Memorial Institute in Columbus began pondering alternatives to microgravity after the space shuttle Challenger exploded in 1986. By 1988, the team was looking under the microscope at excitingly large zeolite (silicalite) crystals—labyrinthine catalyst crystals used in such key industrial processes as cracking big oil molecules into the smaller components making up gasoline—grown in a centrifuge at 20 to 50 times Earth's gravity.

Without the centrifuge, the team had been able to convert roughly 10% of their starting materials into silicalite crystals with diameters in the 200 micron range, already big compared to the 1 micron of standard industrial zeolites. In the centrifuge, "we went to the 500 micron range, and we could make almost all of our starting material usable," Hayhurst says. The team has applied for a patent on high-gravity zeolite making, and Battelle is now investigating ways of transferring the technology to industry.

Shlichta also hopes to make high-gravity processing a commercial reality; he now heads his own company, Crystal Research, in San Pedro, California. Right now he's taking aim at scintillation crystals, which are central to medical imaging technologies such as positron emission tomography (PET) scanners. Growth in a centrifuge, he thinks, should yield higher quality scintillation crystals, less riddled with sensitivity-limiting defects.

Shlichta sees no end to the possibilities of gravitational materials science, which he thinks will be played out all across the gravitational spectrum. He divides that spectrum into several regions—low gravity in space, normal gravity on Earth, a range of about 1 g to about 20 g in large centrifuges like Regel's, and a range of hundreds to many thousands of times normal gravity in ultracentrifuges. Wilcox adds another exotic gravity region—between 1 g and the micro-g of space—that could be produced in a centrifuge spinning on a spacecraft. Each region of the spectrum, Shlichta suspects, will turn out to be best for processing specific kinds of materials. For example, microgravity in space might be best for making most protein crystals and the 1-50 g range for growing more perfect semiconductors.

For Shlichta and his cohorts, the lack of theoretical guidance about what they will find as they explore the gravitational spectrum only adds to the excitement: "We have a brand new baby here and we don't yet know what it will grow into," he says. But then Shlichta's tone turns grave as he reflects on how long it has taken for scientists to realize that the value of g is an experimental condition—something to fool around with. "We should have just had our fiftieth [high-gravity] conference, not the first one," he laments. ■ IVAN AMATO

Three Li'l Pigs and the Hunt for Blood Substitutes

Human hemoglobin can now be made in pigs. But will it provide an easy path to a human blood substitute?

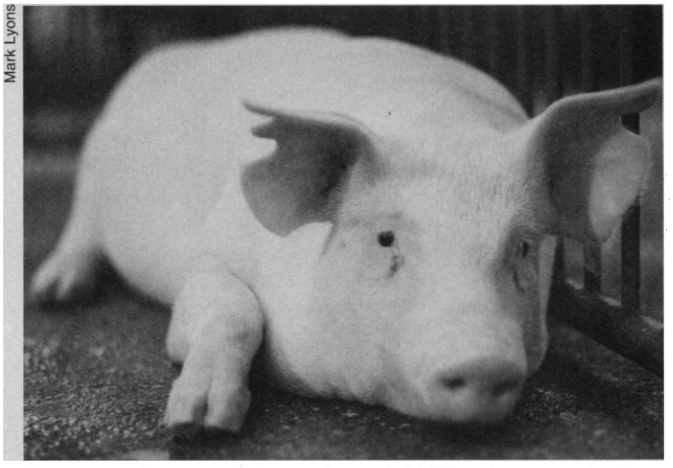
LAST MONTH WHEN DNX, INC., A BIOtechnology company in Princeton, New Jersey, reported that its scientists had created genetically engineered pigs that produce human hemoglobin, the achievement was widely hailed—most notably in a front page story in *The New York Times*—as a milestone in the effort to develop a human blood substitute. Medical researchers have been trying to produce such a substitute for about 50 years. Ideally, it would have several major advantages over the fresh human blood now used for transfusions. It would have a long shelf life and not require refrigeration. And it would be unlikely to trigger possibly lethal immunological reactions or to transmit AIDS, hepatitis, and other viral diseases.

But little heralded in the news accounts about the transgenic pigs were a number of significant obstacles that DNX will still have to overcome before the medical community gets its long-sought blood substitute. "The production of transgenic pigs represents a genetic feat," says hematologist Joseph Baron of the University of Chicago's Pritzker School of Medicine, "but it does not address the key issues in medical therapy."

