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STATOCONIA FORMATION IN MOLLUSCAN STATOCYSTS

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Abstract

The gravity sensors of all molluscs phylogenetically below the cephalopods are spherical organs called statocysts. The wall of the sphere contains mechanosensory cells whose sensory cilia project into the lumen of the cyst. The lumen is filled with fluid and dense "stones", the statoconia or statoliths, which sink under the influence of gravity to load, and stimulate, those receptor cells which are at the bottom. The statoconia of Aplysia californica are shown to be calcified about a lamellar arrangement of membranes. Similar lamellar membrane arrangements are seen within the receptor cells, and their possible role in the formation of the statoconia is discussed. SEM of unfixed statoconia reveals plate-like crystallization on their surface. Elemental analysis shows a relatively high Sr content, which is of interest, since others have recently reported that Sr is required in the culture medium of several laboratory-reared molluscs in order for the statoconia to develop.

Key Words: Statocyst, statoconia, otocyst, sensory receptor, gravity receptor, hair cell, cilia, Aplysia, calcification, strontium.

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Introduction

In all molluscs studied to date, gravity reception is mediated by bilateral paired statocysts. The general form of the statocysts is that of a fluid-filled sac with ciliated mechanoreceptor cells along its wall. The ciliated surface of the receptor cells faces the lumen of the cyst. The gravitational stimulus is transduced by the interaction of "stones" in the lumen with the cilia of the receptor cells. Since the stones have a greater specific gravity than that of the fluid, gravitational forces are normally exerted on the receptor-cell cilia. In some species, either a single stone or a concretion of many smaller stones exists, in which case the mass is referred to as a "statolith". In other species, many individual stones move independently under the influence of gravity, animal movement and beating of the sensory cilia; in these cases, the stones are referred to as "statoconia". In those statoliths made up of multiple adherent stones, the individual stones are also referred to as statoconia. Thus, the term "statoconia" is usually used to denote relatively small (1-50 µm diameter), independent paracrystalline elements. It will be the aim of this paper to review the literature suggesting different sites and mechanisms of generation of these stones, as well as the emerging body of experimental evidence pertinent to this problem. In order to put this material in perspective, the structure and function of various molluscan statocysts will first be reviewed.

Materials and Methods

Specimens of Aplysia californica were obtained from Pacific Biormarine Laboratories, Venice, CA. Animals were maintained in the laboratory in artificial sea water (Instant Ocean), which lacks Sr (Bidwell et al. 1986). Specimens were either 20 - 30 grams (those illustrated in Figures 2, 5, 6, 8 - 10) or 75 - 125 grams (Figures 3, 4 and 7). For light and transmission electron microscopy (TEM), statocysts were dissected free from live circumesophageal rings of ganglia and immersed directly into fixative. For the material for Figures 2, 5, 6 and 8, the specimens were fixed for 4 hours with 3% glutaraldehyde in 0.15M sodium cacodylate
buffer solution prepared with artificial sea water (Instant Ocean) (1,004 mOsm, pH 7.35). Specimens were post-fixed in 1% osmium tetroxide for 1 h, dehydrated in a graded series of ethanols and embedded in epon.

Thick sections (1 µm) were cut at 10 - 20 µm intervals through the cyst and stained with 0.5% toluidine blue in 1.0% sodium borate for examination by light microscopy. For TEM, 90 nm sections were cut and stained with uranyl acetate and Reynold's lead citrate. Thin sections were examined in a Philips 301 transmission electron microscope. Statoconia in Figure 8 were decalcified prior to dehydration by immersion in cacodylate buffer with the pH adjusted to 5.3 for 24 hours. For the thin section in Figure 7, fixation was in 2.5% glutaraldehyde in 250 mM of 2,4,6-trimethylpyridine (s-collidine) buffer (pH 7.4) for 12 - 24 h. No additional decalcification was performed. For SEM of whole statocysts (Figures 3 and 4), this same fixative was applied to an exposed statocyst still in the circumesophageal ring of ganglia and the whole ring was fixed for 12 - 24 h. The cyst was then bisected and most of the statoconia washed out when these blocks were returned to the fixative. These specimens were dehydrated through a graded series of ethanols, followed by ethanol-amyl acetate solutions, critical point dried using liquid carbon dioxide, mounted on copper studs and coated with a 25 nm thick layer of 60/40 gold/palladium. These specimens were examined in a JEOL JSM-U3 SEM at 15 kV. For the SEM of isolated statoconia (Figures 9 and 10) statoconia were isolated into deionized water which was blotted away, rinsed and blotted once more, on carbon planchets. The mounted specimens were coated with 60/40 gold/palladium at 20 nm thickness and examined in a JEOL JSM 35 SEM at 20 kV. For elemental analysis, similarly prepared but uncoated statoconia were examined in the same electron microscope using a Tracer Northern energy dispersive X-ray detector and a Tracer Northern NS880 X-ray analysis system.

