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Crystal Orientation and Plate Structure in Echinoid Skeletal Units

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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  $\text{CuK}\alpha$  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 sponge-like 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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12. Dr. H.-U. Nissen (Eidg. Technische Hochschule, Zürich, Switzerland) kindly made SEM photographs of skeletal elements.
13. J. Halpern (University of Miami) and Miss H. E. S. Clark (Victoria University, New Zealand) collected and treated live starfish for the NaCl study.
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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 Perischoechnoidea 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 *Sphaerichinus granularis* (Fig. 1) are a typical example of the "mammillate structure" formed by innumerable trabeculae with-

in the plates. Very similar SEM photographs were obtained with ambulacral and interambulacral plates of *Strongylocentrotus franciscanus* and *Echinocyamus* cf. *megapetalus*, as well as with the thin scalelike platelets of *Tromikosoma hispidum*. The system of trabeculae in echinoid spines is similar. A

typical example is given in Fig. 2 taken of a spine of *Sphaerechinus granularis*. In *Dendraster excentricus* (and in some parts of the lantern of *S. granularis*) the material is much less porous, leaving only relatively small rounded channels for the protein permeating the entire stereom. The only different trabecular texture

found occurs in coronal plates of *Cidaris rugosa* (Fig. 3), which have a more regular network of trabeculae and lack mammillate shapes. Since *Cidaris* is the only Recent representant of the Perischoechinoidea (9), this seems to indicate that large systematic and phylogenetic differences are accompanied by

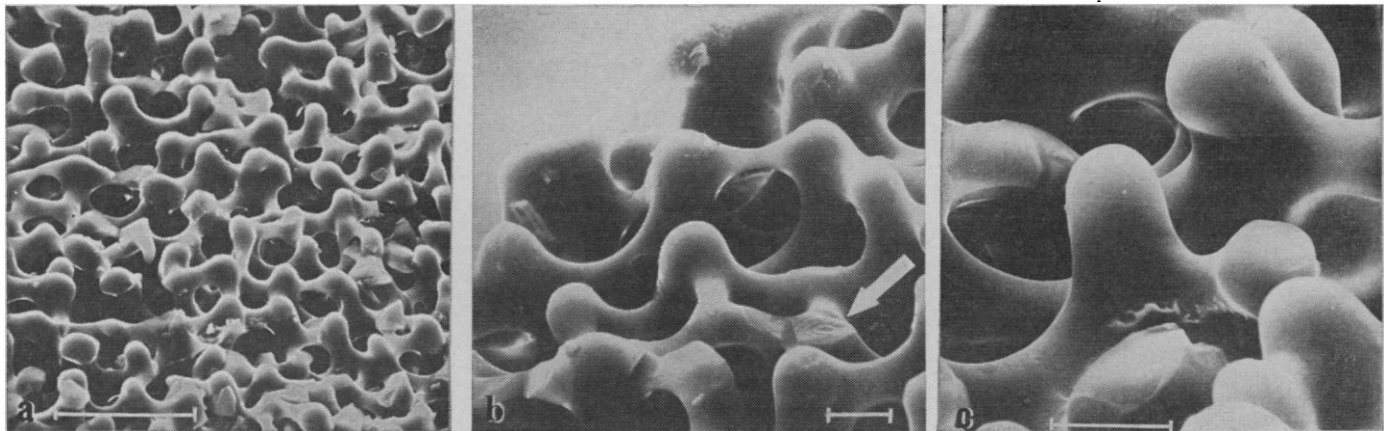


Fig. 1. Fracture surface of echinoid interambulacral plate (*Sphaerechinus granularis* from Cannes, France). Even at high magnifications in an electron microscope, the crystal surface is rounded and shows no indications of faces determined by crystal geometry. Individual trabeculae are connected by bridges. Zonal layer-structure in broken trabecula shown in (b) is marked by arrow. Scale: (a) 0.05 mm, and (b) and (c) 10  $\mu$ m.

