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OTOCONIA AS TEST MASSES IN BIOLOGICAL ACCELEROMETERS: WHAT CAN WE
LEARN ABOUT THEIR FORMATION FROM EVOLUTIONARY STUDIES AND FROM WORK
IN MICROGRAVITY?

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Abstract

This paper reviews previous findings and introduces new material about otolith end organs that help us to understand their functioning and development. In particular, we consider the end organs as biological accelerometers. The otoconia are dealt with as test masses whose substructure and evolutionary trend toward calcite may prove significant in understanding formation requirements. Space-flight helps illuminate the influence of gravity, while right-left asymmetry is suggested by study of certain rat strains.

Introduction

This paper considers otolith end organs, particularly in mammals, as biological accelerometers. We shall focus on otoconia, the test (seismic) masses of the organs. Our emphasis, both in review of previous work and in sections dealing with new findings, will be on contributions made by scanning electron microscopy. Some results obtained by application of other techniques are included for completeness.

Review

Macular otolith end organs: Otolith end organs are found in the saccule and utricle in Eutherian mammals. They consist of a patch of sensory neuroepithelium, a macula, and a suprastructure that includes one large (otolith) or several dust-like (otoconia) mineralized particles. Other vertebrates, except for lamprey, have an additional, lagenar macula. Lampreys have a "macula communis" but parts of it correspond functionally to maculas of the saccule, utricle and lagena according to Lowenstein (Lowenstein et al., 1968). Another macula, macula neglecta, located in the utricle, is not considered here. It has been described in many vertebrates but is non-otolithic and may function in addition in some species (see Corwin, 1981, for discussion). Mammalian otolith organs detect gravitational and translational linear acceleration; in some other vertebrates one or another macula may also have an auditory function.

Otolith end organ organization is fundamentally similar across vertebrates. The macular neuroepithelium consists of sensory cells interspersed among supporting cells. The sensory cells are hair cells, so-named because they have elaborate surface modifications called stereocilia, and a single kinocilium. The stereocilia are arranged in a tuft of several rows of graded height. The kinocilium is at the center of one edge of the tuft, where the stereocilia are longest. The arrangement is most beautifully shown by scanning electron microscopy (Figure 1). Birds and mammals have two kinds of hair cells, type I and type II (Wersäll, 1956), but most other vertebrates have only type II cells.

Above the sensory neuroepithelium there is a gelatinous-appearing substance which, in mammals, supports (or contains) a great number of small, crystalline otoconia. The membrane is the otoconial (otolithic) membrane. The otoconia of the membrane

KEY WORDS: otoconia, otoconial membrane, macula, accelerometer, evolution, development, piezoelectricity, mosaic biomineral, microgravity, right-left asymmetry.

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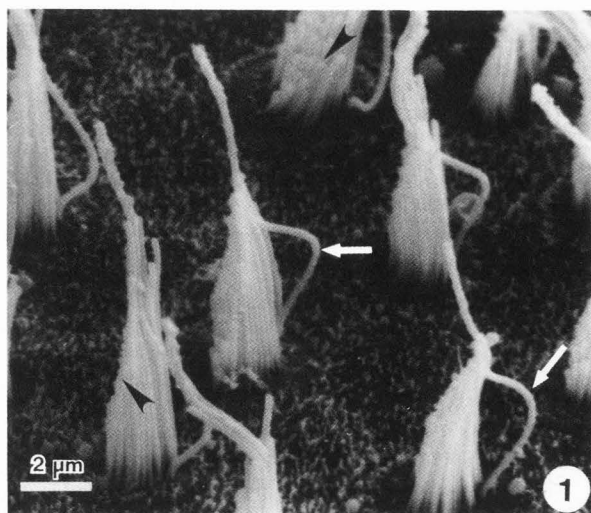


Figure 1. This scanning electron micrograph illustrates the configuration of kinocilia (arrows, center and lower right) in fixed material, and the stepped heights of the stereocilia (arrowheads). Rat saccular macula.

are highly ordered mosaics of organic and inorganic substances (Ross and Peacor, 1975) that mimic the appearance of single crystals (Mann et al., 1983). In mammals, the otoconia contain calcite as the mineral phase. This content is reflected in the outward morphology of the otoconia, which exhibit the three-fold symmetry of natural calcite (Figure 2).

Otoconia are attached to one another and to the underlying otoconial membrane by organic material resembling that of the membrane (Figure 2). It is still unclear whether the organic material between the otoconia is continuous into the otoconia themselves, or merely attaches to their surfaces. Nevertheless, the two can be considered together as a functional unit, an otoconial complex. This complex, in turn, is coupled to the hair cells of the macula, but not to the supporting cells (Ross et al., 1986). Finger-like processes extend from the underside of the otoconial membrane to attach to the kinocilia and to the longest stereocilia of the hair cells. Evidence for this comes in part from scanning electron microscopy but is shown more convincingly by transmission electron microscopy. A second substance fills the space between the macular surface and the otoconial membrane. We have referred to this material as supramacular substance (Ross et al., 1986). It is characterized by the presence of loosely organized macromolecules. These macromolecules are continuous with material of the otoconial membrane, and even extend through the membrane in places to surround the otoconia. Usually, supramacular substance is not observed in scanning electron micrographs. It has a broad distribution and may, therefore, simply prove to be endolymph, or a modification of it.

