

INTRACELLULAR CRYSTAL-BEARING VESICLES IN THE  
EPIDERMIS OF SCLERACTINIAN CORALS, *ASTRANGIA*  
*DANAE* (AGASSIZ) AND *PORITES PORITES*  
(PALLAS)

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Initial mineralization of the coral skeleton is thought to occur either in the extracellular milieu or at an intracellular site of calcium carbonate (aragonite) crystallization. As evidence for the former alternative, an organic secretion from the skeletogenic epithelium appears in light microscopic preparations (von Koch, 1882; Duerden, 1904). This secretion is characterized as an acidic mucopolysaccharide (Goreau, 1959) and is amorphous in ultrastructure (Vahl, 1966). It may serve as a template upon which skeletogenesis ensues.

The latter option, an intracellular site of crystal formation (von Heider, 1882; Ogilvie, 1897), recently received support following ultrastructural identification of electron-dense vesicles within the skeletogenic epithelium containing 50 Å threads (Kawaguti and Sato, 1968). Nevertheless, in the absence of critical and definitive evidence for an intracellular site of mineralization, most investigators prefer the extracellular alternative (Vandermeulen and Muscatine, 1973; Vandermeulen, 1975).

Data sufficient to resolve this controversy requires identification of the primordial crystal population itself. The evidence of the site of those crystals would extend previous observations from both extracellular and intracellular supporters and would reassert the significance of skeletogenic tissues during crystal formation in Scleractinian corals (Hayes and Goreau, 1976a). Such were the objectives in this ultrastructural and radiochemical investigation of the Caribbean reef-building hermatype, *Porites porites* (Pallas), and of the North Atlantic ahermatype, *Astrangia danae* (Agassiz).

MATERIALS AND METHODS

Specimens of *Astrangia danae* were collected by dredging in Vineyard Sound off the coast of Woods Hole, Massachusetts, in the United States during the summer of 1975. Water conditions at the collection site at that time were typically 55-70 feet in depth, 18-21° C and 3-5 knot maximum bottom currents. The low illumination, extremely poor visibility and strong water movement made diving with SCUBA impossible. Small rocks encrusted with the coral were transported to the laboratory for maintenance in a running seawater table. For morphological and radiochemical assay, tissues were utilized within one to two days of collection.

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Preparation for microscopy involved the slow addition of 5% glutaraldehyde fixative in filtered seawater (FSW) to yield a final concentration of 2.5% aldehyde. Tissues were fixed for one to two hours at 4° C. Following a series of washes in cold FSW, post-fixation with 1% osmium tetroxide in FSW was conducted. Dehydration of the tissues through graded ethanol solutions continued at 4° C until reaching absolute alcohol. Tissues were then transferred to propylene oxide and embedded in a 50:50 Epon-Araldite resin mixture. The plastic was cured by incubation at 60° C for 48 hours. Silver sections (*ca.* 600 Å thickness) were cut on an LKB Ultratome II for electron microscopy. Sections were viewed unstained or were stained with 7.5 per cent uranyl acetate in 50 per cent ethanol followed by lead citrate. A Philips EM200 was used for ultrastructural observations. Orientation of all tissues was confirmed using Toluidine Blue-stained 1–2 μ thick sections.

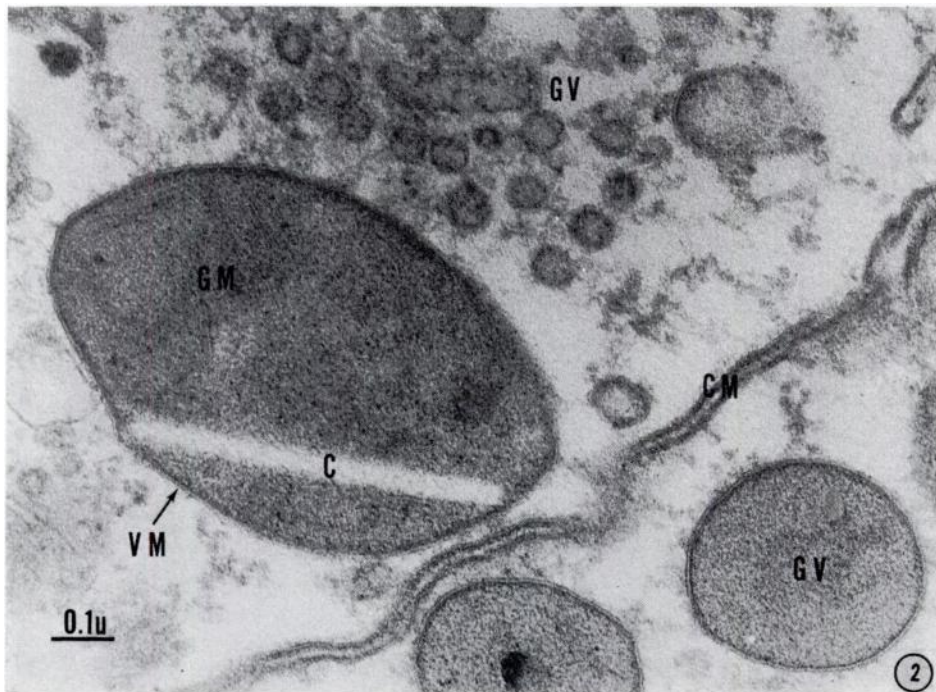
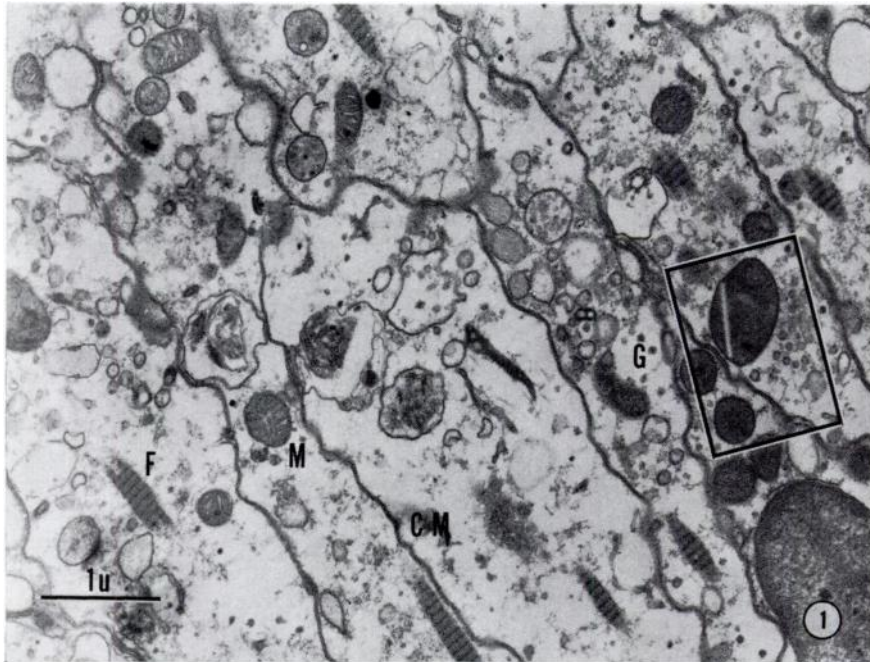
Adult colonies of *Porites porites* were collected from waters adjacent to Port Royal Marine Laboratory (Kingston Harbour Cays) and from the fore-reef near Discovery Bay Marine Laboratory in Jamaica. Water conditions during collection in late winter and spring 1976 were depths of 10–60 feet, temperature averages of 24–26° C and negligible currents. Visibility underwater was excellent, and specimens were collected using SCUBA or snorkel diving techniques. All corals were kept immersed in sea water *en route* to the laboratory.