Structure of Statocysts

The general plan of molluscan statocysts will be described using that of Aplysia californica as an example, because it is representative of the "simplest" form (Coggleshall, 1969; McKee and Wiederhold, 1974). The statocysts are paired, one being located between the pedal and pleural ganglia (parts of the circumesophageal ring of ganglia) on each side of the animal. Figure 1 is a schematic drawing of the organ. Each statocyst is a sphere, approximately 200 µm in diameter. The wall of the cyst is made up of a basal lamina surrounding thirteen large receptor cells. Each receptor cell has its own axon and these thirteen axons join to form the statocyst nerve, indicated schematically in the figure as proceeding to the right of the cyst. There are also a larger number of small supporting cells peripheral to and interspersed between the receptor cells, which are not indicated in this figure. The lumenal surface of the receptors is covered with many (approximately 700 in several examples counted) long cilia (approximately 12 µm long, as illustrated in Figure 2 below). The statoconia are indicated schematically as ellipsoids. They vary from approximately 3 to 20 µm in diameter. The number of statoconia varies from only one in larval Aplysia (Bidwell et al. 1986) to as many as 1,000 in an adult (McKee and Wiederhold, 1974). The cilia all have the typical morphology of motile cilia, possessing a central pair of microtubules as well as nine doublet microtubules around the periphery of the cilium. In fact, these cilia are motile, as well as sensory, as will be discussed below.

Figure 1. Schematic drawing of Aplysia californica statocyst. Note that supporting cells between receptor cells are not indicated. Modified from Gallin and Wiederhold, 1977.
Statoconia Formation in Molluscan Statocysts

Figure 2. Light micrograph of lower portion of an Aplysia statocyst. 1-µm thick, undecalcified section, stained with toluidine blue. Bar = 30 µm.

Figure 3. Scanning electron micrograph of a bisected, fixed statocyst with most statoconia washed out. Note ciliated surfaces of 6 sensory receptor cells. Most statoconia have been removed. Bar = 60 µm.

Figure 4. Scanning electron micrograph of a portion of a statocyst receptor cell showing three statoconia lying on the bed of cilia. Bar = 2 µm.

Figure 5. Transmission electron micrograph of a statocyst receptor cell (bottom left) abutting several supporting cells. Curved arrow indicates the basal body of one cilium. Cyst lumen in upper left-hand portion. Bar = 5 µm.

Figure 6. Transmission electron micrograph of two statocyst receptor cells with several supporting cell processes interposed between them. Note prominent lamellar bodies in both receptor cells. Bar = 5 µm.

to 15 µm thick and up to 100 µm on a side. From the size of the statoconia, relative to the spacing between cilia, illustrated in Figure 4, it is apparent that when a single statoconium strikes the cell surface, it will interact with several cilia nearly simultaneously.
In order to clearly distinguish cell types and their demarcation, it is necessary to employ transmission electron microscopy. Figure 5 is a TEM showing the border region between two receptor cells, with several small supporting-cell processes separating them at the luminal surface. Several characteristic intracellular organelles can be seen in this figure. Extensive smooth endoplasmic reticulum is seen in the receptor cell, approaching its border with the supporting cells. A lamellar body is seen within this receptor cell near its bottom right-hand edge. Lamellar bodies are frequently seen in this position, near the junction with the supporting cells. Multiple lamellar bodies are frequently seen. Figure 6 illustrates another junction between two receptor cells, again with several supporting-cell processes interposed. Here, six lamellar bodies are seen in the left-hand receptor cell and two in the right-hand cell. The large lamellar body in the left-hand cell is 6.3 µm in diameter. The basal body of one cilium, with a process pointing toward the supporting cell, is indicated with an arrow on the luminal surface of the receptor cell in Figure 5.

