The origin of the intercellular membranous complexes containing this blood is uncertain but frequently endoplasmic reticulum and Golgi vesicles are seen in close proximity (Fig. 10).

FIGURE 5. Central region of a photocyte showing blood (bd) in the lumen. The hemocyanin molecules align to form paracrystalline arrays, some of which have a chain-like appearance. Sectioned at different angles the pattern is still characteristic enough to make identification of blood possible.

FIGURE 6. Connection between a photocyte and blood vessel. The paracrystalline pattern is evident (bd) in the vessel and the lumen of the microvillous region. The basement membrane (bm) is continuous with the sheath (sc) of the photocyte. The endothelial cell and pericyte are also evident.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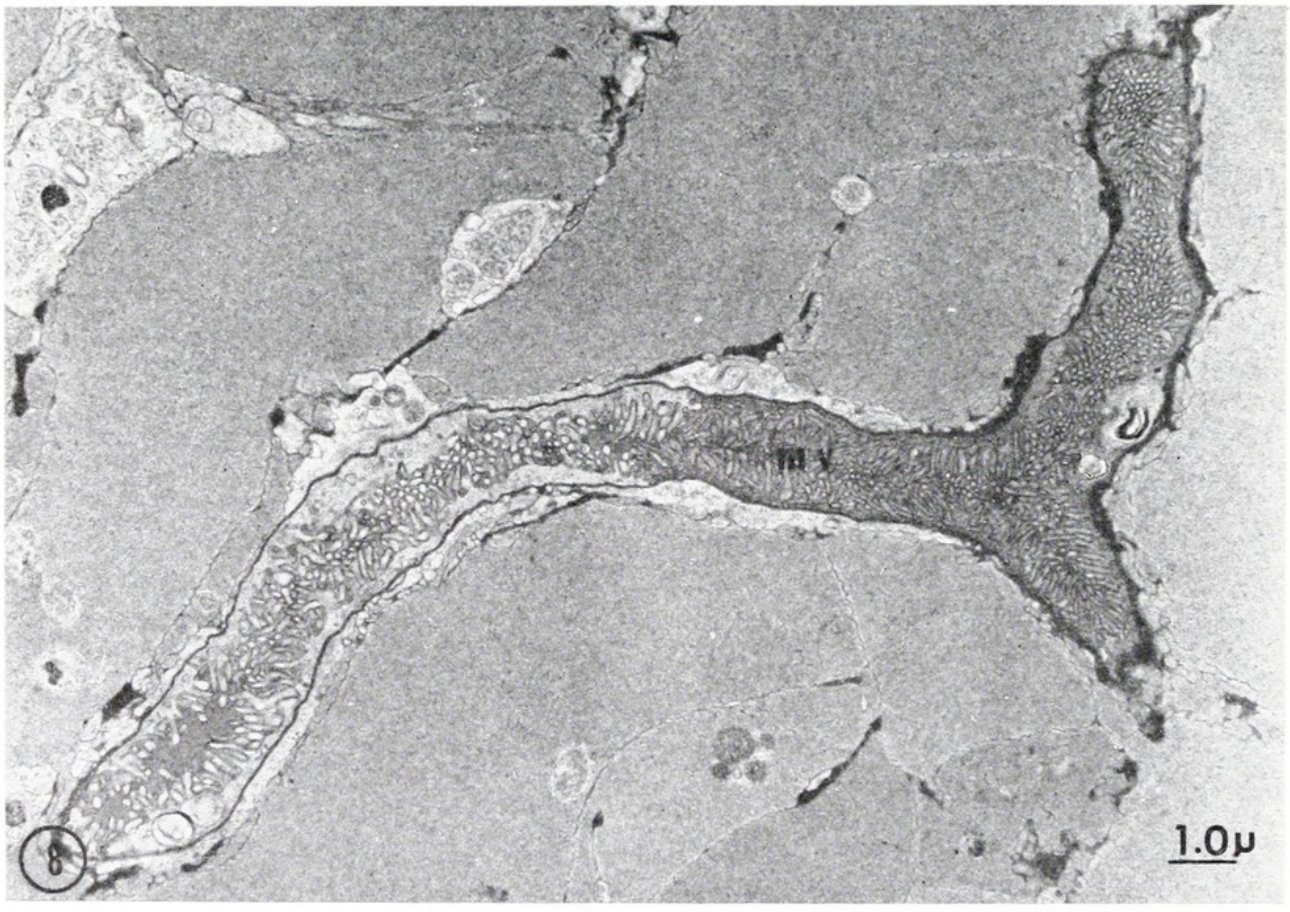
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Sheath cells