With the aid of a dissecting microscope, planulae (larvae) were gently separated from the mesenteric tissues within which they develop. Viability of these planulae was established by their swimming behavior. The active larvae were transferred to a finger bowl containing FSW and further selected on the basis of vitality and morphological integrity for transfer to small aquaria for settlement experiments. Substrates provided for larval attachment included clean glass microscope slides and thin slices of bleached, cleaned and salt water-equilibrated skeletons of *Porites*. Aquaria with planulae were equipped with either a capillary pipette or a hypodermic needle as an aerator port, and a slow discharge of bubbles was regulated so as not to disrupt larval adhesions.

Once attachment was firmly secured, the larvae were removed and fixed either with 2.5% glutaraldehyde or 1.0% osmium tetroxide in FSW at 4° C. Free-swimming planulae and planulae attached for periods of less than one day through one week were preserved for morphological study. Procedures followed duplicate exactly those described above for *Astrangia* adults, with the exception that electron microscopy was accomplished using an AEI instrument.

Sections of all plastic-embedded coral tissues were cut with a Dupont diamond knife. In an attempt to preserve normal morphological relationships, no effort was made to decalcify specimens. For free-swimming planulae, this was not a concern; for attached planulae and adults, tissues were embedded with skeletal substrates and skeletons intact. Blocks of embedded tissues were faced and trimmed leaving small portions of the skeletal matrix for orientation both within the block itself and within thick and thin sections. The presence of this calcified material provided an opportunity to investigate the ultrastructure of the skeleton in ultrathin section.

Several experiments were conducted to determine incorporation of radioactive <sup>45</sup>Ca by adult *Astrangia danae*. Aerated sea water, filtered through a Nalgene filter unit with a 0.2 μ membrane, was prepared with 1.0 μCi/ml of <sup>45</sup>CaCl<sub>2</sub> (New England Nuclear). Volumes of 200 ml were dispensed into clean glass finger



bowls and small colonial growths of *Astrangia* were added. Skeletal weights of these colonies did not exceed 0.1 g. Temperature was maintained at 24° C by setting the aquaria in a seawater table; aeration was constant.

Following exposure to radioactive sea water, corals were rinsed at least five times in cold filtered sea water. Each was wrapped in parafilm envelopes and frozen to terminate metabolic activity. Further analysis consisted of extracting tissue from the skeleton by treatment with concentrated ammonium hydroxide (14.7 M). Skeletons were rinsed two to three times to complete release of tissue. The skeletal fraction was then solubilized in 1.5 N hydrochloric acid and dried in an oven.

The soft tissue fraction in basic solution consisted of the original supernatant to which skeletal washings were added. This was allowed to settle, and the liquid fraction removed and evaporated in an oven. The basic residue was treated with 1.5 N HCl to extract the acid soluble fraction which was decanted and saved. The insoluble residue was then added to the original basic supernatant to integrate the basic tissue fraction. Summarily, three compartments of the original coral were separated for incorporation analysis: a basic tissue fraction, an acid tissue fraction and a skeletal fraction. Each of these was dried to a powder and counted in a Nuclear-Chicago planchet scintillation counter.

## RESULTS

### *Light microscopy*

The body wall of the free-swimming planula of *Porites* comprises two epithelia cemented and supported by a thin connective tissue lamina or mesoglea. The innermost epithelium or gastrodermis is low cuboidal and is easily distinguished by large dense cytoplasmic zymogen granules. This epithelial surface lines the coelenteron or digestive cavity and throws itself into a complex of mesenterial septae, all involved in nourishing the organism. The gastrodermis is also the residence of zooxanthellae or symbiotic algae in hermatypic corals.

