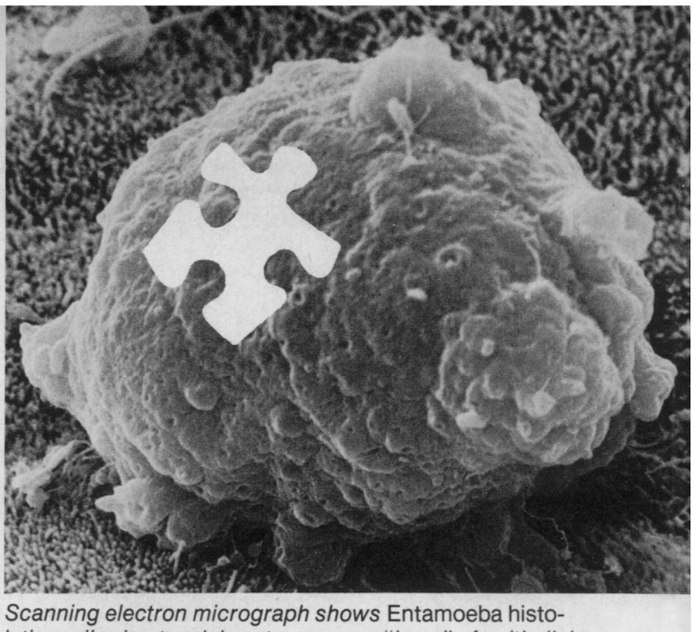
# Mystery Amoeba

## Parasitologists struggle to decipher a puzzling microbe's true identity

By STEPHEN HART



Photos: Martinez-Palomo

*Scanning electron micrograph shows* Entamoeba histolytica *adhering to a laboratory-grown "lawn" of epithelial cells that line the gut. The amoebas range from 10 to 40 microns in diameter.*

The tiny creatures look innocent enough in the cool green light of a microscope. Smaller, rounder versions of the freshwater amoebas studied by high school students, they ooze through the cloudy fluid, eating specks of ground rice. But their looks are deceiving. These amoebas can eat their way through a human's intestinal wall, lodging in the liver and creating abscesses severe enough to kill.

*Entamoeba histolytica* infects the lower intestine of nearly 500 million people — a tenth of the world's population — and kills at least 40,000 of its human hosts each year. Scientists rank the disease, called invasive amebiasis, as the world's third deadliest parasitic ailment, surpassed only by malaria and schistosomiasis. In the United States, 3 to 5 percent of the population harbors the organism, including 30 percent of male homosexuals in some cities.

Most of those infected, however, do not develop the disease. Despite a century's worth of research, scientists still can't explain why invasive amebiasis strikes fewer than 10 percent of the parasite's hosts.

In laboratories around the world, parasitologists using the latest techniques of molecular biology have now reached one conclusion: Though benign and invasive amoebas of this species look alike under the microscope, they differ biochemically. But the stability of those differences remains controversial. Some think recent research shows that people who get sick harbor a genetically different strain of the same species. Other researchers remain skeptical, insisting that the amoeba can change from benign to virulent, like Dr. Jekyll changing to Mr. Hyde.

Scientists haven't completed the *E. histolytica* jigsaw puzzle, but they do know the amoeba's natural history. The organism appears to multiply only asexually. It spreads from one person to another by changing into well-protected dormant cysts that pass into the outer world along with feces. When a person swallows the cysts with fecally contaminated food or water, the amoeba becomes active again, taking up residence in a new colon.

Surprisingly, *E. histolytica* can usually plead innocent to the charge most frequent leveled against it — causing traveler's diarrhea. Even in regions of the world where the organism abounds, amoebic dysentery accounts for fewer than 1 percent of the cases of traveler's diarrhea. And the World Health Organization doesn't rank it high among causes of dysentery in Third World countries, according to Kenneth E. Mott of the organization's Parasitic Disease Program in Geneva, Switzerland. But that's not to say its consequences are trivial for an afflicted individual. "If you ask a tourist who's had amebiasis," Mott adds, "he will assure you that dysentery is a very serious disease."