The transgenic pigs produced by DNX make normal human hemoglobin—and so far efforts to adapt normal human hemoglobin for use as a blood substitute have not panned out for several reasons (also see *Science*, 21 December 1990, p. 1655).

A blood substitute has to perform one critical function: It must pick up life-supporting oxygen in the lungs and deliver it to the tissues of the body. In the bloodstream, of course, that's done by the hemoglobin carried within the red blood cells. But researchers learned early on that hemoglobin alone doesn't work. The free hemoglobin protein binds oxygen all right—but with such high affinity that it can't release enough to the tissues.

And there's another serious problem that also prevents use of plain, purified hemoglobin as a blood substitute. When hemoglobin is removed from the confines of the red blood cell, the molecule, which is composed of four protein chains, breaks into two halves. These dimers are rapidly filtered out of the blood by the kidneys, which may become irreparably damaged for reasons that aren't fully understood.



Mark Lyons

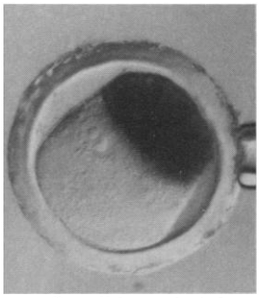
Down on the gene farm. *One of DNX's pigs, genetically engineered to make human hemoglobin, takes it easy.*

In recent years, a number of companies, including Northfield Laboratories, located north of Chicago, and Biopure Corp., an Upjohn subsidiary in Boston, have tried to surmount these problems by chemically modifying hemoglobin so that the protein chains are crosslinked or polymerized. That produces a molecule that is both stable and capable of releasing the oxygen that tissues need. But while animal studies of the modified hemoglobins have been encouraging, human trials haven't fared well.

Last year, for example, Biopure conducted a successful trial of its modified bovine hemoglobin in Guatemala, but this spring the company's parent, Upjohn, abruptly halted a similar trial it was conducting in Kalamazoo, Michigan, for reasons that company officials haven't explained. Similarly, Northfield successfully administered its polymerized human hemoglobin to healthy

volunteers in 1987, but 1 year later had to stop a trial of the product in trauma patients after two of them complained of shortness of breath and chest tightness. "The trials," says Northfield's CEO Richard Dewoskin, "are like dealing with an onion. We peel away one set of problems and another set appears."

Enter DNX, which is developing its own, somewhat different strategy for dealing with the numerous problems that have cropped up during efforts to use hemoglobin preparations as a blood substitute. The ultimate goal, according to John Logan, the company's vice president for research, is to produce a genetically engineered form of human hemoglobin with the same features as the chemically modified hemoglobins: The protein chains would be crosslinked to give the molecule stability and the molecule's affinity for oxygen would be reduced so that it could release oxygen to the tissues. The production of the transgenic pigs, described by Logan at the World Congress on Cell and Tissue Culture,* was the first step toward achieving that goal.



Robert E. Hammer/University of Penn. School of Veterinary Medicine

Small target. *A fertilized pig egg.*

And as Chicago's Baron said, it was quite a genetic feat. Although molecular biologists have been genetically engineering large animals such as pigs and sheep to produce foreign proteins for about 3 years, the techniques are far from routine. The foreign genes have to be injected into the small, fragile, newly fertilized eggs of the recipient animals. Typically, less than 0.5% of the injected eggs develop into animals in which the transferred gene is functional.

And because mammalian hemoglobins are composed of two different proteins (two copies of each in the intact molecule), the DNX workers had to introduce two genes into the pigs to get them to make human hemoglobin. The genes also had to carry the correct regulatory sequences so that the hemoglobin proteins were made only in red blood cells. Despite these difficulties, the DNX group got three transgenic pigs that make human hemoglobin out of hundreds of eggs that were injected.

But that's only the beginning, Logan says. For one thing, the efficiency of the human hemoglobin production needs to be improved; it's made by only about 15% of the animals' red cells. For another—and this brings us back to what many researchers look upon as the critical hurdle—there's no reason to expect that normal human hemoglobin made in pigs will be any better in terms of stability and the ability to release oxygen than ordinary human hemoglobin. So the next step, Logan says, is to create transgenic pigs that contain genes for mutant human hemoglobins that are both stable and have a reduced oxygen affinity.

Meanwhile, another company has already beaten DNX to the punch in producing such mutant hemoglobins—albeit not in pigs. In the January issue of *Bio/Technology*, Gary Stetler and his colleagues at

*The congress was held in Anaheim, California, on 16 to 20 June.

