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# Photic Vesicles[1]

by

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*Abstract.* A summary is presented of various investigations on unique cytoplasmic organelles—termed photic vesicles—in the eyes of gastropod mollusks. Emphasis is given to the research of the author and his associates and to that to Tomiyuki Hara and his colleagues at Osaka University. Some unpublished findings of the Osaka workers are included. The following aspects of the vesicles are covered: structure, origin, fate, and function as transporters of the photopigment retinochrome and calcium. The data are synthesized into a scenario, with a heretofore unpublished diagram, on the cycling of retinochrome in which photic vesicles play a strategic role.

Photic vesicles are remarkable cytoplasmic organelles in the eyes of gastropod mollusks. They are unique in aggregating in very large numbers in the cell bodies of type I photosensory cells (Figures 1, 3), and in carrying a photopigment, hence the term "photic vesicles." They have been studied mostly in pulmonates (*e.g.,* snails and slugs—referenced below), but they occur also in opisthobranchs (*e.g.,* nudibranchs—EAKIN *et al.,* 1967), and in prosobranchs (*e.g.,* periwinkles—MAYES & HERMANS, 1973).

## Early History

The vesicles were first observed by two groups of European investigators studying the ultrastructure of the eyes of a snail, *Helix pomatia*: RÖHLICH & TÖRÖK (1963), who described how the vesicles—called *Elementarkörperchen*—form a crystalline mass (*Biokristall*) and SCHWALBACH *et al.* (1963) who regarded the vesicles as a foam (*Schaumstruktur*). The truly vesicular nature of the organelle was confirmed and further described in another snail, *Helix aspersa*, by Mrs. Jean Brandenburger, my research associate for many years, and me (EAKIN & BRANDENBURGER, 1967a).

Recently I learned in a personal communication from Professor Tomiyuki Hara of Osaka University that he and two colleagues, Y. Koshida and A. Tanaka, had studied the eyes of a land snail, *Euhadra callizona amaliae*, in 1963 and observed masses of vesicles in the cell bodies of the photosensory cells. Their findings on the vesicles, however, were not published. I have now received from him an unpublished manuscript with six plates of electron micrographs. It contains much information on the structural and biochemical features of a snail's eye that was regrettably not entered into the scientific literature. The authors had measured the vesicles (40–50 nm in diameter) and had shown the beautiful paracrystalline arrangement of them when compacted.

Dr. Jane Westfall and I had observed the vesicles in *Helix aspersa* in a survey of invertebrate photoreceptors that led to a 1962 symposium paper (EAKIN, 1963), but it was not until 1967 that a description of them was published (EAKIN & BRANDENBURGER, 1967a). In that year we suggested the possibility that the vesicles may carry a photopigment (EAKIN & BRANDENBURGER, 1967b). At the same time a similar function was postulated by HARA *et al.* (1967) in an excellent paper on photopigments in the retina of an octopus. In the discussion they analogized the gastropod vesicles with lamellated bodies in cephalopod photosensory cells shown by them to contain a new pigment termed retinochrome. We gave to the vesicular organelle the name photic vesicle (EAKIN & BRANDENBURGER, 1970).

## Structure

The vesicles are spherical when not compressed, and uniform in size (diameter 80–85 nm in *Helix aspersa*). When tightly massed in the perinuclear region of a photosensory cell as normally seen (Figure 1), or when compressed by centrifugation of an eye (REED & EAKIN, 1976), the vesicles become oval and packed into a paracrystalline array. Normally, large granules (35 nm or more) and a few mitochondria are dispersed among the vesicles. All other cytoplasmic structures appear to be shunted aside as the vesicles are produced and accumulated. The granules

[1] Dedicated to Dr. Rudolph Stohler, Founding Editor of *The Veliger*.

are considered beta particles of glycogen because of their size and digestibility by alpha amylase (EAKIN & BRANDENBURGER, 1967b). These features characterize only type I sensory cells of *H. aspersa*, not type II cells (BRANDENBURGER & EAKIN, 1974; BRANDENBURGER, 1975).

