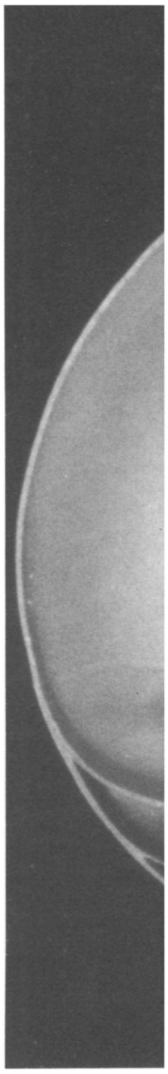
Natureworks

Making minerals the biological way



By ELIZABETH PENNISI

For beach lovers, the seashell symbolizes sand, surf and sun. Children quickly learn to tap its hidden talents. By putting one close to their ears, they can listen for that familiar ocean roar.

But for materials scientists, the seashell symbolizes something much more elusive. They cherish the seashell's integrity: Though made from simple stuff, mollusk shells are quite tough and strong. In fact, organisms create a whole host of hard materials, each suited to a particular purpose.

Out of seawater, oysters fashion pearls, the nautilus crafts chambered compartments, snails make intricately spiraled shells, sea urchins grow long, piercing spines and bacteria piece together internal compass needles that guide them to suitable environments (see sidebar, p.330). In a matter of hours, a hard, protective shell begins to encase a hen's developing egg. Vertebrates make bones that they repair and remodel throughout their lives and teeth sculpted to meet their particular grinding, gnashing, ripping or munching needs.

Organisms fashion all these biominerals with great precision and under conditions that would make many processing engineers envious. Over and over they demonstrate that whatever engineers can do, nature can do better and on a much finer scale. "We haven't tried the molecular engineering that exists in nature," says Arthur H. Heuer, a materials scientist at Case Western Reserve University in Cleveland.

As researchers strive to create and customize new materials, they come to appreciate the prolific artistry of biological systems. "The cells are really kind of amazing little workhorses," says David J. Fink, a chemical engineer with CollaTek, Inc., in Columbus, Ohio. Sometimes these cells work like master artists, taking years to create a finished product. Other times, they dash off a new layer of biomineral quite quickly.

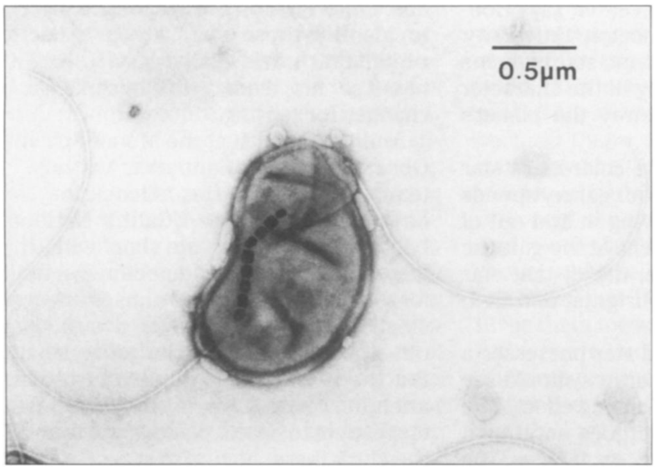
Slow growth yields layers of thin inorganic plates sandwiched in an even thinner organic matrix, a "glue" of proteins and other molecules, says Heuer. These layers lie parallel to the substrate upon which they form. With fast growth, the matrix and mineral build columns perpendicular to the substrate surface. Each organism shapes this hard stuff while depositing it, often by incorporating building blocks into a hierarchical architecture that repeats itself from microscopic to macroscopic dimensions. Different levels of the hierarchy impart different properties to the finished material.

Biominerals offer almost everything a materials scientist could want – without requiring any special processing environment. Seashells consist of calcium carbonate, the stuff of chalk. Yet "the toughness of the abalone and the pearl oyster is twice as high as any high-tech ceramics," says Mehmet Sarikaya, a materials scientist at the University of Washington in Seattle. "Also, the shells are tough and strong at the same time." Most synthetic materials fail because even tiny cracks grow, compromising the material's integrity. But in biominerals, small cracks tend not to grow, says Arthur Veis, a biochemist at Northwestern University Dental School in Chicago.

