FINE STRUCTURE OF THE EYE OF A NUDIBRANCH MOLLUSC, HERMISENDA CRASSICORNIS

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SUMMARY

The eye of a nudibranch, Hermissenda crassicornis, was studied by light and electron microscopy. Three kinds of cells were observed: large sensory cells, each bearing at one end an array of microvilli (rhabdomere) and at the other end an axon which leaves the eye by the optic nerve; large pigmented supporting cells; and small epithelial cells, mostly corneal. There are five sensory cells, and the same number of nerve fibres in the optic nerve. The receptor cells contain an abundance of small vesicles, 600-800 Å in diameter. The lens is a spheroidal mass of osmiophilic, finely granular material. A basal lamina and a capsule of connective tissue enclose the eye. In some animals the eye is 'infected' with very small bodies, 4-5 μ in diameter, thought to be symbionta.

INTRODUCTION

This study was suggested to the senior author by Professor Donald Kennedy of Stanford University to provide a structural basis for the interpretation of electrophysiological investigations being conducted on the eye of the nudibranch Hermissenda crassicornis (Barth, 1964; Dennis, 1966).

A second objective was a contribution to the analysis of structural patterns in molluscan photoreceptors. According to the hypothesis of Eakin (1963, 1966a, 1968), photoreceptors in the mollusc–annelid–arthropod line are predominantly rhabdomeric (i.e. consist of arrays of microvilli arising from the cell membrane), whereas those in the coelenterate–echinoderm–chordate lineage are basically ciliary derivatives. Some studies have revealed, however, ciliary-type receptors in molluscan eyes (Miller, 1958; Yanase & Sakamoto, 1965; Barber, Evans & Land, 1967). Additional studies on the eyes of other molluscs, especially gastropods and pelecypods, are needed to clarify the picture in this large and diversified phylum. As the eye of a nudibranch had not been examined previously with the electron microscope it was hoped that this investigation would be useful to anatomist and evolutionist as well as to neurophysiologist.

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MATERIALS AND METHODS

Adult specimens of the aeolid nudibranch *Hermissenda crassicornis* (Eschscholtz, 1831 (see MacFarland, 1966)) were collected on the floats in Monterey harbour, California. The brain, eyes and statocysts were removed from living animals and fixed. Best preservation was obtained with 2% osmium tetroxide in 0.2 M cacodylate buffer at pH 7.4, or with 2% acrolein and 6.5% glutaraldehyde (redistilled) in 0.1 M Sorensen's phosphate buffer (Sandborn, Koen, McNabb & Moore, 1964) or Veronal acetate buffer at pH 7.1. The period of fixation was overnight in the former and 2 h in the latter, and for both the temperature was just above 0 °C. The specimens were rinsed in the respective buffer, post-fixed (if needed) in cold 2%OsO₄ in Veronal acetate buffer for 2 h, rapidly dehydrated in ethanol, and embedded in Epon in which all but a small part of the brain was removed with microknives (Eakin & Westfall, 1965a) before the plastic was slowly cured. Ultrathin sections were cut with a diamond knife in a Westfall-Healy section mounter, stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined in an RCA EMU3G electron microscope. Complete serial thick (1.0 μ) sections (Westfall, 1966) of two eyes were cut and stained with toluidine blue in borate for light microscopy.

OBSERVATIONS

**Light microscopy**

Eye, optic ganglion, and statocyst of *H. crassicornis* lie adjacent to one another in this order from anterior to posterior (see Russell, 1929; Barth, 1964). The present study concerns only the eye. It is ovoid in shape, measures 80 μ on the longest axis, and appears black, owing to pigment in the retina. A transparent lens bulges dorso-laterally from the anterior end of the organ. Light microscopy shows three types of cells: non-pigmented sensory cells (Figs. 2, 3, sc); pigmented supporting cells (pc); and small epithelial cells (ec), mostly in the cornea (c). The first two elements make up the retina. The eccentrically placed opticoel is filled with the lens (ls), a large (40 μ) spheroidal homogeneous mass of finely granular material. The eye is enclosed in a capsule of connective tissue through which passes the optic nerve (on) to the nearby optic ganglion (og).