The general statocyst form described above has been found in several gastropod molluscs, including the opisthobranchs Aplysia californica (sea hare) (Coggeshall, 1969; McKee and Wiederhold, 1974), Aplysia limacina (sea butterfly) (Wolff, 1973), and Stone Timacina (Tsirulis, 1974), the nudibranchs Hermissenda crassicornis (Grossman et al. 1979) and Rostanga pulchra (Chia et al. 1981), the pulmonates Lymnaea (pond snail) (Zaitseva et al. 1980), Pomacea paludosa (Stahlschmidt and Wolff, 1972) and Pterotrachea (Barber, 1968; Barber and Dilly, 1969). In Pterotrachea, there are few receptor cells on the luminal surface of the cyst but receptors are concentrated around one central, large receptor, forming a sensory macula at the bottom of the cyst. This organ possesses a single spherical statolith which appears to be a single paracrystalline body rather than a concretion of statoconia.

Morton (1985) describes several types of statocysts in the bivalve molluscs. Most have many receptor cells along the cyst border and contain either a single statolith in each cyst (e.g., Thracia villosiuscula), or numerous statoconia along with a statolith (e.g., Mya dolton). In the cuspipariidae, a small number of receptor cells (possibly a reversion back to 13) is found, with one large statolith filling most of the cyst lumen.

By far the most evolved statocysts in the molluscs occur in the dibranchian cephalopods, including Loligo (squids), Sepia (cuttle-fish) and Octopus (Barber, 1968; Budelmann et al. 1973; Budelmann, 1978; Colmers et al. 1984). In octopus, there is a macula in each statocyst with approximately 5,000 receptor cells (here termed "hair cells" because of their closer analogy with those in vertebrates). A gelatinous substance covers the hair-cell cilia and connects the sensory cells to a large statolith, which is a concretion of statoconia (Colmers et al. 1984; Dilly, 1976). This macula serves as a gravity receptor. In addition, there are three cristae, or strips of hair cells, in each larva, numbered in nearly orthogonal planes which detect angular acceleration, analogous to the arrangement of the semicircular canals in vertebrates. In the decapods, including Sepia, there are four cristae and up to 11 or 12 antimaculae (Barber, 1968). Rather than each receptor cell having its own axon, in these cephalopods there are synapses between the hair cells and afferent axons, as well as efferent axons from the brain which innervate both hair cells and afferent nerve terminals within the statocyst (Colmers, 1977).
Thus, the cephalopods possess a very sophisticated acceleration-detecting system which is probably necessary to serve the exquisite repertoire of locomotion which these animals exhibit.

From this brief review, it can be seen that there is a great variety of gravity sensors within the molluscs. However, they all appear to function by using mechanosensory cilia to sense the gravitational force on dense calcified statoconia. The physiological responses of the receptor cells in the simpler statocysts of the gastropod molluscs will be reviewed below to clarify the function of these organs.

Function of Statocysts

Molluscan statocysts have provided useful preparations for the study of mechanoelectric transduction, or a tilting table. When the table is tilted from a position in which the recorded position is above the level of the statoconia to a position where the recorded cell's cilia are in contact with the stones, large depolarizations and generation of action potentials are observed. The action potentials carried in the statocyst nerve fibers travel to the cerebral ganglia which give the animal orientation information. Only those cells at the bottom of the cyst are activated, giving the central nervous system information concerning which direction is "down". The depolarization in response to this physiologic stimulus can be as large as 50 mV. By passing small current pulses through the receptor-cell membrane, through the recording electrode, it is also possible to measure changes in membrane conductance associated with the response. Although the electrical characteristics of the receptor-cell membrane are very non-linear, which can complicate the analysis (Wiederhold, 1977), it can be shown that the depolarization is due to an increase in membrane conductance caused by the mechanical stimulus. By changing the ionic composition of the artificial sea water bathing the preparation, it has been shown that the conductance increase caused by stimulation results primarily from an increase in conductance to sodium, presumably either on the ciliary membrane or the luminal surface membrane of the receptor cell (Gallin and Wiederhold, 1977). By eliminating the sodium in the bathing solution, responses to tilting are eliminated within 15 to 20 minutes, suggesting a relatively unrestrained exchange between the cyst lumen and the external medium. This is consistent with the finding that a microelectrode in the lumen records no standing potential and virtually no electrical resistance, relative to a reference electrode in the bath. Thus, ions and trace elements in the extracellular spaces of the animal will have access to the cyst lumen and the luminal surfaces of the receptor and supporting cells.