By looking in great detail at the structure of a few biominerals, Sarikaya, Veis and others have begun to fathom what makes the shell so tough and strong (SN: 12/9/89, p.383). At the March meeting of the American Physical Society in Indianapolis, Sarikaya described a recipe for mimicking nature that may help scientists improve synthetic materials. Eventually, he and others hope to learn enough of nature's secrets to duplicate what organisms seem to accomplish with so little effort.

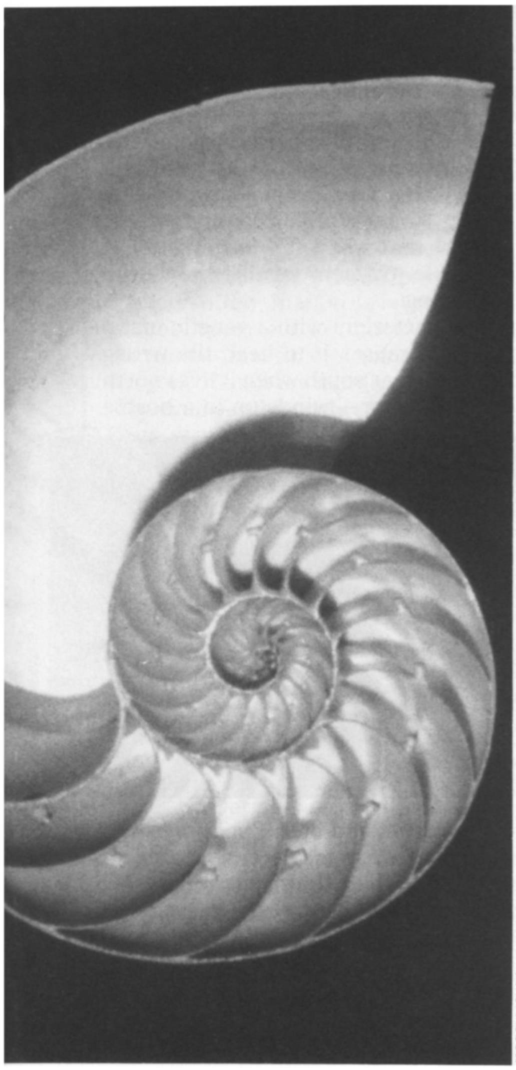
"You know that a complex ceramic material got made somehow, so there's incentive to learning how that is done," says Fink. "The key is to be able to isolate and reconstruct what happens."

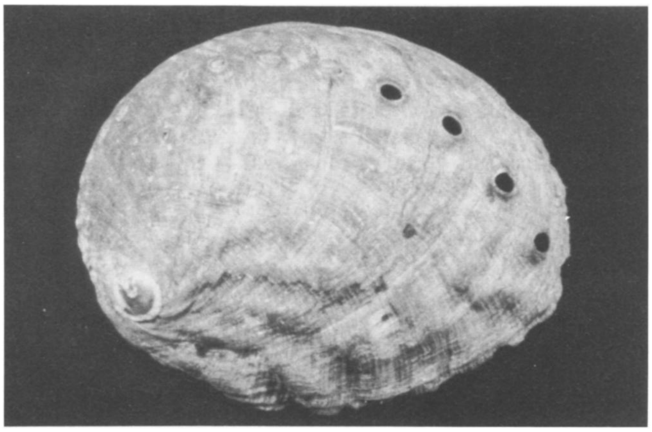
The secret lies in the way organisms



Electron micrograph shows bacterium with its chain of rectangular, membrane-encased magnetic particles, which help orient the microbe as it uses its two flagella to swim.

Bazylinski/Virginia Tech





Though quite different in appearance, the thick-shelled red abalone (near left) and the delicate nautilus (far left) build their shells in a similar way, one that materials scientists hope to mimic.

Photos: R. Tucker Abbott

put a mineral's molecules together. Organisms seem to rely on proteins and other large molecules to order inorganic molecules and to control the size and shape of the resulting crystal. Last month, geologists used the atomic force microscope to observe the formation of one such crystal, calcite (SN: 4/18/92, p.246). Other research groups are now working to determine which molecules direct that process.

"The kind of understanding that we will get from biology certainly will give us more insight into how to do a better job," says Heuer, who with several colleagues summarized current understanding of biomineralization in the Feb. 28 SCIENCE. That understanding has developed only after years of effort.

