Research in Progress

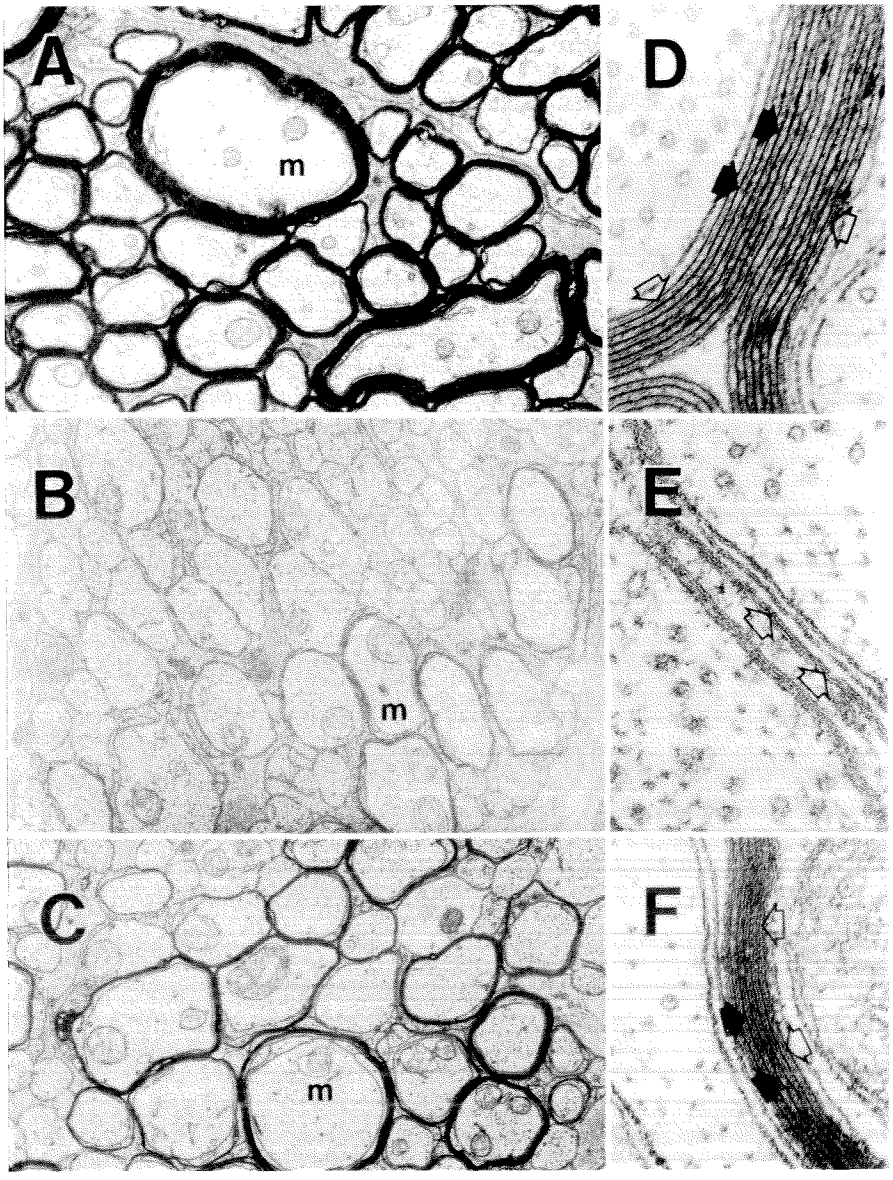
Genes, Incorporated

Right: The shiverer mouse (left) suffers from a genetic neurological disease that causes tremors and premature death. Genetic surgery has cured the shiverer mouse on the right.

THE TWO MICE appear virtually identical in still photos. They're both brown, and they're both about the same size, since they are, in fact, cousins. But in a moving picture their differences become immediately apparent. One looks normal, and the other shivers uncontrollably. If we were to follow these two mice throughout their lives another difference would reveal itself: The normal-looking mouse will, in all likelihood, live out its normal lifespan of two to three years, while the shivering mouse will develop convulsions and die at just three to six months of age.

The mouse who shivers does so because it is homozygous for a defective gene, the gene that normally codes for myelin basic protein (MBP). MBP is a major component of the myelin sheaths that insulate central nervous system axons, and in its absence the transmission of electrical impulses along those axons becomes severely disrupted. The amazing thing about the apparently normal mouse is that it too is homozygous for the defective MBP gene. But, in a stunning feat of genetic engineering, Caltech scientists have managed to insert correct copies of the MBP gene into its chromosomes. Although for unknown reasons these genes produce only about 25 percent of the normal amount of MBP, this is enough to prevent the appearance of the shivering disease.