The sheath cells characteristically surround the whole developing photocytes encasing them in a layer of cytoplasm of varying thickness (Fig. 7). The nucleus of the sheath cell invariably occurs in proximity of the photocyte nucleus. The cytoplasm is considerably less dense than that of the photocyte and contains few scattered organelles. Mitochondria and membranous vesicles are occasionally encountered but mainly the cytoplasm is homogeneous with small aggregations of granular "background" material. Where the sheath cell contacts a blood vessel, vesicles suggestive of pinocytosis are apparent. The nuclei of the sheath cells are also less dense than those of the photocyte and appear to have fewer regional densities (Fig. 7).

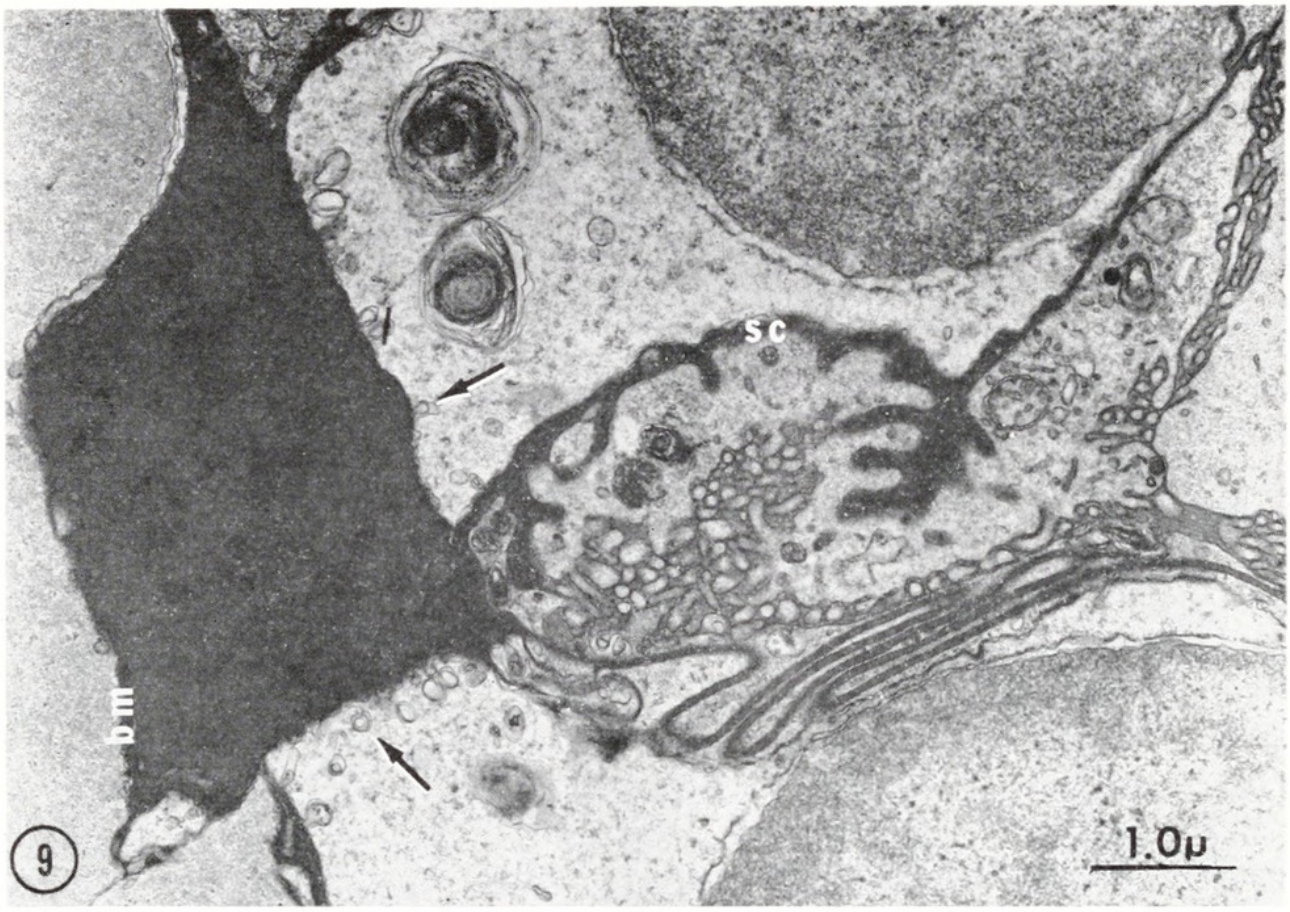
The sheath itself (Figs. 4, 8, 9) is homogeneous, amorphous, and electron dense. It appears to be entirely extracellular because it occurs outside the plasma membrane of both the sheath cell and the photocyte. It can be seen to be continuous with the basement membrane of the blood vessels although it is usually of less uniform thickness. On mature photocytes, it is quite irregular in thickness and shape (Fig. 8). The sheath cells are quite similar in appearance to the pericytes of the blood vessels (Barber and Graziadei, 1965) and in some instances it is hard to separate them on morphological criteria other than position (Fig. 6). As the photocyte matures the association of the sheath cell and photocyte is ended apparently by retraction of the sheath cell so that the photocyte with its surrounding sheath comes into direct contact with the surrounding packing cells (Fig. 8). The eventual fate of the sheath cell could not be determined from our micrographs.

