the imbalance left by the removal of Mare Orientale and Mare Marginis, but the results reported here eliminate the possibility of any presently verifiable scheme.

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X-ray Diffraction Studies of Echinoderm Plates

Abstract. *X-ray diffraction studies confirm that, with few exceptions, each skeletal element of echinoderms is a single crystal of magnesium-rich calcite and that a relation exists between the shape of the element and the crystallographic* a- *and* c-*axes. The exceptions include the teeth of echinoids, and the calcareous ring as well as the anal teeth of holothurians. The tubercles of an echinoid plate begin their growth as parts of the single crystal of the plate; under the mechanical action of the spines that are attached to them, they become partly polycrystalline, as shown by scanning electron microscopy and by x-ray powder diffraction. The interface between inorganic crystalline and organic amorphous matter in the skeletal element appears to be the first example reported in nature of a periodic minimal surface.*

The echinoderm skeleton is known to be composed of magnesium-rich calcite; no aragonite has been reported, no other crystalline inorganic or organic phase is present, and even evidence for a solid amorphous phase is lacking (*1*, *2*). However, there persists in the literature (*3*) confusion on the crystallographic nature of the skeletal element: Does it consist of a single crystal, subparallel crystals, or a polycrystalline aggregate? In its external shape a skeletal element, as strikingly shown recently by scanning electron microscope photographs (*4*), is completely noncrystallographic, consisting of mammillated surfaces that show neither planes nor straight edges, leave alone interfacial angles! Nevertheless, it must be crystalline if it diffracts x-rays. It is monocrystalline, that is, made up of "a single crystal," if all its parts diffract coherently to give discrete spots on the film—proof that it must be referred to a single continuous lattice. It will be polycrystalline, if its parts diffract incoherently—smearing the spots into smooth continuous powder-diffraction lines if the aggregate is finely crystalline and into gritty-looking lines if it is coarsely crystalline. The grain size, which is the size of the component crystals, can be estimated from the appearance of the diffraction lines: diffuse, sharp, "gritty," corresponding to ~ 0.1 μm or less, ~ 1 μm, and ~ 10 μm or more, respectively.

We used the precession camera (with $MoK\alpha$ radiation), as well as the Weissenberg and back-reflection Weissenberg cameras (with $CuK\alpha$ and $FeK\alpha$), the powder camera ($CuK\alpha$), the petrographic microscope, and the electron probe. The rhombohedral calcite crystal is referred to its hexagonal triple cell, [$a \sim 5$ Å, $c \sim 17$ Å, $6(Ca,Mg)CO_3$ per cell, cleavage {10.4}]. The directions of *a* and *c* are used to describe the crystal orientation with respect to plate shape. The accuracy with which such an orientation relation can be determined is limited by the variation in the plate shape itself and by the range of orientations of the plates on the body, which can be considerable. The orientation relations we are reporting (Table 1) are accurate enough to insure that the first orientation pattern obtained on the precession camera will yield the desired crystallographic directions. In other words, perfect adjustment of these directions will not require angular corrections larger than 7° or 8°. At least three plates were tested from each species. They always agreed in *c* direction. The a directions were occasionally found to be somewhat more variable, although they too were found to be oriented when the plate offered a reference direction, be it a row of holes in a holothurian plate or the flattening of a spine in an ophiuroid. We have been warned (*5*) that generalizations based on so few samples may be hazardous; our present aim is mainly to induce more zoologists to explore this field of biocrystallography.