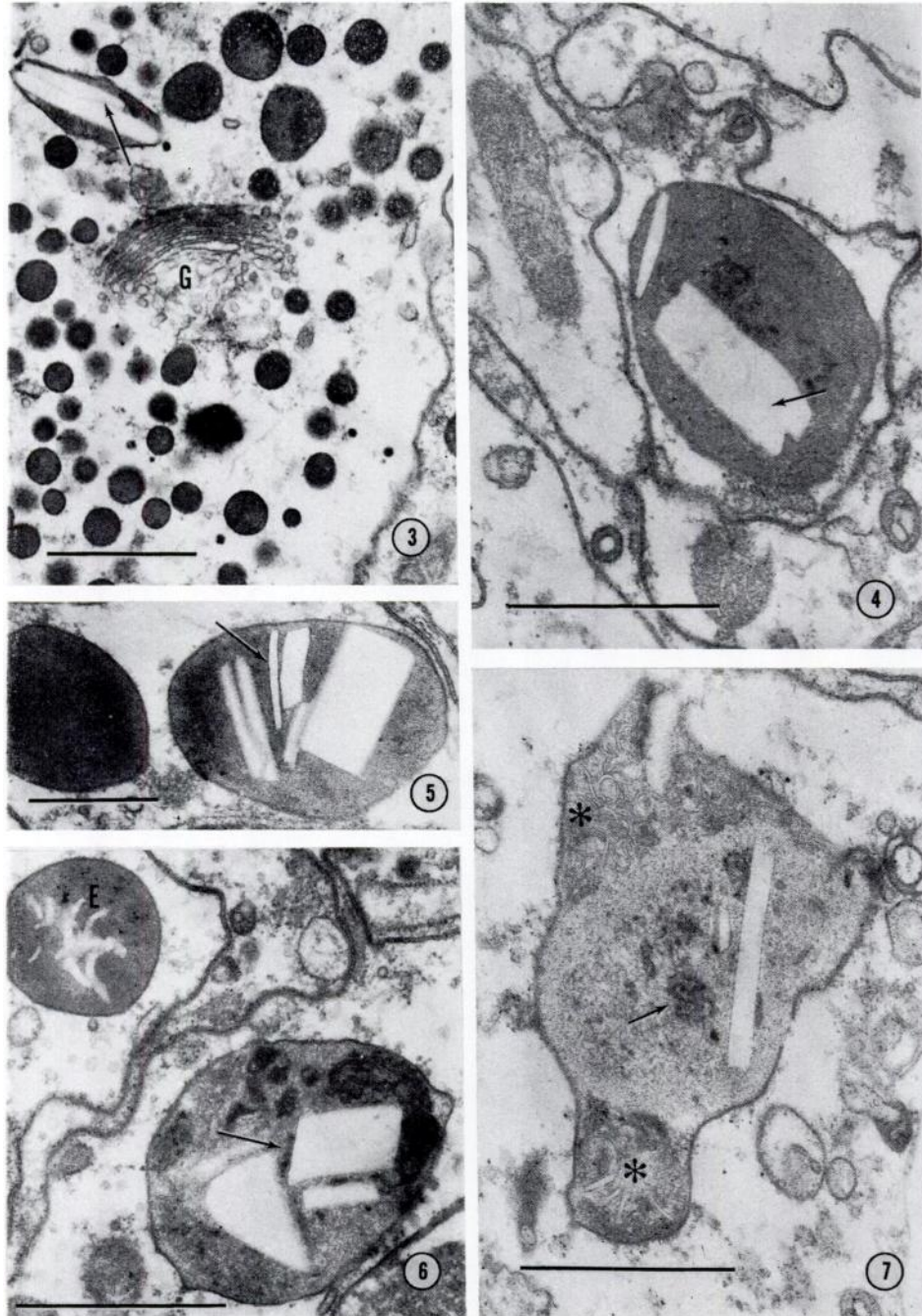
The superficial epithelium or epidermis is high pseudo-stratified columnar in all regions, except in surfaces of attachment of the coral organism to a solid substrate; whereas the free-swimming larva has only a pseudo-stratified columnar epithelium, morphosis of that epidermis occurs upon settlement, and the resulting epithelium assumes a squamous cellular configuration which may or may not be stratified. Because of its juxtaposition to sites of the coral skeleton, this transformed squamous epithelium is termed the skeletogenic or "calicoblastic" epidermis. Epidermal differentiation into a free columnar zone and a skeletogenic squamous zone persists in the settled, attached planula of *Porites* and in the adults of *Porites* and *Astrangia*. These epidermal zones are morphologically contiguous through a transitional zone at the edge of the organism, if solitary, or at the edge of the colony, if colonial.

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FIGURE 1. Apical cytoplasm of epidermal epithelium of adult *Astrangia danae*. One crystal-bearing vesicle appears within insert. Other cytoplasmic organelles include mitochondria (M), flagellar axial filaments (F), glycogen (G) and lateral cell membranes (CM).

FIGURE 2. Enlargement of crystal-bearing vesicle in Figure 1 insert, revealing detail of trilaminar membrane (VM), granular matrix (GM) and crystal rodlet (C) along with several Golgi vesicles without crystals (GV). Note similarity in matrix compared with other vesicles.





FIGURES 3-7. Assortment of crystal-bearing vesicles in *Astrangia danac*. Scale bars are 1  $\mu$ .

The principal cell type in the free epidermis is a columnar cell with a single flagellum at its apical surface and a striated border. Other cell types recognized are nematocytes, mucous goblet cells and granular cells. These cells persist in the skeletogenic epidermis although in remodelling during settlement, the apical specialization of the principal cell type, including the flagellary apparatus, disappears.