Mammalian saccular and utricular macular end organs as linear accelerometers: Man-made accelerometers utilize a seismic mass (vibration and shock sensitive) that is spring-loaded to a case. A damping medium improves sensitivity to signal over noise. A transducing element (frequently a piezoelectric material)

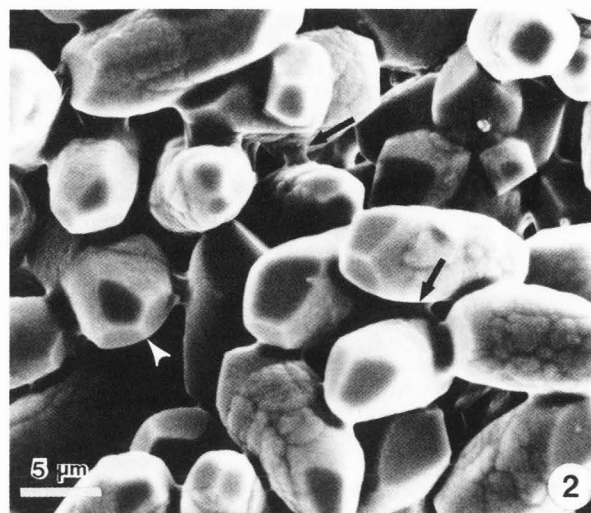


Figure 2. Mammalian otoconia have the three-fold symmetry of calcite crystals (arrowhead, center left). Organic material extends between the otoconia, linking them together (arrows). Rat saccular otoconia.

produces an electrical output that is proportional to the acceleration-induced motion of the base of the case. In some of the most sensitive accelerometers, piezoelectric elements are multiple and used in parallel.

It is intriguing that macular end organs are organized along these same principles. That is, the end organs consist of vibration-sensitive (seismic) masses (otoconia, Figure 2) that are spring-loaded (through strands of the otoconial membrane) to the hair cells (detectors) beneath. The otoconia are also connected to one another by similar, spring-like material (Figure 2). A second, possibly softer spring (supramacular substance), lies between the otoconial membrane and the surface of the macular neuroepithelium. This "spring" could serve as a damping agent to isolate the system from spurious stimulation (noise). The basic concept of spring-loaded seismic masses as the macular suprastructure was previously discussed (Ross, 1983), but the fact that the otoconial membrane is attached to kinocilia and the longest stereocilia of the hair cells, across the entire macula, is a recent discovery (Ross et al., 1986).

A difference in motion between the seismic mass (otoconial complex) and the underlying base (sensory neuroepithelium), from either gravitational or translational linear acceleratory force, is thought to stimulate the hair cells. The hair cells detect and then transmit direction and rate of acceleration to the nerves leading to the central nervous system. However, the exact mechanisms involved in detection of the stimulus, and the site of initial transduction of mechanical into electrical energy, cannot be said to be known with certainty. In part, this is because otoliths have been shown to be piezoelectric (Morris and Kittleman, 1958), and otoconia may also have this property (Ross and Pote, 1984). If the seismic masses of macular end organs are piezoelectric, then transduction occurs initially at the level of the otoconial complex. The otoconia would not have to move, only be stressed, to produce an electrical signal. If otoconia

are not piezoelectric, then a mechanical force is transmitted to the detector hair cells where transduction to an electrochemical signal takes place. The matter of piezoelectricity is, therefore, crucial to resolve.

Another issue is the question of kinociliary motility. Wersäll (1956) and others have suggested that kinocilia are immotile. Our own current notion, based upon ciliary ultrastructure and fixed appearance, is that the kinocilia are motile and stress the masses above them for constant detection of gravity (Ross, 1985). Translational acceleration would produce additional strain which would be interpreted against a background of activity related to gravity detection (vector summation). Because hair cells are polarized in the direction of the kinocilium (Lowenstein and Wersäll, 1959; Hudspeth, 1982, 1983), their response will depend upon the direction of applied force. The end result would be a change in kinociliary activity that is related to the direction as well as the magnitude of applied force.