Packing cells

The packing cells are characterized by being filled with a homogeneous granular matrix which occupies the position of the cytoplasm in a more typical cell (Fig. 11). The membranous components of the cell are in direct contact with this matrix and there is no evidence that the matrix is contained in a special vacuole. Occasionally mitochondria are encountered but they appear to be randomly distributed and the outer mitochondrial envelope is in direct contact with the matrix (Fig. 6). Golgi material and a few membranes suggestive of smooth endoplasmic reticulum are present infrequently (Fig. 6). The nuclei have large regions of dense material which primarily occupies the periphery of the nucleus. The packing cells tend to be globular in shape but assume the shape of the more highly structured elements around them.

FIGURE 7. Developing photocyte within a sheath cell (sc). Note that the photocyte is completely enclosed within the sheath cell.

FIGURE 8. Retraction of the sheath as the photocyte matures. The microvilli (mv) completely fill the mature portion of this photocyte branch and the sheath cell no longer surrounds this area. Where the microvilli are less dense and not as well differentiated the sheath cell enclosed the photocyte branch.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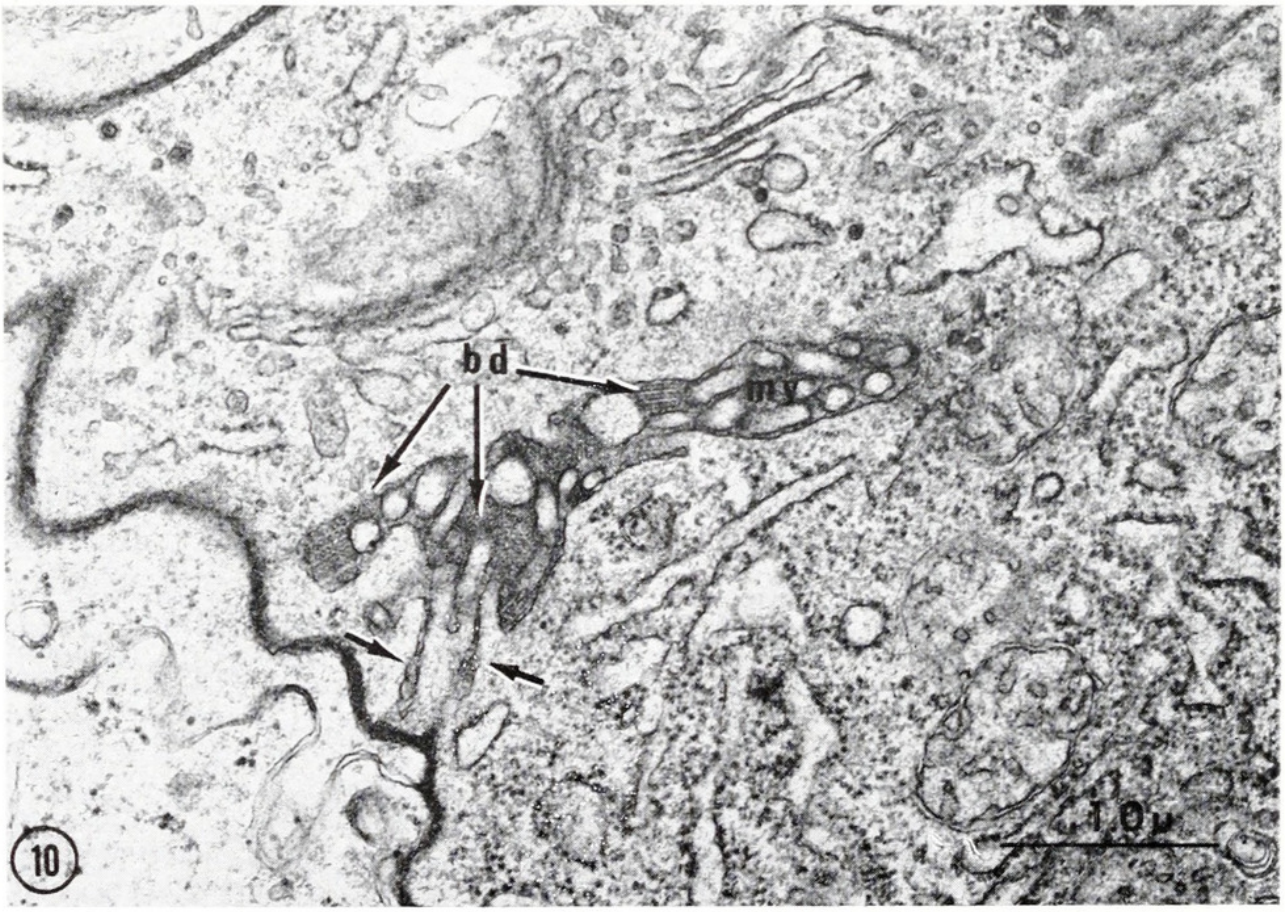
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Mitochondrial cells