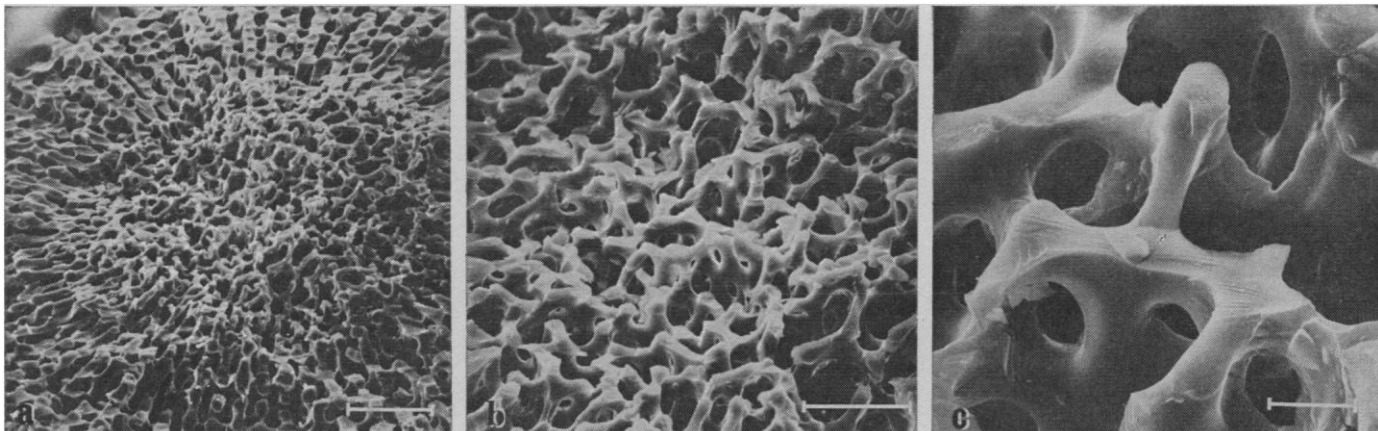


Fig. 2. Interior of fracture surface normal to the length of a monocrystalline echinoid spine (*Sphaerechinus granularis*). Scale: (a) 0.1 mm, (b) 0.05 mm, and (c) 10  $\mu$ m.

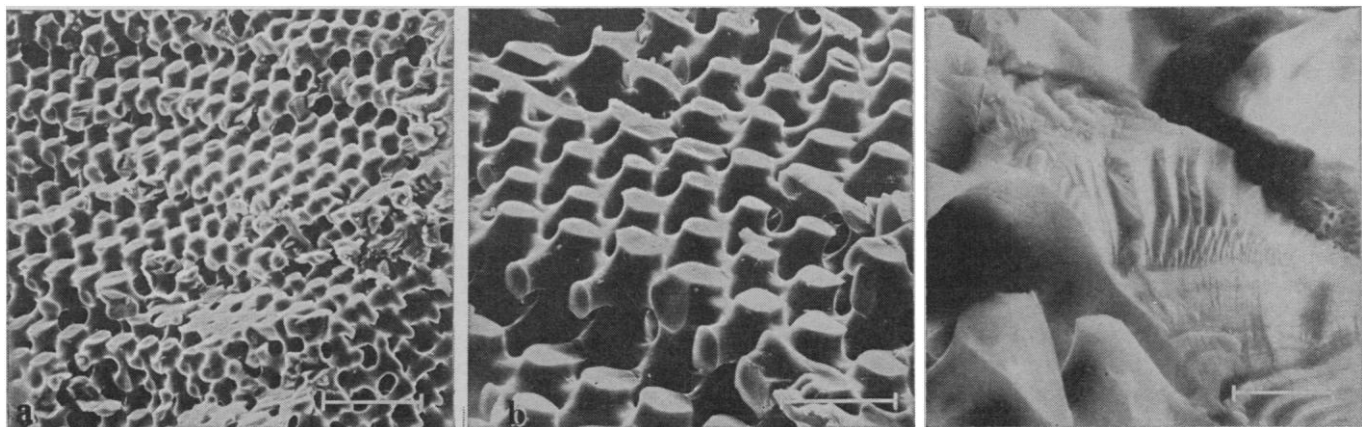


Fig. 3 (left). Fracture surface of coronal plate of *Cidaris rugosa*. Scale: (a) 0.1 mm and (b) 0.05 mm. Fig. 4 (right). Zonal layer-structure interrupted by cleavage planes in skeletal carbonate of *Dendraster excentricus*. Scale: 10  $\mu$ m.

differences in the morphology of the system of trabeculae, as visible in the SEM. This assumption is strengthened by comparing the corresponding structures of holothuroids, crinoids, asteroids, and ophiuroids, examples of which were also photographed in the SEM.