The research group was led by Leroy Hood, the Ethel Wilson Bowles and Robert Bowles Professor of Biology, and it included Carol Readhead, member of the professional staff; Brian Popko, Carmie Puckett, Raul A. Saavedra, Eric Lai, and Stephen W. Hunt, III, research fellows; Naoki



These electron micrographs show cross-sections of the optic nerve of a normal mouse (A and D), a shiverer mouse (B and E), and a cured shiverer mouse (C and F). Inserting correct copies of genes for myelin basic protein, which the shiverer mouse lacks, into fertilized eggs results in myelin sheaths that are far better formed than those of the shiverer mouse, but not as well-formed as those of the normal mouse.

Takahashi of Tokyo University; and H. David Shine and Richard L. Sidman of Harvard.

The researchers accomplished their feat by using recombinant DNA techniques to attach the MBP gene, including flanking regulatory sequences, to circular pieces of DNA known as cosmid vectors. They then injected these cosmid vectors into fertilized shiverer mouse eggs. The eggs were then implanted into foster mothers and allowed to develop normally.

This form of genetic surgery is known as germline therapy, since the genes were inserted into an animal's germ cells — its ova or sperm. The other form of genetic surgery is called somatic-cell therapy — the insertion of genes into specific body tissues, such as the bone marrow or the pancreas. Germline therapy will not be used in humans for the forseeable future. For one thing, only about one in 150 injections "takes." But, more significantly, germline therapy in humans is prohibited by ethical considerations. Extraneous genes inserted into the germline can be passed on forever, from generation to generation, with consequences that are impossible to predict. It is widely expected, however, that somatic-cell therapy will be attempted within the next year or so in efforts to cure people suffering from specific genetic diseases.

In addition, the Caltech researchers have, in the shiverer mice and in various strains of "cured" shiverer mice, a convenient experimental system for studying the consequences of decreased levels of myelin and myelin basic protein. The shiverer mutation is not a direct analog of any human neurological disease, but there are several human diseases in which demyelination is an essential characteristic; these diseases include multiple sclerosis and Guillain-Barré Syndrome.

Says Leroy Hood, "In a sense the shiverer mouse is a test tube by which we can come to understand the basic functioning of the gene for myelin basic protein. We'll use these mice to understand how this protein is expressed and how it functions. Beyond that, we've learned that we can actually construct mice that have differing levels of myelin — 5 percent, 20 percent, or 30 percent — and we'll use these specially constructed mice to understand 1) how the myelination process, which is very complex, works, and 2) the normal roles that myelin plays in facilitating nerve impulses."

These mice will also be valuable in studies of gene regulation. When preparing the gene for insertion into the cosmid vector, the researchers were careful to include large portions of DNA from areas flanking the area actually encoding myelin basic protein. They did this because they know that these areas contain "promoter" sequences and other regulatory elements that ensure that the MBP gene will be transcribed and expressed. Yet, even mice that were specially inbred to be homozygous for the inserted gene (and its accompanying regulatory sequences) produced only 25 percent of the normal amount of MBP. Although this is enough to ameliorate the symptoms of the shiverer disease, it presents something of a puzzle: If these mice have two full copies of the correct MBP gene, just as normal mice do, why don't they produce *100 percent* of the normal amount of MBP?

A clue may lie in studies that indi-

cate that the inserts do not become incorporated at the normal location of the MBP gene — on chromosome 18 — but at some other, most likely random, location. And this may indicate that some as yet undiscovered regulatory sequences are necessary for the full expression of the gene. It is entirely possible that these regulatory sequences are as much as 10 kilobases from the gene's normal location. Another possibility is that the inserted gene may simply have been incorporated at an unfavorable location within the genome. Further research already underway with shiverer mice may well resolve these questions.