Another interesting feature of the physiological responses of statocyst receptor cells is the large increase in fluctuations in membrane potential associated with depolarizing response to tilt or rotation (Gallin and Wiederhold, 1977; Grossman et al. 1979). When an Aplysia statocyst preparation is viewed under a dissecting microscope, the statoconia can be seen to be in continual, random movement. This has also been noted in Clione limacina (Tsirulis, 1973), Helix Pecten maximum larvae (Cragg and Nott, 1977) and Hermisenda crassicornis (Grossman et al. 1979). Direct observation of active ciliary beating is reported in the statocysts of Helix (Laverack, 1968), Lymnaea stagnalis (Guezé, 1968) and Hermisenda (Stommel et al. 1980). When nickel chloride (10 mM) or serum from patients with cystic fibrosis (both of which can block ciliary motility--see Lindemann et al. 1980; Danes and Bearn, 1972), were added to the sea water bathing an Aplysia statocyst, the random motion of statoconia ceased. The motion is thus, in all likelihood, imparted by the active beating of the receptor-cell cilia. Application of nickel or cystic fibrosis serum also greatly reduces the voltage fluctuations and the magnitude of the depolarizing response to tilt. The fact that the depolarizing response of the receptors is greatly reduced by the same treatments that block active ciliary beating led Wiederhold (1976, 1978) to conclude that the actual transduction mechanism involves the cilia actively striking the statoconia during their ongoing beating, rather than the statoconia passively deflected by the cilia, as is thought to be the case in vertebrate hair cells.

From this synopsis, it can be seen that the physiology and biophysics of sensory transduction in molluscan statocysts is understood in reasonable detail. The manifestation of collisions between receptor-cell cilia and individual statoconia, in the prominent Voltage fluctuation associated with the responses, suggests that the physical and structural characteristics of the statoconia could appreciably affect the physiologic responses of these gravity receptors. These aspects will be treated in more detail in the next section.

Formation and Composition of Statoconia

Most molluscan statoconia described to date have forms similar to that illustrated in Figures...
1 - 3. The major exceptions are some of the bivalves, which have large "single-crystal" statoliths or statoliths which are concretions of statoconia (Morton, 1985), and the dibranichian cephalopods (squid, cuttlefish and octopus) in which the normal statolith has a complicated shape and is made up of a concretion of small spindle-shaped crystals, as is discussed below (Dilly, 1976; Colmers et al. 1984). Cragg and Nott (1977) review extensive literature dating from 1880 to 1959 on the statocysts of pediveliger bivalves, including 12 species among the palaeotaxodonta, pteriomorpha and heterodonta, with a single statolith in each statocyst and six species of pteriomorpha, including mussels and oysters, which have numerous statoconia, presumably similar to those of Aplysia described here. The finding that in the same species, Aplysia californica, there is only a single statolith in larval animals (although its structure is not illustrated) (Bidwell et al. 1986) and approximately 1,000 statoconia in adults (McKee and Wiederhold, 1974) indicates that stones are added to the cyst lumen during development. The statocysts are one of the first components of the Aplysia nervous system to develop. Fully formed statocysts are present in larval animals when they hatch, ten days after fertilization. At this stage the only other components of the nervous system which can be identified are the cerebral and pedal ganglia (Kriegstein, 1977a and b). Bidwell et al (1986) note the presence of a statolith in normal 5-day embryos. Coggeshall (1969) emphasizes that the diameter of the statocyst varies little between the smallest (1 g) and the largest animals studied (300 g) and that the number of receptor cells (13) is constant. It is not known when in the development of these animals the number of statoconia increases or whether this is an ongoing process throughout their lives.

There are several suggestions in the literature concerning the site of generation of statoconia. Laverack (1968) was one of the first to apply electron microscopic techniques to the investigation of molluscan statoconia. He formed the large ciliated cells, of which there are 13 also in Helix, "giant" cells, but concluded that these could not be receptor cells, largely because all sensory receptors described to that time were small columnar cells. Thus, Laverack concluded that the supporting cells were the receptors. However, he does illustrate "fully formed" statoconia, approximately 7 µm along their major axis, within a giant cell. He states that "the bulk of the calcareous material leads to herniation of the capsule...when the cell contains a number of statoliths they are released into the lumen of the statocyst, by disruption of the cell". Laverack also notes striking lamellar bodies, similar to those illustrated in Figure 6, in the giant (receptor) cells, near intracellular statoconia, but concludes that the lamellar bodies do not seem to be related structurally to the statoconia. He states that in regions of the giant cell where lamellar bodies are numerous, the cell frequently becomes detached from the capsule and "disintegrates into the lumen of the statocon". We have occasionally seen such disruption with impressive amounts of regular, but more loosely arranged membranes than those illustrated in Figure 6, within the lumen. However, we could not be assured that this was not a form of degeneration, perhaps due to trauma associated with either dissection or preparation of the specimen.

