



Random Walk

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HOW NOW MAD COW?

Two small spots, the modest signature of two proteins in a sea of others, have led to an unambiguous diagnostic test for Creutzfeldt-Jakob disease (CJD), a rare but inevitably fatal neurological disorder. Developed recently by researchers at Caltech and the National Institutes of Health, the test involving the same two proteins appears also to have great potential for diagnosing bovine spongiform encephalopathy (BSE). Better known as mad cow disease, BSE has ravaged Britain's cattle industry and created considerable political uproar in the European Union since the March announcement of the hypothesis that the disease had entered the human food chain. As many as 15 British cases of a new variant of CJD have been tentatively linked to the consumption of BSE-infected beef, and several hundred cows a week are still being diagnosed with BSE in Britain, despite measures to contain the disease.

Although only suddenly a very hot property, the two

proteins were originally discovered more than a decade ago. Michael Harrington, a member of Caltech's Beckman Institute, spotted them during an NIH project examining the spinal fluid of 541 subjects—about 100 of them normal and the rest suffering from a variety of neurological diseases, including 21 with CJD. Harrington was screening the fluid for about 100 proteins, using two-dimensional gel electrophoresis, a technique that applies an electric field to separate a complex mixture of proteins, which end up as distinct spots of different size and charge in different positions on the gel.

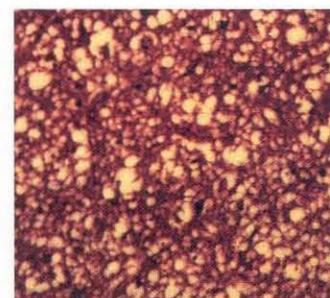
The overall goal of the project was an objective description of various diseases of the brain and nervous system, including Alzheimer's disease, Parkinson's disease, Huntington's chorea, and schizophrenia, by the spinal-fluid population of particular proteins that might serve as markers for the diseases. Harrington, who is a physician as well as a biologist, refers to the spinal fluid as the "urine of the brain" for its diagnostic versatility.

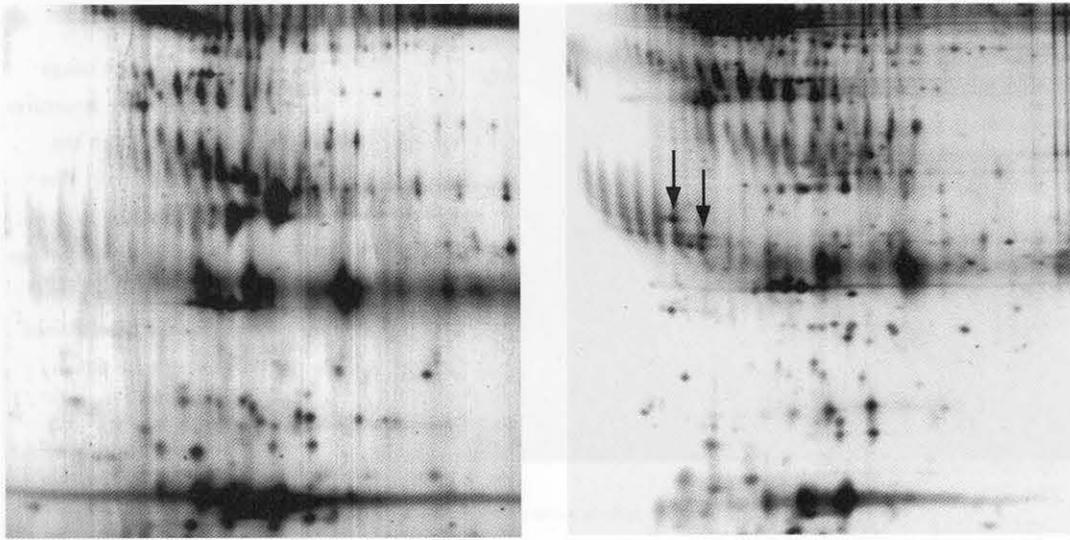
The two spots, known only as 130 and 131, turned up in this spinal "urine" of all the

CJD patients and of only the CJD patients; that is, they described CJD with 100-percent specificity and selectivity. Harrington thought the characteristic spots would make a useful diagnostic marker for the disease, and in 1986 he published this in a paper.