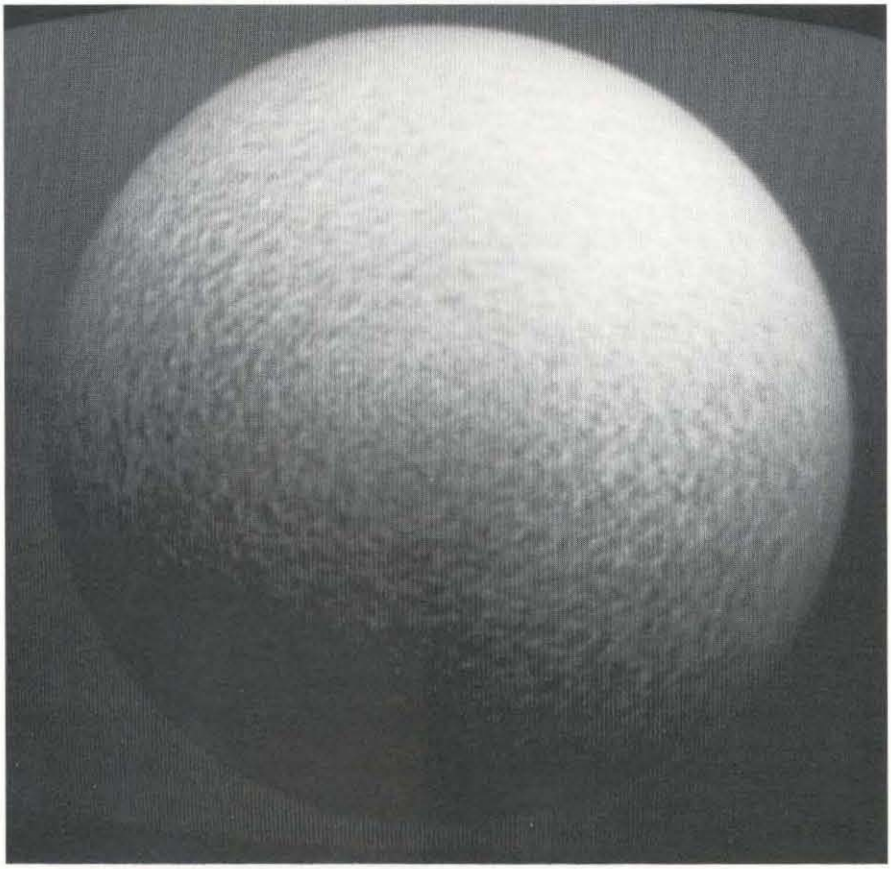
So even though the cure of the shiverer mouse can make no *direct* contribution to the cure of human disease, its *indirect* contribution is likely to be sizable. Not only will the shiverer mouse help scientists understand how myelin works in the nervous system, it will advance the art of genetic surgery and it may go a long way toward increasing our fundamental knowledge of gene regulation.
□ — *RF*

Sounding the Sun

"The sun is a star, the only one we can study in detail."
George Ellery Hale

UNTIL VERY RECENTLY, almost all detailed studies of the sun were confined to its surface features and outer atmosphere. Even questions as basic as the rotation rate of the solar interior remained nearly as mysterious as if the earth's nearest star were 8 thousand light years away instead of 8 light minutes. So did a host of other conditions in the solar interior, including the strength of its magnetic fields, the relative abundances of hydrogen and helium, and numerous details of the thermonuclear fusion reactions in the solar core. Nearly all work on these topics was carried out through theoretical modeling, for which there were little observational data or support.

In the last five years, however, the field of helioseismology has opened the sun's previously inaccessible interior to observation. Just as geologists study seismic waves to map the interior of the earth, solar astronomers are now investigating solar acoustic waves to



In this photo of the oscillating solar surface, Libbrecht's Doppler shift data have been processed so that light regions represent material moving toward earth, and dark regions represent receding material. The variations in brightness are caused by the sun's rotation.

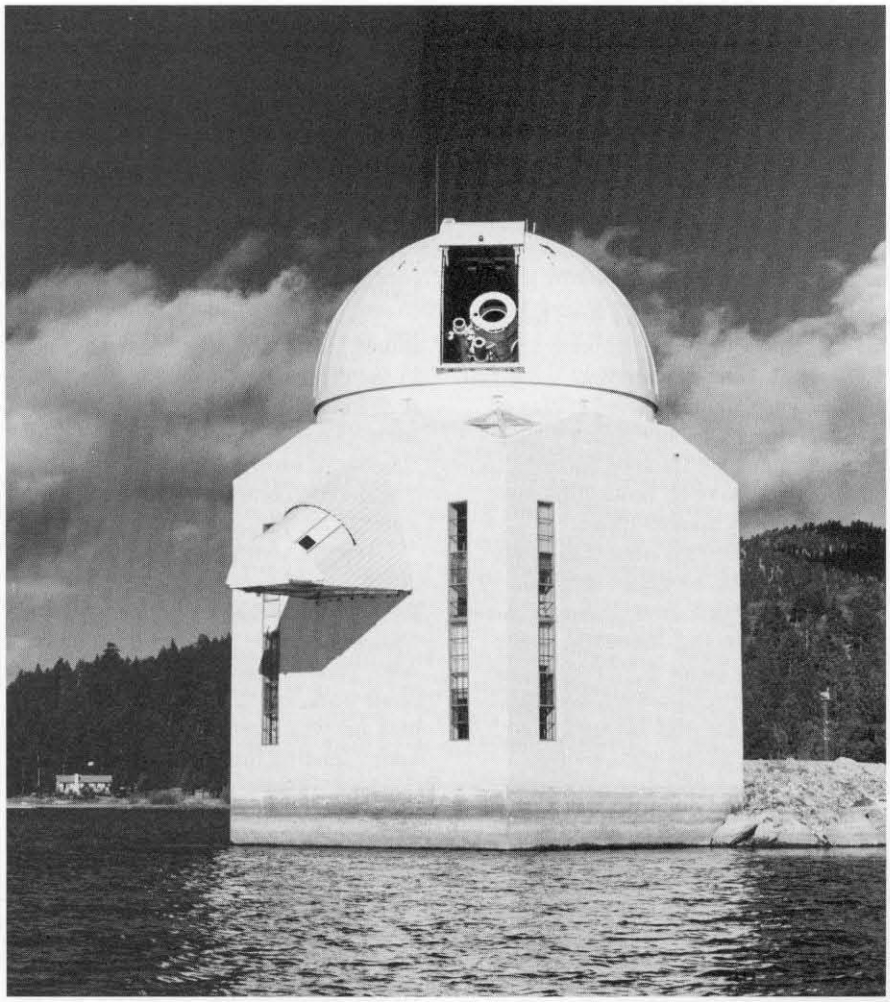
probe the dynamics and internal structure of the sun.