In contrast to Laverack's suggestion that the statoconia are generated within what we now believe to be the sensory receptor cells, several investigators have illustrated small statoconia in small invaginations of the luminal surface of supporting cells between receptor cells, near the region where the nerve leaves the cyst. Tsirulis (1974) illustrates five statoconia, up to 12.5 µm along their long axis, within one supporting cell and another, 4 µm long, apparently emerging from a supporting cell in Clione limacina. Geuze (1968) presents evidence that in both normal and regenerating statocysts of Lymnaea stagnalis, the statoconia are produced by supporting cells. After puncturing the statocyst and removing both the statolymph and statoconia, the statocysts completely returned to normal appearance in 48 h. At 12 h after puncture, statoconia of low electron density were seen within vacuoles at the apical surface of poorly differentiated cells. In "subadult" normal animals, examples are shown of statoconia in broken vacuoles, in the cyst lumen, adjacent to the apical surface of supporting cells. Kuzirian et al (1981) also demonstrate what is described as a forming statoconium within a supporting cell in Hermissenda crassicornis. However, it is difficult to distinguish this from a portion of a lamellar body. The example illustrated is near the junction between supporting and receptor cells, where processes of the two frequently interdigitate, and it is difficult to distinguish one cell type from the other. Cragg and Nott (1977) describe one cell in each statocyst of the pediveliger Pecten maximus which is comparable in size to the ciliated receptor cells, but contains no cilia itself. This cell is said to contain inclusions resembling the variety of statoconia they describe in the cyst lumen and they suggest that this cell generates these stones and expels them into the cyst lumen. No one else has described such a cell. To date, we have not identified indisputable statoconia within either receptor or supporting cells in Aplysia californica. Of course, all of these studies suggesting that the statoconia are generated within either the receptor or supporting cells are based on static anatomical methods and the possibility that the stones penetrated into the cells from the lumen, possibly as a post-mortem artifact, cannot be excluded. In fetal rat, Salamat et al. (1980) demonstrate that developing otoconia are released from vesicles on the surface of cells in the sensory epithelium of the saccule.

It is clear that the mineralization of molluscan statoconia is laid down on a biological membrane structure. Vinnikov et al. (1980) generalize that the "otoliths" of the "lower" invertebrates, the coelenterates and ctenophores are formed by an "endogenic" intracellular mode,
while those of the molluscs and arthropods are formed by an endogenous deposition upon an extracellular structure. Figures 7 and 8 illustrate TEM's of statoconia within the lumen of Aplysia californica statocysts prepared and partially decalcified by different methods. Similar figures have been shown by Coggeshall (1969) for Aplysia and Kuzirian et al. (1981) for Hermissenda crassicornis. Concentric membranous rings can be seen in most of the stones in Figure 7 and in the left-hand stone of Figure 8, but not in the other statoconium in Figure 8. The lack of a visible ring structure is not uncommon. Presumably the whole stone is made up of concentric ellipsoids and if such a structure were cut near an end, the ring structure could be missed, as in cutting a section from the side of an onion rather than cutting across its center. It would seem likely that the statoconia grow after their initial formation by adding successive layers. If this is the case, the lamellar bodies (Figures 5 and 6) could provide the membrane for such deposition. As noted above, Laverack (1968) described "disintegration" of the receptor cells, in the region of numerous lamellar bodies, into the cyst lumen, a phenomenon which we have observed in aplysia statocyst cells of uncertain condition. Williams (1977) describes a process by which lamellar bodies, which appear similar to those in Figure 6, in the alveoli of fetal rat lung, exocytose at the cell surface to produce the tubular myelin which is thought to serve as a store of phospholipids for pulmonary surfactant. Thus, there is a precedent for similar structures being exocytosed to provide extracellular membrane. In both Figures 7 and 8, the outer membrane layer appears more irregular than do the inner rings. Perhaps as the outer layer calcifies, the membrane is packed into a more confined space. Vinnikov et al. (1980) illustrate exceptionally thick and electron-dense outer membranes in thin sections of statoconia from Acmaea pallida (limpet).
All of the statoconia described above have gone through one form or another of fixation, which could alter the elemental composition and surface structure. To minimize such changes, we have examined statoconia from one preparation in which the statocysts were dissected free from a live specimen (Aplysia californica) and bisected in a drop (at least 1,000 times the volume of the statocyst) of deionized water, directly on a carbon planchet. The water was blotted away and another drop placed and blotted to rinse the stones a second time. SEM's of several stones prepared in this manner are shown in Figures 9 and 10. It is clear that the surface of these stones is more planar and angular than those seen in Figures 3 and 4 and those previously illustrated in the literature (e.g., Kuzirian et al. 1981). The smaller stones in Figure 9 (1.5 to 4.5 µm along their long axis) appear to have much sharper corners than the fixed stones. These and the large stone (14 µm long) in Figure 10 all show plate-like irregularities on their surface which appear to have cleaved at a preferred angle, suggesting a single-crystal formation.