Occasionally, *E. histolytica* shows its ugly face in unexpected places. In 1980, at a chiropractic clinic in Colorado, 36 patients got amoebic cysts while undergoing colonic irrigation therapy — an unproven, enema-like treatment used by alternative health practitioners for a variety of complaints. Six of the patients died.

Yet the parasite continues to act like Jekyll rather than Hyde in about 90 percent of the people it infects, living peacefully in the colon and producing no symptoms. Even in AIDS patients, whose ravaged immune systems succumb to many weak parasites, the amoeba often causes no problems.

Does the amoeba's dual nature stem from two separate strains — a harmless Dr. Jekyll and a deadly identical twin? Or does the good doctor sometimes change to Mr. Hyde, making every *E. histolytica* infection potentially dangerous? At stake is the high cost, especially in Third World countries, of current attempts to treat the 430 million carriers who pass cysts but suffer no disease.

"It's a hot topic, very debatable," says parasitologist Sharon L. Reed of the University of California, San Diego. The United States can afford to treat every *E. histolytica* carrier, usually with the drug metronidazole (Flagyl), Reed says. But in developing countries, she adds, "if there's a reliable test that can say there's less than 5 percent chance that this person is ever going to get any symptoms from this particular *E. histolytica,* then you can save [the cost of] the therapy, which might be equivalent to the [government's] whole health budget for that patient that year."

As early as 1925, when the species was first grown in the laboratory, parasitologists suggested the existence of a separate, pathogenic strain of *E. histolytica.* And much modern research backs up that idea. By separating four amoeba enzymes, each of which comes in varieties called isoenzymes, researchers have identified 22 different combinations. A group of amoebas sharing a particular isoenzyme pattern constitutes a "zymodeme." Because only eight zymodemes come from patients with amebiasis, many parasitologists say the isoenzyme test clearly separates invasive from benign strains. After five years of routinely isolating and growing the parasite in her own lab, Reed says, "we've had probably 150 isolates, and there's been a

100 percent correlation between the isoenzyme pattern we've gotten and [patients'] clinical syndrome."

Reed and others have also found other differences between pathogenic and benign amoebas. Cell adhesion properties, cell surface antigens and monoclonal antibodies all distinguish the two. In addition, Reed finds differences in the amoebas' vulnerability to the immune system's cell-rupturing proteins, collectively known as the complement system. Pathogenic amoebas withstand the body's assault, while benign amoebas succumb. And in an ironic twist, the pathogenic amoebas secrete a protein-dissolving enzyme that not only damages colon cells but also activates the complement system, turning the body's defenses against its own cells. Complement-caused inflammation may help the parasites penetrate the colon, Reed suggests in the July JOURNAL OF IMMUNOLOGY.

But another puzzle piece doesn't fit into this picture of two strains. In 1961, Louis S. Diamond of the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Md., pioneered a new technique to grow *E. histolytica* in the laboratory. He developed "clean cultures," coaxing pathogenic amoebas to flourish on finely ground rice in a nearly sterile fluid – without the hordes of bacteria they normally eat. Diamond has grown them directly from the colon and, by selecting a single amoeba, has raised a clone. But for years, he and others failed in attempts to grow *benign* amoebas without their lush flora of colon bacteria. Diamond assumed he hadn't found the right conditions. "I had never given it a really honest try," he says.

In 1984, David Mirelman of the Weizmann Institute of Science in Rehovot, Israel, joined Diamond for an all-out attempt to wean benign amoebas from a bacterial diet. They tried for four months and still failed.