In contrast to the packing cells, the fourth cell type in the photogenic tissue, the mitochondrial cells, tend to have their cytoplasm occupied mainly by mitochondria (Fig. 11). These cells tend to be concentrated in the axial cone and in rare cases form a more or less continuous layer. However, because they branch and ramify throughout the photogenic tissue they are present in the spherical knob and are seen in contact with all of the other cell types including the iridophores and no preferential association is obvious. The mitochondrial themselves have the "somewhat empty" appearance typical of cephalopod mitochondria but typically occupy better than 50% of the total cell volume. In contrast to the nuclear condensation seen in the packing cells, the nuclei of the mitochondrial cells seem homogeneous although the cisternae of the nuclear envelope are infrequently expanded (Fig. 11).

Vascular and neural elements

Vascular elements are frequently encountered entering the photogenic tissue. They are easily identified by the characteristic basement membrane, endothelial cells, and pericyte (Barber and Graziadei, 1965). The hemocyanin frequently shows a paracrystalline pattern which also aids in identification (see above). Where vessels pass through the posterior cup, the iridophores are displaced or modified in shape to accommodate them. Inside the photogenic area the endothelial cells and pericytes are frequently reduced or even lacking (Fig. 9), thus allowing the blood to come into intimate contact with the cells of the photogenic tissue. The lumen of the photocytes is frequently seen filled with blood (Figs. 5 and 6) which is in direct contact with the microvilli and occasionally blood can be found within intracellular membranes in developing photocytes.