Unfortunately, 10 years ago such a diagnostic test took three days of highly skilled labor, and since only about 200 CJD cases occur annually in the U.S., it didn't exactly catch on in everyday medical practice. Still at the NIH, Harrington did continue to follow up on

Brain tissue that resembles a sponge is characteristic of all the transmissible spongiform encephalopathies; this sample is from a person who died of Creutzfeldt-Jakob disease.





Two-dimensional gel electrophoresis, which separates a complex mixture of proteins by size and charge, turned up two spots, originally called proteins 130 and 131, that were present in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease (right, arrows) but not present in normal cerebrospinal fluid (left).

pathology samples sent to him from all over the world and diagnosed 260 cases of CJD with 99-percent accuracy (one false positive and one false negative). But for all their diagnostic accuracy, proteins 130 and 131 were still unidentified. From their position coordinates on the two-dimensional gel, you can assign a charge and a size—that's all. So Harrington decided to try to find out what they were, a task that became possible through the protein-sequencing techniques that had been developing in the years since his first CJD paper was published.

By 1988 Harrington was working at Caltech, where biologists had pioneered much of the work in protein sequencing. (Image-processing algorithms developed at Caltech's Jet Propulsion Laboratory also made comparison of spot patterns in the gel much easier; see *E&S*, Fall 1990.) But in order to sequence proteins, you first had to isolate them, and even this proved difficult for 130 and 131. Because

they exist in such trace amounts in spinal fluid, it proved impossible to obtain a sufficient amount of CJD-spinal fluid to purify a sample large enough to sequence. No amount of spinal fluid that Harrington could obtain would yield enough of a protein sample.

Then Harrington speculated that 130 and 131 might exist in normal brain tissue and only leak out into the spinal fluid from a brain damaged by CJD. This turned out to be correct, enabling Harrington, postdoc Kelvin Lee, and researchers at NIH to purify sufficient protein for sequencing from a mere gram of normal brain tissue. From the peptide fragments that emerged from this process, a partial sequence was obtained—enough to feed into a protein database to look for a match. Protein 130 turned out to be a well-known neuronal protein, 14-3-3, for which commercial antibodies were available. These antibodies should bind to the protein and light up, indicating its

presence. When Harrington screened the spinal-fluid proteins from a CJD patient with the antibodies, both 130 and 131 lit up. Harrington and his colleagues now had a simple, rapid, clinical test for CJD; they published it in *The New England Journal of Medicine* at the end of September.

The implications of proteins 130 and 131 reach wider, however, than a diagnostic test for a rare disease, or even for a politically and economically significant one. The two turn out to belong to a highly conserved (in evolutionary terms) family of brain proteins that have already attracted the interest of biologists, says Harrington, because they appear to be involved in a wide variety of functions, including the transmission of signals—and in protein folding. The latter function is of particular interest to Harrington: CJD and BSE are thought to be caused, not by an infectious agent such as a bacterium or a virus, but by an abnormal prion—a protein that propagates out of control and

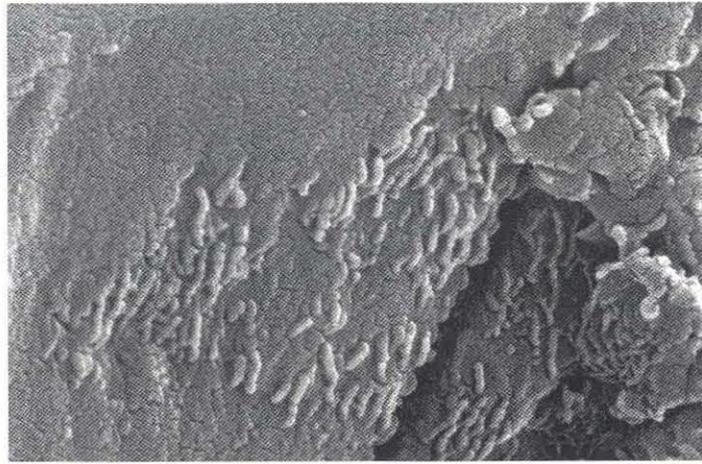
accumulates in the brain. Unlike normal proteins, prions in CJD and BSE are conformationally abnormal. This leads Harrington to speculate that his two proteins that play a role in the conformational stabilization of other proteins might be more than just markers and could perhaps be implicated in the development of the disease. And by manipulating them, you might be able to stop the prion's progress.

