Turning Blood Cells into Oyster Shells

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The general consensus has been that the shells of mollusks form outside of cells. Akin to bone formation in vertebrates, an organic matrix is secreted by cells and the inorganic material is deposited within this matrix in an organized way. Andrew Mount, A.P. (Hap) Wheeler, Rajesh Paradkar, and Dennis Snider have now found convincing evidence that blood cells (specifically a class of granulocytic hemocytes) may be directly involved in shell crystal production for oysters.[2]

In vitro studies of molluscan shell formation usually require supersaturated levels of calcium carbonate, much higher than levels measured *in vivo*. Other studies have shown that secondary nucleation events on matrix-coated crystals *in situ* are probably not involved in shell formation.

Mount *et al.* reasoned that one alternative explanation of the lack of these extracellular events may be that crystallogenic cells could supply crystals to the mineralization front. Such circulating amoeboid hemocytes could account for a large nonexchangeable pool of calcium known to exist in these animals. In oysters, there are two major types of amoeboid cells in the hemolymph: agranulocutes and granulocytes. The agranular cells tend to spread out as thin cells and secrete collagen fibers. Granular cells are more motile, having more of a macrophage-like function. A subclass of these granulocytes contains birefringent granules and is known as refractive (REF) granulocytes. The function of these particular cells has not been fully elucidated.

In order to determine if REF granulocytes play a role in oyster cell formation, Mount *et al.* cut a notch in the cell margin to induce rapid (a matter of a few days) shell growth. There are three distinct layers observed in the adult oyster shell, and observations with the scanning electron microscope (SEM) revealed that the regenerating layers do resemble their normal counterparts with some minor exceptions. Some of the elements are not as closely packed in the regenerating shell and some of the surfaces are not identical to the adult.

Interestingly, REF granulocytes increased from 5% to 15% of the total hemocyte population 48 hours after notching. Furthermore, SEM revealed crystal-shaped inclusions in these cells. X-ray microanalysis (SEM-EDS) confirmed that these crystals contain higher levels of calcium than surrounding regions of the cell. These crystals appeared to be released at the mineralization front. These and other observations, including observations of living cells with a vital fluorescent stain, suggest that REF granulocytes are capable of delivering calcium-containing crystals to the site of shell formation.

Crystals released at the regeneration front quickly remodel to resemble normal shell structure. Hemocytes may even participate in this process. Additional studies established that the number of REF granulocytes and their crystals are more than sufficient to account for sustained calcification of the shell.

Although direct cellular involvement has been shown in other mineralization events, such as spicule formation, this is the first report that hemocytes can initiate shell formation. Mount *et al.* conclude that crystal formation in molluscan shells involves complex interactions between organic phases and cells. ■

[1] The author gratefully acknowledges Dr. Andrew Mount for reviewing this article.

[2] Mount, A.S., A.P. Wheeler, R.P. Paradkar, and D. Snider, Hemocyte-mediated shell mineralization in the eastern oyster, *Science* **304**:297-300, 2004.

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From a collection of wine images by Tom Saunders, see the article on page 36. Residual evaporation crystals from a mixture of "Cabernet Franc" wine and vitamin B6 imaged by a Zeiss Axiophot; 5x/0,15 Plan-Neofluar lens utilizing cross polarization with a rotating analyzer slider. A quartz plate Red 1 Lambda insert and an adjustable cellophane strip was inserted above the condenser. Image captured with a 6.1mpx digital camera. Image adjustment with Photoshop 7: unsharp mask and slight contrast increase -- no color adjustments. Final image 4x6 feet on archival canvas with archival pigments. Part of the Art of Science Collection by T.H. Saunders©2005.

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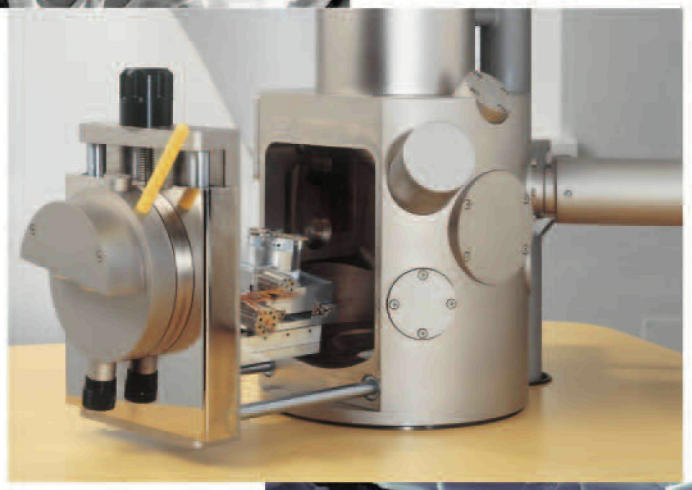