Six years ago, while visiting the Olympic Peninsula in Washington, Sarikaya bought a red abalone shell from a street vendor. The abalone survives for decades, adding about an inch a year to its armor until the shell measures about a foot across. The shell, a natural ceramic consisting of 95 percent inorganic material, fascinated Sarikaya. Until then, biologists had studied shell structure by first removing the inorganic component. Sarikaya wondered what he would see if he examined the shell from a materials scientist's perspective – with the inorganic part intact.

He and University of Washington colleague Ilhan A. Aksay observed that the shell consists of two layers. Calcite makes up a rough outer layer, while aragonite makes up the inorganic portion of the inner layer, called the nacre.

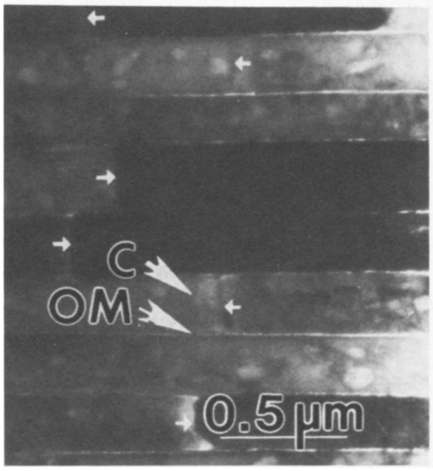
They expected abalone aragonite to resemble the aragonite found in metamorphic rock in Aragon, Spain. But the two versions looked quite different. This means that the abalone cell somehow controls crystal development—a feat that still eludes materials scientists, says Sarikaya.

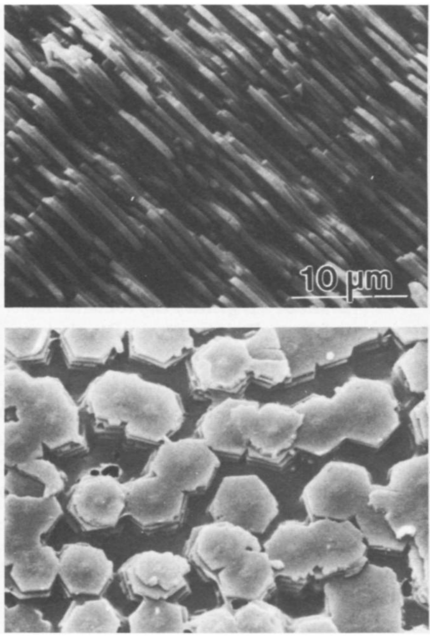
In abalone, they saw a brick-and-mortar configuration: stacked layers of six-sided calcium carbonate bricks, with a thin organic "mortar" in between. Each brick layer is about 0.25 micron high, while the mortar ranges from 10 to 50 nanometers thick.

The mortar itself seems layered. Large, acidic, protein-like molecules surround a core, which probably consists of a tough carbohydrate substance called chitin. Giant soluble molecules form a top coating. The acidic molecules assemble themselves in such a way that they form a template for crystal growth, says Sarikaya.

To their surprise, the researchers also found this brick-and-mortar arrangement in the free-swimming chambered nautilus and in pearl oyster shells, even though these mollusks shape the material quite differently. "Although these organisms diverged [evolutionarily] from each other hundreds of millions of years ago, they have exactly the same structure at the nanometer level," says Sarikaya. The abalone makes flat, impact-resistant layers one-half-inch thick so that otters and other predators must work quite hard to crack the shell. The nautilus, a

Micrographs show side (top right) and face-on (bottom right) views of nacre's aragonite bricks, with a close-up of brick-and-mortar construction (below), made up of calcium (C) and organic matrix (OM).





Sarikaya/Univ. of Washington

A biological orientation

Almost 800 years ago, Arabian sailors used a fish-shaped iron leaf suspended in water to guide their journeys northward on cloudy nights. But nature first harnessed Earth's magnetic field for navigation long before that. For millions of years, "magnetotactic" bacteria have made their own internal compasses.

Richard P. Blakemore, a microbiologist at the University of New Hampshire in Durham, discovered these organisms in mud samples almost 20 years ago, when he noticed that they tend to gather at the north end of water droplets. Since then, researchers have sought to understand how the bacteria make and use their microscopic compass needles. Recent interest in mimicking nature's handiwork has made some researchers wonder about recruiting these microbes to make magnetic particles for commercial applications.