The study was begun with the intent of testing, by x-ray diffraction, a hypothesis proposed by Towe (*6*)—that the primary deposit of calcite in echinoid skeletal plates is polycrystalline, the shape of the deposit being thus readily adjusted to the needs of the organism. Recrystallization resulting in a single crystal would take place in the solid state as a secondary process, the shape of the polycrystalline plate being preserved. This is an intriguing proposal for the formation of a pseudomorph; it is the reverse of a process that is common in mineralogy: a single crystal transforms to, or is replaced by, another

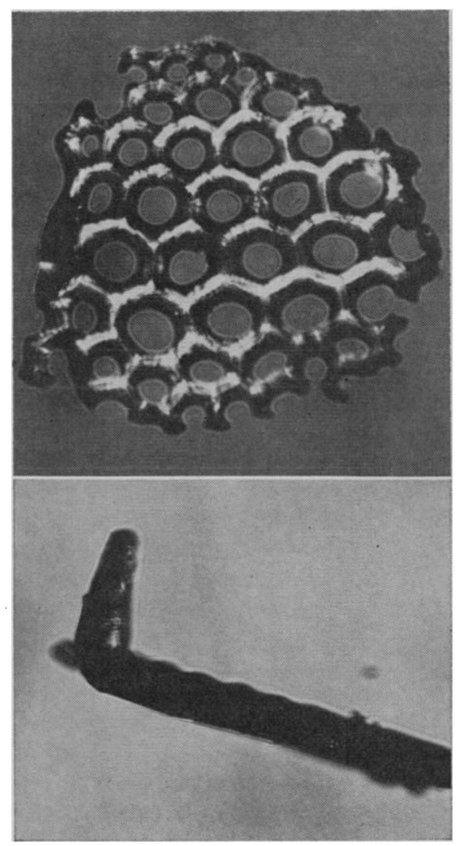

Fig. 1. (Top) Plan view of plate from holothurian *Echinocucumis hispida* (Barrett) between crossed nicols with *c* perpendicular to plane of viewing. It is imperfectly isotropic. In hexagonal net of holes, the *a* directions lie along the rows of holes. Largest horizontal width of plate: 0.42 mm. (Bottom) Profile view of the same plate showing spine at right angle to the plane of the plate. Spine and plate belong to one single crystal, at extinction between crossed nicols. Note perfect extinction: the *c* direction, along the spine axis, lies in the plane of the microscope stage.

single crystal or polycrystalline aggregate while the external shape of the original crystal is perfectly preserved.

We examined five coronal plates, taken from one interamb in a series adjacent to the apical system, from the echinoid *Strongylocentrotus droebachiensis* (Müller) (*7*). The smallest plate, about 0.7 mm in longest dimension, was of most recent origin, increasing size corresponding to increasing age. A polycrystalline deposit, at least about 800 Å thick, is detectable by the appearance of powder diffraction lines (that would show up as concentric circles on a precession film) and a comparison of the diffraction patterns from the different plates would show different stages of recrystallization.

The youngest plate, when completely immersed in the x-ray beam, gives a perfect single crystal pattern without any trace of powder lines. A 100-hour exposure of a zero-level reciprocal lattice net containing the strongest reflections 10.4 shows thermal diffuse scattering around the Bragg reflections, but no diffuse scattering that would indicate disorder in the crystal structure. No film blackening is visible except at the nodes of the reciprocal lattice. All crystalline parts of this small plate were able to contribute to the diffracted beams since the linear absorption coefficient of calcite for $\mathrm{MoK}\alpha$ is 2.19 mm^{-1} so that, after traversing the longest dimension of the plate, the emergent beam still has one fourth of the incident intensity. If a polycrystalline layer were present it would have to be less than approximately 0.1 μm thick to escape detection by x-ray diffraction. Such a thin layer might well be observable by low-energy electron diffraction (LEED), but it appears unlikely that it could exert any controlling influence on the shape and texture of the plate.

X-ray diffraction patterns leave no doubt that all of the plate, including the budding tubercle, forms one single crystal. The patterns are indistinguishable from those of optically clear calcite cleavage-rhombohedra of comparable weight. This statement applies to all specimens described below as single crystals. The cell dimensions are smaller, however, owing to the appreciable amounts of magnesium substituting for calcium in the crystal structure. We found $a = 4.933 \pm 0.001$ Å, and $c = 16.773 \pm 0.003$ Å at 23°C with wavelength $\mathrm{CuK}\alpha_1 = 1.54051$ Å, which corresponds to 13.5 atomic percent magnesium according to the calibration curves in the literature (*8*).