While the outer shapes of the trabeculae are generally rounded, the fracture surfaces of single trabeculae are in part strictly planar, and their position with regard to the plate coordinates and the crystal axes known from the x-ray studies indicates that they are rhombohedral planes  $\{10\bar{1}1\}$  (Fig. 4). The areas showing these cleavages are up to 0.1 mm in diameter and thus within the range of optical microscopy, especially in the specimens with relatively little "pore volume." However, the fracture surfaces show mainly what is usually called "conchoidal fracture" (Fig. 2c). This is very unusual for calcite, fragments of which are normally completely bounded by cleavage faces, and it may explain why echinoid spines and plates show no preferred macroscopic fracture surfaces. A possible explanation is seen in the area marked by an arrow in Fig. 1b as well as in Fig. 4—on this cross section a zonal structure is visible. Nothing is known as yet about the chemical or crystallographic differences (or both) of these zones, which may be growth structures. It is likely that this layering gives rise to the unusual morphology of fracture surfaces of the trabeculae.

For evaluation of relations between the crystallographic symmetry and the shape symmetry of the biocrystal the lattice position must be fixed in space relative to the organism's morphology. Echinoid plates usually have their  $c$ -axes either parallel or perpendicular to the plate surface (10). In *D. excentricus*, for example,  $c$  is normal to the plate surface within  $1^\circ$  of approximation. Nine aboral plates were investigated in a juvenile specimen of this species. The plates were left in their mutual orientation relation, that is, the entire bottom of the skeleton was mounted on a Laue camera. Two photographs were taken in such a way that the x-ray beam could pass through parts of two adjacent plates. In spite of an astonishing parallelism of  $c$ -axes (the bottom in this species is rather flat), the  $a$ -axes vary considerably in their position, though generally one of them points approximately toward the

anus. In one specimen a pair of adjacent plates, lying on the morphological symmetry plane, show the two lattices rotated with respect to each other  $60^\circ$  around the  $c$ -axes, which approaches a twin relation to a high degree of approximation. This orientation relation is unusual because in some hundred echinoderm skeletal units investigated the crystals were always found to be single crystals without any twinning.

Measurements of lattice orientation were made by using the texture x-ray camera (6) because, besides the common cases where  $c$ -axes are parallel or perpendicular to the plate surface, certain species exist (4) in which the  $c$ -axis is inclined to the plate surface and the angle of inclination varies from plate to plate (2). In two plates selected respectively from *Sphaerechinus granularis* and *Strongylocentrotus franciscanus*, having nearly identical  $c$ -axis orientations with respect to morphology, the  $a$ -axes differ by a rotation around the  $c$ -axes of approximately  $60^\circ$ . These examples show the great variety of lattice orientations with regard to shape and symmetry elements of the skeletal units which must be expected in the echinoids. Many more species must be investigated before these measurements can be used successfully for the evaluation of phylogenetic relations.

Recent physiological findings (10) are compatible with the assumption, which is offered as a working hypothesis, that the collagen fibers found in the hollow spaces between the trabeculae act chemically as a substance that inhibits further secretion of calcite around them. These fibers, therefore,

are assumed to act in an opposite way to the organic envelope, secreted by the cells at an earlier stage, that promotes calcite growth. The layering shown in Fig. 4 seems to indicate changes in the secretion conditions during growth of a plate.

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## Cystine: Compartmentalization within Lysosomes in Cystinotic Leukocytes

**Abstract.** *The large amount of cystine compartmentalized in cystinotic leukocytes cosediments in isopycnic sucrose density gradients with dense lysosomal particles, within which it is presumably contained. Such cystine appears to be primarily noncrystalline in these organelles.*

Nephropathic cystinosis, cystine-storage disease of childhood, is a recessive heritable biochemical disorder characterized by a high intracellular concentration of cystine and accumulation of cystine crystals in many organs of the body (1). Such accretion within the kid-

ney is associated with renal tubular dysfunction and glomerular damage leading to death in uremia usually before puberty.

The precise subcellular location of the cystine has not been determined. In cystinotic leukocytes and fibroblasts, the