In his continuing study of CJD, Harrington now has 15 protein markers for the disease, and markers that may turn out to be even more descriptive of other diseases such as Alzheimer's disease or Parkinson's disease. Harrington has also characterized about 2,000 normal spinal-fluid proteins in his attempt to create a complete profile of proteins in the nervous system, in which specific changes indicating pathology can be observed and therapies monitored. "We can build up a molecular picture of disease," Harrington believes, through proteins, which are more descriptive of disease than genes. He even envisions a massive "Human Proteome Project" to complement the Human Genome Project. "Proteins are what we're made of," he says. "It's how our genes have produced different proteins that make us what we are."

In the meantime, however, Britain's beef disaster may provide the first direct application of Harrington's work. During his research for the 1986 paper, he did look at scrapie (a disease of sheep that is presumed to be the precursor of BSE) to see if a form of proteins 130 and 131 occurred, because the CJD and scrapie brain pathology was similar. All of the sheep proteins, however, lodged at coordinates on the gel different from the human ones,

rendering a comparison with a sheep form of 130 and 131 impossible at the time. But the antibody that binds to and lights up the two CJD-marker proteins now changes all that and has enabled Harrington to trace successfully the now-known marker proteins in several different transmissible spongiform encephalopathies that affect sheep, cattle, and chimpanzees.

Harrington has not yet been able to test spinal fluid from any mad cows, but "the data obtained from experimentally transmitted disease in cattle suggest that it might be a useful test for BSE," he says. In the meantime he's been hounded by the British media more tenaciously than any other Caltech scientist in recent memory. □



This electron microscope image shows the tiny tube-like structures (the largest being 1/100th the diameter of a human hair) that may be fossils of ancient bacteria. They were found within the orange carbonate globules (below) that are believed to have been formed in the Martian rock 3.6 billion years ago.

MICROBES IN MARTIAN METEORITE?

Ancient bacterial life on Mars? Announcement of its possible manifestation leaked to the press a couple of weeks before the scientific paper actually appeared in the August 16 issue of *Science*. A team of scientists from NASA, McGill University, the University of Georgia, and Stanford had found several types of evidence for these bacteria in a 4.5-billion-year-old Martian

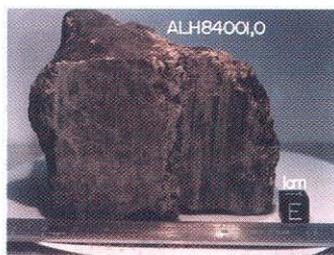
meteorite, named ALH84001 for the Allen Hills region of Antarctica, where it was found after it was blasted off Mars by the impact of another meteorite millions of years ago. While any one bit of evidence might be explained by other means, the concatenation of the data led the authors to conclude that the most reasonable explanation was that it had

been produced biologically about 3.6 billion years ago.

A NASA news conference trumpeted the results. The thrill of discovering that perhaps "we are not alone," even if our ostensible companions are microscopic organisms that ceased to exist 3.6 billion years ago, clearly touched a collective emotional nerve. Reactions ranged from jubilant hype to cold-fusion sneers; sci-fi fans cried "I told you so," and the devout made haste to reconcile it with the Almighty's plan; scientists, on the whole, took the paper seriously but maintained skepticism. *E&S* asked some of Caltech's scientists for their opinions.