Monocrystalline skeletal elements. Of the other species studied (Table 1), only a few need special mention. Every spine was found to be one single crystal, elongated parallel to *c*, even when slight changes in cell dimensions indicate variations in magnesium content along its length. The striations along the spine of *Evechinus chloroticus* (Valenciennes) and the rows of holes along the spine of *Apatopygus recens* (Milne-Edwards) could not be related to the *a* direction because there are too many such rows and striations. Stem ossicles of the crinoid *Neocrinus decorus* Wyville Thomson show a five-rayed articulum containing a fivefold axis of morphological pseudosymmetry directed along the stem; an *a*-axis lies along one of the five ridges of the articulum. Adjacent plates do not always have the same ridge as an *a*-axis direction.

Cleavage. All attempts to induce cleavage were unsuccessful. We wedged, to no avail, a razor blade into the crystal parallel with the 10.4 plane, whose direction had been determined by x-ray study; we fractured crystals with a hammerblow, and could not find cleavage fragments with the microscope. Neither macroscopic nor microscopic cleavage could be observed. If any cleavage did exist, the cleavage steps would have to be submicroscopic in area.

Table 1. Orientation of calcite crystallographic axes in skeletal elements of echinoderms.

Species studied*	Skeletal element	*c*-Axis orientation	*a*-Axis orientation
	Echinoidea (sea urchins)		
Strongylocentrotus droebachiensis (Müller)	Interambulacral plate	Perpendicular to plate	Unpredictable
Heterocentrotus mammillatus (Linnaeus)	Interambulacral plate	Perpendicular to plate	Not determined
Evechinus chloroticus (Valenciennes)	Interambulacral plate	Perpendicular to plate	Along line connecting mouth and plate Along line connecting anus and plate
	Primary spine	Along long axis	Not identified
	Holothuroidea (sea cucumbers)		
Placothuria huttoni (Dendy)	Scale	Perpendicular to plane of flattening	Perpendicular to long axis
	Plate	Perpendicular to plate	Along a row of perforations
Pentadactyla longidentis Hutton	Plate	Perpendicular to plate	Along a row of perforations
Psolidium diomedeae (Ludwig)	Oral and anal valves	Perpendicular to plane of valve	(Discussed in text)
Echinocucumis hispida (Barrett)	Plate	Perpendicular to plane of plate	Along a row of perforations
Heteromolpadia marenzelleri (Theel)	Anchor plate	Perpendicular to plane of plate	Along a row of perforations
	Asteroidea (sea stars)		
Patiriella regularis (Verrill)	Actinal interradial plate	Perpendicular to plate	Along long axis
	Actinal intermediate spine	Along long axis	Perpendicular to plane of flattening
Asterina miniata (Brandt)	Adambulacral spine	Along long axis	Along plane of flattening
Astrostole scabra (Hutton)	Adambulacral spine	Along long axis	Along plane of flattening or perpendicular to it
	Ophiuroidea (brittle stars)		
Ophionereis fasciata (Hutton)	Arm spine	Along long axis	Along plane of flattening or perpendicular to it
	Ventral arm plate	Along long axis of plate	Perpendicular to plate
	Crinoidea (sea lilies)		
Neocrinus decorus Wyville Thomson	Stem ossicle	Along axial canal	Along a ridge of articulum
Above species plus *Notocrinus virilis* Mortensen	Arm ossicle	Along axial canal	Not determined
	Pinnule	Along axial canal	Perpendicular to ridge
	Cirrus	Along axial canal	Perpendicular to long axis

* The orientations given are not necessarily the same for other species of the given classes.