Still another question involves the precise extent of otoconial linkage across the macula. It is often assumed that the entire otoconial mass resembles an otolith in functional organization; that is, all the otoconia are ultimately linked to one another in such a way that there is a single center of gravity. One might ask then, what advantage is there in a multitude of tiny seismic masses of various sizes over a single, large one? And why distribute the masses very precisely by size over the sensory area? Nature tried otoliths, but otoliths today are confined to certain fishes (mostly, teleosts) while all other living vertebrates have otoconia. One possible advantage to otoconia might be that they do not act as a single seismic mass, but are grouped in clusters with differing thresholds to applied acceleratory force. In such a case, several masses of different sizes could act on patches of sensors (hair cells) over more limited areas (see Lindeman, 1973; and Ross, 1983). It must also be noted that, regardless of extent of otoconial linkage across a macula, the fact that otoconia are linked at all means that the masses affect one another's response to a given acceleratory force. They dampen or enhance the motion of their neighbors (see Ross, 1983).

It is clear that several issues should be resolved to complete our analogy between macular end organs and man-made accelerometers. Nevertheless, our theory that they are analogous lets us consider otoconia in a particular way: as seismic masses that are spring-loaded to one another and to hair cells. As a consequence, we ask: What are the unique properties of these masses that Nature selected them for the job at hand? How do these interesting biogenic crystals form? Does gravity influence their formation or their final structure? To begin to answer these questions, we must determine the composition and structure of otoconia, learn where the materials come from, and explain how the organic and inorganic phases interact to shape the otoconium. We must also study the effects of microgravity on their genesis and maintenance.

The composition of otoconia: the mineral phase: Carlström (1963) conducted the most complete comparative study of the crystallographic properties of otoconia and otoliths in vertebrates. More recently, scanning electron microscopic work (Lim, 1969, 1973, 1974, 1984; Lindeman, 1969, 1973; Ross and Peacor, 1975; Ross et al., 1976; Wright and Hubbard, 1978; Harada et al., 1978; Salamat et al., 1980; Wright et al., 1982; and others) and use of ultra-high resolution transmission electron microscopy (Mann et al., 1983; Ross and Pote,

1984) have added to our knowledge.

Vertebrate otoconia, with few exceptions, contain a polymorph of calcium carbonate as the mineral phase. In lampreys, one of the exceptions, otoconia are spherical. Their shape likely reflects their composition, for the mineral is in the form of amorphous calcium phosphate (amorphous apatite, Carlström, 1963). In warm-blooded vertebrates (birds and mammals) and in certain cold-blooded species (Carlström, 1963), the mineral is calcite. Most cold-blooded vertebrates have otoconia containing aragonite. Holosteans (gar pike and bowfin), however, have vateritic otoconia and otoliths, even though vaterite is a highly unstable form of calcium carbonate in nature.

Although otoconia most often resemble single crystals in outward form, they are highly ordered mosaic biominerals (Ross and Peacor, 1975) instead. This has been demonstrated conclusively by lattice imaging and by electron diffraction work (Mann et al., 1983). Teleost otoliths are also mosaics, although they were previously described as polycrystalline (Carlström, 1963).

Results obtained by ultra-high resolution transmission electron microscopy and by electron diffraction studies (Mann et al., 1983; Ross and Pote, 1984) demonstrated differences between aragonitic otoliths, aragonitic otoconia and calcitic otoconia even though all were mosaic biominerals. Rat otoconia (*Rattus norvegicus*, calcitic) were most clearly mosaics, with crystallite subunits that were platelets 80-100 nm in diameter and a few hundred angstroms thick (Mann et al., 1983; Ross and Pote, 1984). Fish otoliths (plaice, *Pleuronectes platessa*) and frog (*Rana pipiens*) otoconia fragments showed more extensive areas of lattices but the fragments were also more sensitive to beam damage. Otoliths contained impurities which vaporized in the electron beam, leaving an aragonitic lattice of lowered symmetry. Frog otoconia were least stable of all. The fragments contained amorphous material about 50 Å in diameter. When this material was affected by the beam, the entire fragment would become disorganized (polycrystalline) before one's eyes. The findings suggest an evolutionary trend toward more perfect crystallites and a more definite mosaic structure, with more organic material separating the subunits. Why is the evolution of more perfect mosaics important?

A further finding of this research was that the biominerals did not resemble their natural crystal counterparts (calcite and aragonite) in fragment shapes or in faces exposed by cleavage. For example, the biominerals fractured into fragments which often had rounded edges, but natural crystals cleaved into particles with sharp edges. The conclusion seems inescapable that organic material is involved in the genesis, structuring, and maintenance of otoliths and otoconia, whose crystallographic properties differ from those of minerals produced inorganically.

The composition of otoconia: The organic phase: Scanning electron microscopy is not useful in determining the composition of the organic material, but has been helpful in demonstrating its distribution. In agreement with findings that go back to the last century (Henle, 1873), scanning work on rat otoconia (Ross and Peacor, 1975) showed that organic material is present throughout the otoconia. Moreover, pie-shaped subdivisions that are laminated extend from the surface toward the center of the units. This and other (Lim, 1973) scanning research also confirmed the existence of a central particle (Herzog, 1925; Kolmer, 1927; among

others), later found to be well-demarcated in fetal rat otoconia (Salamat et al., 1980). Only recently, a similar situation was demonstrated in mature rat otoconia (Pote and Ross, unpublished) by transmission electron microscopy. The core is significant developmentally because it is related in some way to the size of the mature otoconium (Salamat et al., 1980).