To investigate the elemental composition of the statoconia, uncoated preparations similar to those shown in Figures 9 and 10 were studied by energy-dispersive X-ray microprobe analysis. Figure 11 is an X-ray energy spectrum obtained from a whole statoconium. Note the prominent calcium K-alpha peak, which would be expected for calcium carbonate. There is also a significant strontium peak. The Sr peak, as well as those corresponding to several other elements can be better resolved in Figure 12, a replotting of the data from Figure 11 on an eight-fold more sensitive scale. Small but significant amounts of sodium, magnesium, strontium, sulphur and chlorine are present. The aluminum peak is an artifact from the X-ray detector housing. Note that no phosphorus peak is seen, indicating that there is probably no hydroxyapatite in the statoconia. In an attempt to quantitate the relative molar amounts of Ca and Sr present in the statoconia, a deconvolution algorithm was used to determine the contributions of these two elements to the spectra. To calibrate these measurements, standards of pure calcium fluoride and pure strontium fluoride were analyzed. The average Ca/Sr molar ratio derived from six statoconia was 97:1 +/- 3 (Mean +/- S.E.M.). The average number of counts for the Ca K-alpha peak was 90,900 and that for Sr L peak was 1,300 (the molar ratio was also derived from these counts, corrected for the relative emission efficiencies of pure CaF2 and SrF2 crystal standards). The Ca/Sr ratio is very similar to that obtained by Bidwell et al. (1986) in the single statolith of larval Aplysia californica and is close to the molar ratio of sea water (103:1) given by Nicol (1967).

The presence of strontium in the statoconia is of interest since Bidwell et al. (1986) have recently shown that the Sr content in the medium in which embryos are reared has a profound effect on the development of both the shell and the statoliths in a number of molluscan species. Normal sea water has 8 parts per million (ppm) of Sr. If fertilized Aplysia californica eggs are raised in artificial sea water with no Sr, all larval animals fail to develop any statoliths. Statoliths were also absent in all animals reared in 2 ppm Sr, but at 3 ppm Sr some animals had no statoliths, while others had either one or both statoliths present. Above 4-ppm-Sr, all animals had both statoliths. Embryos reared in Sr-free sea water during their 10-day developmental period except for a 24-h pulse of 8-ppm Sr sea water on day 4 all developed both statoliths. Pulses of Sr either before or after day 4 were not effective. The necessity for Sr seems to be specific, since basal medium with Mn, Li, Rb, Al and K salts added would not correct the deficiencies noted in the absence of Sr. A similar requirement for Sr in order to develop normal statoconia was noted in Hermisenda crassicornis and the bivalve Bankia Gould (shipworm) (Bidwell et al. 1986). Roger Hanlon at the Marine Biomedical Institute in Galveston has found similar
results in cephalopods (personal communication). We had previously studied the statoliths of individuals of several species of hatchling cephalopods which exhibited a behavioral anomaly, in that they followed a "corkscrew" pattern while swimming or "somersaulted," i.e., were generally unable to swim in an oriented manner (Colmers et al. 1984). Such individuals were termed "spinners." The only structural abnormalities in these animals were in their statoliths. Hanlon has now found that in two species of octopus (large- and small-egged species), Sepia and two species of squid, if there is no Sr in the sea water in which the embryos are raised, all animals are spinners. If the Sr level is raised to 4 ppm, approximately one half of the individuals are spinners and when the Sr is raised to the normal 8 ppm, all animal exhibit normal behavior. This requirement for Sr to obtain normal development of molluscan statocysts would appear to be analogous to the requirement for manganese for normal otocystal development in mammals (Erway et al. 1970; Lim and Erway, 1974).