## Origin and Fate

We believe that photic vesicles arise in the cell bodies of type I sensory cells by abscission of the ends of the Golgi cisternae—a conclusion based upon electron micrographs (EAKIN & BRANDENBURGER, 1967a) and upon osmium staining (EAKIN & BRANDENBURGER, 1970 see below). KATAOKA (1975), however, ascribes their origin to agranular endoplasmic reticulum (ER) in the slug *Limax flavus* because vesicles were sometimes observed in continuity with the smooth ER and because the vesicles, ER, and the convex side (but not the concave side) of the Golgi apparatus were stained with osmium tetroxide (see symposium discussion in EAKIN & BRANDENBURGER, 1976). In our experience, osmium staining revealed fine granular deposits in the smooth ER, whereas the Golgi apparatus and photic vesicles were intensely blackened. This observation accords with the prevailing dogma on the secretory activity of these organelles. After formation, photic vesicles appear to accumulate in large masses, described above. Then the vesicles move distally within the sensory cells. The movement is probably caused by pulsations of the eye resulting from contractions of unique musculo-secretory cells in the optic capsule (EAKIN & BRANDENBURGER, 1972; MORTENSEN & EAKIN, 1974). The final fate of the vesicles appears from our electron micrographs (EAKIN & BRANDENBURGER, 1982) and from osmium staining (Figure 2, EAKIN & BRANDENBURGER, 1970) to be fusion with smooth ER lying beneath the photoreceptoral microvilli.

## Transporters of Photopigment

One of the functions of photic vesicles is transport of photopigment along the pathway just described from perinuclear ER to the microvilli (EAKIN, 1972). Experiments, now presented in approximate chronological order, attest to this function.

**Autoradiography:** Snails (*Helix aspersa*) injected pericardially with tritiated vitamin A exhibited sequential uptake by Golgi, photic vesicles, and microvilli (EAKIN & BRANDENBURGER, 1968; BRANDENBURGER & EAKIN, 1970). Although not definitively shown, it was assumed at that time that a snail's eye contains a photopigment whose chromophore is a vitamin A derivative. GILARY & WOLBARSHT (1967) had found by electrical recording that the eye of another snail, *Otala lactea*, exhibited a spectral sensitivity in close agreement with the absorption curve of rhodopsin. Moreover, the Osaka workers, cited earlier, had discovered that green light (525 nm) was absorbed by the microvilli of a land snail (KOSHIDA *et al.*, 1963). Ultimately, OZAKI *et al.* (1986) demonstrated that the vesicles contain the photopigment retinochrome (discussed below). In our papers on the autoradiography of the eye of *H. aspersa*, mentioned above, we postulated that the aggregations of vesicles store a photopigment.

**Osmium staining:** In contrast to osmium fixation, staining with osmium tetroxide involves impregnation of specimens with the metal by 1–3 days of immersion in unbuffered 2% aqueous solution of $OsO_4$ at 40° C. When this procedure was applied to the eyes of *Helix aspersa*, the Golgi cisternae and photic vesicles were intensely stained (EAKIN & BRANDENBURGER, 1970). The so-called osmium black is believed to result from $OsO_4$ reduction by an unsaturated compound such as a vitamin A-containing photopigment. The rod disks of an amphibian (*Xenopus laevis*) were similarly stained. The distal ER beneath the microvilli and the bases of villi in the eyes of *H. aspersa* also showed intense osmiophilia (Figure 2), but distally the villi exhibited only a dusting of osmium black.

**Light- and dark-adaptation:** The detectable effects of light and darkness on photic vesicles suggest that the vesicles are transporters of photopigment (EAKIN & BRANDENBURGER, 1974). Snails (*Helix aspersa*) kept in total darkness for one to four months exhibited a breakup of the aggregations of vesicles, a marked increase in the production of lysosomes that incorporated the vesicles, and a deposition of multilayered membranous capsules and partitions around and within the masses of vesicles. These features indicated cessation in formation of photic vesicles and a removal of those in storage—a picture not observed in normal (wild) or light-adapted (control) snails. Moreover, in the latter an accumulation of vesicles distally near the microvilli was discernible (EAKIN & BRANDENBURGER, 1967c). The microvilli—the other principal organelles involved in photoreception—also showed ultrastructural changes in response to the presence or absence of light.