Returning to Israel, Mirelman thought of a way to fool the amoebas. Again, he started with amoebas belonging to a benign zymodeme and suppressed their bacterial flora with antibiotics. But this time he also fed them radiation-weakened bacteria, which could not reproduce but still supplied nutrients. "This trick seemed to work," Mirelman says, "because the [amoeba] cultures did not die." He eventually weaned the amoebas of even the debilitated bacteria. The benign amoebas were now thriving with no bacteria. "But to my surprise," Mirelman says, "they were more active. So I checked the zymodeme, and to my bigger surprise, I found that it had changed."

Mirelman says both he and Diamond initially doubted this result but became convinced as they repeated the zymodeme switch. Diamond even flew to Israel to repeat the experiment himself. Not only did the zymodeme change, but the amoebas gained virulence, infecting the livers of lab hamsters. When Mirelman returned the newly pathogenic amoebas to their bacterial flora, they reverted to the original isoenzyme pattern and lost their virulence.

Mirelman then successfully repeated the experiment with cloned amoebas supplied by another lab, reducing the possibility that he had started with a mixture of pathogenic and benign amoebas. He concluded that the biochemical differences between zymodemes don't remain stable and that the amoebas can change from one state to another.

His results, published in 1986 in both EXPERIMENTAL PARASITOLOGY and INFECTION AND IMMUNITY, have not brought a stampede of *E. histolytica* researchers into his camp. Echoing many of the skeptics, Reed says the zymodeme changes have "never been observed to happen by most of the researchers who work with clinical isolates" from infected people. Molecular biologist John Samuelson of the Harvard School of Public Health and Brigham and Women's Hospital in Boston adds that Mirelman's experiment is "the only contradiction to the hypothesis of two strains."

Diamond and Mirelman say parasitologists in India and Norway have repeated the switch from benign to invasive and back to benign. But no laboratory has yet published a repetition of Mirelman's experiment.

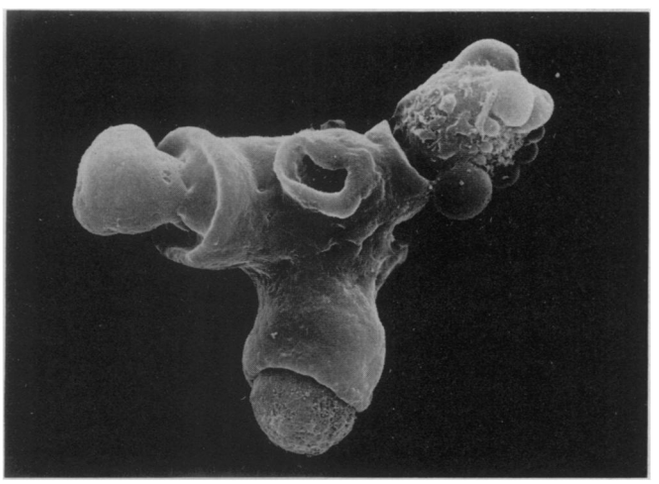
Scientists applying genetic engineering techniques to the amoeba *have* published results, which some say reinforce the picture of two separate strains.

Instead of focusing on isoenzyme patterns, which represent only the expression of genes, several of these researchers have directly compared genes from pathogenic and benign amoebas. Using genetic engineering techniques, they have isolated and cloned sections of the parasite's DNA. These radioactively labeled DNA fragments bind specifically to matching DNA, so that the probe itself becomes a label, identifying the test DNA. Working with Reed and others, Samuelson made such a probe that distinguishes *E. histolytica* from other parasites in stool samples. Samuelson's group reports its results in the April JOURNAL OF CLINICAL MICROBIOLOGY and suggests that DNA probes may offer a quick and accurate way to detect *E. histolytica* in large numbers of people.

At about the same time, a group at the Weizmann Institute created DNA probes that not only recognize the species but also distinguish pathogenic amoebas from benign ones, demonstrating a genetic difference. Reporting in the March INFECTION AND IMMUNITY, Leonard I. Garfinkel and his co-workers, including Mirelman, echo Samuelson's suggestion that DNA probes may prove useful in detecting amoebic infections.