#### *Electron microscopy*

Ultrastructural examination of the supranuclear region of the epidermal cell reveals numerous assorted cytoplasmic organelles. Richly endowed with spherulitic mitochondria and displaying pronounced vacuoles and glycogen rosettes, this cytoplasmic zone also accommodates the Golgi membrane system of flattened sacs and assorted dense granular vesicles (Figs. 1, 8).

Within the larger vesicles, electron-lucent crystalline configurations appear (Figs. 1–12). The location and structure of these crystal-bearing vesicles indicates that they are derived from the Golgi system. Measurements on the crystals establish that they are flattened plates or rodlets with maximum dimensions of  $0.7 \mu$ , by  $0.1 \mu$  by  $0.3 \mu$  in both the adult *Astrangia* and the larval *Porites*. Crystallographic description of these structures with three unequal axes at right angles to each other conforms to the orthorhombic configuration. Such geometry is characteristic of the aragonitic polymorph of calcium carbonate (cf. Palache, Berman and Frondel, 1944). Further analysis of these crystals is in progress to confirm the lattice configuration which is expected to be that of aragonite.

In many of these vesicles, multiple crystalline profiles are observed, and some appear coupled or closely linked together (Figs. 3–5). Again, in a crystallographic context, this resembles a twinning phenomenon, a common occurrence in aragonite (cf. Palache *et al.*, 1944).

From their appearance in ultrathin plastic sections, it is concluded that these crystalline inclusions are electron-lucent, that is, freely penetrable by the electron beam in  $600 \text{ \AA}$  plastic sections. The crystalline zones reveal a subtle texture and compare favorably with the electron density of the background cytoplasmic matrix. Examination of adult coral skeletons, coral substrates used for larval attachment and settled larval primary skeletons establishes electron-lucency of aragonite crystals in thin sections viewed with the electron microscope. In this technical procedure, the crystals are not solubilized during tissue preparation, and the analysis of unstained sections containing crystals eliminates the possibility of crystal removal following exposure to uranyl acetate as reported in the literature (Wilbur, Colinvaux and Watabe, 1969).

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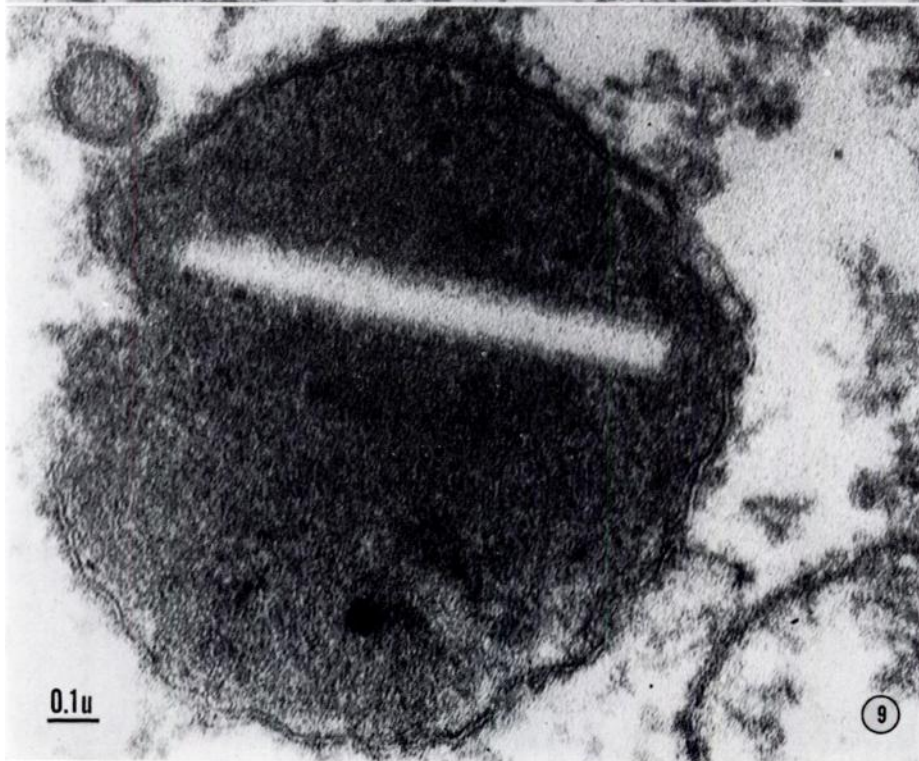
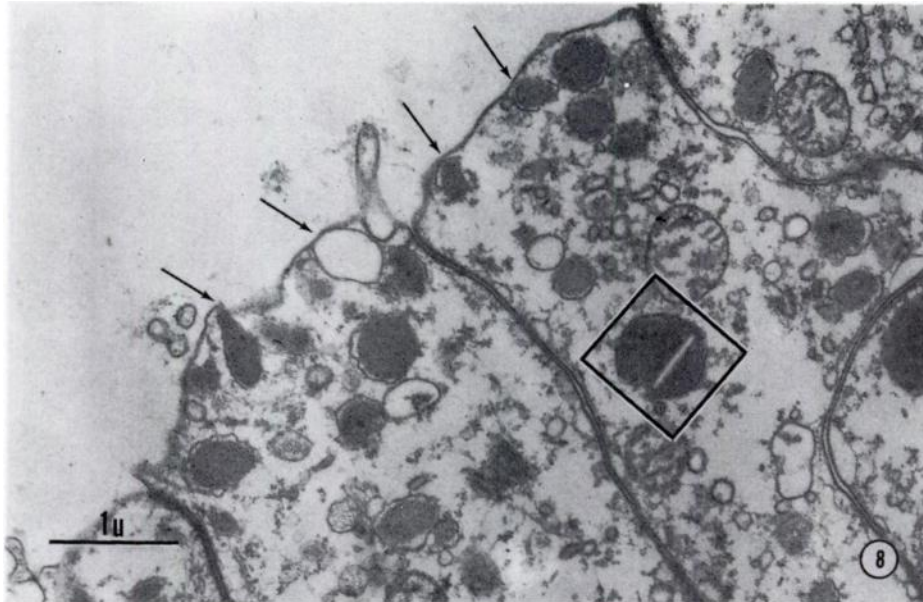
FIGURE 3. Twinning configuration of crystals (arrow) with thin sheet of matrix between units. Note proximity to and structural similarity with Golgi membrane system (G).