Norman Horowitz (PhD '39), professor of biology, emeritus, and former chief of the bioscience section for JPL's Mariner and Viking missions (which concluded that life was impossible on the present Martian surface) found the paper technologically impressive, "a serious paper by competent investigators using the most advanced analytical methods." He notes that the authors "don't claim to have proof of Martian life, only that that is the best explanation for their findings," and that they are





The 4.5-billion-year-old rock ALH84001, believed to be part of a Martian meteorite, was found in Antarctica in 1984.

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cautious. “In my view there is much to be cautious about.”

“In the first place, the idea of past life in a piece of igneous rock—a rock that crystallized from a melt, which is what the meteorite is—is unusual. Evidence of past life is normally found in sedimentary rock. The authors’ position is that fissures in the rock became a home for microbes a billion years after the rock was formed.

“Then, the evidence they present is based almost entirely on inorganic chemistry. Carbonates, magnetite, and iron sulfide found in the fissures of the meteorite are of biological origin, they argue. Their argument is a sophisticated one, based on the particular minerals found, their morphology, and their association in the rock. I know little mineralogy, but I am skeptical of this argument because these substances, and/or their close chemical relatives, are common in the solar system. They are found in meteorites and on the surface of Mars, and they are formed by well-known chem-

ical reactions. I am sure that a knowledgeable chemist could produce a credible nonbiological model for the occurrence of these same minerals in the Mars meteorite involving fewer assumptions than those made by McKay et al.

“The only organic substances that the authors report in the meteorite are polyaromatic hydrocarbons. They are aware that polyaromatics of nonbiological origin are found in carbonaceous meteorites and that they are readily produced in the laboratory. Their argument for a biological source of these meteoritic polyaromatics is based on their mass distribution and other characteristics, none of them biodiagnostic. The one aspect of their finding that I find interesting is that these hydrocarbons are the first organic matter that, as far as I know, has been identified with Mars. The Viking Lander found no organic matter in the surface of Mars.

“Finally, electron microscopy of the chips showed tiny structures that, except for their minute size, resemble

ordinary rod-shaped bacteria. The authors suggest that these may be microfossils. No supporting evidence for this interpretation is given.”

So Horowitz remains unpersuaded. What would it take to bring him around? He thinks that fossil evidence is required to make the case convincing. The distinctive chemical features of life—such as optical activity—could not survive for 3.6 billion years, and, if found, would indicate contamination.

Joseph Kirschvink (BS, MS '75), professor of geobiology, is an expert on biomineralization. In addition to making the initial prediction and discoveries of earthly magnetofossils, he has found magnetite in the brains of whales, tuna, and humans (the phenomenon of biologically produced magnetite was discovered at Caltech by the late Heinz Lowenstam in the early sixties and confirmed when magnetotactic bacteria were observed in 1975).

Kirschvink was quoted in *Science* as believing that a Martian biogenic source for the meteorite’s magnetite was “not unreasonable at all.” He has written that the “putative Martian magnetofossils. . . look interesting, perhaps the most convincing of all the evidence marshaled in the paper.”

Kirschvink claims that the hypothesis of biogenic Martian magnetite can be tested quite easily. Characteristic of terrestrial magnetofossils and true bacterial magnetosomes (groups of magnetic crystals), is the alignment of the magnetite crystals in linear chains. Experiments could show whether the Martian magnetite occurs in linear chains. Also, depending on what the strength of Mars’s original magnetic field might have been (Kirschvink believes it was probably substantial) and whether it shifted in direction, and depending on the conditions under which the magnetite-

FEYNMAN’S MISPLACED LECTURE

In Feynman’s Lost Lecture, which appeared in our last issue, David Goodstein recalled Richard Feynman giving a guest lecture (not the lost one, but the one in which Feynman spoke of “his” supernova) to Goodstein’s freshman physics class shortly before Feynman died. David A. Edwards, BS '90, PhD '94, who was in the freshman physics class (and so was his wife) in December 1987, when this lecture allegedly took place, writes that it wasn’t so—that neither Feynman nor Goodstein taught the class—and sent along his homework assignment (listing McKeown and Frautschi as instructors) to prove it. Goodstein researched the class records, and found that indeed his memory was faulty, and that Feynman had delivered his last guest lecture to Goodstein’s class on March 13, 1987, just a month after the supernova, but 11 months before Feynman’s death.