To ascertain the number of sensory cells a complete series of 1-μ sections was cut and drawings were made of the nuclei of the receptor cells in each section. A count of the individual nuclei could be determined without reconstruction of models. In two large nudibranchs the number was five. In one additional animal five nerve fibres were counted in electron micrographs of the optic nerve (see below).

**Electron microscopy**

*Receptor cells.* Each sensory cell has many microvilli extending from its inner end toward the lens or laterally toward a supporting cell (Figs. 1, 4). We postulate that the villi are light-sensitive. They are about 10 μ in length and 0.08 μ in diameter. They are closely ordered into arrays which in many instances appear undulating, as a result,
perhaps, of shrinkage during fixation. The interior of each villus contains granular material. Cross-sectional views show a roughly hexagonal pattern (see left centre, Fig. 4), presumably the consequence of close packing. There is little space between the
diagram of a receptor cell and parts of two pigmented (supporting) cells from eye of *Hermissenda crassicornis*.
Another characteristic of a receptor cell is a massive aggregation of small, clear, spherical vesicles (600–800 Å in diameter) around the nucleus and in the peripheral part of the cell (Figs. 1 and 5, v). Similar vesicles occur less abundantly in other areas from the base of the microvilli to the nerve fibre which leads from the outer end of the sensory cell. Between the vesicles are numerous aggregations of glycogen granules, mostly of the alpha type (Fig. 5, g), namely, rosettes of subunits (beta granules). Free beta units, however, occur interspersed among the alpha granules. Additionally, there are smaller, less dense particles which are probably ribosomes (r) judging from their similarity to granules on the rough endoplasmic reticulum. In some places the vesicles and granules are so abundant that other cytoplasmic structures appear to be pushed aside. In addition to the many small, thin-walled vesicles just described there are a few larger ones ranging in size from 900 to 1400 Å in diameter and some with thick membranes that are distinctly trilaminar at higher magnification. Additionally, some appear rough-coated externally. There are indications that these vesicles arise from Golgi saccules (Fig. 6). Mitochondria are prominent in the vicinity of the nucleus.

Near the posterior pole of the eye the receptor cells narrow abruptly and give rise to neurites, one per cell, which bundle together and upon leaving the eye become the optic nerve (Fig. 3). At the origin of a fibre, the axonal hillock, there is an unusually large number of mitochondria embedded in a mass of small vesicles and granules described above. Additionally, there are neurotubules oriented lengthwise within the neurite, and often an aggregation of lipid droplets. Following an axon distally one observes a marked increase in the density of the cytoplasm owing to an increase in granules. The 600–800 Å vesicles, however, decrease in number (see below).

Pigmented cells. As noted earlier, large pigment-bearing supporting elements interdigitate with the receptor cells (Figs. 1, 4). The cytoplasm of the former is less dense than that of the latter owing to fewer organelles and to an absence of the small vesicles found in the sensory cells. Pigment granules are membrane-bound bodies, assumed to be melanin, which are usually ovoid but often irregular in shape. The inner surface of the supporting cell possesses microvilli (Fig. 7, arrows) which project toward the lens or intermingle with the villi of a sensory cell. The villi of a supporting cell differ from those of a sensory cell in being fewer, much shorter, and slightly thicker. An occasional rudimentary cilium was noted among the villi of pigmented cells. Beneath the microvilli is a terminal web of fine tonofilaments. Below this layer, which is absent in the receptor cell, one finds in addition to pigment granules a scattering of small particles and membrane-bound droplets presumed to be formed in Golgi centres deeper in the cell (Fig. 8). Although these secretory droplets and pigment granules are of similar density they appear to be different types of inclusions. The former are smaller (usually less than 0.3 μ in diameter) than the latter and commonly exhibit a granular halo between the droplet of secretion and the vesicular membrane. The vesicles presumably move through the terminal web and...
release their contents into the opticoel (not shown in Fig. 7) where they become the humour of the eye. One extruded droplet may be seen among the microvilli (Fig. 7, upper right).