Optics. Thin translucent plates taken, for example, from the body wall of the holothurian *Echinocucumis hispida* (Barrett), show strain (or form) birefringence when immersed in oil in parallel light, between crossed Nicols. Such a plate, found by x-rays to be a single crystal of calcite, is not perfectly isotropic when viewed along its *c* direction (Fig. 1). Spines of echinoids also show this effect, but give sharp extinctions in planes containing *c*, thus showing that their uniaxial index ellipsoid has been deformed into a biaxial one. Since our results were obtained on untreated skeletal elements, we are sure that they are not due to mechanical deformations that could have been induced by the method of sample preparation, such as the grinding of a thin section.

Polycrystalline skeletal elements. Echinoid teeth have long been known to be composed of several discrete parts (*9, 10*). The teeth of *Evechinus chloroticus* (Valenciennes) are subparallel aggregates of monocrystalline "whiskers" and small packages of curved sheets, with *ca* planes approximately perpendicular to the length of the tooth. The normals a^* to the *ca* planes all lie within a cone of 4° half-angle around the direction of tooth elongation. The whiskers have a length-to-diameter ratio ranging from 50 to 150. They are readily separated from one another. Since the whiskers are aligned parallel to the tooth axis, their *c*-axis is normal to their length. The few curved, strongly adhering, monocrystalline sheets in one package tightly clasp the whiskers together. The sheets are in subparallel orientations and simulate a single crystal. No two packages, however, show even approximately similar crystallographic orientations. These packages are the only example we found of random orientation.

On the basis of histological studies, Moss and Murchison (*11*) called attention to the unusual structure of the anal teeth and calcareous ring of the holothurian *Actinopyga mauritiana* (Quoy and Gaimard). Using optical techniques, they found multiple *c*-axes in both of these structures. An x-ray study of the anal teeth of *A. mauritiana* showed that each tooth contains several large (100 μm) monocrystalline grains randomly oriented with respect to one another and to the shape of the tooth. In addition, a "gritty" powder pattern shows the presence of much smaller grains (10 μm) with random orientations. Thus we confirm the findings of Moss and Murchison for *a* as well as for