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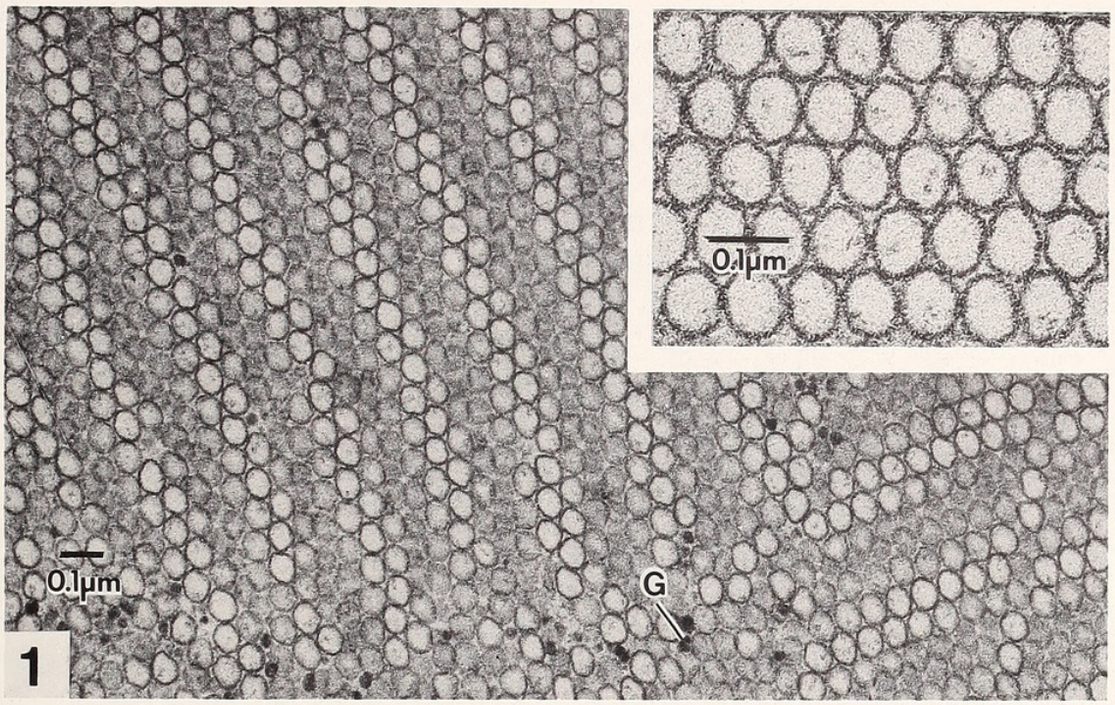
Explanation of Figures 1 and 2

