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11. Whole blood hemolyzates assayed in this study contained a variety of nonhemoglobin erythrocyte and plasma proteins, some of which influence immunodiffusion reactions between HbF and antiserum to HbF. To minimize this effect and to maintain comparability between HbF standards and the hemolyzates being assayed, standards from the HPFH homozygote were diluted with whole blood hemolyzate from a normal adult; the resulting standards uniformly contained $\sim$ 55 mg of adult hemoglobin per milliliter. The diluent hemolyzate came from an individual who had $\leq$ 0.03 percent HbF, based on assay of a sample with a total hemoglobin content of 87 mg/ml. Immunodiffusion reaction areas obtained with such standards, after correction for the assumed maximum of 0.03 percent HbF in the diluent, were slightly larger than those developed when corresponding amounts of HbF were diluted with water or buffer. Although the origin of this augmentation is uncertain, it is not attributable to HbA which, when purified and used as a diluent, has no influence on reaction areas.
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15. Ratios of phenylalanine to isoleucine for the HPFH heterozygote were measured in hydrolyzates obtained directly from washed erythrocytes. This procedure differs from that previously reported (*12*) insofar as the hemolyzates used here were not otherwise fractionated. The validity of our procedure, when applied to the HbF-rich hemolyzate from the HPFH heterozygote (88 percent HbF during an episode of iron-deficiency anemia; MCH, 14.4 pg per cell) who is also heterozygous for thalassemia, was established by a parallel finding of nearly perfect agreement between observed (3.84) and expected (3.75) phenylalanine/isoleucine ratios when erythrocytes from the HPFH homozygote (100 percent HbF; MCH, 23.7 pg per cell) were examined in the same way.
16. Our resolution of the diameter of each pericellular immunoprecipitate (Fig. 1) was abetted by repeated changes in focal plane, a maneuver not easily illustrated. Although many reaction diameters still remained hard to define, two different observers (S.H.B. and T.K.B.), looking at unidentified preparations, repeatedly obtained similar measurements; furthermore, the mean reaction diameter of F cells from any one subject was distinctive and remained grossly constant in day-to-day sampling. In any case, the crude nature of diameter estimates does not affect our conclusions. For example, among six subjects whose F cell contributions of HbF lie above the dashed line in Fig. 2, arbitrary twofold depreciation of mean pericellular diameters would only lead to a closer fit to that line. Accordingly, diameter estimates, while the least reliable of our measurements, weigh no more heavily in their errors than the other forms of measurement used.
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Vaterite: A Mineralization Product of the Hard Tissues of a Marine Organism (Ascidiacea)

Abstract. *X-ray diffraction and infrared absorption spectra show that the spicules of the common tropical ascidian,* Herdmania momus, *are mineralized with vaterite. These are the first strictly marine organisms known to normally precipitate vaterite. The biomineralization of vaterite may constitute another link between the urochordates and vertebrates. The vaterite of ascidian spicules immersed in natural seawater remains mineralogically unchanged for 1 year, which indicates that vaterite may be preserved transiently in marine sediments.*

Vaterite ($CaCO_3$) is a metastable polymorph of crystalline calcium carbonate. It is easily precipitated in the laboratory, but it is rare in nature as it usually converts to one of the more stable carbonate phases, aragonite or calcite, and possibly to monohydrocalcite ($CaCO_3 \cdot H_2O$) (*1*). Vaterite is precipitated in nature by a variety of organisms, both normally and under pathologic conditions (*2*, *3*). It has been found also in artificially induced skeletal repair and in laboratory cultures under abnormal conditions (*4*, *5*). In the sea vaterite is synthesized by two fish species: *Acipenser sturio*, a sturgeon, which migrates occasionally from rivers to marine waters, and *Gadus morrhua*, a cod, which deposits vaterite under pathologic conditions (*3*).

Here we report the normal skeletal mineralization by vaterite of a sessile, benthic marine animal, the tunicate, *Herdmania momus* (Savigny, 1816) (Urochordata: Ascidiacea), and comment on its possible evolutionary and sedimentary implications. *Herdmania momus* is a highly variable species (or species complex), widely distributed in warmer seas. It is most abundant in nearshore waters 0 to 30 m in depth, but occasionally occurs below 500 m (*6*). Its mineralized hard parts consist of fusiform spicules, fringed by rows of