The molecular basis for the Sr requirement for otocystal development is unknown. Carlstrom (1963) states that strontium is known to favor the formation of the aragonite form of calcium carbonate. However, aragonite can be formed in the absence of Sr. Whereas the otocyst of all mammals, aves and some reptilia are all made of calcite, many fish and some amphibia have their otocysta made up of aragonite (Carlstrom, 1963; Ross and Pote, 1984; Mann et al. 1983). The lizard Podarcis s. sicula has calcite otocysts in the lagena, aragonite otocysta in the endolymphatic duct and sac and a mixture of the two in the saccule (Marmo et al. 1981). Lowenstam et al. (1984) have recently shown that the otocyst of tetrabranchian cephalopods (Nautilus) as well as the statoliths of all of the dibranchian cephalopods (cuttlefish, squid and octopus) are all aragonite. The otocysts of the nautilus are about 1% by weight Sr, whereas the statoliths of the other cephalopods are about 0.5% Sr by weight. The shell and other hard parts of nautilus are also made up of aragonite, but their Sr concentration is less than that of the statoliths. Richards et al. (1965) have shown that the Ca/Sr ratio is 4.5 times greater in the shell of Nautilus, compared to that of sea water. Thus, Sr is more effectively excluded from the shell than from the statocysts, even though both are made of aragonite. Aragonite is felt to be advantageous for Nautilus, in that it is less brittle than calcite, and thus makes the shell and its chambers better able to withstand the large hydrostatic pressures at the depths of 500 to 900 meters where these animals frequently live. The crystal structure of the Aplysia statocysta is not known, but the plate structure, particularly evident in Figure 10, suggests that they too are made of aragonite. This type of formation is typical of aragonite but not of calcite in the lizard (compare Figures 1,2 and 3 of Marmo et al. 1981). However, Ross and Peacock (1975) do illustrate plate-like features on rat otocysta, which are Sr++, due to its ionic radius, will fit into the aragonite form of calcium carbonate, but not readily into the calcite crystal. Thus, Sr can substitute freely for Ca in aragonite; that is, the Ca/Sr molar ratio will be the same as that of the medium in which the aragonite crystals are formed. In the case of calcite, Sr will only partition into the crystal at 0.2 times its relative concentration in the medium (Kinsman, 1969). The fact that Sr appears to partition into the statocysta in the same molar ratio to Ca as is found in sea water, is, thus, further indication that they are made of calcium carbonate in the aragonite form.

The advantage of having statocysts made of aragonite rather than calcite is not obvious. Perhaps in species with independent statocysts there is enough friction as the stones tumble over one another that the physical properties of aragonite would make them less susceptible to "wear" than if they were made of calcite. In mammals and other species in which the otocysta are embedded in a gelatinous membrane, the stones are prevented from striking one another. In some spinner cephalopods which have abnormal statocysts, the crystal structure is grossly different from that of normal animals. Whereas in normal octopus, the statoliths are a concretion of spindle-shaped crystals 2 to 3 µm long (Dilly, 1976; Colmers et al. 1984), the case of a spinner Octopus joubini illustrated in Colmers et al. (1984), is a large mass of tightly packed prismatic crystals. Thus, changes in crystal structure of the developing statoliths in the low-Sr sea water probably contributed to the spinner trait. In those specimens which failed to produce any statoliths, the lack of Sr could have been sufficient to prevent the precipitation of any stable form of calcium carbonate.

Conclusions

In light of the profound influence of the trace element strontium on molluscan statocysta formation and of manganese on the formation of mammalian otocysts, it will be of interest to see if future research reveals similar mechanisms responsible for the abnormal crystal structure and shapes of aberrant otocysts seen in normal (Johnsson et al. 1980) or abnormal (Ross and Peacock, 1975) laboratory animals and in cases of human otopathology (Johnsson et al. 1982). If the membranous framework upon which the molluscan statocysts are built is generated within the sensory receptor cells, this opens the possibility for an interaction between the physiology and activity of the sensory structures and the formation and growth of the statocysts. This would imply that the maintenance of the appropriate number and form of statocysts is a dynamic, rather than passive, process.

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References


Discussion with Reviewers

D.B. Spangenberg: How do nickel or serum from patients with cystic fibrosis cause cessation of ciliary motility? Could they affect nerve cells or transduction in receptor cells?

Authors: The mechanism of ciliary stasis caused by these agents is not understood, but they do not have direct effects on the passive or active properties of the receptor-cell membrane. Resting membrane potential, input resistance, action-potential threshold and amplitude remain unchanged in the presence of either Ni ions or CF serum (see Wiederhold, 1976, 1978). Thus, it is thought that the only effect on transduction is through blocking the active beating mechanism.

D.B. Spangenberg: Do receptor cells have ciliary rootlets?

Authors: The ciliary basal bodies appear continuous with rootlets which radiate out in all directions from each cilium. Each outer microtubule doublet appears to give rise to its own rootlet, which spreads out to become nearly parallel to the surface membrane (see McKee and Wiederhold, 1974).