*Symbionts.* The eyes of some of the nudibranchs studied contain small bodies, about 4–5 μ in diameter, within the cells of the eye, and these have also been observed in the optic ganglion. These structures are usually spheroidal but sometimes irregular in outline, and in some instances they possess lobate protrusions suggestive of pseudopodia. They may be seen in the light microscope (Fig. 3, sy), especially in the clear epithelial cells and in the pigmented cells in which each body is surrounded by a ring of pigment granules (Figs. 3, 9). The cytoplasm of these bodies, thought to be symbionts (see Discussion), contains many vesicles, varying in size and density of contents, and a perinuclear endoplasmic reticulum, the profiles of which often parallel the nuclear envelope. Ribosomes occur on the membranes of the endoplasmic reticulum and free in the cytoplasm (Fig. 5). Mitochondria and a recognizable Golgi apparatus were not observed. Frequently the bodies are in contact with the mitochondria of host cells.

We speculate that the symbiont shown in Fig. 5 is in the process of entering a sensory cell. It is situated just under the basal lamina of the eye and seems in places to be separated from the cytoplasm of the receptor cell by membranes, as though the body lay between folds of the sensory cell or within a double-layered vacuole. In other places the investing membranes give the impression of transforming into strings of vesicles (300 Å in diameter) or rows of microtubules so that in these areas the body is separated from the cytoplasm of the host cell only by its own plasma membrane, as in instances of bodies more deeply embedded within the retinal cells. One should be mindful, however, of the probability that these vesicles are artifacts of osmium fixation.

*Epithelial cells.* Although all cells of the optic vesicle are epithelial elements, this heading excludes the receptor and supporting cells just considered. The remaining cells are relatively small, non-pigmented units, most of which cover the anterodorsal aspect of the lens as a corneal layer one cell in thickness (Fig. 10). The inner ends of the cells are joined together by desmosomes and their surfaces facing the lens are covered with short microvilli, similar to those of the pigmented cells.

At the junction of the cornea and retina the epithelial cells differ slightly from those just described. They are larger, the ergastoplasm is more abundant and there are more bundles of tonofilaments. A few epithelial cells lie at the emergence of the optic nerve from the retina and enclose the neurites like sheath cells. They are packed with filaments and glycogen.

*Accessory structures.* The large spheroidal lens is remarkably uniform in appearance in our electron micrographs although thick sections stained with toluidine blue or methylene blue show concentric rings of varying intensity of stain. The lens is finely granular and very osmiophilic. It is not bounded by a membrane and its surface is uneven (Fig. 10), owing perhaps to fixation.

The eye is enclosed in a dense basal lamina (Figs. 5, 10, bl), about 600 Å in thickness,
which is presumably formed by adjacent sensory, supporting and epithelial cells. Outside this layer lies a loosely organized collagenous capsule (cc) which is continuous with the surrounding connective tissue.

**Optic nerve.** Cross-sections of the optic nerve near its exit from the eye show five neurites (Fig. 11, 1–5) which vary a little in size. The second fibre from the right in the upper row measures 1.5 by 3 μ along the short and long axes, respectively. The fibres contain many vesicles, most of them about 600 Å in diameter, granules of glycogen, numerous mitochondria, neurotubules (not discernible in Fig. 11), and an occasional dense granule or multivesicular body. The bundle of five axons is almost entirely enclosed by a glial (sheath) cell filled with tonofilaments among which are scattered large granules. Outside the glial cell is a broad perineurium containing basal lamina and collagenous fibres.