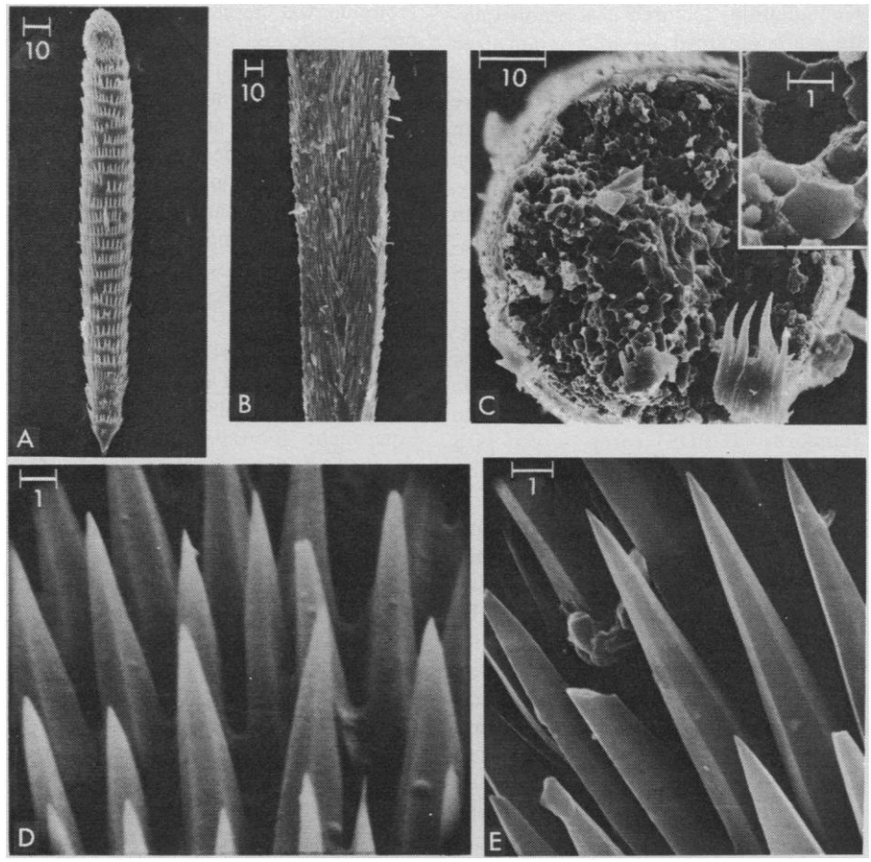

Fig. 1. Scanning electron micrographs showing spicule morphology (A); crystal fabric of the spicule core as seen in a lengthwise cut (B) and in cross section (C); arrangement and crystal habits of fringing spines (D); and crystal shapes of fringing spines of a spicule after exposure to seawater for 1 year; note the patchy, crystal coatings (E).

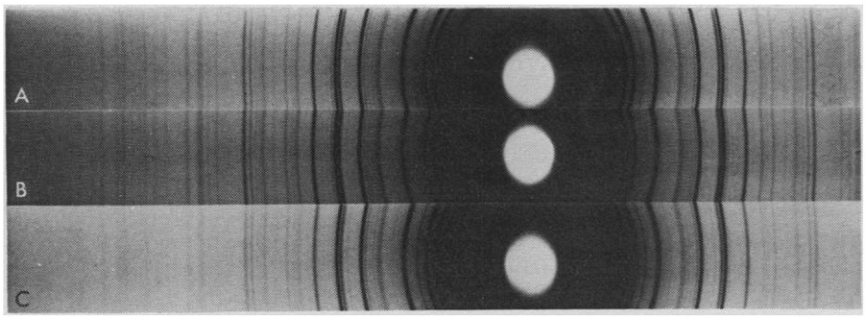

Fig. 2. X-ray diffraction patterns of (A) reference vaterite; (B) spicules from Tasmania; (C) spicules from Hawaii.

appressed spines (*6*). The spicules in our samples range in length from 170 to 1710 μm and in width from 20 to 60 μm. This study is based on samples obtained from the Atlantic, Pacific, and Indian oceans (*7*) and preserved in 70 percent ethanol.

Spicules were extracted from the body tissues with a 5.25 percent solution of NaClO. Scanning electron micrographs were obtained with an Etac Autoscan and a Cambridge Mark II instrument. X-ray diffraction analyses were performed on powdered samples in milligram quantities with a Debye-Scherrer camera and nickel-filtered Cu Kα radiation. Infrared absorption spectra were obtained on microgram-sized samples with a Perkin-Elmer 180 spectrophotometer. Semiquantitative chemical analyses were made with an ETEC-MAC V SM3 automated electron microprobe. Spicules weighing 2 mg, extracted from a single specimen with NaClO, were stored at room temperature in 700 ml of sand-filtered natural seawater in a sealed Pyrex container for 12 months (*8*). Artificially precipitated vaterite crystals, matching the x-ray diffraction pattern indexed on Joint Committee Powder Diffraction Standards (JCPDS) cards, served as a reference sample in x-ray diffraction and infrared analyses (*9*).