D.B. Spangenberg: Could low levels of strontium affect transduction in receptor cells?

Authors: The Instant Ocean artificial sea water, in which the animals were maintained, and in which the preparation was bathed for some of the physiological experiments, contains no Sr, thus this element is not required for normal function. We have not tested the effects of adding Sr to this medium.

D.B. Spangenberg: The lamellar bodies in receptor cells illustrated in Figure 6 resemble whorled bodies found in hair cells of jellyfish and in other organisms. Is it possible to distinguish the lamellar bodies in the Aplysia from the whorled bodies of other organisms?

Authors: The whorled bodies demonstrated by Hündgen and Biela (J Ultrastruct Res. 80:178-184, 1982) do appear to be similar to those we see in the Aplysia statocyst, although the membrane packing is tighter in Aurelia (20-30 nm spacing) than in Aplysia (60 nm spacing in the example of Figure 6). Similar structures have also been seen in purported photoreceptors in a ctenophore (Horridge GA. Quart J Micro Sci. 105:311-317, 1964) and a mollusc (Wiederhold ML, MacNichol EF, Bell AL. J Gen Physiol. 61:24-55, 1973), so their function is not well established, and probably not unique.
V.C. Barber: Were the specimens male or female? This is relevant because of my recent article showing gender differences in inner ears (Cell Tiss Res. 241:597-605, 1985).
Authors: Aplysia are hermaphrodites.

L.C. Erway: Are there any data for the number of cilia on the sensory cells of the molluscan statocysts? Are the basal bodies interconnected in any way?
Authors: In Aplysia californica, each receptor cell has approximately 700 cilia. Their basal bodies appear to radiate in all directions, without interconnection. See McKee and Wiederhold, 1974.

L.C. Erway: Given the evidence for effects of Na, Ni, and CF factor on ciliary movement of statocoria, is there any other biophysical evidence, analogous cases, or postulated basis for the transducing effect of statocoria on the cilia or sensory cells?
Authors: Grossman et al. (1979) and Stommel et al. (1980) have also concluded that ciliary motility is involved in the transduction process in the statocyst of Hermisenda crassicornis. In Wiederhold, 1976, evidence is reviewed, showing that membrane potential does not change in synchrony with active beating in many ciliated cells in their normal extracellular fluid environment. It is argued that a mechanical stimulus, or the presence of an inertial load such as a statocormum, could disrupt the "molecular program" associated with ciliary beating, stress the membrane and thus lead to conductance and Voltage changes.

L.C. Erway: Is there any evidence for the continuous motility of cilia in the statocyst, or of changes in ciliary patterns, with and without contact between the statocoria and cilia?
Authors: Grossman et al. (1979) have measured ciliary beat frequency in Hermisenda and report that the beat frequency is decreased when they are loaded by the statocorial mass.

L.C. Erway (Comment): Given the evidence for effects of Sr on formation of statocoria, aragonite is "...formed at ordinary temperatures through the action of organic agencies or by precipitation from saline water containing sulfates or small amounts of carbonates of strontium or lead (Kraus, Hunt and Ramsdell, Mineralogy, 1951)". This information, together with the commercial use of Sr(OH) in crystallization of beet sugar, may suggest the importance of Sr in nucleation sites for aragonite-containing statocoria. Although Al, K, Li, Mn, and Rb were ineffective in replacing Sr, one might test for effectiveness of sulfates, and of Ba, Pb, and Zn. Mn appears to be essential for biosynthesis of organic matrix for mammalian otocoria. The studies in molluscs may have implications for formation of gigantic crystals in saccule of mutant mice (see Erway et al., 1986).

J. Ballarino: The surfaces of calcium carbonate crystals are susceptible to dissolution in deionized water. Although no noticeable dissolution in deionized water can be detected in the larger otocoria of juvenile and adult terrestrial vertebrates, the small otocorial crystals of developing embryos are etched by deionized water. What evidence do you have that your material was not subject to dissolution by deionized water?
Authors: We attempted to minimize such effects by dissecting the live statocysts in a drop of deionized water, immediately blotting it away with a small wedge of filter paper, and then repeating this procedure to remove the salts from the sea water. The statocoria were not in deionized water for more than 30 to 60 s. As you have shown (Am J Anat. 174:131-144, 1985), glutaraldehyde can also greatly alter the surface appearance of otocoria. This is presumably why the statoconia in Figures 3 and 4 (which were fixed in glutaraldehyde) appear to have smooth surfaces, whereas those in Figures 9 and 10, which were unfixed, have distinct sharp edges where the facets meet.

Additional Reference