The Great Crystal Caper

Chemists debate whether it makes scientific sense to try to grow macromolecular crystals in space

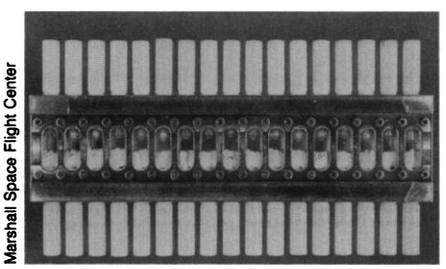
When Spacelab 2 is launched this summer, it will carry with it a tiny experiment that has become the focus of a debate among crystallographers. In a small corner of a lab pigeonhole rented by McDonnell Douglas are minute droplets of protein solutions. By the end of the week-long flight, if all goes well, the solutions should have formed crystals.

Researchers have for years been growing crystals of small molecules in space and there are theoretical reasons to hope that materials such as proteins and nucleic acids might also form bigger and purer crystals in a weightless environment. Crystal structures can help resolve lots of unanswered questions about how proteins and nucleic acids interact in three dimensions. Given a crystal, determining the three-dimensional structure is now relatively easy. However, growing large, pure crystals is a problem that hampers research now. Thus, the space experiment in space.

But a number of investigators have serious reservations about the whole project. It is not scientific, they say. The experiments are hastily conceived and poorly thought out. The chances of success are slim. Space experiment enthusiasts, while not contradicting the critics, take a somewhat different view. "The kind of people who get excited about this are the people who like toys. That's my kind of stuff. I have no qualms whatsoever," says Ponzy Lu, a biochemist at the University of Pennsylvania who, with his colleague Donald Voet, hopes to grow DNA crystals in space on an August shuttle flight.

The biological-crystals-in-space project was first conceived about a year ago by Charles Bugg, Lawrence DeLucas, and Frederick Suddath at the University of Alabama, in collaboration with Robert Naumann and Robert Snyder at NASA's Marshall Space Flight Center. Bugg, who is the principal investigator, says his interest was sparked by the realization that crystals of small molecules grow well in space. Then he and other crystallographers were intrigued by a report published in the 13 July 1984 issue of *Science* (p. 203). German investigators, Walter Littke and Christina John of the University of Freiburg, reported that two proteins they sent up on Spacelab 1 grew enormously. They grew lysozyme crystals that were 1000 times larger than those they grow on earth, and crystals of β-galactosidase that were 27 times larger than those on Earth. Although they did not give the actual sizes of the crystals they grew in space nor of the earth-grown crystals that they compared them to, their report was hard to dismiss.

"We were already working with Marshall when *Science* published the paper by Litke," says Bugg. "That paper is what got everyone interested. There are an awful lot of frustrated crystal growers out there. We now have 35 coinvestigators and that's just by word of mouth." These coinvestigators include major pharmaceutical firms as well as academic scientists.



Marshall Space Flight Center

Too many experiments?

One of two racks of tubes sent up in April's Spacelab flight. Each rack is 1 inch by 5 inches by 10 inches in size and contains 17 samples.

A major problem that besets crystallographers is how to make complex biological molecules form relatively large and pure crystals. Everyone agrees that crystal growing is more an art than a science and that there are precious few artists. The biggest difficulty, says Alexander Rich of the Massachusetts Institute of Technology, is probably the very nature of the biological material. "You may, for example, have a protein with a loose end. That loose end may be an important part of the molecule—it's supposed to be loose. But if the loose end is in the lattice-building part of the molecule, you won't get a crystal," Rich said.

Among the most difficult molecules to crystallize are segments of DNA. When James Watson and Francis Crick discovered the structure of DNA, they were not working from crystal data. Instead, they had DNA fibers, which only give information on two-dimensional structure. To go from fiber diffraction data to a three-dimensional structure, says Helen Berman, a DNA crystallographer at the Fox Chase Cancer Center in Philadelphia, Watson and Crick had to do some "very, very sophisticated and clever model-building." They came up with a model that represents an overall picture of DNA. But it does not tell anything about how the local structure varied around specific sequences. Richard Dickerson of the University of California at Los Angeles, who with Mary Kopka is the only one to have crystallized and solved the structure of a DNA segment that represents more than one turn of the helix, explains: "Proteins recognize specific DNA sequences. We want to look at how small changes in those sequences affect the structure of the helix. And a lot of drug molecules bind to DNA. The only way to understand that binding is to look at crystals."

One reason that DNA crystals are inordinately difficult to grow, says Berman, is that "there is a tendency to get disorder in the position of the atoms of nucleic acids. You lose resolution because you have different conformations of the same structure. The larger the [DNA] rod, the greater the chance of disorder." Although a few groups of investigators have been able to get crystals of a few short sequences of DNA, there are, says Berman, "a lot of unreported nonresults."

But once crystallographers obtain a crystal, it is now a relatively easy and straightforward process to determine its three-dimensional structure. With the advent of area detectors to collect data, synchrotron x-ray sources that allow data to be produced much more quickly and with smaller crystals than in the past, and the current computing power that is available to crystallographers, it is sometimes possible to get crystal structures in a matter of weeks. In contrast, Lu points out, Max Perutz took 30 years to get the structure of hemoglobin.

Pharmaceutical firms are extremely interested in biological crystals because of their promise for rational drug design. Many want the crystal structure of renin,

for example, which regulates blood pressure. They also want the structure of purine nucleoside phosphorylase, which is a bacterial enzyme used to make nucleoside analogs. These analogs are used as chemotherapeutic agents, as immunosuppressants, and as antiviral agents. The demand for good biological crystals has never been greater. But, says Rich, "we are not much better at growing crystals now than we were 10 or 20 years ago."