AND A MISPLACED PERSON

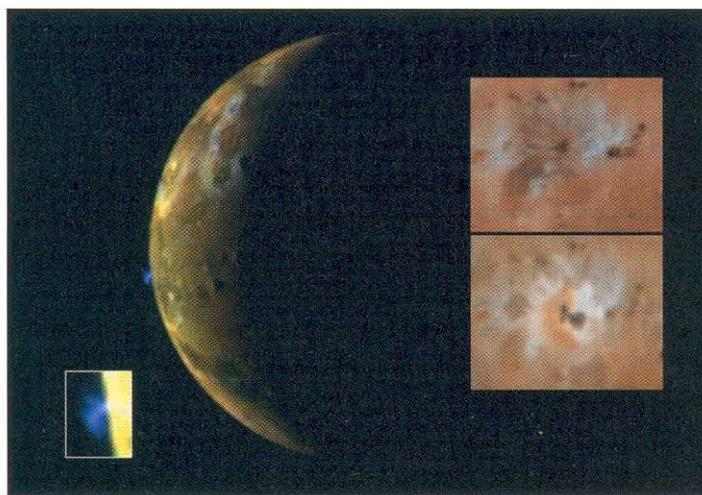
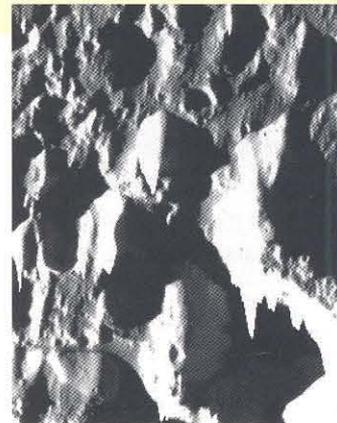
In the last issue of *E&S*, the caption on page 10 accidentally transposed the names of the two graduate students. Brett Doleman is, in fact, on the left, and Erik Severin is on the right.

containing carbonate was formed, various experiments might be able to determine whether the magnetization direction of the magnetite in ALH84001 is different from what would be expected of its host rock.

Kirschvink's own lab is the only magnetically shielded, clean-lab facility in the world housing a superconducting magnetometer system, which, although designed for studies of biogenic magnetite in animal tissue, could also accommodate a piece of rock. And his 15-year-old SQUID (superconducting quantum interference device) moment magnetometer was recently rebuilt and equipped with new sensors that would easily be able to measure tiny amounts of Martian magnetite. Hojatollah Vali, of McGill University, one of the *Science* paper's coauthors, was a visiting associate in this lab in 1989, and Kirschvink has offered his facility for further tests to help determine whether life did indeed exist on ancient Mars. □

GALILEO SHOOTS THE MOONS

On July 27 the Galileo orbiter spacecraft flew by Jupiter's moon Ganymede within 519 miles (70 times closer than Voyager 2), sending back stunning images of incredible sharpness. Ganymede, the largest moon in the solar system, is believed to be about half water ice and half rock. Galileo's experiments revealed that Ganymede has a magnetosphere, and its pictures show the moon's surface to be pock-marked by impacts with comets and asteroids and wrinkled and fractured by the same internal forces as Earth. While it was in the neighborhood, Galileo also snapped some shots of Europa from about 96,300 miles and Io from 604,000 miles. The spacecraft will pass closer to Europa in December.



Left: A new volcanic plume, colored blue by sulfur dioxide and extending 60 miles into space, erupts at Ra Patera on the constantly changing surface of Io. The inset at right shows how this volcano has changed since Voyager's visit in 1979 (top). The new plume has covered a surrounding area the size of New Jersey with the volcanic deposits seen by Galileo (bottom).