And this summer, one more genetic piece of the two-strain picture fell into place. Egbert Tannich of the Bernhard Nocht Institute for Tropical Medicine in Hamburg, West Germany, describes two new DNA probes in the July PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (Vol. 86, No. 13). Like Garfinkel's probes, these appear to distinguish invasive and benign amoebas. Tannich says the test is sensitive: "I only need 20 or 30 amoebas to distinguish [pathogenic from benign amoebas]." He told SCIENCE NEWS he has since tested amoebas isolated from 40 people – some with disease symptoms, others without – and still sees only two types, which he views as distinct strains.

In Mexico City, Adolfo Martínez-Palomo of the National Polytechnic Institute and his co-workers may have discovered the same DNA sequence. During their search for a probe, "apparently we stumbled into the same thing," Martínez-Palomo told SCIENCE NEWS. Their ongoing work remains unpublished.



*This voracious amoeba simultaneously engulfs three epithelial cells while a fourth "mouth" prepares to suck in another cell.*

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Infrared Mapping Spectrometer will map the global distribution of chlorofluorocarbons and other atmospheric gases thought to contribute to global warming through the greenhouse effect. It will also seek thunderstorm-generated clouds punching up into the mesosphere. At the same time, Galileo's Ultraviolet Spectrometer will study the ozone hole over the South Pole and take what Clarke and Fanale call the first measurements of Earth's "airglow," or ultraviolet fluorescence, ever made by a spacecraft coming toward it from deep space.

On the first of Galileo's two Earth encounters it will fly straight up the tail of Earth's magnetic field, allowing a Plasma Science Experiment to measure the electrically charged particles trapped there. Even the mere existence of Galileo's radio beam, independent of whatever scientific data it carries, will be valuable, letting researchers measure subtle changes in the craft's trajectory and thus determine Earth's mass more accurately than has been possible with any past spacecraft.

While passing the Earth, Galileo will probably get good spectral measurements of Earth's moon, enabling comparisons of mineralogical differences between the moon's near and far sides, including photos of a previously unmapped strip on the near side south of huge Orientale basin.

**Oct. 29, 1991: *Gaspra.*** Nine months after the Earth encounter, the craft will take the first close look at an asteroid, passing within about 1,000 km of Gaspra. So far, as with all asteroids, space scientists have little to go on in studying Gaspra except spectral measurements from Earth. They believe the asteroid measures about 15 km in diameter and resembles a stony meteorite in composition. Zipping by at about 29,000 km per hour, Galileo will have time to gather only a few photos and other data. Yet whatever they show will be unprecedented.

**Dec. 8, 1992: *Earth again.*** The first Earth flyby will have placed the spacecraft in an orbit that takes about two years, including the Gaspra visit, to circle the sun. NASA has designed the carefully calculated second Earth trip to enlarge Galileo's orbit into one with a six-year period, big enough to reach all the way to Jupiter. The craft this time will pass only 300 km from Earth.

This flyby will also carry Galileo directly over the moon's north pole, offering a chance to look for signs of what some scientists believe may be ice left in the shaded parts of lunar craters by comet impacts. This controversial idea suggests a possible source of water on the moon, a valuable resource in proposals for a permanently inhabited lunar base.

**Aug. 28, 1993: *Ida.*** Outbound toward Jupiter, Galileo is scheduled to take a close look at the asteroid Ida, about 30 km in diameter (estimated, as in the case of Gaspra, from Earth-based measurements and from assumptions about the composition of its surface). This time the spacecraft will fly past even faster, with mission scientists hoping to grab a few data as the craft hurtles by at about 45,000 kph.