"The bacteria are able to control the [particle's] size, shape and placement in the cells. This is submicron technology. We would like to know how they do it," says Richard B. Frankel, a biophysicist at California Polytechnic State University in San Luis Obispo.

Many kinds of bacteria make magnetic particles. Some make an iron sulfide crystal, such as greigite; others make magnetite, an iron oxide. Scientists are evaluating the phylogenetic relationship between these two types to understand better how – and how often – magnetic biomineralization evolved.

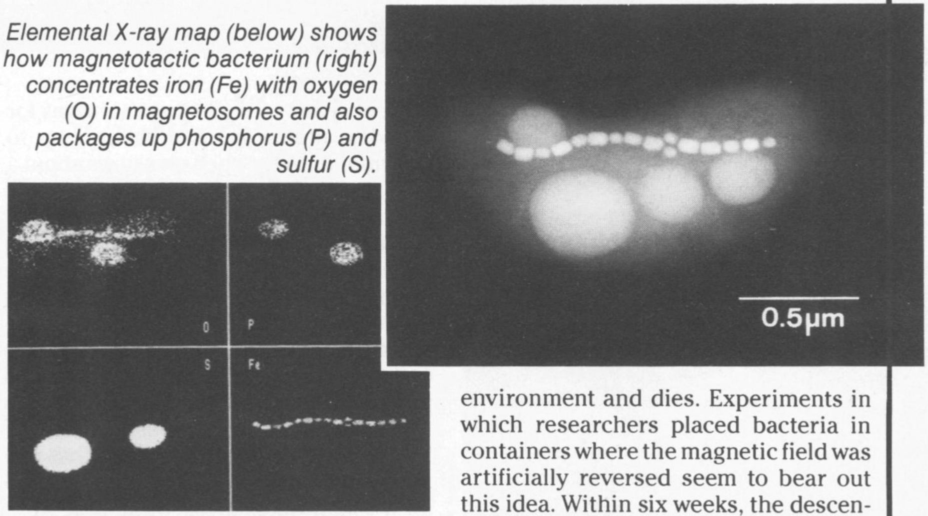
He thinks the magnetite makers collect iron and convert it to a stable iron oxide to create the magnetite crystal. But at least three species that have poor access to oxygen combine iron with sulfur instead, Frankel reported at the March 1991 meeting of the American Physical Society in Indianapolis. Iron accounts for 2 percent of the organism's total weight, making these bacteria "the most prodigious iron accumulators in the world," he says.

But each kind of bacterium customizes its particle, says Frankel. Some adopt cubic-octahedral arrangements, while others build six-sided or rectangular prisms. "This tells us that the mineralization part in these bacteria is very highly controlled," says Dennis A. Bazylinski at Virginia Polytechnic Institute and State University in Blacksburg.

"It's the equivalent of our producing bones and teeth. For a long time, people thought only higher organisms could do this [biomineralization]," says Frankel. But the closer researchers look, the more the microbial process seems to resemble that occurring in vertebrates. A membrane encases each particle, indicating that an organic component plays a key role in forming the inorganic component. That membrane probably contains a protein that lures iron compounds out of solution and concentrates them inside the membrane.

"What the membrane seems to do is not only regulate the deposition of the particle, but also control its position relative to the cell's other particles," says Frankel.

A microbe makes about 20 particles, each some 50 nanometers long – just big enough to have the internal polarization needed to orient in a magnetic field, but not so big that the particle has multiple regions of polarization, which might weaken the magnetic response. Then the particles line up north to south, and this chain polarizes the cell. "It creates a hierarchical structure," says Frankel. While each particle will orient itself, the series of 15 or 20 makes the magnetic orientation 20 times as strong. Thus, the Earth orients the cell along a north-south axis as the organism swims.



Elemental X-ray map (below) shows how magnetotactic bacterium (right) concentrates iron (Fe) with oxygen (O) in magnetosomes and also packages up phosphorus (P) and sulfur (S).

Bazylinski/Virginia Tech

Although a shovelful of mud can yield thousands of kinds of magnetobacteria, it took 15 years for microbiologists to grow one of these microbes in the lab, says Bazylinski, a microbiologist. Most bacteria either thrive in air or require an absence of oxygen. But most magnetic types are more finicky: Too much or too little oxygen can kill them. They won't grow in a petri dish in air.