CALTECH ALMOST MAKES THE MOVIES

Sharp-eyed moviegoers amongst us may have picked up on the Caltech presence in last summer's brace of alien-invasion flicks. An early scene in *Independence Day* shows the military brass trying to figure out why all their satellites are going off-line. During the conversation, a badly pixellated, purple-and-red photo—obviously an infrared-telescope image—is handed round. The image shows a mysterious round object, the size of a minor planetoid, orbiting Earth. (This, of course, is the alien mother

ship, but only the audience knows that then.) A couple of scenes later, a second photo shows a set of thin, flat objects (the just-launched invasion fleet) underneath the mother ship. It's only on the screen for a few seconds, but emblazoned across the bottom of the photo is "10-METER KECK TELESCOPE, MAUNA KEA, HAWAII." No other affiliation is given, however.

And the folks who took in *The Arrival* saw Caltech but didn't know it. Caltech's Owens Valley Radio Observatory played the part of Oro

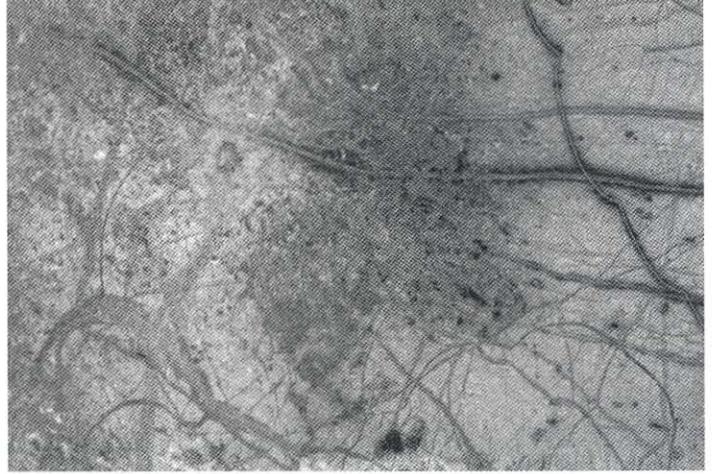
Valley Observatory in the film. Several exterior shots were filmed there, including those of the actors clambering about in the 130-foot dish. On the other hand, the scenes that purported to be of Caltech's Jet Propulsion Laboratory, where radio astronomer and SETI (Search for Extra-Terrestrial Intelligence) expert Zane Ziminski (Charlie Sheen) worked, were shot elsewhere. And to round out the "things are not what they seem" category, Tony Award winner Ron Silver plays Gordian (as in knot), the head of JPL who's also

One Of Them.