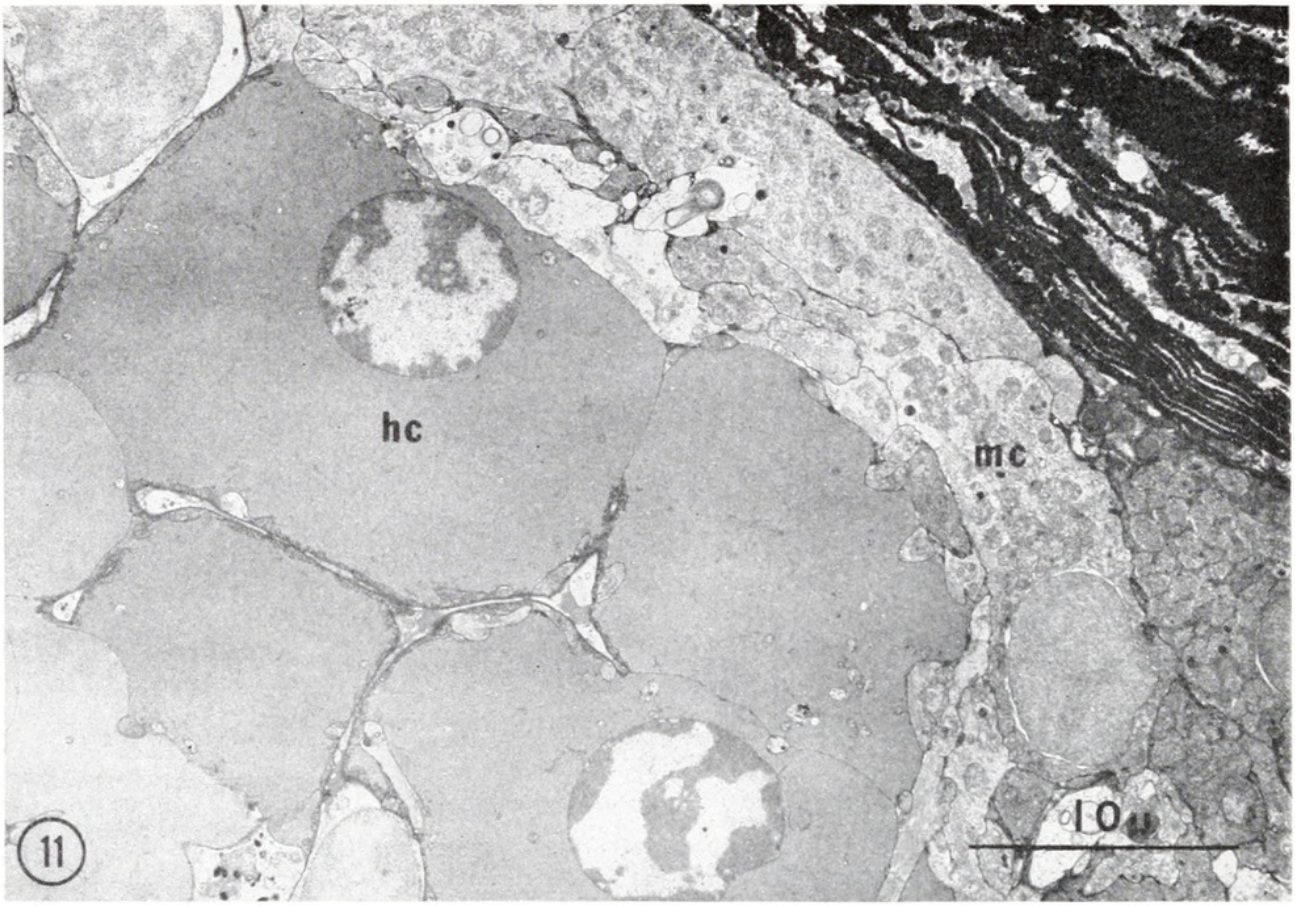
Neural elements are represented primarily by single nerve processes which occur among the photogenic cells. These nerve fibers apparently form synapses with the photocytes and in favorable sections gaps in the sheath surrounding the photocytes can be found which suggest the possible transport of synaptic vesicles (Fig. 12). We have not encountered any synapses with other cell types of the photogenic tissue.