The chemical nature of the organic material is unknown, but Shrader et al. (1973) have suggested it is (sulfated) mucopolysaccharide (proteoglycan). The otoconial membrane shows high periodic acid Schiff (PAS)-reactivity (Wislocki and Ladman, 1955), which would suggest that the organic material of the otoconia and otoconial membrane contains glycoprotein. Biochemical work, employing high performance liquid chromatography (HPLC) (Ross et al., 1985b) and lectin-binding (Gil-Loyzaga et al., 1985), has not yet resolved this issue. It is known only that the organic material is high in acidic amino acids and low in basic amino acids; and that its sugar content is compatible with either proteoglycan or glycoprotein (Ross and Pote, 1984; Ross et al., 1985b; Gil-Loyzaga et al., 1985).

The amount of organic material in rat otoconia is remarkable, judged upon transmission electron microscopic results alone (see also Marco et al., 1971). The organic materials of the central core and the more peripheral parts are organized differently, when observed in transmission electron micrographs. Whether they are chemically identical is not yet known. The center of an otoconium demineralizes before the outer portions when subjected to acid or to natural causes of demineralization (i.e., aging in humans, Ross et al., 1976). This suggests that the core is less perfectly mineralized than the peripheral zone and/or is less well protected by organic material.

It is highly likely that the proportion of organic material differs in otoconia of different classes or species, and between vateritic, aragonitic, and calcitic otoconia. Ballarino (1985) found little protein in otoconia of *Rana pipiens* and of *Thamnophis sirtalis* (garter snake). The nature of the organic materials involved in the genesis of otoconia containing the various polymorphs of calcium carbonate may also be different. It would be of interest to know whether morphological changes in otoconia, alluded to above, are the end result of primary alteration of the organic phase. To resolve these issues, comparative analytical research is required.

Evolution toward the calcitic form: Work with electron diffractograms demonstrated that certain frog otoconial fragments yield diffractograms identical to those of rat (001 face in calcite). This raised the question whether the aragonite (in frog) mimicked calcite crystallographically, or whether calcitic crystals were intermixed with the aragonitic. The finding led to further questions concerning the evolution of otoconia toward the calcitic polymorph, and what this might mean in functional terms.

The questions are far from answered at the present time. A scanning electron microscopic study demonstrated, however, that there is an apparent evolution toward calcitic-type otoconia by macular site (Ross and Pote, 1984). The most interesting examples occur in reptiles, in turtle and in alligator. These vertebrates were studied in detail because they are close to the evolutionary stem-line which gave origin to mammals and birds, respectively. Both turtle (western painted, *Chrysemys picta bellii*) and alligator (American, *Alligator mississippiensis*) have calcitic-type

otoconia in their lagenas. In turtle, the calcitic type is mixed with otoconia of aragonitic appearance in the utricle, but only otoconia with aragonitic morphology are present in the saccule. (Aragonitic otoconia have real side faces and often closely resemble faceted, natural crystals. Calcitic otoconia have trigonal end faces and rounded bodies). In alligator, otoconia with calcitic morphology have replaced the aragonitic type in the utricle and are common in the saccule. In related, unpublished work, we have found a large complement of calcitic-type otoconia in lagena of frog (*Rana pipiens*), and a rare few in the utricle. It is still not known whether the calcitic shape means that the mineral calcite is present, although Carlström's results (Carlström, 1963) by the powder diffractogram method gave strong lines for aragonite and some lines for calcite in turtle otoconia. The matter is under investigation.

The reason for the presence of a specific polymorph of calcium carbonate in a particular vertebrate class or in a given species is enigmatic. Neither is it clear why there is a progression by site, lagena-utricle-saccule, toward the calcitic-type otoconia in the turtle and alligator. Minor differences in composition of the organic material would be the simplest explanation for the occurrence of two polymorphs of calcium carbonate side by side in the same end organ, since variation in environmental factors (i.e., pH, temperature) would be improbable over the small areas involved.

While there are doubtless at least slight differences in the chemistry of the organic phases which determine the polymorph laid down, there is also the likelihood that some similarities exist. Intertwining, or possibly twinning, of otoconia in the crossed configuration can be demonstrated for each polymorph (Figures 3-5). It is of further interest that such intertwining can be demonstrated even for otoconial masses in lamprey (Ross and Pote, 1984), in which the mineral is apparently non-crystalline apatite (Carlström, 1963). The findings suggest that something fundamental about the structure of the organic phase of otoconia was established from the very beginning in the vertebrate series.