FIGURE 4. Multiple crystals within one vesicle. Note twinning of crystals (arrows).

FIGURE 5. Crystal-bearing vesicle revealing condensation of matrix substance around crystal surface (arrow).

FIGURE 6. Organization of matrix constituents into myelinised configuration at crystal surface (arrow). Crystal embryo (E) in vesicle.

FIGURE 7. Bizarre configuration of swirling membrane-like profiles (asterisk) and heterogeneity of matrix substances (arrow) in crystal-bearing vesicle.



Other components of the crystal-bearing vesicle include its limiting membrane and its osmiophilic matrix. The limiting membrane conforms to the unit trilaminar configuration characteristic of eukaryotic cells and is equivalent in thickness to the plasma membrane. The inner osmiophilic leaflet of the vesicle membrane reveals increased thickness in comparison to the osmiophilic leaflet of the membrane nearest the cytoplasm (Figs. 2, 9). The matrix material within the vesicle stains with osmium tetroxide and further with uranyl acetate. The consistency of the matrix is granular, and 30–35 Å particles are resolved within the densely packed internal structure (Figs. 2, 9). A clear zone appears around the granular matrix in some vesicles (Figs. 11, 12). No further substructure appears in the matrix of the vesicle in the absence of crystalline inclusions. The crystal-bearing organelles, however, manifest increased complexity in the vesicular matrix. Dense clumps of matrix substance, swirling patterns and myelin configurations are associated with the vesicles possessing crystals (Fig. 7). The myelin figures appear as stacked sheets of alternating light and dark lines and are always organized or oriented parallel to one or more of the crystal surfaces (Fig. 6). Often a single dense osmiophilic line delimits the contour of the electron-lucent crystal surface (Fig. 5) suggesting that the crystal itself imposes organization within the matrix substance.

Distribution of the crystal-bearing vesicles, although limited to the apical cytoplasm of epidermal cells, exceeds the range of the attached or skeletogenic epithelium in both the adult and larval corals. Cells of the entire epidermal surface of the organism generate granular vesicles with the exception of the stomodeum, oral disc and tentacles proper. Once the granular vesicle appears, the potential for crystal formation is established. Several crystal-bearing vesicles may appear in the cell just as adjacent cells may contain crystals. Also, the crystals may appear singly or as multiple clusters within a single vesicle. Sampling error in the electron microscope unfortunately disallows any distribution plot of these structures.

Random spatial distribution accounts for association of the vesicles with other cell organelles in the apical cell cytoplasm. However, intimate contact with mitochondria suggests significant metabolic interplay (Figs. 8, 10 and 12). Other non-crystalline vesicular elements of the Golgi system, interpreted to be developmental stages in the maturation of the vesicle population, cluster around the crystal-bearing structures (Figs. 2 and 3). Granular matrix constituents of all such vesicles are identical. However, secondary modification of the vesicle matrix results from coalescence of vesicles (Figs. 11, 12) and from the physical presence of crystals as discussed above. Although the vesicle normally assumes a spherical shape, the presence of crystalline elements may distort that profile resulting in ovoid, bulbous or flattened contours.

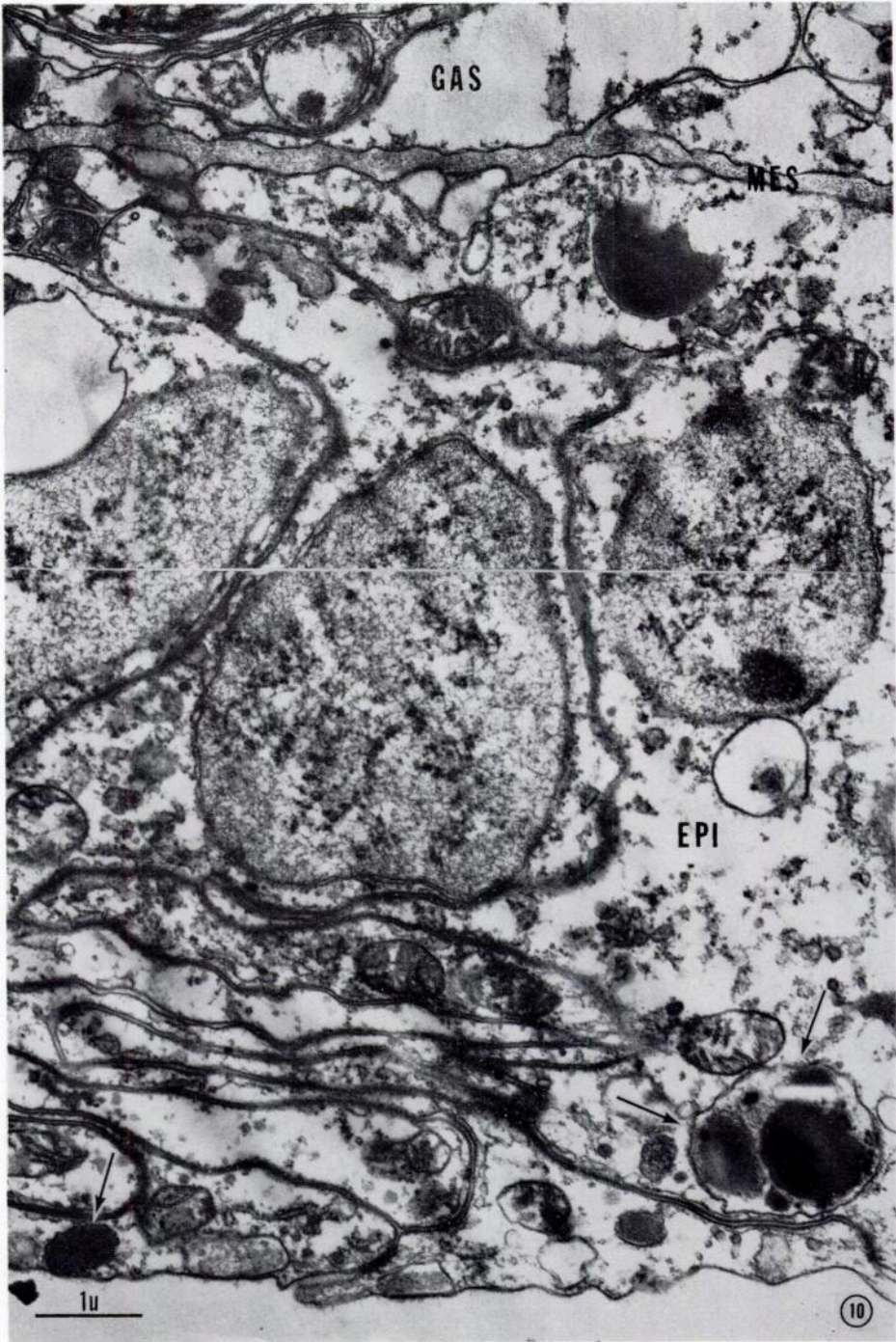
Efforts to recognize these crystals within the vesicles at the light microscope level have been unsuccessful. Because of the size of the crystals, it is unlikely that they would be easily resolved in thick sections due to the masking influence of