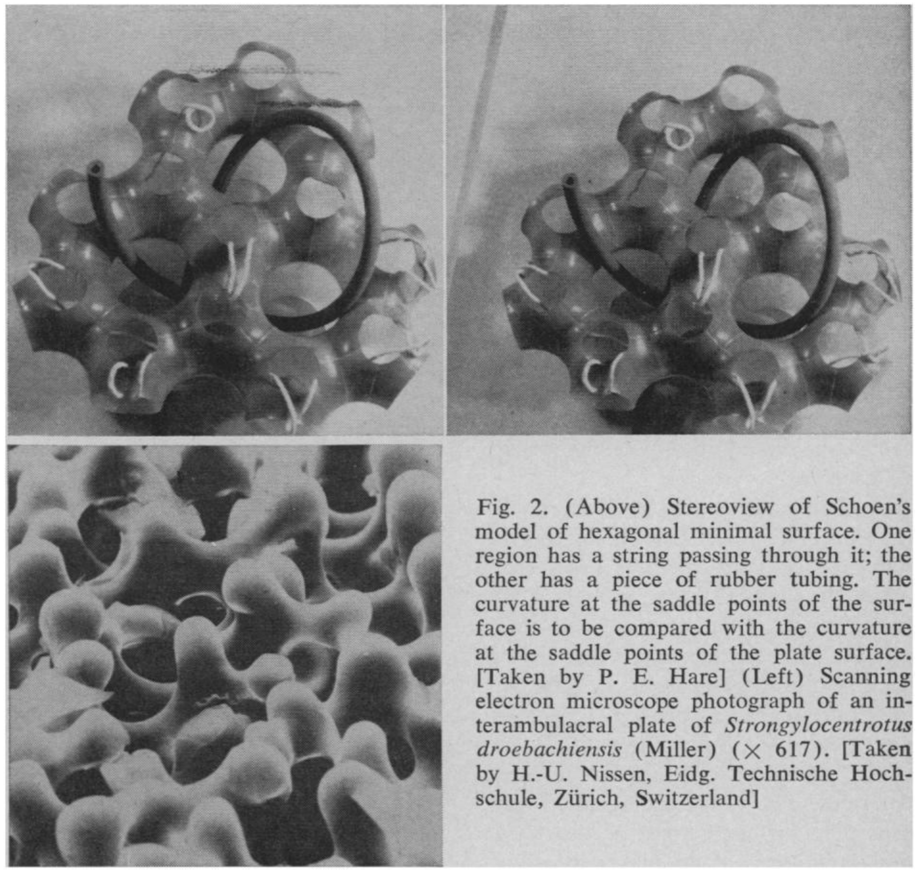

Fig. 2. (Above) Stereoview of Schoen's model of hexagonal minimal surface. One region has a string passing through it; the other has a piece of rubber tubing. The curvature at the saddle points of the surface is to be compared with the curvature at the saddle points of the plate surface. [Taken by P. E. Hare] (Left) Scanning electron microscope photograph of an interambulacral plate of *Strongylocentrotus droebachiensis* (Miller) (× 617). [Taken by H.-U. Nissen, Eidg. Technische Hochschule, Zürich, Switzerland]

c directions. The x-ray pattern of a portion of the calcareous ring of the same species shows only a gritty powder pattern without preferred orientation. A grain size of 10 to 100 μm in longest dimension is indicated. Since the ring serves as an area of insertion for several muscles, it is subject to stress. Mechanical action may be responsible for breaking up the few original crystals into many small grains.

We could find nothing to support Moss and Murchison's hypothesis that the condition of the ring and anal teeth may be taken as evidence for crystal twinning. The only twinning we observed in any of the specimens here studied occurred in a test plate of *Strongylocentrotus purpuratus* (Stimpson) from which the primary tubercle had been removed with a razor blade; x-ray precession photographs taken before and after the operation proved that twinning had been mechanically induced; the twin law is (0001).

Oral and anal valves of the holothurian *Psolidium diomedeae* (Ludwig) show only one *c*-axis direction per plate but four distinct *a* directions in their common 0001 plane covering a small angular range, which was measured to be 6° for one plate and 8° for another. Such an edifice can be described as a monoperiodic twin, here a fourling, with twin axis *c* and with arbitrary twin angle.

As mentioned above, the budding tubercle on the young plate of *Strongylocentrotus droebachiensis* appeared as part of the single crystal of the plate, whereas previous optical and electron microscope studies, by Raup (*3*) and Towe (*6*), respectively, on spine-bearing tubercles of echinoid plates showed them to be polycrystalline. We therefore studied numerous tubercles of different ages, including the non–spine-bearing rudimentary tubercles of the cidaroid *Cidaris rugosa* (Clark). Young spine-bearing tubercles and non–spine-bearing tubercles of any age were found to be part of the single crystal of the plate on which they grow. The mechanical action of the base of the spine upon a tubercle gradually pulverizes the spongelike parts of the single crystal, a process analogous to the mylonitization of the petrologists. Spine-bearing tubercles of juvenile echinoids thus become polycrystalline as the animals grow; rudimentary tubercles of cidaroids, which do not bear spines in adults, remain in crystalline continuity with the plates that carry them and do not change with age. Scanning electron microscope photographs of tubercles (*12*) strikingly confirm the above conclusions. The base of the spine, which grinds the tubercle to a powder, does not itself show evidence of mechanical breakdown.

The only other crystalline phase we encountered in some of the plates was NaCl. Sharp, uniform powder lines became visible, for example, on rotation patterns taken for 5 hours or more with CuKα radiation. Much longer exposures are needed for precession films taken with Mo radiation. Soaking the specimen in water, or household bleach, or alcohol for 1 week did not affect the powder-line intensities. Spines of animals taken live out of seawater and placed immediately into alcohol showed no trace of crystalline NaCl (*13*). Spines from animals that had been allowed to dry in air after being taken from the sea gave the NaCl pattern, as did the spines from animals first allowed to dry and then placed into alcohol. We conclude that the living organism prevents salt from crystallizing in the interior of its skeleton. Crystallization takes place when the animal dies and the seawater contained in its shell evaporates. The randomly oriented salt grains then formed cannot be dissolved from the intact calcite plates, possibly because the solvents used have high enough surface tension so that they cannot penetrate the interior of the spongelike crystals.