Development of otoconia: Whether an organic template in the shape of an otoconium forms first and is subsequently mineralized (Nakahara and Bevelander, 1979); or if laying down of both mineral and organic phases is simultaneous (or nearly so) (Salamat et al., 1980) is a matter of some controversy. Literature pertaining to this subject was recently well-reviewed by Lim (1984). Fermin and Igarashi (1986) propose that otoconial templates are formed first in the chick, through a process of segmentation of organic material above the macula. They indicate that these templates subsequently mineralize. They have not, however, demonstrated all the stages between "segmentation" and the final product, a mineralized otoconium. This is essential to support their hypothesis. Ballarino et al. (1985), in contrast, using polarized light microscopy, demonstrated that developing chick otoconia are mineralized. The problem of conflicting results possibly stems from the fact that fetal otoconia are extremely prone to demineralization (Peacor et al., 1980; Ballarino, 1985). Salamat et al. (1980), for example, had to micro-dissect fresh otoconia from fetal rats and embed them directly to preserve calcium carbonate within them. Even the collection of sections in water may lead to some loss of mineral, depending upon how thoroughly the embedding medium has penetrated the tissue and protects the mineral against solution. Experience has

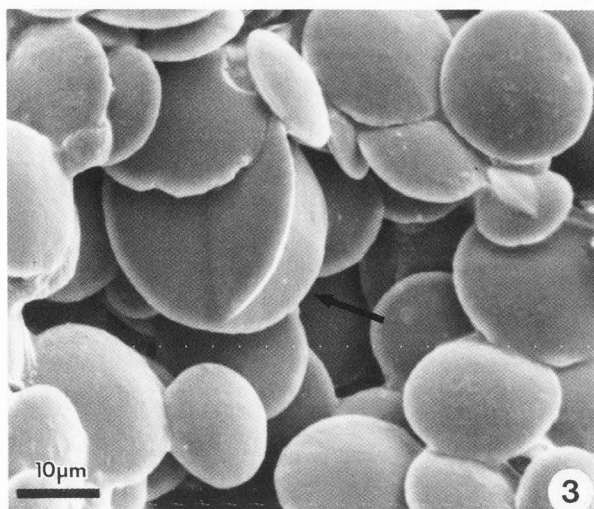


Figure 3. Vateritic otoconia of gar pike have a discoid appearance. A crossed otoconium is shown at upper center, left [arrow]. Otoconia from sacculus.

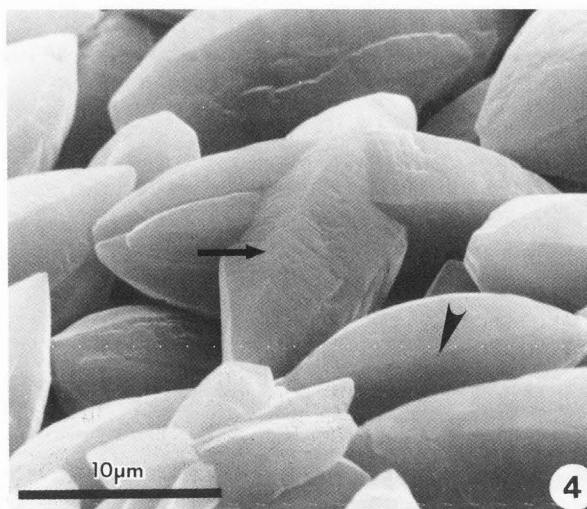


Figure 4. Aragonitic otoconia of the frog (*Rana pipiens*) have faceted side faces [arrowhead]. A crossed otoconium is featured here [arrow, center]. Sacculus otoconia.

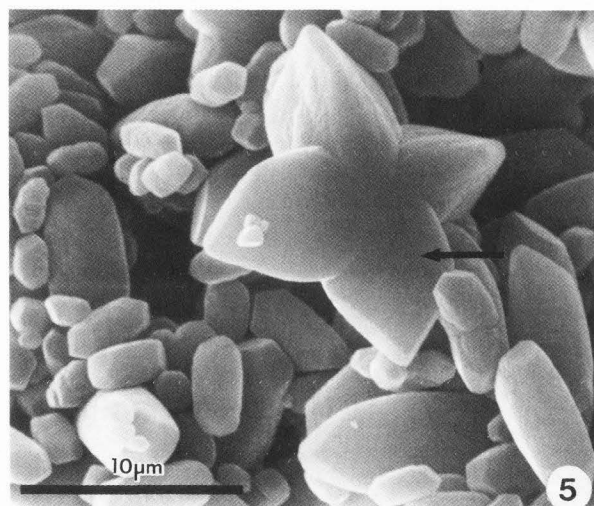


Figure 5. The crossed configuration [arrow] is common in calcitic otoconia of mammals. Sacculus otoconia of monkey are illustrated.

shown that exposure of fetal otoconia to fluids used routinely for tissue preparation for transmission electron microscopic study results in their demineralization, even though nothing is done willfully to remove mineral (Salamat et al., 1980).