Scanning electron micrographs show that spicules in samples from all three oceans have the same basic building plan: a fusiform axis, surrounded by rows of equidimensional, anteriorly inclined, tapering spines (Fig. 1A). Spicules of samples from different localities differ in the shape, size, and morphology of their fringing spines. Lengthwise cuts show that the spicule core is composed of acicular crystals, which radiate at an angle from the center (Fig. 1B). Transverse sections of the core indicate that the crystals vary in diameter and have hexagonal outlines (Fig. 1C). The crystal faces are rarely equidimensional in densely packed aggregates (Fig. 1C insert, upper right corner). The fringing spines of the spicules are surface extensions of the acicular crystals, with basal prismatic sections and pyramidal terminations (Fig. 1D). The dimensions and shapes of their prismatic and pyramidal sections are always the same within single individuals but show considerable variations in spicules from different populations.

Electron probe analyses show that Ca is the major constituent (average 39.4 percent) and Sr, Mg, P, Fe, Na, and K are present in trace amounts (totaling 0.3 percent) (samples from one Atlantic and two Pacific localities). X-ray diffraction patterns of spicules from Tasmanian and Hawaiian specimens (Fig. 2, B and C, respectively) match that of the reference vaterite sample (Fig. 2A) in both spacings and intensities. A comparison of the infrared spectra of the synthetic vaterite standard and of spicules from samples from seven other localities shows them to be identical.

The spicules stored in seawater for 1 year were sampled quarterly. Scanning electron micrographs obtained after the experiment showed no morphological changes of the spicular crystals (Fig. 1E). Localized surface coatings, composed of micrometer-sized crystals, developed on the crystal faces of the fringing spines during the experiment (Fig. 1E). Infrared absorption spectra showed the presence of vaterite and, after 6 months, the addition of trace amounts of calcite.

The data show that the spicules in adults of *H. momus* are mineralized entirely with vaterite. These ascidians are the first strictly marine organisms known to normally precipitate vaterite in nature.

Ascidians are invertebrate members of the phylum Chordata. They share with the vertebrates a common plan of embryonic development, a notochord, a dorsal tubular nerve cord, a postanal tail, pharyngeal clefts, an endostyle (homolog of the vertebrate thyroid gland), and the ability to synthesize thyroxine. At a more tenuous level, even the production of cellulose by ascidians has its vertebrate parallels (*10*).

We suggest that the biomineralization of vaterite may indicate another such parallel. Among invertebrates, vaterite occurs only as a precursor of the more stable polymorphs, aragonite and calcite, in the repair of exoskeletons of adult gastropods and bivalves (*4, 5*). In the chordates, vaterite, while rare, occurs as a stable strengthening agent of tissue-enclosed hard parts in adults, that is, in the form of spicules of certain ascidians and the otoliths of some fish. Available data indicate that skeletal mineralization of vaterite in adults is a transient, short-lived phenomenon in the invertebrate phyla; in the chordates this carbonate phase is a stable constituent. Future investigations should show whether this distinction is more than a coincidence (*11*).

Vaterite has been precipitated from a solution approximating seawater (*12*), but not from natural seawater (*13*), nor has vaterite been found in marine sediments (*13*). The occurrence of vaterite in otoliths of two fish species indicates biosynthesis of this carbonate polymorph in the sea (*3*). *Acipenser sturio* is a transient marine dweller, and it is not known whether vaterite accretion continues during its brief incursions into the open sea. Our study of *Herdmania momus* provides the first conclusive evidence that vaterite is widely synthesized by a strictly marine animal.

Vaterite precipitates of individual indigenous and transient marine chordates range from 55 μg to about 200 mg (*11, 14*). At present, *H. momus* appears to be the most important agent of vaterite mineralization. While it is not yet possible to estimate the volume of vaterite biologically synthesized in the sea, it is doubtless quite small compared to the enormous volume of biologically precipitated calcite and aragonite. Our experiment shows that the vaterite spicules of this ascidian remained mineralogically unchanged after immersion in natural seawater for 1 year. In the absence of evidence to the contrary, we interpret the calcite deposits on the spicular crystal faces as products of direct precipitation from seawater. If the experiment approximates natural conditions, one can expect vaterite-bearing hard tissues to reach the sea

floor mineralogically unchanged. This supports the possibility of short-lived vaterite preservation in the initial phase of its incorporation into tropical marine sediments at shallow depth. To determine whether vaterite-bearing hard parts become transient constituents of the depositional environment under these conditions will require careful search for them in shallow-water marine deposits.