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FIGURE 8. Free surface of settled planula, *Porites porites*, indicating presence of crystal-bearing vesicle within cytoplasm of epidermis (insert). Note exocytosis of vesicles without crystals (arrows).

FIGURE 9. Enlargement of crystal-bearing vesicle in Figure 8 insert, revealing similar structural detail as in *Astrangia danae* Figure 2.





the matrix substance and the osmiophilic quality of that material. Identification of the vesicles themselves in light microscopy is uncomplicated since they average 1.5  $\mu$  in diameter and are easily resolvable. Furthermore, the vesicles may be stained in thick sections with routine basophilic dyes such as Toluidine blue or Methylene blue.

In the free epidermis, there is extensive development of the apical cytoskeletal complex consisting of desmosomal tonofilaments, the terminal web of cytoplasmic fibrils and lateral extensions from the flagellar axial fibers (Hayes and Goreau, 1976b). The absence of these specializations in the skeletogenic epithelium would allow crystal release at that site (Figures 10–12). The granular matrix of the crystal-bearing vesicle, as well as the matrix material of vesicles bearing no crystals, contribute to the formation of the organic phase of the exoskeleton. Following release from the cell by exocytosis this matrix material would provide an important, perhaps the only, source of calcium for continued crystal growth and nucleation.

#### *Incorporation of radioactive $^{45}\text{Ca}$*

The soft tissues of *Astrangia danae* incorporate ionic  $^{45}\text{Ca}$  rapidly as shown in Table I. More than 90% of that radioactivity is extractable in the basic-soluble tissue fraction. The acid-soluble tissue fraction, which can be interpreted as representing  $^{45}\text{Ca}$  bound into acid-soluble protein and/or into crystals of acid-soluble calcium carbonate (cf. Palache *et al.*, 1944), is derived from the cytoplasm and contributes a small percentage of total incorporated radio-activity, never exceeding 5%. Skeletal incorporation in six hours or less constitutes the smallest fraction of the radio-calcium pool at 3% or less of total uptake.