Our findings favor the concept of nearly simultaneous deposition of the two materials. Primitive otoconia of rat have the calcitic morphology (three-fold symmetry) from the very beginning. Maturation is completed in several phases. A core develops first, and the peripheral zone, only later, at least in mammals. Whether or not a core is present in submammals is under investigation. It is possible that maturation involves further changes in degree of mineralization of various parts of the otoconium (Ross and Peacor, 1975). The end product is a mosaic (Ross

and Peacor, 1975) that is neither a single crystal nor polycrystalline (Mann et al., 1983). It does appear that in mammals otoconia form in situ. The sources of the organic and inorganic materials are unknown, but organic components could be released into the labyrinth from vesicles of macular or other cells. Formation of otoconia from bleb-like protrusions of supporting cells (Harada, 1979; Anniko, 1980) is a less attractive hypothesis. As Lim (1984) noted, blebs are common as fixation artefacts. It is clear that certain elements, such as zinc and manganese (Erway et al., 1971; Purichia and Erway, 1972; Lim and Erway, 1974; and Erway et al., 1986), and carbonic anhydrase (de Vincentiis and Marmo, 1968) are essential to the normal production of otoconial constituents.

Scanning electron micrographs of fetal rat otoconia (Salamat et al., 1980) first demonstrated the fact that otoconia, once a core is established, continue to grow by the seeding of multiple subunits. Only later do the subunits merge into structures that have the outward appearance of single crystals. It is not expected that otoconia of other mammals grow in a fundamentally different way. We do not yet, however, have comparable studies of the development of otoconia in amphibia or reptiles to note whether the process appears to be similar or is different.

It is evident from studies on human material covering the lifespan that otoconial development is largely a fetal event, but is not confined to that period (Ross et al., 1976; Wright and Hubbard, 1978). Utricu- lar masses, in particular, appear to be capable of seed- ing new otoconia; but saccular otoconia are more likely to grow in size. We do not yet know whether otoconia seeded postnatally have a central core, or grow by seeding on the face of an already established otoconium (see section immediately below). Post- natal growth occurs mostly through infancy and child- hood in humans, and is less prominent after puberty. Recently, we learned that a strain of rats also shows postnatal changes that take place during early life. The findings are considered below, because they are related to control studies for the effect of micro-

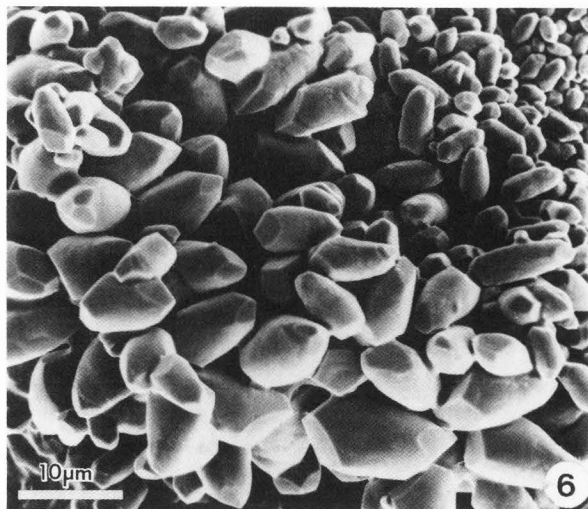


Figure 6. This figure illustrates the lateral border of the utricular patch in a control rat, weight-matched with the space-flown rat whose utricular otoconia are shown in Figure 7.

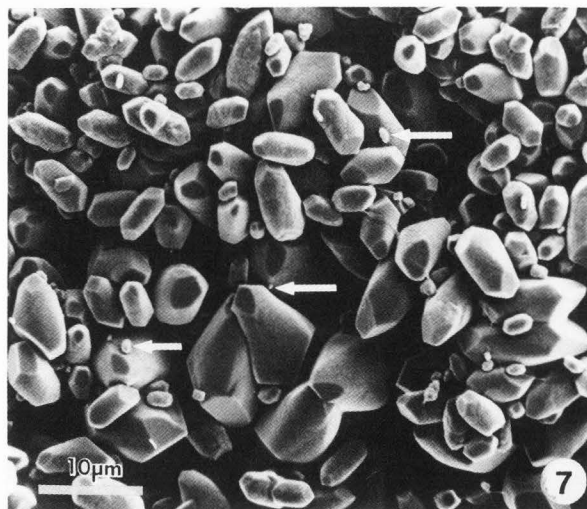


Figure 7. This scanning electron micrograph shows otoconia at the lateral border of a utricular patch taken from a space-flown rat. Note the large number of very small otoconia in the field (arrows indicate a few).



Figure 8. This figure shows otoconia at the lateral border of the left utricular patch taken from a young rat from Taconic Farms. Otoconia of the right side are illustrated in Figure 9. Note difference in sizes of large otoconia, and the numerous small otoconia on the left side. The two micrographs were taken from equivalent portions of the maculas.