Thus, in nature, most of these fussy bacteria thrive where the water forms stable layers and where each layer contains a certain oxygen content. In fact, they may use magnetism to help them find the perfect layer. As the microbes propel themselves along with their whip-like flagella, Earth's magnetic field turns them toward the poles. Southern-hemisphere bacteria head south; their northern counterparts head north. The closer they are to the poles, the more steeply Earth's magnetic field orients them downward as well as poleward, Bazylinski explains. That downward tilt pulls them away from oxygen-rich surface water. Bazylinski and others suspect that when the bacteria sense favorable oxygen levels, they stop swimming.

Any bacterium with a genetic mutation that causes it to head the wrong way – such as south when it lives north of the equator – winds up in a hostile environment and dies. Experiments in which researchers placed bacteria in containers where the magnetic field was artificially reversed seem to bear out this idea. Within six weeks, the descendants switch the direction in which they swim, says Bazylinski. Thus, natural selection seems to segregate north-seekers in one hemisphere and south-seekers in the other.

But orientation may be a side benefit of particle formation, Bazylinski suggests. He believes some bacteria may oxidize the iron to get energy. In addition, iron sulfide particles may be important in the cycling of sulfur through the environment, he says.

Growing these bacteria in the lab has proved quite a challenge. To create the right environment, Bazylinski puts hydrogen sulfide in the bottom of a test tube, then seals it and lets the sulfide gas and the air spread out. Inside, an oxygen-sensitive strip turns pink, letting him know the location of the air-gas boundary. That's where he starts his colony of magnetotactic bacteria. "I've got six strains now," he says.

Most of the interest in these microbes stems from a fundamental amazement that such simple organisms can accomplish something as complex as magnetic orientation. So even though some scientists envision creating microbial factories to produce biomaterials, neither Frankel nor Bazylinski thinks bacterially grown magnetic particles will ever coat cassette or computer tapes. "You've got to grow hundreds of gallons of cells to make a little magnetite," Bazylinski says. "It's not worth it."

– E. Pennisi

relative of the octopus, keeps its highly curved shell paper-thin and builds in chambers filled with air to make its body buoyant. This shell holds up even at depths of 1,800 feet, where pressures reach 55 atmospheres.

Such different properties may arise from the way the organisms lay their bricks. Like many other biological systems, abalone nacre exists in a hierarchical arrangement, Sarikaya and several colleagues reported at last December's meeting of the Materials Research Society, held in Boston. The Seattle team determined this after looking at the orientation and makeup of each aragonite crystal – a 5-micron-wide hexagonal brick – with respect to its vertical and horizontal neighbors. They saw that the crystals are imperfect. Each brick contains "twinning" defects that divide the crystal into four to six wedges, and each wedge is the mirror image of the one next to it. The researchers find that twinning also exists on smaller and larger scales. Within each brick's wedges, 1- to 4-nanometer-wide regions twin with their neighbors; twinning also occurs between bricks.

"For the first time, we find a hierarchical structure for hard tissue in a biological material," Sarikaya told SCIENCE NEWS. The twins are all touching each other, with no space in between. This hierarchy may confer abalone's toughness and strength. Sarikaya also thinks that similar defects somehow allow the nautilus to shape its developing shell and to make the highly curved chambers without sacrificing strength.

Now that they know what the abalone shell looks like on a microscopic scale, Sarikaya and his coworkers want to learn how the organism creates this hierarchy. Other researchers are investigating how other organisms build their bones, shells and teeth. "The key is that it's matrix-regulated," says Fink. "The matrix constrains the crystal in certain regions."

Proteins and carbohydrate-like molecules make up the organic mortar. These may set themselves up in an orderly formation such that one protein provides a flat surface. Chemical side groups stick out of the protein and cause dissolved molecules to precipitate and start forming a crystal. In teeth and mother-of-pearl, the matrix creates large biomineralization compartments where long crystals can form. But in bone, the compartments package the biomineral in very small, discrete crystals that wind up confined within collagen fibrils, says Heuer.