Discussion

Although the inability to maintain living material in the laboratory imposes certain limitations on the interpretation of the observations presented here, it is possible to speculate on the function of the various cell types presented here on purely morphological grounds. The photocytes seem to be the only possible

Figure 9. Developing microvilli and acellular region of blood vessel. The developing microvilli arise inside the photocyte but are in contact with the surface. A small acellular blood vessel is in contact with the sheath cell and developing photocyte. The sheath (sc) is continuous with the basement membrane (bm). Blood is evident inside the cell in the future lumen.

Figure 10. Developing microvilli (mv). Note the membrane continuity between the future microvillous region and the surface (arrows). Blood is clearly evident.



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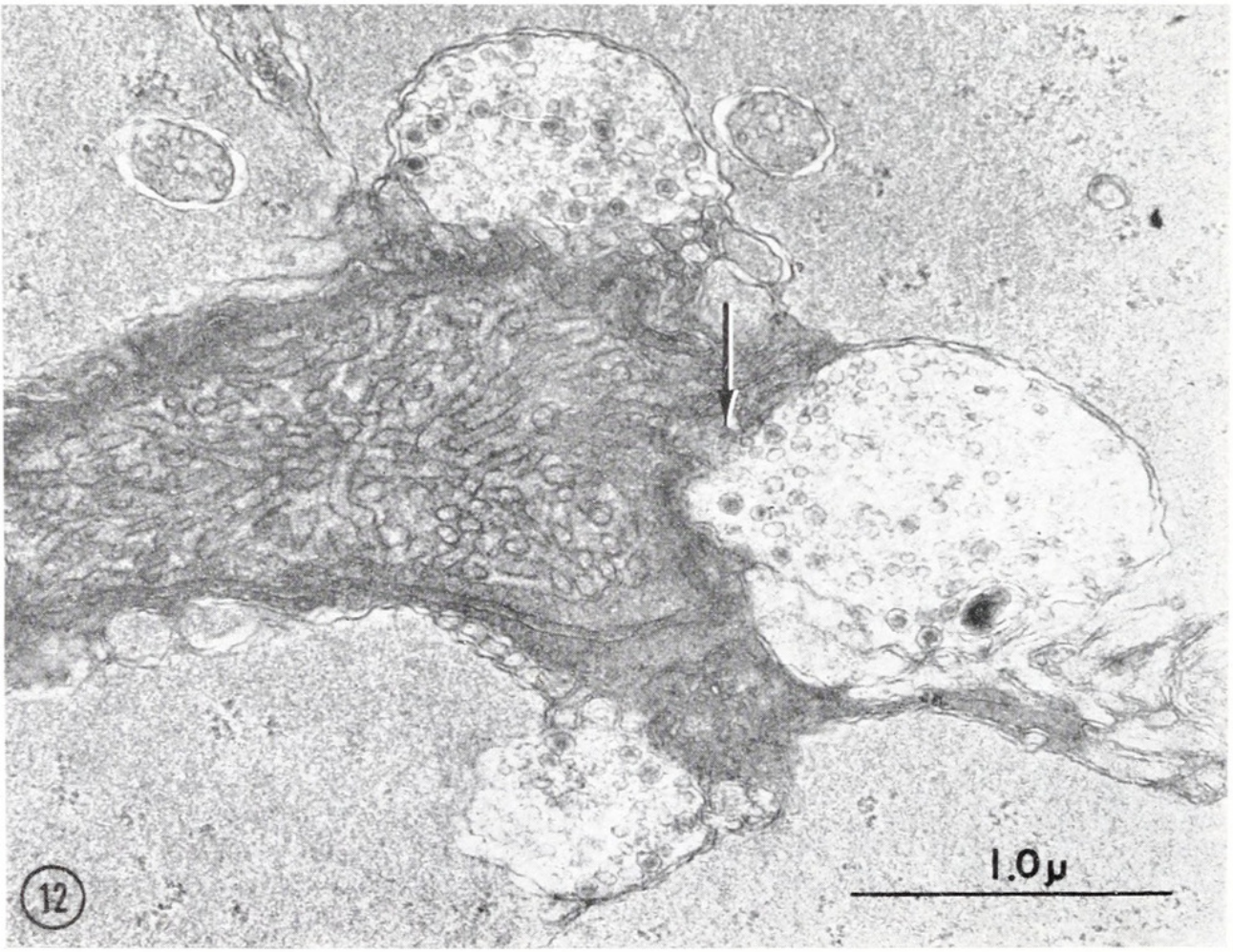