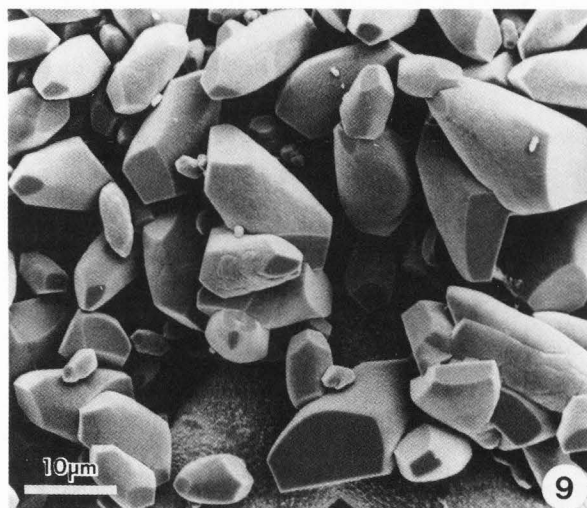


Figure 9. Otoconia at the lateral border of the right utricular patch of the same young Sprague-Dawley rat illustrated in Figure 8.

gravity on otoconia.

Does gravity influence otoconial morphology?:

Because otoconia are essentially seismic masses used to determine gravitational and translational linear accelerations, it is reasonable to ask whether gravity has any influence on their structure. A basic question is whether microgravity will affect their development, and another is whether it can cause otoconial demineralization during short- or long-term space flight. Experiments in space are required to answer these questions, because there are no methods on Earth to produce an environment comparable in all ways to that of space.

In 1985, we obtained inner ear tissues from 9 Sprague-Dawley rats (Taconic Farms) that were space-flown for a period of approximately 7 days. Three were ~51 days of age and weighed 200-265 gms. The remaining 6 rats were ~93 days old and weighed 375-400 gms. Otoconial complexes from the experimental animals and from an equal number of weight-matched controls were studied by scanning electron microscopy (Ross et al., 1985a). Upper and under sides of (different) membranes were examined, for completeness. There were no signs of otoconial demineralization present in either saccular or utricular otoconia; nor were any other deleterious effects evident. This was the only unequivocal result of the research. The finding confirms recent Soviet results reported by Lychakov and Lavrova (1985).

There were other interesting observations, how-

ever. Saccular otoconia of flown rats tended to have smoother surfaces than those of matched controls; and utricular complexes showed many very small otoconia among the largest of the otoconia, located at the lateral border. These very small otoconia were not fragments, but were miniatures of typical otoconia found in rat. Few such miniature otoconia were present in the controls (compare Figures 6 and 7). The tiny crystals always appeared to grow along faces of already existing otoconia. This might suggest that, in keeping with findings *in vitro*, it is easier to spawn new biomineral on stereochemically analogous faces of proteins attached to a rigid surface than by precipitation (Addadi and Weiner, 1985).

It seemed possible that saccular otoconia had shown growth by accretion and utricular otoconia, by neogenesis. This would be in agreement with previous observations in human material (Ross et al., 1976). However, we had turned some otoconial complexes upside down, to observe more basal as well as more superficial otoconia. Thus, we did not have a pair of patches from the same animal turned in the upright direction for comparison, even in controls. Even though right and left sides had been turned over at random, we believed it essential to compare the upper sides of right and left otoconial complexes of the same animal. It remained possible that we had evidence of asymmetry rather than new growth, and that chance had favored our turning over sides (in each case) that would otherwise have demonstrated this. We had not observed such miniature otoconia in our previous studies of otoconia obtained from rats of various ages supplied by a different vendor (Charles River). It seemed essential, therefore, to pursue the matter further through a study of Sprague-Dawley rats of the Taconic strain.

Right-Left Asymmetry of Otoconial Masses

Materials and Methods

Nine Taconic rats (3 each, 51 days, 200-212 gms; 72 days, 270-300 gms; 93 days, 360-400 gms.), were used. Oldest and youngest animals were closely age- and weight-matched to flown rats. A third group of rats of intermediate age and weight was included to better determine whether miniature otoconia are normally present in early life but decline with age. Rats were decapitated and their bullas were rapidly removed to a dish containing a solution of 2.5% glutaraldehyde + 0.5% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The cap of the cochlea was removed and the vestibule was gently perfused with fixative. Further procedures simulated those employed on flight animals, from which both macular tissue and otoconial patches were removed, to permit valid comparisons. Thus, overnight fixation and postfixation with 1.0% osmium tetroxide were carried out even though neither step is essential for otoconial study alone. After washing in buffer, samples were dehydrated to 70% alcohol and microdissected. Otoconial patches were carefully removed to copper grids, which were then mounted on aluminum stubs and dried over desiccant. Specimens were coated with gold-palladium in a Polaron E5100 sputter-coater. Entire otoconial patches were photographed, for comparison purposes, in an International Scientific Instruments (ISI) DS130 scanning electron microscope. Montages of the area

of special interest (the lateral border of the utricular patch) were made at higher magnification. Right and left sides were then compared.