Figure 1. Electron micrograph of photic vesicles massed near the nucleus of a type I photoreceptoral cell in an eye of the snail *Helix aspersa*. G, glycogen granules. ×50,000. *Inset*: Some photic vesicles at higher magnification. ×100,000. Figure 2. Osmium staining of distal part of a type I photosensory cell in an eye of *Helix aspersa*. Note dense deposits of osmium black in photic vesicles (PV), endoplasmic reticulum (ER), and bases of some microvilli (MV). ×20,000. From EAKIN & BRANDENBURGER, 1970, with permission of *The Journal of Ultrastructure Research*.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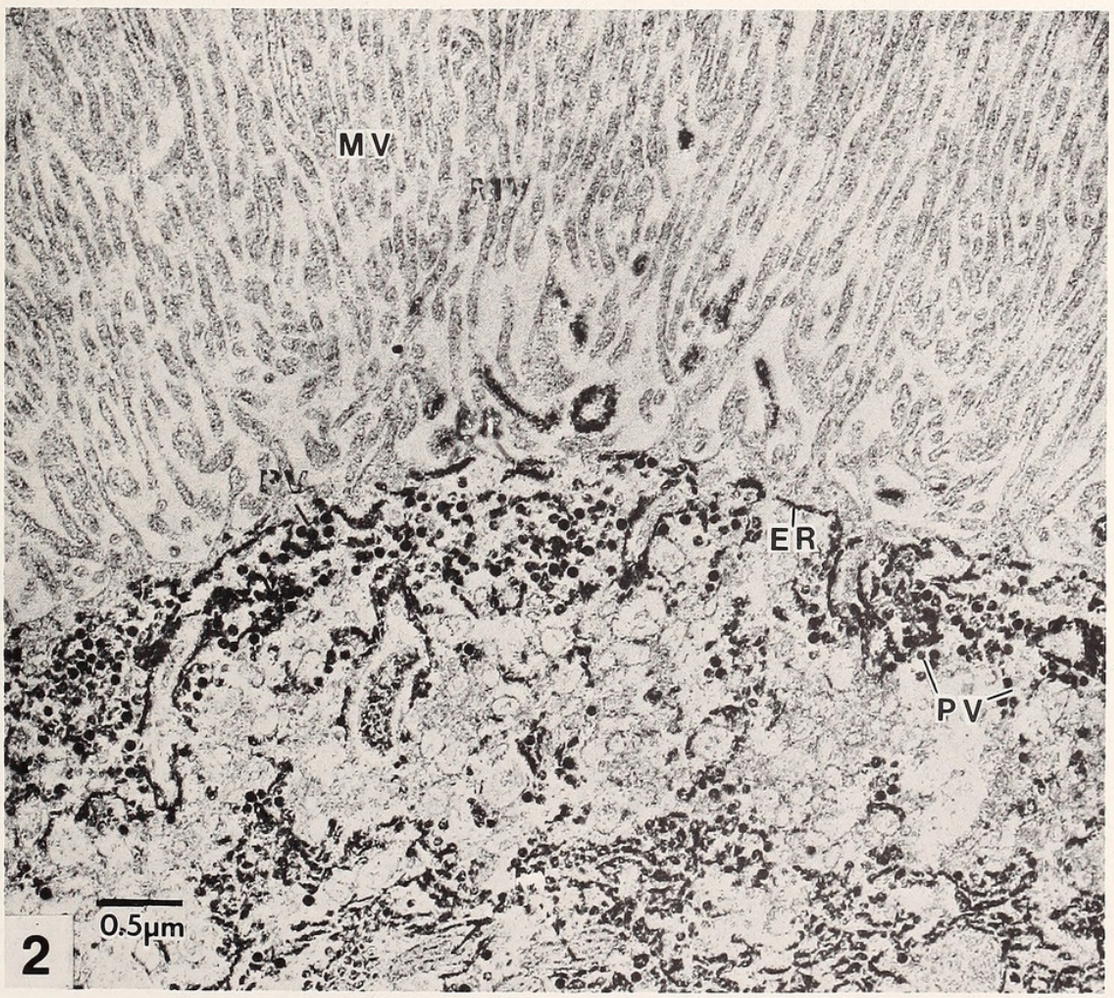
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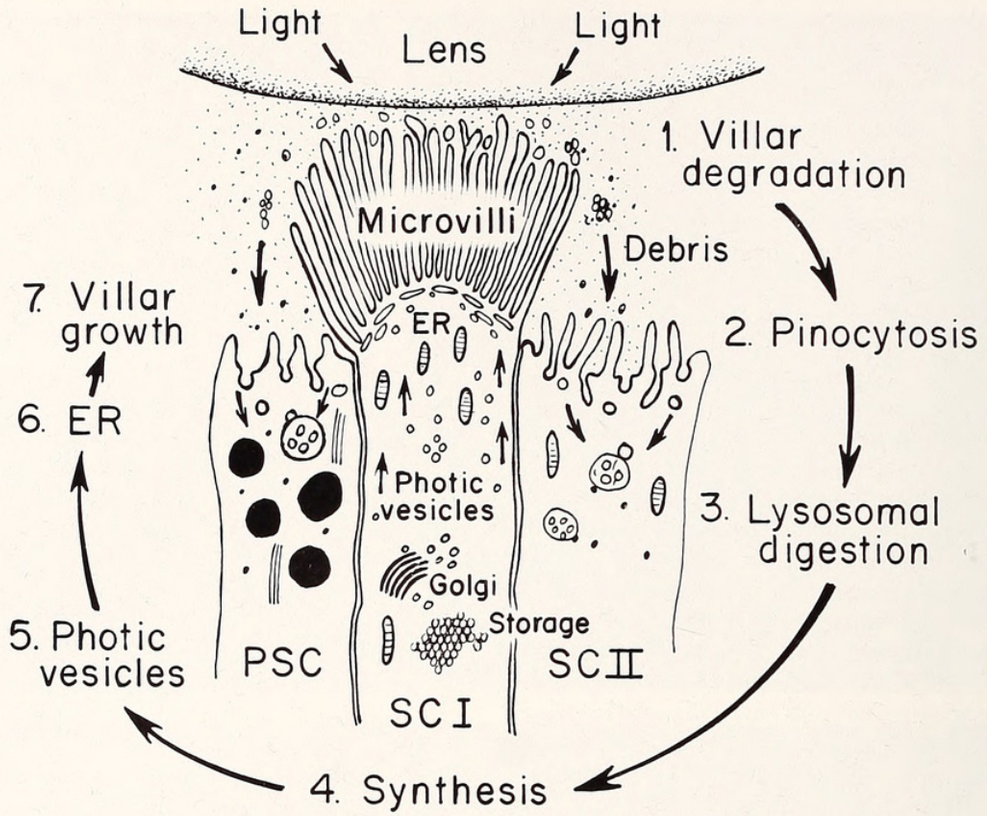