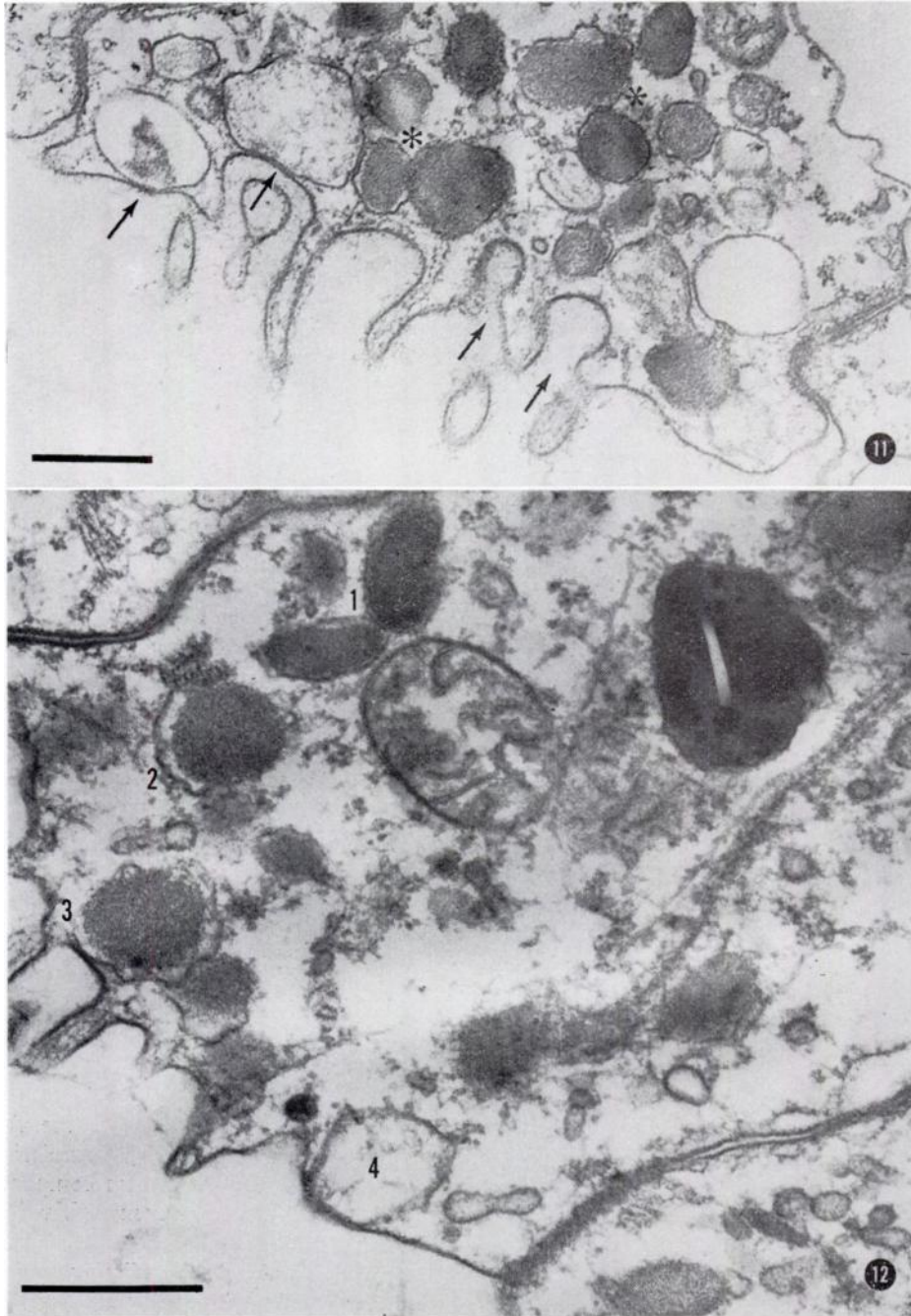
#### DISCUSSION

Ultrastructural data from epidermal cells of Scleractinian corals, supported by radiochemical assay, substantiate the presence of a crystalline structure which resembles the aragonitic polymorph of calcium carbonate. This intracellular crystalline product is demonstrable following preparation of tissues for electron microscopy, as well as in unfixed and treated samples from  $^{45}\text{Ca}$  uptake studies. Organization of the organic matrix of the crystal-bearing vesicle evidences the existence of crystalline inclusions *in vivo* prior to fixation or subsequent processing of the tissue. The preparative techniques used in this investigation are similar to procedures employed by other investigators of coral ultrastructure (Kawaguti, 1964; Kawaguti and Sato, 1968; Vandermeulen, 1974, 1975; Vahl, 1966) in which crystalline inclusions are denied.

The question of electron-lucency of the aragonite crystal has led to considerable investigation on our part as to the appearance of calcium crystals in transmission electron microscopy. Reports in the literature of mineralized systems composed of aragonite have indicated these calcium crystals as electron-lucent (Vander-

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FIGURE 10. Montage of attached epidermal epithelium in the planula, *Porites porites*, showing localization of crystal-bearing vesicle (arrows) along surface of contact of organism with its substratum. A vesicle without crystals is being released in lower left of field (single arrow). Epidermis (EPI), mesoglea (MES) and gastrodermis (GAS) as designated for orientation. Compare surface appearance with Figure 8.



FIGURES 11 and 12. Attached surfaces of epidermis of *Porites porites* evidencing release of contents of Golgi vesicles by exocytosis. Scale bars are  $0.5 \mu$ .

TABLE I  
Incorporation of  $^{46}\text{Ca}$  by adult *Astrangia danae*.

Exposure	Fraction					
	NH <sub>4</sub> OH tissue extract		HCl tissue extract		HCl skeletal extract	
	cpm	% total cpm	cpm	% total cpm	cpm	% total cpm
20 min	40,626	94.2	1,340	3.1	1,165	2.7
2 hr	57,406	92.9	2,501	4.0	1,972	3.1
6 hr	89,023	92.8	4,453	4.6	2,583	2.6

meulen and Watabe, 1973), as well as electron-dense (Wilbur *et al.*, 1969). Another author (Anderson, 1973) has categorically stated that calcium is electron-dense in transmission microscopy. Our experience has been that the section thickness is critical for determination of electron penetrability. Sections with silver-gray interference color reveal electron-lucent crystals in the vesicles of adherent coral planulae and adult polyps.

Identification of an intracellular crystalline product contributed by the epidermal cells to the site of skeleton formation prompts emergence of a novel concept of cellular regulation of events culminating in coral skeletogenesis. Mechanisms by which cells of the organism dictate specific construction or design of their skeleton must now encompass regulatory roles for the vesicle membrane, the plasmalemma, the protein synthetic machinery (rough endoplasmic reticulum), the packaging system (Golgi membranes, agranular reticulum) and, in general, the entire physico-chemical character of the apical cell cytoplasm.

The membrane-bound Golgi vesicle serves as a miniature crystal fabrication station as well as a vehicle for transport of the crystal to an appropriate site for discharge. Release of the crystal, embedded within an organic matrix and endowed with a pre-established affinity for ionic calcium and a charge of lipoprotein-bound calcium, allows continued calcification extracellularly. The charged organic matrix is especially important since skeletal exchange of calcium with the environment is improbable (Goreau and Goreau, 1960). Microprobe analysis of cytoplasmic vesicles without crystals in another reef-building coral has established that they are a site of accumulation of calcium (Vandermeulen, 1975). The vesicular membrane gains significance as a site of regulation of calcium ion transport and of generation of carbonate ion perhaps through enzymatic action of carbonic anhydrase as suggested by Goreau and Goreau (1960). The phosphatase

FIGURE 11. Vesicles in cell cytoplasm become confluent with one another (asterisks), fuse with plasma membrane and discharge contents as vesicle membrane is integrated into cell surface (arrows).