**DISCUSSION**

**Receptor apparatus.** The array of microvilli in *H. crassicornis* bears no discernible anatomical or physiological relationship to the few rudimentary cilia which may be found at the inner ends of the sensory cells. Incidentally, the pigmented or supporting cells likewise exhibit rudimentary cilia. We draw the inference that the embryonic optic vesicle probably consisted of simple ciliated ectodermal cells which subsequently differentiated microvilli on their inner ends by repeated folding of the plasma membranes. The villi of the presumptive corneal and supporting cell remained relatively short whereas those of the receptor cells lengthened and presumably acquired a photopigment which endowed them with photosensitivity. This hypothesis is supported by three embryological studies on other forms, namely, an analysis of the origin of the receptor cell villi in the eyes of the gastropod mollusc *Helix aspersa* (Eakin, 1963, 1965a; Eakin & Brandenburger, 1967), of an onychophoran, *Macroperipatus geayi* (Eakin & Westfall, 1965b; Eakin, 1966a), and of a polychaete worm, *Nereis succinea* (Eakin, 1963; Eakin & Westfall, 1964). Accordingly, the eyes of these animals are regarded as rhabdomeric. We believe that an embryological study of the eye of *Hermissenda* would yield the same conclusion.

**Receptor cell vesicles.** The presence of 600–800 Å vesicles in the sensory cells of the eye of *H. crassicornis* is a highly characteristic feature of these retinal units. They are absent from the supporting, corneal, and other epithelial cells. Although they are found throughout the cells, except in the microvilli, they accumulate in large masses about the nuclei and at the axonal hillocks. Their number decreases markedly as one traces a cell into its neurite. Physiological investigations (Dennis, 1966) have revealed that inhibitory connexions exist between all of the sensory cells. The fact that the postsynaptic potentials are conducted passively into the soma suggests that the responsible synapses are situated either on the soma or in the axonal hillock. The time course of the synaptic potentials indicates that the mechanism of transmission is chemical. It is therefore possible that the vesicles contain synaptic transmitter, even though no conclusions as to the site of transmitter release may be drawn from the micrographs. On the other hand, the vesicles might contain photopigment or pre-
cursors thereof as postulated by Röhlich & Török (1962) for vesicles in the photoreceptor cells of the flatworm *Dendrocoelum lacteum*.

The origin of the 600–800 Å vesicles is not clear. Numerous pictures like that in Fig. 6 were obtained, which suggest that the vesicles are formed from the ends of the Golgi saccules. Often, however, the vesicles in the vicinity of these organelles, as in Fig. 6, are not identical with the spherules massed about the nucleus and in the axonal hillock, being less uniform in size and shape and having thick, distinctly trilaminar membranes which are sometimes coated externally. Maybe the vesicles change in physical features and perhaps also in chemical composition as they aggregate, acquiring thinner walls, spherical shape and a fairly uniform size (Fig. 5). Similar vesicles abound in the sensory cells in the eye of a pulmonate snail, *Helix aspersa*, and these also appear to arise in Golgi centres (Eakin & Brandenburger, 1967). In this form and in the European snail *Helix pomatia* (Schwalbach, Lickfield & Hahn, 1963; Röhlich & Török, 1963) the spherules are arranged in rows, giving masses of them a paracrystalline appearance.

**Origin of lens.** We believe that humour-forming materials are synthesized on the rough endoplasmic reticulum of corneal and supporting cells and released as secretory vesicles by the Golgi apparatus (Fig. 8). The vesicles then move to the inner ends of the cells (Fig. 7), fuse with the cell membranes, and discharge their contents into the opticoel. We surmise that the substances expelled into the lumen of the optic vesicle then disperse and become the humour which bathes the microvilli and forms the lens. Perhaps in the embryo the sensory cells are also active in the secretion of the humour. As yet the development of the eye of *Hermissenda* has not been examined. The evidence for this interpretation consists simply in the similarity of material in Golgi saccules and secretory vesicles in the corneal and supporting cells with the droplets among the villi and the substance of the lens (see Figs. 4, 7, 8 and 10). Further support for the above hypothesis is to be found in a similar picture in the developing eye of *Helix aspersa* (Eakin, 1965b; Eakin & Brandenburger, 1967).

**Symbionts.** The identity of the bodies found within the sensory, pigmented, and corneal cells of some, but not all, specimens of *H. crassicornis* is unclear. They appear to be true cells judging from the presence of nuclei with a double nuclear envelope and nucleoli and a ribosome-studded endoplasmic reticulum. It is noteworthy, however, that mitochondria are seemingly absent. Among the several possible interpretations of the nature of these cells we favour the hypothesis that they are intracellular symbionts. Several botanists whom we consulted have agreed that the symbiont could be a fungus.