One of the researchers in this new field is Ken Libbrecht, assistant professor of astrophysics at Caltech. Working at the Institute's Big Bear Solar Observatory, Libbrecht is measuring the frequencies of solar seismic waves to study the temperature, rotation speed, and chemical abundances of the solar interior. His research is being supported by the National Science Foundation, a fellowship from the Alfred P. Sloan Foundation Program, and a Presidential Young Investigator Award.

The foundations for Libbrecht's research were laid at Caltech more than two decades ago by Robert Leighton, now Valentine Professor of Physics, Emeritus. In 1962 using a camera he had developed for solar studies, Leighton discovered that the sun's exterior was not static but agitated by waves of gas rising and falling at five-minute intervals across the entire solar surface, a pattern that was dubbed the "five-minute oscillations."

Leighton's findings provided a new picture of a bubbling solar exterior that resembled a wave-covered ocean. But it was another ten years before astrophysicists discovered that these oscillations were not unrelated and incoherent surface waves, but the surface projections of an organized network of sound waves, or acoustic modes, resonating throughout the solar interior. The sun is now known to harbor at least 10 million of these modes, each with a unique "pitch" or frequency that depends on the sound velocity in the solar interior. Because the speed of sound in turn depends on the temperature and chemical composition of the solar plasma, the frequencies of these surface oscillations can be used to investigate such internal properties as temperature and the abundance of helium relative to hydrogen in the solar core.

To measure these frequencies, Libbrecht has developed a technique based on the Doppler shift of solar light. Viewed through a filter that splits sunlight into its component frequency bands, light from the rotating solar surface is shifted into the blue, or high-frequency, end of the spectrum as it approaches an observer on earth, and shifted into the red, or low-frequency range as it recedes from



The helioseismology telescope is the nose-like projection on the Big Bear Solar Observatory.

view. The measurement of the Doppler shift gives the actual velocity of the oscillating gas, which can then be used to calculate the frequencies of the acoustic modes reverberating through the solar interior.

Using a specially constructed helioseismology telescope at Big Bear Solar Observatory and an extremely narrow filter that limits the entering light to a narrow range of wavelengths, Libbrecht has been able to measure the frequencies of more than a thousand such modes, to an accuracy of one part in 10,000. His measurements have been found to match theoretical projections of the frequencies to an accuracy of one percent, a rare occurence in the field of astronomy.

Surface oscillations are also being applied to studies of the sun's internal magnetic field, long believed to be the source of solar flares and the 22-year sunspot cycle. Sound wave frequencies are altered in the presence of a magnetic field, and the stronger the field, the more pronounced the effect. By measuring the extent to which the sun's seismic wave frequencies deviate from their predicted values, Libbrecht believes it should be possible to determine the accuracy of the standard "solar dynamo" sunspot model.

Although the scientific importance of solar oscillations is a recent discovery, the sun is by no means the only pulsing star known to astronomers. The best known such stars are the Cepheid variables. Their periodic and regular fluctuations in brightness, originally spotted in the 1920s, enabled astronomers to make the first accurate measurements of the relative distances between earth and stars outside the Milky Way galaxy. As astrophysicists begin to apply helioseismology's observational findings about the solar interior to the dynamics of stars, the scientific significance of the sun, originally foreseen by Hale, will at last come into its own. □ — *Heidi Aspaturian*