With this motivation, Bugg and his associates believe there is nothing to lose by trying to grow macromolecular crystals in space. And there are at least some reasons to believe the lack of gravity in space might be a real advantage. Bugg explains: "Without gravity you lose tremendous convective flow currents. As a crystal is growing in solution, high density solutes move down and low density solutes move up. This creates tremendous flow patterns and tremendous convective plumes. The effects are to generate large differences in the concentration of the crystallizing material at different places in the solution so you no longer have a homogeneous growth medium. In addition, you actually create physical turbulence. It's like trying to grow a crystal in a river rather than a lake."

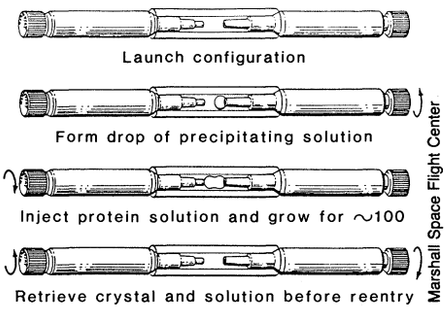
Since density differences in the weightlessness of space do not cause convection currents, then "the only thing that determines crystal growth is simple diffusion," Bugg says.

Another advantage of space, according to Bugg, is the effect of weightlessness on droplets of solution. Crystals of macromolecules are grown in droplets, either hanging off glass or on the surface of a microscope cover slip. But, says Bugg, "on the ground, droplets drop. Also, droplets are not usually spheres. They usually spread out if you put them on glass. In space, you can take a pipette, extrude a solution, and form a drop the size of a grapefruit. You can do containerless crystal growth."

A third reason to believe crystals might grow better in space is that they will remain suspended in the solution as they grow. On Earth, a growing crystal is either more or less dense than the solution it is growing in. It floats to the top or the bottom of the solution and is not bathed fully in solution.

So, in April, Bugg and his 35 coinvestigators tried to crystallize macromolecules on space-shuttle flight 51-D. It was a disaster. For one thing, the experiment was overly ambitious. They tried to crystallize 34 samples, consisting of 12 proteins and three DNA sequences, in a space of only 100 cubic inches. "Scientifically, that is not what we should have done," says Bugg. "We should have done just two or three samples. We built the hardware in only a few weeks and we hoped that one or two or a few of the samples would survive."

What happened instead was, Bugg remarks, "the worst possible scenario." Droplets were lost at every stage. First, the shuttle shook enormously on takeoff, and a number of droplets fell out of syringes that had no stoppers on them. Then the shuttle had to pursue Syncom, an errant communications satellite, which meant the shuttle had to accelerate in space. More samples were lost, the droplets smashed against the inside of the containers. Some of the syringes turned out to be designed exactly the opposite way from what Bugg and his colleagues now believe is optimal, so, says Bugg "we lost quite a few drops". (They now are making pipettes with large blunt ends that are treated with silicon, which seems to be the best way to preserve the droplets.) Finally, on landing, the shuttle blew a tire. It was like "flinging your wrist while you are holding a test tube," says Bugg. More samples were lost.



Growing crystals in space

A schematic diagram of the apparatus used to grow macromolecular crystals. Astronauts have to turn the tubes during flight.

Of the 34 samples, five survived takeoff and the satellite chase and all of those were lost on landing. But there was one gratifying surprise. One crystal, lysozyme, grew so large that it physically lodged in the tip of the syringe. The opening in the syringe was 1 millimeter and the plan was to suck in any crystals that formed before the shuttle landed. But the lysozyme crystal was 1.6 millimeters across. "I don't know of any case where a lysozyme crystal has grown so big in just 5 days," says Bugg. "The results of that one crystallization are extremely encouraging but not definitive. It confirms what we thought possible."

On the Spacelab 2 flight, Bugg is trying to crystallize lysozyme again and also purine nucleotide phosphorylase, supplied by Burroughs Wellcome Company, and human c-reactive protein, a protein that occurs at high concentrations in serum in response to infections or injury. The proteins were chosen because they crystallize in 2 to 3 days on Earth but, except for lysozyme, they never grow very large. "Our fingers are crossed that we will get much higher quality crystals than we get on the ground. That would be tremendously important," Bugg says.

But a number of biochemists are more than a bit skeptical. A key spokesman for the critics is Thomas L. Blundell of the University of London, an Englishman who recently voiced his concerns at a meeting of crystallographers. Basically, his concerns are twofold. First, he says, crystallography is still an art. "There are problems of *p*H, salt concentrations, and the concentrations of various agents in the solution. It is a complex system and there have been very, very few advancements [in the art of making crystals] over the past 20 years. We need a concerted approach. If we are serious about crystallography, we should be serious about doing the background work first. Then we could try varying one or two factors in space. But the background experiments are being ignored."

Blundell's second concern is that the experiments to grow crystals in space may be used as credits to the space program in general. "It is not a scientific program at the moment," Blundell says. "One must be careful that one is not justifying a huge program of space exploration on the basis of marginal experiments." The idea is that NASA is under great pressure to show the commercial value of its space program, which could result in its exploiting the crystallization experiments—a prospect that Blundell and others find extremely distasteful.

Rich takes a more moderate view. He does not expect anything to come of the crystallization in space experiments because, he says, "my belief is that gravity is a very small variable in the development of disorder in macromolecular crystals." Still, even if they fail to produce bigger and better crystals, the experiments might bring some new ideas to the field. "It is possible that the experiments will excite enough interest in the problem to bring in some good physicists or physical chemists. Some scientific principles may emerge," Rich says.

And, of course, there is still that outside chance that the experiments will be successful. Maybe gravity is more important than he realizes, Rich speculates. "You never know unless you try."

—GINA KOLATA