**Functional significance.** Intracellular microelectrode recordings from the eye of *H. crassicornis* (Dennis, 1966) have revealed two distinct physiological classes of cells. Units of the first type show resting potentials, action potentials and slow receptor potentials upon illumination. Such recordings are presumed to be from sensory cells. Units of the second type have resting potentials, but show no electrical response of any kind to illumination. The latter recordings are almost certainly from pigmented cells, a conclusion which agrees with the suggestion made above that these cells serve...
only a supporting function, as judged by the abundance of tonofilaments which they contain and the absence of neurites.

The knowledge of the exact number of sensory cells is of value in determining the physiological influence of the units upon one another. Since there are so few, it may be possible to monitor the activity of all five cells by recording from three, for example, and observing the synaptic potentials in these resulting from action potentials in the other two.

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REFERENCES


Structure of nudibranch mollusc eye


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Fig. 2. Diagram of eye, optic ganglion (og), and statocyst (s) of *Hermissenda crassicornis* seen in longitudinal section. c, cornea; ls, lens; on, optic nerve; pc, pigmented (supporting) cell; sc, sensory cell with microvilli above and neurite below; tl, statolith; sm, statocyst nerve.

Fig. 3. Light micrograph of 1-μ thick section of eye and associated structures. ec, epithelial cells; og, optic ganglion; on, optic nerve; pc, pigmented (supporting) cells; sc, two of the five sensory cells; sy, symbionts. Lens not included in section. Stained with toluidine blue. × 730.

Fig. 4. Low-power electron micrograph of an eye showing an array of microvilli (mv) or rhabdomere from a sensory cell (sc). Fingers of a pigmented cell break up the sensory cell into columns. h, humour between microvilli. Fixed with glutaraldehyde/acrolein in phosphate buffer. × 8500.
Fig. 5. Part of sensory cell near its nucleus (n) showing masses of 600–800 Å vesicles (v) and glycogen granules (g) and a symbiont (sy). bl, basal lamina; cc, collagenous capsule; er, endoplasmic reticulum; m, mitochondrion; r, ribosomes; sn, nucleus of symbiont. Arrows indicate small tubules or vesicles resulting possibly from breakdown of cell membranes of sensory cell. Fixed with osmium tetroxide in cacodylate buffer. × 33000.

Fig. 6. Golgi apparatus (gd) and endoplasmic reticulum (er) of sensory cell showing possible origin of vesicles from Golgi saccules. Fixed with osmium tetroxide in cacodylate buffer. × 45000.
Fig. 7. Microvilli (mv) from a sensory cell (out of figure above) adjacent to pigmented cell (below). Short villi project from pigment cell (see arrows) and interdigitate with those of receptor cell. g, granules of glycogen; sd, secretory droplets, one released among villi (upper right); te, terminal web. Fixed with glutaraldehyde in cacodylate buffer. x 35000.

Fig. 8. Golgi centre of pigmented cell showing probable origin of secretory droplets (sd) of humour-producing material from Golgi saccules. Fixed with glutaraldehyde/acrolein in phosphate buffer. x 31000.

Fig. 9. A symbiont (sy) with nucleus (n), nucleolus, double nuclear envelope, and sparse endoplasmic reticulum within a pigmented cell (pc). Fixed in paraformaldehyde/glutaraldehyde in cacodylate buffer. x 18000.
Fig. 10. Cornea above lens (l); bl, basal lamina; cc, collagenous capsule; d, half desmosome; mv, microvilli; n, nucleus of corneal cell. Fixed with osmium tetroxide in cacodylate buffer. × 10000.

Fig. 11. Cross-sectional view of optic nerve near its exit from eye. The five axons composing the nerve are numbered (1-5). gc, glial or sheath cell; p, perineurium. Fixed with glutaraldehyde/acrolein in phosphate buffer. × 19500.