Bumper Transgenic Plant Crop

Although it may seem hard to believe, it's been almost 10 years since researchers showed that they could use gene transfer technology on plants. Since then the plant genetic engineers have taken great strides. With several dozen field trials already under way, they may soon achieve their original goal—the development of high-yielding plant varieties with enhanced resistance to herbicides, disease, or insects. So now the researchers are branching out, beginning to design plants with improved consumer appeal, such as tomatoes that hold up better to freezing, as well as creating plants that can serve as factories for pharmaceuticals and industrial oils, just as researchers are now attempting to use pigs to make human hemoglobin (see story). Here is a rundown on some of the weird and wonderful plant varieties being developed.

■ Researchers at the Scripps Clinic and Research Foundation, La Jolla, California, have genetically engineered tobacco plants to produce functional human antibodies. Plant biologist Mich B. Hein says that these "plantibodies" are not quite the same as those produced by traditional monoclonal antibody techniques, but test tube studies show that the plantibodies behave enough like more typical monoclonals to suggest that they can be used for diagnosing and treating human diseases. This summer Hein and his Scripps colleagues will start pharmacological testing to see how the plantibodies behave when they are injected into mice.

Scripps is also expanding its plant efforts. The institute has recently brought in Roger Beachy, a pioneer in plant genetic engineering, from Washington University to start up a plant science section. The ultimate goal for such work is to harvest high-value pharmaceuticals from common crops, such as alfalfa.

■ Plants may also become more efficient production factories for oils. Two Iowa State University researchers, geneticist Eve Wurtele and biochemist Basil Nikolau, have discovered a carrot gene that controls the quantity of oils produced. By introducing extra copies of the gene, which encodes an enzyme called acetyl-CoA carboxylase, into plants such as soybeans, it might be possible to induce them to increase their oil production. A more distant goal for this line of research is to switch plants from making the usual cooking and salad oils to producing high-value hydrocarbons, which can be used as petroleum replacements for industry.

■ At the Oakland, California, laboratory of DNA Plant Technology, researchers have cloned the gene for a protein that helps keep the winter flounder from freezing, and inserted it into tomatoes and tobacco. Based on knowledge that freezing and thawing damages plant structures, the company's Gary Warren predicts that fruits and vegetables containing this antifreeze gene will have an improved, firm texture after freezing and thawing. He'll soon know. Field trials of tomatoes transformed to make the antifreeze protein began in northern California the week of 20 June.

■ For people who enjoy a large helping of refried beans, but suffer from the intestinal unpleasantries associated with the digestion of legumes, Agracetus, Inc., of Middleton Wisconsin, has good news. Working with researchers at the University of Wisconsin, Madison, and the University of California, Davis, this biotechnology company has transferred new genes into dry, navy, and green beans. While the company has so far concentrated on improving the plants' disease resistance, another priority is to alter the chemical composition of beans to reduce the flatulence they can cause.

This latter goal, however, may be tougher to realize, says Agracetus' David Russell. He notes that while the traits that affect disease resistance are reasonably well known, the sugars thought to be the culprits in flatulence have not been positively identified, and therefore plant scientists do not yet know how to redesign bean plants to eliminate the undesirable components. But when they do find out the job should be well within reach of the techniques available for the genetic engineering of plants. ■ **A.S.M.**

Somatogen, Inc. in Boulder, Colorado, reported that they had made normal human hemoglobin as well as a mutant with reduced oxygen-binding ability in yeast. In addition, says Stetler, who is vice president for research and development at Somatogen, the company has more recently made a double mutant that not only has the reduced oxygen affinity but is also stabilized to prevent the four chains of the molecule from coming apart. They've produced this mutant hemoglobin in both yeast and in the bacterium *Escherichia coli*.

The Somatogen groups prefers producing the customized hemoglobins in yeast or bacteria, Stetler explains, because the proteins are easier to purify. They don't have to be separated from pig hemoglobin. And besides that, there is little hazard of microbially produced hemoglobins carrying potentially dangerous animal pathogens, whereas hemoglobins produced in pigs might.

DNX's Logan counters that his company has devised an efficient method of separating human and porcine hemoglobins. He also notes that while both red blood cells and microorganisms contain endotoxins that can cause fever and other side effects in humans, it should be more economical to remove any contaminating red blood cell endotoxins. Lastly, pigs should make admirable production factories for proteins such as hemoglobin. They breed fast, have big litters, and produce large volumes of blood that can be removed without causing ill effects.