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candidate for light production since they are in the proper position, are highly specialized, and have direct communication with both the nervous system and circulatory system. The microvillous areas of these cells would seem to be the most likely site of light production. In many invertebrate photoreceptors, light is converted to chemical energy in strikingly similar structures (Clark, 1967). The availability of nutrition and oxygen, innervation, and mitochondrial energy would strongly support this assumption. The possibility that the "photophore" might be some type of a secondary photoreceptor could be raised but in other genera organs with quite similar structure are undoubtedly photoproductive (Berry, 1920a, 1920b; Hoyle, 1902, 1904). The morphological similarities between organs of photoreception and photoproduction imply that interconversion of light and chemical energy may have a necessary basic subcellular mechanism.

Although the observations on the development of the photocyte are not sufficient to reconstruct the complete pathway of development a few interesting points may be made. Apparently early in the life of a photocyte, it must be isolated from surrounding tissues; first by developing within another cell (the sheath cell) and later by being enclosed in a dense sheath continuous with the basement membrane of the circulatory system. This would imply that insulation from other cell types is necessary for either photocyte differentiation or function. It would be tempting to speculate that the sheath (and sheath cell) chemically or electrically insulate the photocyte but more likely is the possibility that the sheath functions in keeping the photocyte in intimate preferential contact with the circulatory system thereby insuring an adequate nutrient and respiratory supply. The microvilli of the photocyte apparently arise from an intracellular membranous system that very early in development is in direct contact with blood. The retraction of the sheath cell as the photocyte presumably matures implies that the control of differentiation of the photocyte is somehow directed or enhanced by the sheath cell.

The simple structure of the packing cells suggest that they primarily function in occupying space between more metabolically active tissues. In formalin preserved, unstained photophores, the packing cells are clear and would thereby not impede or modify light produced by the photocytes. It would seem most likely that the packing cells function in dispersal and separation of the photocytes and have a low metabolic level.

Conversely, the mitochondrial cells, by the sheer volume of mitochondria seem to be very metabolically active. Because they branch and ramify throughout the photogenic tissue and make contact with many cells, they probably provide metabolic energy to the other cell types and in particular the photocytes.

The structure of the circulatory system in the photogenic tissue is interesting because blood comes into direct contact with the photocytes and because in some areas vessels are apparently reduced to a basement membrane containing blood with neither pericytes nor endothelial cells present. Barber and Graziadei (1965)

FIGURE 11. Packing cell and mitochondrial cell. Most of the cytoplasmic region of the packing cells (hc) is occupied by a homogeneous material. In the mitochondrial cell (mc) most of the cell's volume is devoted to mitochondria.

FIGURE 12. Synapse with the photocyte. Note the sheath is broken in one region and the synapse appears to be in direct contact with the microvillous region (arrow).

described four types of blood vessels in the circulatory system of *Octopus* and *Sepia,* two of which had the basement membrane exposed directly to the blood. A logical extension of this would be a completely "acellular vessel" composed of basement membrane only. Such a vessel is shown in Figure 9. These "acellular vessels" are frequently encountered and always are smaller than the cells they contact. However, since there always appears to be a basement membrane except where the blood directly contacts the photocytes this is not a true open circulatory system. In the case of the photocyte the basement membrane (=sheath) contains the whole cell suggesting the photocyte is intimately related to the circulatory system.