At a recent symposium one of us (G.D.) listened to a lecture by Schoen on the partitioning of space by periodic minimal surfaces of which, so far, no example has been reported in nature (*14*). Such a surface divides space into two interpenetrating regions each of which is a single multiply connected domain. The two regions have no connection with each other. A plastic model built by Schoen of one of these surfaces, with symmetry *P6/mmm*, bears a striking resemblance to the scanning electron microscope photographs of echinoderm plates (Fig. 2). Here one region could be filled with stroma, the other with calcite; the surface would be the interface between the inorganic crystal and the amorphous organic matter. Maximum contact for crystal growth exists, and the single crystal nature of the shell is explained by the connected nature of the crystalline domain.

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Crystal Orientation and Plate Structure in Echinoid Skeletal Units

Abstract. *The submicroscopic morphology of magnesian calcite skeletal units of echinoids, revealed by scanning electron microscopy, was compared with crystal orientation data obtained by x-ray methods and with macroscopic morphology. The Perischoechinoidea and the Euechinoidea differ with regard to the shapes of their trabeculae. Nearly all plates and spines are single crystals. A variety of different directional relations of* c- *and* a-*axes to the main morphological directions are found for different species; adjacent plates with identical* c-*axis orientation differ strongly in orientation of their* a-*axes. Fracture surfaces of single trabeculae show cleavage planes and zonal layers attributed to changes in secretion conditions.*

The skeletal parts (stereom) of all Echinoidea, which have been a favorite object of study with the polarizing microscope (*1*), are composed of crystalline units of calcite. Each skeletal plate and most spines behave optically like a single crystal, as do also the recrystallized fossil skeletal units (*2*). On the other hand, the earliest detailed microscopic description of these parts (*3*) stressed the fact that they have a spongelike internal structure so that only a small fraction of the plate volume is occupied by crystalline calcite. The lack of planar crystal faces in the morphology of the stereom did not appear compatible with the optical result that these plates were single crystals. However, optically only the *c*-axis and not the complete orientation (that is, at least one other crystallographic direction) had been determined (*2*). Single crystal x-ray methods corroborated the assumption of single crystals: The skeletal elements of adult, juvenile, and larval stages of recent species of echinoids were found to be monocrystalline (*4*). The only exceptions are the complicated polycrystalline teeth of echinoids (*5*).

The spongy nature representing an external shape not bounded by single crystal faces, on one hand, and the single crystal nature of the plates, on the other, lead us to the conclusion that the living part (stroma) of the echinoid acts on the growing stereom in such a way as to inhibit growth in certain directions. Thus the living matter determines the overall plate shape. The problem of plate and spine formation is, therefore, a special and possibly relatively simple case of the general biological problem of form determination, whereby the product is a single crystal with a known atomic structure while the stroma causing the crystallization is part of a chemically complicated animal.

In order to collect more information necessary for the understanding of the genesis of such "spongelike" crystals, more x-ray single crystal data have been obtained with the use of Laue and precession cameras, as well as an x-ray texture camera that can also be successfully used for polycrystalline materials, such as lamellibranchs and gastropods (*6*). The same objects investigated by x-rays were then studied with regard to their outer shape and their fracture surfaces in a scanning electron microscope (SEM) (*7*), which is particularly well suited to the investigation of objects with complicated surface morphology (*8*).

The fracture surfaces of *Sphaerechinus granularis* (Fig. 1) are a typical example of the "mammillate structure" formed by innumerable trabeculae with-