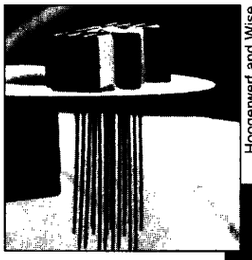
But even if genetically engineered hemoglobins can be made that are sufficiently stable and capable of releasing oxygen to be used as blood substitutes, a troubling question remains about the toxicity that has cropped up in the human studies of the chemically modified hemoglobins. "The central issue is whether the toxicity is caused by hemoglobin itself or a contaminant," says Robert Winslow, a pioneer of blood substitute research, who is currently moving his laboratory from the U.S. Army's Letterman Institute of Research in San Francisco to the University of California, San Diego.

Winslow and most other blood substitute researchers think that the toxicity has been caused by contaminants. "Red blood cells have all sorts of things that must be gotten rid of," says hemoglobin expert Sam Charache of Johns Hopkins University School of Medicine. "It is a tough problem." Tough, but Winslow suggests, ultimately solvable. Still, the difficult history of artificial blood research suggests that the solution may not come readily—or soon. ■ ANNE SIMON MOFFAT

Engineers Open a Dialogue With Neurons

"WE WANT A DEVICE WE CAN USE TO TALK TO nerves," says Gregory T. A. Kovacs, an electrical engineer in the Center for Integrated Systems at Stanford University. He was describing his own work on a tiny, perforated electrode meant to be implanted in regenerating nerves. But he might just as well have been speaking for a wider group of engineers who are looking for a way to put silicon microengineering in touch with nerves and muscles.

Kovacs and other engineers who spoke in San Francisco last week at Transducers '91, the Sixth International Conference on Solid-state Sensors and Actuators, are sculpting minute electrodes that can eavesdrop on the electronic chatter of small clusters of nerve cells or even join in the conversation. The work will equip neuroscientists with new research tools; eventually, it may open the way to a technological endrun around paralysis.



Hoogerwerf and Wise

Listening device. *Arno Hoogerwerf's 16-probe neural-recording array shown from the side and from above.*

Microelectrodes that can record or stimulate single neurons are nothing new, but the deft workmanship of these micromachinists is yielding sensors in entirely new forms. For example, University of Michigan graduate student Arno C. Hoogerwerf, who works with microengineering specialist Kensall D. Wise, described how he microfabricated a cage-like, 16-probe neural recording array. Hoogerwerf hopes the half-millimeter-wide array will be able to listen in on the activity of ensembles of tens or hundreds of neurons. Such ensembles are neglected by existing microelectrodes, which monitor several cells at most, and by global brain monitors such as positron emission tomography, which monitor millions or billions of cells.

Tayfun Akin of the University of Michigan's Center for Integrated Sensors and Circuits described another kind of silicon probe—one meant to have more intimate interactions with its target cells. Akin, Khalil Najafi, and workers in the University of Michigan's dental school have built electrodes shaped like tiny, flat sieves. When such an electrode is slipped between the severed ends of a peripheral nerve, regenerating axons find their way through the holes in the sieve, making it possible to monitor the electrical activity of several axons at once. Such a tool could prove useful for neuroscientists trying to understand how the nervous system transduces environmental stimuli into neural signals.

In a first test of the sieves, Akin says, the cut ends of taste fibers in rats' glossopharyngeal nerve successfully regenerated through the holes. Future studies will include recording from such regrown axons and stimulating the axons electrically—a step toward micromachined devices that might someday restore movement to paralyzed limbs. Kovacs has designed a similar perforated electrode, with which he has recorded and stimulated axons in a leg nerve of a rat.

Still other devices described at the meeting would take the place of nerves altogether. University of Michigan engineer Babak Ziaie envisions remotely controlled silicon prostheses that would stimulate paralyzed muscles directly. Ziaie, working with Yogesh Gianchandani and Najafi, is trying to build silicon strips small enough to slide through a hypodermic needle into strategic sites in the muscle. Each strip would host microelectrode arrays for jolting muscle cells into action, electronic circuitry for controlling the electrodes, and a tiny coil for receiving power and instructions from the outside world.

Ziaie and his colleagues are well on their way to completing several elements of their "implantable microstimulator." One is an array of electrodes, 450 strong, crowded together in a strip measuring 1.2 mm by .3 mm at one end of a thin silicon slab. The workers have tested the remote-control concept as well: Last year, Ziaie and Najafi, working with Akin, sent power and data to a different device using radio-frequency telemetry. Even so, Najafi thinks a full-fledged, implantable microstimulator will take a few more years of work. ■ IVAN AMATO