It is possible to present an integrated speculative picture of the function of the cell types in the photogenic region of this photophore. Light is produced by energy conversion on the microvilli from precursors provided by the circulatory system and energy derived from the mitochondrial cells. The photocytes are probably stimulated to luminesce by the numerous synapses which penetrate the sheath. The photocytes are separated by packing cells and contained within the sheath. Although the above model of light production by one type of photophore of a deep sea cephalopod is hypothetical and theoretically tenuous, it is hoped this model can serve as a tentative structural basis for further comparative and physiological study.

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Summary

One type of photophore of the deep sea squid *Pterygioteuthis microlampas* was examined with the electron microscope and its fine structure described. The photogenic tissue is composed of four cell types each with distinctive morphology which suggests their function. The photocytes branch and ramify throughout the central region of the photophore and have an extensive system of microvilli (the photogenic organelle) which are arranged about a central blood filled lumen. The photocytes apparently develop inside a sheath cell and are surrounded by a sheath which is continuous with the basement membrane of the blood vessels. The photocytes and associated sheath cells are surrounded by packing cells whose cytoplasm is replaced with a homogeneous granular material. Finally, cells containing many mitochondria branch and ramify throughout the photogenic area. Apparently the circulatory system is in direct contact with the photocytes, and acellular blood vessels, composed only of basement membrane, are found throughout the photogenic tissue. The similarity between photoproductive organelles and photoreceptive organelles is striking.

LITERATURE CITED

BARBER, V. C. AND P. GRAZIADEI, 1965. The fine structure of cephalopod blood vessels. I. Some smaller peripheral vessels. *Z. Zellforsch.* **66**: 765–781.

BELL, A. L., S. N. BARNES, AND K. L. ANDERSON, 1969. A fixation technique for electron microscopy which provides uniformly good preservation of the tissues of a variety of marine invertebrates. *Biol. Bull.,* **137**: 393.

BERRY, S. S., 1920a. Light production in cephalopods. I. An introductory survey. *Biol. Bull.,* **38**: 141–170.

BERRY, S. S., 1920b. Light production in cephalopods. II. An introductory survey. *Biol. Bull.,* **38**: 171–195.

BOLETZKY, S. V., 1970. Biological results of the University of Miami deep sea expeditions 54. On the presence of light organs in *Semirossia* Steenstrup 1887. (Mollusca, Cephalopoda). *Biol. Mar. Sci.,* **20**: 374–388.

CLARK, A. W., 1967. The fine structure of the eye of the leech, *Helobdella stagnalis. J. Cell Sci.,* **2**: 341–348.

CLARKE, M. R., 1965. Large light organs on the dorsal surface of the squids *Ommastrephes pteropus, Symplectoteuthis oualaniensis,* and *Dosidicus gigas. Proc. Malacol. Soc. London,* **36**: 319–321.

CLARKE, W. D., 1963. Function of bioluminescence in mesopelagic organisms. *Nature,* **198**: 1244–1246.

HOYLE, W. E., 1902. The luminous organs of *Pterygioteuthis margaritifera,* a Mediterranean cephalopod. *Manchester Mem.,* **46**(16): 1–14.

HOYLE, W. E., 1904. Reports on the dredging operations off the west coast of Central American by the Albatross, etc. Reports on the Cephalopoda. *Bull. Mus. Comp. Zool.,* **43**: 51–64.

NICOL, J. A. C., 1960. *The Biology of Marine Animals.* Interscience, New York, 707 pp.

YOUNG, R. E., 1972. Function of extra-ocular photoreceptors in bathypelagic cephalopods. *Deep-Sea Res. Oceanog. Abstr.,* **19**: 651–660.

YOUNG, R. E., 1973. Information feedback from photophores and ventral countershading in mid-water squid. *Pac. Sci.,* **27**: 1–7.

YOUNG, R. E., 1975. *Leachia pacifica* (Cephalopoda:Teuthidae) spawning, habitat and function of brachial photophores. *Pac. Sci.,* **29**(1): in press.