As part of his 30-year quest to understand biomineralization, Northwestern's Veis thinks he has a crystallization protein in hand for dentin, which makes up the inner bulk of teeth. The protein has phosphorus-containing side chains that may act as templates for crystal formation. Different organisms seem to rely on different proteins. The protein in mollusks, for example, may use lots of amino acids with acidic side groups as templates. With these observations, some scientists have begun making their own template proteins, some of which cause crystal growth that resembles biomineral crystallization, Veis notes.

To get a better grip on this process in other types of hard materials, Heuer, Arnold I. Caplan of Case Western Reserve and Jose L. Arias of the University of Chile in Santiago have chronicled the formation of chick eggshells. In about 20 hours, the cells in the lining of a hen's reproductive tract incorporate 2 grams of calcium carbonate into the rapidly forming shell. The process begins as egg white surrounds the yolk and then is cloaked by two fibrous membranes. The outer membrane seems critical to shell formation.

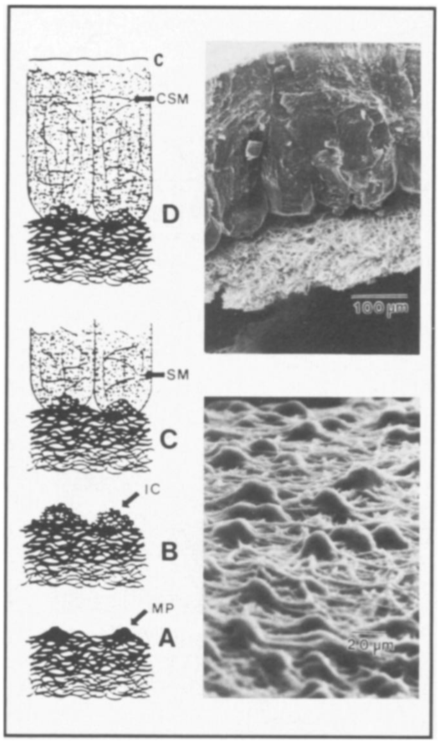
The scientists remove this outer membrane, which contains nodes of organic material thought to be the starting point for crystallization. Using the membrane as an organic film, they then examine details of mineralization.

Calcium carbonate crystals seem to grow up and out in all directions from the nodes. "When we grow calcium carbonate, it starts around the knobs but not between the knobs," says Fink, who is collaborating with the Cleveland group. As the crystals grow, organic molecules continue to assemble into the scaffolding that guides this growth.

In some experiments, the researchers added extracts from the organic matrix to the membrane preparation. The extract caused finer crystals to develop, they reported at last December's Materials Research Society meeting. They also think that at least one type of fibrous molecule, a collagen, may function by inhibiting biomineralization where it is not wanted. But like Sarikaya, they have yet to pin down the proteins that initiate mineral formation.

Sarikaya finds that by working backward, he can deduce the probable blueprint for the organic layer by the shape and structure of the crystals. On the basis of work done in part by Stephen Weiner at the Weizmann Institute of Science in Rehovot, Israel, and on his own observations of the aragonite microstructure, he hypothesizes that one type of protein, always folded the same way, is responsible and forms an orderly array. "They must organize in a pseudohexagonal fashion," he predicts.

Sarikaya has enlisted biologists to analyze the molecules, particularly proteins, present in this mortar. Once Clement E. Furlong, a geneticist at the University of Washington, knows which proteins are there, he can make antibodies for them. By labeling the antibodies, each of which attaches to a specific protein, Furlong can then determine which proteins wind up at the mineral-mortar interface and start the mineralization. Then he and microbiologist James T. Staley will genetically program bacteria to mass produce this protein. Once produced, the protein should provide a starting point for synthetic biomineralization, says Sarikaya.



Heuer et al/SCIENCE

On an eggshell membrane's knobs (a, lower micrograph), calcium crystals form (b). Then a matrix (c) guides the building of calcite columns (d, top micrograph).

This biological approach represents the cutting edge for materials scientists, who hope to duplicate, not just mimic, nature's artistry. "We want to use biology directly to do the chemical processing for us," says Paul D. Calvert, a materials scientist at the University of Arizona in Tucson. Someday, researchers may harness biomineralization to make entirely new materials with electrical, magnetic or optical properties as well as the seashell's toughness, adds Veis.

But achieving that goal is decades away. "People do not know where to start," says Sarikaya. "You need [experts in] five or six different disciplines to do significant research in this area."

"That's hard to do," he adds. ☐