Figure 3

Diagram of recycling of photopigment in eye of *Helix aspersa*. See text for explanation.

In another study, of a light-tolerant slug (*Ariolimax californicus*) and a nocturnal one (*Limax maximus*), we found a difference in the distribution of photic vesicles (EAKIN & BRANDENBURGER, 1975a). In the nocturnal species the vesicles were primarily in the perinuclear regions of the sensory cells, whereas in the diurnal species many vesicles occurred in the distal halves of the photoreceptoral cells, as well as in masses in the cell bodies. All these observations support the hypothesis that light stimulates production, migration, and utilization of photic vesicles.

**Autofluorescence:** Knowing that vitamin A fluoresces (POPPER, 1944) and that the eyes of *Helix aspersa* contain this vitamin (EAKIN *et al.*, 1974), we examined sections of eyes of that snail by a fluorescence microscope to determine where the vitamin A was situated (EAKIN & BRANDENBURGER, 1978). The microvilli and the masses of photic vesicles immediately fluoresced, emitting a brilliant orange color that faded within 20–30 sec. Sections exposed to white light prior to examination under ultraviolet radiation did not fluoresce. As a control, sections of eyes of an amphibian (*Xenopus laevis*) were similarly treated and studied. The outer segments of the rods and cones fluoresced promptly, but the bright silvery color faded in 20–30 sec. Sections first exposed to white light did not fluoresce. Conclusion: the photic vesicles may possess a vitamin A-containing compound such as a photopigment. In 1986 OZAKI *et al.* conducted a fluorescence microscopy study of the eye of a marine conch, *Conomulex luhuanus*, and observed autofluorescence of both microvilli and photic vesicles. There was a difference, however, in the light emitted by the two types of organelles.

**Freeze-fracture:** Carbon-platinum replicas of the surfaces of fractured, frozen eyes of *Helix aspersa* were studied by electron microscopy (BRANDENBURGER *et al.*, 1976) to obtain information on size and amount of intramembranous particles of photic vesicles and of photosensory microvilli. In light-adapted specimens (versus dark-adapted ones), 70 Å particles on the internal or protoplasmic (P) leaflet of the microvillar membranes were numerous. The particles, in the size range of rhodopsin, were interpreted as photopigment. Most photic vesicles cross-fractured. The vesicular membranes were usually not separated into P and E (external) leaflets, probably because of the small size of the vesicles. In favorable instances, however, in which the vesicular membrane was split, we found very few particles attached to either P or E surfaces. From this investigation I draw the conclusion that the putative photopigment, now known to be retinochrome (see below), is not carried in the vesicular membrane but in solution or colloidal sus-

pension *within* the vesicles. This hypothesis agrees with our earlier demonstration that osmium staining blackened the contents of the vesicles (see Figure 2).

**Cell fractionation, biochemistry, microphotometry:** My associates and I succeeded in obtaining a cell fraction containing the photic vesicles in *Helix aspersa* (EAKIN *et al.*, 1974). We were unsuccessful, however, in obtaining publishable biochemical and microphotometric data about the contents of the vesicles.

OZAKI *et al.* (1984, 1986) proved the vesicular pigment to be retinochrome by cell fractionation, biochemistry, fluorescence microscopy, and microphotometry in a brilliant multifaceted investigation of the eyes of a marine gastropod, *Conomulex luhuanus*. Moreover, the authors determined that the photopigment in the microvilli is 11-*cis* rhodopsin. Additionally, they found a difference between the photic vesicles in the perinuclear masses and those more distally situated in the pigmented layer of the eye and perhaps also—my conjecture—in the tips of the photosensory cells. The distal vesicles contain only retinochrome, whereas the aggregated vesicles possess retinochrome *and* aporetinochrome. The investigators speculated that the distal vesicles "act as a direct supplier of retinal to the closely located microvilli, whereas the [perinuclear aggregation of vesicles] serves as a storage place for retinal in retinochrome and for newly formed aporetinochrome."