HEINZ A. LOWENSTAM
Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena 91125
DONALD P. ABBOTT
Hopkins Marine Station, Stanford University, Pacific Grove, California 93950

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Immunoglobulin-Positive Cells: Their Appearance in the Mixed Lymphocyte Reaction

Abstract. *In mixed lymphocyte cultures prepared with thoracic duct lymphocytes from allogeneic rats, approximately 20 percent of all blast cells that appeared at the end of 72 hours of incubation had surface receptors for rabbit antibody to rat immunoglobulin.*

It is generally accepted that in the mixed lymphocyte reaction (MLR) small lymphocytes from allogeneic donors transform into blast cells, synthesize DNA, and proliferate (*1, 2*). Most of the evidence to date indicates that the responding lymphocytes and the resulting blast cells are thymus-derived (T) cells (*3*). However, evidence suggesting that bone marrow–dependent cells (B cells) with immunoglobulin (Ig) receptors on their surfaces may also undergo blastogenesis in the MLR (*4*). In view of the fact that the MLR represents a cell-mediated immune response (*5*) and that such responses may possibly involve both T and B cells (*6*), our study was undertaken to further explore the possibility that B cells respond in the MLR.

To prepare MLR's, equal numbers (5×10^6) of cells from the thoracic duct lymph (TDL) of young adult Lewis male rats and F_1 hybrids of the Lewis and Brown Norway (BN) strains were mixed together in 5 ml of culture medium (*2*). Under these conditions, the MLR response is considered to be unidirectional with parental Lewis cells reacting against the BN component of the F_1 hybrid (*7*). For controls, nonmixed cultures were prepared with 10×10^6 TDL cells from either the Lewis or F_1 donor. After 72 hours at 37°C, cells from each culture were treated with 10 μg of ^{125}I-labeled rabbit antibody to rat Ig for 30 minutes at 4°C (*8*). The cells were then washed, and smears were prepared for light microscopic radioautography. The antibody to Ig (Cappel Laboratories, Inc.) was iodinated by the method of Greenwood *et al.* (*9*) and had a specific activity of 7.0 μc/μg. Although the purity of the antibody to Ig was assured by immunoelectrophoresis, two additional tests were carried out in order to further define the specificity of the antibody. First, 10×10^6 Lewis rat thymocytes were treated with ^{125}I-labeled antibody to Ig. Since less than 0.3 percent of the cells were labeled, we concluded that the antibody to Ig would not bind to cells lacking surface receptors for Ig. Second, the ^{125}I-labeled antibody to Ig was incubated with purified rat Ig prior to its exposure to MLR cells. Because lymphocytes and blast cells failed to label, we concluded that the Ig had reacted with the antibody to Ig, thereby inhibiting the binding of the latter to cell surfaces.

The percentage and types of labeled cells in control and MLR cultures were determined by counting at least 1000 cells per slide. Lymphoid cells were classified as either small lymphocytes (SL) or large and medium lymphocytes (LML) (*10*). Blast cells were defined as those cells greater than 14 μm in diameter and having a nucleus with a fine chromatin pattern, observable nucleoli, and intensely basophilic cytoplasm.

Results indicated that TDL cells from either Lewis or F_1 (Lewis × BN) animals consisted of only SL's and LML's before culture and at the end of 72 hours of incubation. When Lewis and F_1 cells were mixed, the cells present after 72 hours included SL's and LML's as well as a significant percentage (23 percent) of blast cells. Since the total number of cells surviving after 72 hours has been shown to be approximately the same for both mixed and nonmixed cultures

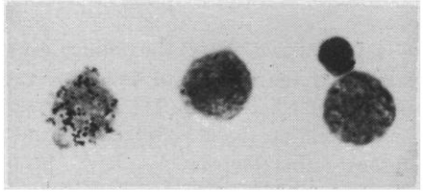

Fig. 1. Light microscopic radioautograph of a blast cell (left) treated with ^{125}I-labeled antibody to Ig. Two unlabeled blasts (center and bottom right) and a typical small lymphocyte (top right) are also shown (× 1000).