FIGURE 12. Assortment of vesicles, one crystal-bearing, at various stages of release of contents by exocytosis. Confluence of vesicles (1), clear zone developed around granular matrix (2), near-fusion between vesicle membrane and plasmalemma (3) and membrane fusion with discharge of vesicle contents (4).



enzymes, especially the alkaline phosphatases, if localized in or on the vesicle membrane, may exert inhibitory influence over crystal formation as suggested by Simkiss (1964).

The physical and chemical status of the apical cell cytoplasm is important with respect to transport and release of the crystalline product. For release into the extracellular milieu contact between the vesicle membrane and the plasmalemma must be established. Beyond that, however, nucleation of the intracellular crystal itself may be dependent upon the environment of the vesicle. Two of the most critical influences upon biological crystallization are pH and ion strength (Bachra, 1973). Abrupt alteration of either or both of these factors with settling of the coral planula could trigger  $\text{CaCO}_3$  clusters or crystal embryos which spontaneously grow into crystals within the calcium-loaded vesicles.

The observation that crystal generation encompasses a larger area of epidermis than just the substrate-contacted surface indicates that changes within the epidermis do not relate to the morphological transformation at that site. Upon settling, cessation of locomotory function by the flagellae may provide the stimulus for local responses in the apical cell cytoplasm inducing crystal formation uniformly throughout the epithelium. That the entire epidermis of the planula with the exception of the juxta-stomodeal epithelium would be competent to generate aragonitic crystals seems logical since the planula has no control over the size, contour or consistency of its settlement site. Whatever the orientation of the planula upon settlement, or whatever the extent of the substratum, crystal-bearing epidermis is available for establishment of the skeleton.

Calcium-accumulating vesicles within the intracellular matrix of vertebrate ossification systems have revealed needle-like crystalline structures identified as apatite (Anderson, 1969; Bernard and Pease, 1969; Bonucci, 1970). These vesicles appear to be the site of initial deposition of calcium phosphate crystals in cartilage, bone and dentin. As early as the seventh day of development of the chick femur, mineralized vesicles have been sighted confirming their physiological significance in embryogenesis. Although the vertebrate vesicles might be derived from chondrocytes, they have only been observed as independent extracellular structures in vertebrate tissues (Anderson, 1973). In contrast to the intercellular vesicles in vertebrate systems, those described in Scleractinia in this study are unquestionably intracellular.

The granular matrix material of the vesicles remains uncharacterized in both vertebrate and invertebrate systems. However, as originally proposed by Anderson (1967) the osmiophilic character of the material strongly suggests that it is lipidic. The observation in Scleractinia of myelinized configurations parallel to the crystal surface suggests that this material is lipo-protein and that it is capable of spontaneous organization along an appropriate interface into trilaminar membranous profiles.

It is important to note that the crystalline structures which have been described in this study in corals are not interpreted as precursor substances nor as building blocks to be utilized in skeleton formation. The process by which the coral skeleton is generated is envisioned as a process of nucleation which utilizes seed crystals liberated from the epithelial cell to initiate skeletogenesis. Once the seed is deposited extracellularly, principles governing crystal growth in a supersaturated



solution may prevail. Extracellular mineralization proceeds with the initial crystal serving as a nucleation catalyst for formation of other crystals by epitaxy. The organic matrix which is liberated along with the seed crystal provides an intrinsic supply of ionic calcium. The organic matrix is eventually engulfed by the mineral product thereby limiting the skeletal growth consequent to one episode of intracellular crystal release. The free-swimming larvae possess granular vesicles, but crystals appear only after the decumbent organism has become adherent and is engaged in skeletogenic function. The adult coral gives evidence that this process is spontaneously continued throughout the life of the organism and supports the interpretation that the skeletogenic potential of the single crystal and its organic matrix is self-limited.

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#### SUMMARY

Orthorhombic aragonitic crystals, embedded with a granular lipo-protein matrix and surrounded by a trilaminar membrane, are localized in the apical cytoplasm of epidermal cells of Scleractinian corals. Adult specimens of *Astrangia danae* (Agassiz) and settled planulae of *Porites porites* (Pallas) contain crystals averaging  $0.7 \mu$  by  $0.1 \mu$  by  $0.3 \mu$  within Golgi-derived vesicles. Short-term labelling with  $^{45}\text{Ca}$  reveals distribution of radioactivity among a basic tissue fraction (92%), an acid tissue fraction (5%) and a skeletal fraction (3%).

Identification of the primordial crystal population within membrane-bound vesicles provides overwhelming evidence for the intracellular mode of calcification in Scleractinia. Moreover, it permits development of a novel concept of cellular regulation over these dynamic events. The membrane-bound vesicle is a miniature crystal fabrication station and a vehicle responsible for transportation of seed crystals and an organic matrix material to sites of discharge from the cell. The vesicle membrane becomes a probable locus of active transport and enzymatic activity as well as a physical barrier to be penetrated for release of vesicle contents into the extracellular milieu. Contact between the vesicle membrane and the plasmalemma would result in exocytosis and the onset of skeletogenesis. Principles governing crystal growth would prevail from then on. The released crystal becomes a nucleation catalyst and the organic matrix, a supply of ionic calcium for self-limiting crystallization. Crystals are produced by the organism spontaneously and continuously from shortly after larval attachment throughout the life of the polyp. Therefore, these membrane-bound vesicles signal the dynamic process by which initiation, differentiation, growth and limitation of the coral skeleton is regulated.

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