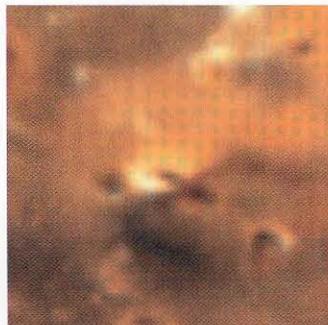
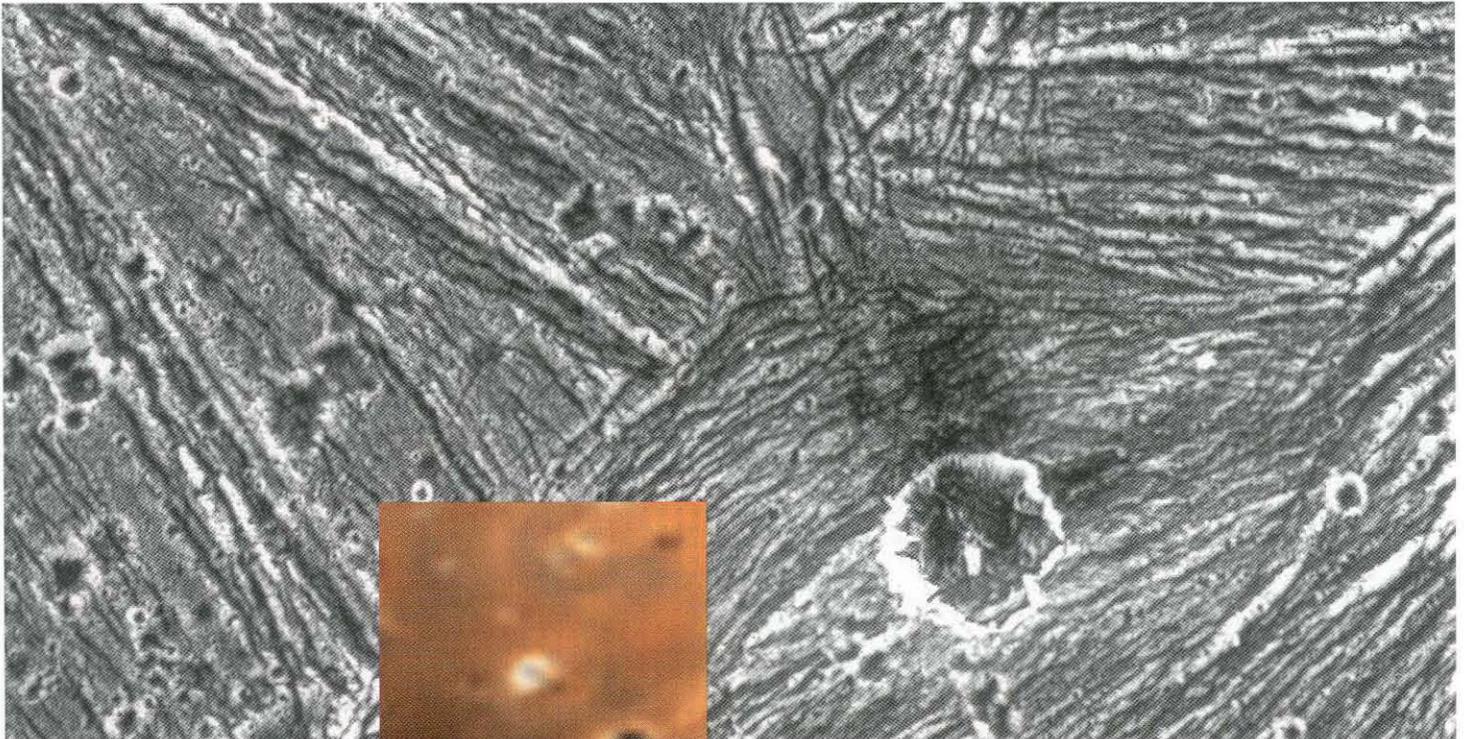
And released shortly before *E&S* went to press is *Infinity*, starring Matthew Broderick as a very young Richard Feynman and Patricia Arquette as Arline Greenbaum. Greenbaum, Feynman's first wife and the great love of his life, died of tuberculosis in an Albuquerque sanatorium while Feynman worked on the Manhattan Project at Los Alamos, a hundred miles of bad road away. Since Feynman didn't come to Caltech until 1950, Caltech isn't mentioned in this movie, either. □

Left: Ice hills in an unnamed region of Ganymede, their western sides lit up by the sun, were seen in 2000-times greater detail than any previous images. The smallest objects here are only 11 meters across.

Below: This terrain in the Uruk Sulcus region is typical of about half of Ganymede's surface. The ancient, pock-marked, cratered terrain to the left (north) is cut by younger striations on the right. Next to the large impact crater at lower right, some dark material has been ejected onto these linear ridges. The smallest features that can be seen here are about 74 meters across.



Above: On Jupiter's moon Europa, a new impact crater (left of center), about 30 km across, scattered light-colored debris over a wide area as it struck Europa's icy crust. The X-shaped pattern at right probably occurred as the icy crust fractured and then filled in with slush.



Galileo's view (bottom) of Euboea Fluctus on Io (seen here in simulated Voyager color for comparison) shows diffuse material that has been deposited around the volcano since Voyager 2 flew by in 1979 (top). It extends over a radius of 285 km.