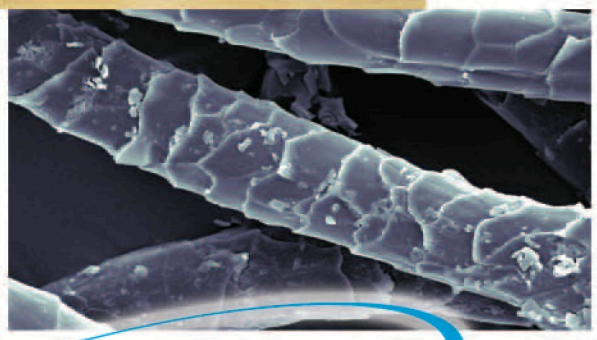

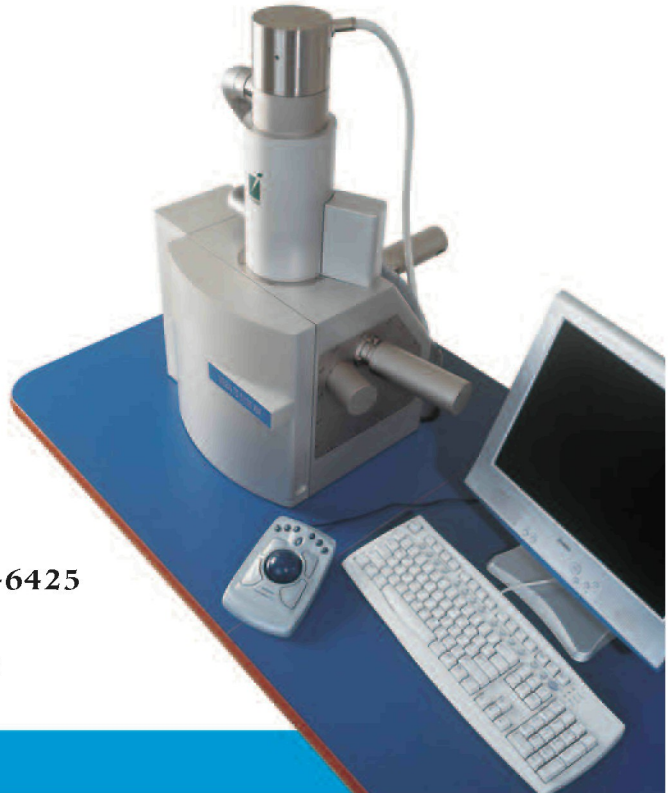

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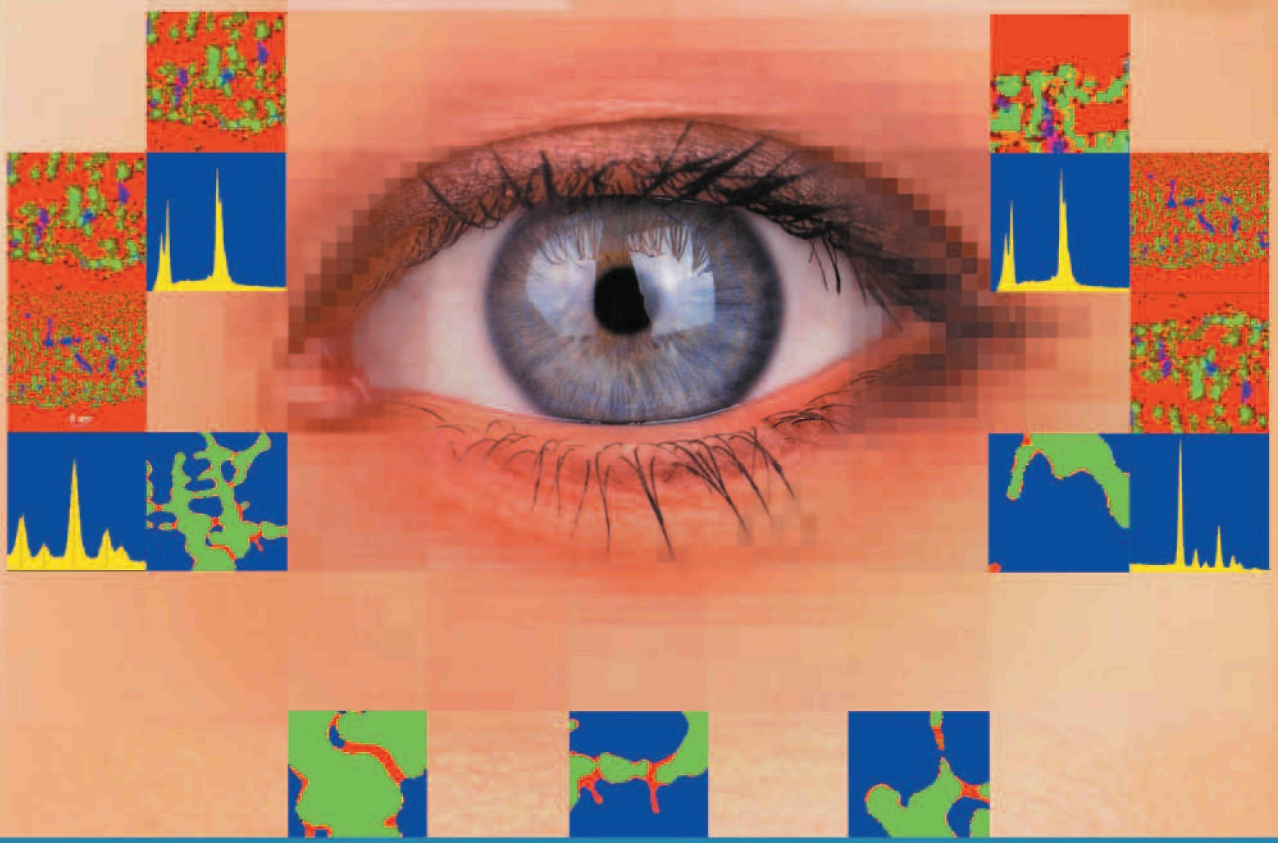

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