Results

Young rats showed a tendency to have numerous small otoconia among the large ones present at the lateral border of the utricular patch, particularly on one side (Figures 8 and 9). Heavier and older rats had many fewer miniature otoconia.

Additionally, signs of asymmetry were present in both young and old rats. That is, otoconia tended to be more numerous and smaller on one side than on the other, along the lateral border. However, we could not uncover a tendency for nascent otoconia to be located on one side more frequently than on the other in the small number of young animals studied. Many more specimens would have to be studied to obtain reliable statistical results.

Discussion

The findings suggest that it is possible that new otoconia were spawned in utricular maculas of space-flown rats; or that otoconial maturation was inhibited. Control rats of comparable weight and age to older animals space-flown lacked the great numbers of miniature otoconia observed post-flight. Asymmetry in otoconial distribution by size is, however, a characteristic present in Taconic rats that must be taken into account. Further experiments in space, with this new finding of asymmetry kept in mind, are required to expand upon our previous results.

Asymmetry in otoconial size may not represent asymmetry in mass, if the otoconia of the two sides are of equivalent, total mass and are linked together into a single functional unit. As explained above, it matters greatly how the masses are coupled, in small groups or in a large mass; how they are coupled relative to the direction of a given acceleratory force; and how the "springs" function (stiff or more yielding). Otoconial linkage can be subjected to computer analysis and mathematical computation to provide some answers to the issues raised, once the precise connections between otoconia have been plotted.

Von Baumgarten and Thumler (1979) proposed that otoconial ("otolith") asymmetry might account for susceptibility to space-motion sickness. We believe that end organ asymmetry, including that of the sensory hair cell region, is probable. Thus, otoconial asymmetry in itself may be only part of the clue to explaining a proclivity for space-related or Earth-bound motion sickness. But the issue of possible right-left asymmetry in macular end organs is of basic interest as well, in developmental and behavioral studies. The universality of asymmetry can only be determined by further, comparative studies.

Summary and Conclusions

Evolutionary studies indicate that there has been a progression toward calcite as the mineral deposited in the test masses of macular otolith end organs, and toward a highly ordered mosaic structure in which the

organic phase becomes more prominent. Comparative studies of the chemical composition of the organic material should, in the future, provide insights to the factors involved in genesis of the specific biominerals observed; while comparative work on end organ organization as a whole could lead to interesting behavioral correlations. In all such research, developmental studies of normal and abnormal otolith end organs are an essential complement. Microgravity is a new and unique tool that will shed new light on the interplay between gravity and the genetic pool that helped produce biological accelerometers so that organisms could orient themselves in Earth's gravitational field. As research on otolith end organs proceeds, scanning electron microscopy will continue to play a major role. The beauty of the structures involved can scarcely be appreciated by light or transmission electron microscopy; and if beauty is truth, scanning electron micrographs reveal much about inner ear biominerals difficult to learn as quickly by any other means.

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Discussion with Reviewers

L.C. Erway: How would you propose to further study the variability in otoconial formation and neurophysiological function based on the model given?

Authors: One of the first things that must be done on a morphological basis is to learn whether there is a relationship between otoconial size and mass and the properties of the underlying macula; that is, the type of stereocilia on the hair cells, the number of cells in the sensory fields and the innervation patterns present. A further important factor is the extent to which otoconia are tied together. This was alluded to in the text. For example, do the otoconia comprise one mass, with one center of gravity? Are there two masses, one over pars externa and a second over pars interna of the macula? Or are there a multitude of masses corresponding to the number of sensory fields? With the answers in hand, it is assumed that the total morphology would be reducible to particular features that are related to the specific physiology of the nerve fibers. This physiology could be learned by actual experiment, through collaboration with others; or by deduction from results already published in the literature. Obviously, knowing the exact characteristics of the nerve fibers considered would be best. The findings should be interpretable in light of the engineering requirements for man-made accelerometers. Perhaps we would even obtain insights as to how we might design better accelerometers! Once we understand the characteristics of normal bioaccelerometers, study of abnormal ones would be fruitful in terms of learning whether predictions from the model could be verified. It would also be interesting to learn whether responses could be predicted for micro- and hypergravitational environments.

J. Ballarino: Do you feel that calcite in itself has an inherent advantage over aragonite? Or do you believe that the phylogenetic trend toward calcite is just the result of production of an organic phase that happened to favor calcite deposition?

Authors: The question might well be reversed to ask whether aragonite has an inherent advantage over calcite in the species in which it predominates. The question is, however, a fundamental one. Since both calcite and aragonite occur in cold-blooded species, even at times side by side in the same end organ, it is readily apparent that there is no simple answer. The organic phase must differ, if it serves as a nucleator and inhibitor, in order to produce one chemical phase in preference to another. Learning how it differs may lead to insights concerning why it differs. Can species with aragonitic crystals mobilize the calcium from such crystals more readily than from calcitic ones? Is this essential in animals spending more time in the water than on land? These are only two of many possible related questions, for which we lack answers.