Similar results in the marine snail *Bulla gouldiana* were reported recently in an abstract of a poster by BOGART *et al.* (1989). Using the fluorescence technique of OZAKI *et al.* (1986) Bogart and her colleagues observed that the "distal segments" of the photoreceptors contained rhodopsin whereas retinochrome was found in the "soma layer" of the receptoral cells. Although photic vesicles were not mentioned, presumably masses of them are situated in the somatic regions of the photosensory cells of *Bulla,* as in other snails.

## Bearers of Calcium

Because calcium is an important catalyst in many physiologic processes including photoreception, we investigated the possibility that photic vesicles transport this element in addition to photopigment. Using a non-dispersive X-ray analyzer we showed that the nuclear layer of a *Helix aspersa* eye, wherein lie the masses of vesicles, contains a high concentration of calcium (EAKIN & BRANDENBURGER, 1975b). This finding does not, of course, prove that calcium is in the vesicles. Then we (EAKIN & BRANDENBURGER, 1980) fixed eyes of *H. aspersa* in glutaraldehyde and treated them with potassium pyroantimonate. Electron microscopy of unstained ultrathin sections of the eyes revealed a dense granule in the center of each photic vesicle. The granules were interpreted as precipitated calcium complexed with pyroantimonate. Credence to this conclusion was provided by the results of experiments in which sections of the same eyes were floated on a solution of the chelating agent EGTA before examining them in an electron microscope. The granules were absent! Conclusion: photic vesicles contain calcium.

## A Scenario

In Figure 3 (heretofore unpublished) I summarize the supposed major events in a gastropod eye in which photic vesicles play an important role.

**Villar degradation:** Light, after passing through the cornea and lens of a snail's eye, strikes the photoreceptoral microvilli. The photoresponse causes the breakdown of the microvillar membranes, especially at the tips (EAKIN & BRANDENBURGER, 1982).

**Pinocytosis:** The debris from the above event is taken up by pinocytosis and phagocytosis by retinal cells, especially type II sensory cells and pigmented supportive cells (EAKIN & BRANDENBURGER, 1982; BRANDENBURGER & EAKIN, 1983).

**Lysosomal digestion:** The internalized pinocytic and phagocytic vesicles fuse with primary lysosomes, which contain digestive enzymes (*e.g.*, acid phosphatase), to form secondary lysosomes (EAKIN & BRANDENBURGER, 1974; BRANDENBURGER, 1977; BRANDENBURGER & EAKIN, 1983).

**Synthesis:** The products of lysosomal digestion reach the synthetic centers—ER and Golgi apparatus—of type I sensory cells where the recycled molecules become incorporated into aporetinochrome and retinochrome and packaged into photic vesicles.

**Photic vesicles:** Photic vesicles released from the ER and Golgi cisternae (accelerated by light) are stored in large masses near the nuclei of type I sensory cells. The vesicles are moved distally, supposedly by pulsations of unique cells in the optic capsule that contain smooth muscle fibers (EAKIN & BRANDENBURGER, 1972). This process is also accelerated by light.

**Smooth endoplasmic reticulum:** Upon reaching the distal ends of the sensory cells, the photic vesicles fuse with smooth cisternae beneath the microvilli, releasing retinochrome and other vesicular contents (EAKIN & BRANDENBURGER, 1982).

**Villar growth:** I speculate that the microvilli are regenerated by basal addition of membrane constituents. Molecules of photopigment and perhaps other compounds become incorporated into the microvillar membranes, now ready for light reception again.

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ology, especially its artist, Phyllis Thompson Spowart, who prepared Figure 3. Integrative Biology is a successor of the Department of Zoology, my academic home for 60 years (1929–1989).

**Added in proof:** A. W. CLARK (1963, Jour. Cell. Biol. 19:14A) reported many "550 Å spheres" in retinular cells of a snail (*Viviparus maleatus*).

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