Nearly two more years will pass before Galileo's probe separates from the rest of the craft and heads off in July 1995 for its deep dive into the atmosphere of Jupiter. The dramatic plunge, expected to take place that Dec. 7 and last for perhaps 75 minutes, will be the first such penetration of an outer planet's dense atmosphere. On the same day, the orbiter will make its one close trip past Jupiter's bizarre, volcanically active moon Io, flying only about 1,000 km away and so deep into Jupiter's radiation belts that the spacecraft was modified from its initial design to include "radiation-hardened," solid-state electronic components. Finally, Galileo will settle in for two years of photographing and measuring the Jovian system, including the planet itself and the three other big Galilean satellites — Europa, Ganymede and Callisto.

Jupiter and its moons are the mission's central goal — but getting there should be a rewarding journey. □

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While the detection of genetic differences may seem to complete the puzzle, the one piece that didn't fit won't go away. Garfinkel's DNA probes did show genetic differences between pathogenic and benign zymodemes. But the group also tried its probes on lab zymodemes that appeared to have changed when weaned of bacteria. A DNA probe specific for pathogenic amoebas recognized amoebas that had changed from benign to pathogenic. The benign probe recognized the amoebas of the same zymodeme after they had returned to a benign state among colon bacteria from which they were isolated.

The Israeli DNA probes appear to tell a curious story. Not only had the enzyme pattern changed — a result that could be due to differences in gene expression — but the genes themselves also seemed to have changed. And the researchers found hints that all amoebas may have at least one copy of both pathogenic and benign DNA. Faint traces of the pathogenic probe bound to benign amoeba DNA, and faint traces of the benign DNA probe bound to pathogenic amoeba DNA.

The group acknowledges a slight possibility that the DNA and zymodeme changes arose from an inadvertent mixing of amoeba types in the lab. But they speculate that *E. histolytica* may be able to amplify, or make extra copies of, certain genes in response to differing conditions. "We were intrigued by the idea that the amoeba has master copies [of genes] that it amplifies," Garfinkel says.

Argues Samuelson, "It's a stretch to imagine how an organism can change its genome depending on its environment." He finds the evidence for two strains "overwhelming" and says Garfinkel's paper itself supplies plenty of support for the two-strain hypothesis.

The genetic evidence for two strains has also convinced Tannich. "Now it's clear that there are two genetically different subspecies and that only 10 percent of the infected people have to be treated — only the group that is infected with pathogenic forms," he says.

Mirelman disagrees. "I think it's premature to say that they are genetically distinct until you preclude the possibility that a master sequence is present," he says.

And parasitologists can't ignore Mirelman's odd puzzle piece, contends Martínez-Palomo. Fitting Mirelman's findings into the body of *E. histolytica* research is "the most important question to be solved in amebiasis right now," he says. Mirelman acknowledges that the experiments are difficult to reproduce, and he doesn't yet understand how the switch works. The process seems to have "some sort of magic in it," he muses.

If, in completing the puzzle, researchers find themselves looking at a clear picture of two strains, they may be able to turn their laboratory DNA probes into diagnostic tools to detect virulent infections before symptoms appear. Such a test could precisely identify the 10 percent of infected people whose lives may depend on the expensive treatment.

As a research tool, Tannich's probe is "very sensitive and rapid," he says. But he adds, "I think in developing countries it is not useful at the moment." Parasitologists in several labs, however, are moving ahead with attempts to create a nonradioactive probe that could be used in Third World villages. Reed hopes to develop such a probe within a year and says it should outperform the current "gold standard" of diagnostic techniques — microscopic examination of stool samples.

"That could change completely the way clinicians treat [the] infection," Martínez-Palomo says. NIAID's Diamond remains unpersuaded. "I don't see molecular biology leading to a diagnostic tool," he says. "But it's about the only way we are going to be able to understand what makes [*E. histolytica*] virulent."

As more genetic pieces fall into place, a clearer picture of *E. histolytica* should emerge. Then parasitologists may finally see whether gentle Jekyll has a dangerous double or transforms into the hideous Hyde. □