Of Mice, Oncogenes, and Rifkin

Activist Jeremy Rifkin seeks to enjoin experiments involving oncogenes, transgenic mice, and the AIDS virus; he also wants NIH to do an environmental impact statement

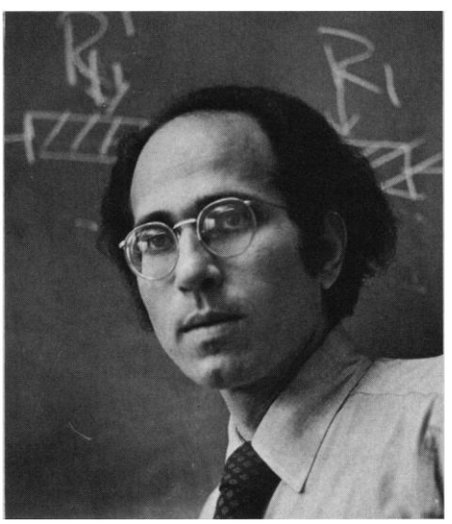
In the worst-case-scenario world of activist Jeremy Rifkin, almost anything could happen. Mice carrying the AIDS virus could escape from an elaborate "mouse jail" and run wild through the streets of suburban Maryland. Human cell lines that have been genetically engineered to carry the AIDS virus could "infect" other cells in laboratories around the world, resulting in "the uncontrolled spread of the AIDS virus genome." And finally, the intestines of well-meaning but foolhardy scientists could become "cancer-producing factories" fueled by the very genes they sought to control.

Even Rifkin admits that taken together these events sound more like the plot of a grade-B horror movie than the normal run of affairs in the country's biomedical research laboratories. No matter. Rifkin and his Washington, D.C.–based Foundation on Economic Trends are suing the National Institutes of Health (NIH) and the Department of Health and Human Services anyway. The suit calls for the immediate suspension of certain experiments involving oncogenes and the human immunodeficiency virus (HIV). Rifkin is also trying to force NIH to produce an environmental impact statement that examines the potential hazards of such research.

Rifkin's suit targets oncogene research in general and the work of two scientists in particular. The first is Richard Axel of Columbia University, who has managed to manipulate a line of human epithelial cells so that they can serve as a target of HIV infection. The second is Malcolm Martin, chief of the molecular biology and microbiology laboratory at the National Institute of Allergy and Infectious Diseases, who recently succeeded in introducing the entire genetic code of HIV into a strain of lab mice. The work is believed to be the first time that the complete genome of a human pathogen has been placed into every cell in a mouse.

Martin's research has raised eyebrows even within the scientific fraternity. A group of immunologists and others at NIH initially raised questions about Martin's proposed "transgenic mouse" experiment. "They were worried about putting the AIDS virus into the germline of mice. Like everybody at NIH, they've walked down the halls at night and have seen mice running around," explains Martin.

The scientists' concerns were real enough to delay Martin's research for several months, during which time there was a debate over the experiment in the office of Joseph E. Rall, the deputy director for intramural research at NIH. To calm the waters, Martin gave a tour of the facility where he planned to keep his mice. Eventually a compromise was reached. Instead of breeding hundreds of mice, Martin would limit the number to 108 and would end his experiment in April.



Richard Axel *says there is no risk in his work on the CD4 receptor in HeLa cells.*

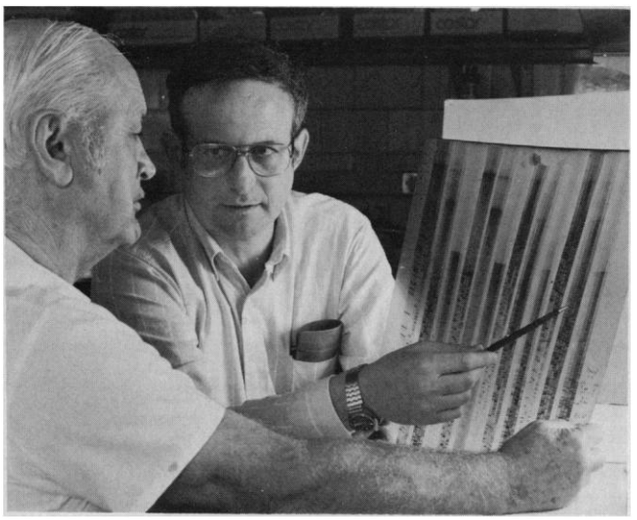
It was not until last November that Martin and his colleagues microinjected into the pronuclei of newly fertilized mouse eggs a sequence of DNA obtained from two strains of HIV, one from the French isolate and one from New York City. A few weeks later, Martin snipped off pieces of the newborn pups' tails and found that of 34 mice tested, 4 were positive for HIV.

Martin plans to breed the mice and to see if any of the transgenic animals express the virus or get symptoms of disease. The effects of the virus in the genome of the mice, Martin notes, will not completely mimic how the AIDS virus operates in humans, since mice do not have cells such as the human T lymphocyte which possesses a special receptor for the virus to bind to. But the custom-built animals may allow scientists to study the virus' latent phase and to see what effect proteins secreted by the virus have on the animal.

One of the difficulties in doing AIDS research is the lack of adequate animal models. Chimpanzees, which may harbor the virus but do not get AIDS, are extremely expensive and in short supply. Says Martin: "If we had a mouse model, even one to study the latency phase of the virus in vivo, I'd be very happy." Martin does not yet know what is happening inside his animals. "At this point, they're perfectly normal-looking mice," he reports.

What is not entirely normal is the facility that houses the new life forms. Martin calls the place "a mouse jail." The facility is actually a laboratory on the Bethesda campus with a Biosafety Level (BL) of 4, the maximum. There are only a half a dozen BL4 facilities in the country. The special buildings are designed to contain microorganisms, often extremely dangerous and exotic ones, and as such have elaborate security and containment systems, such as fumigation chambers, double-doored autoclaves, filtering devices, and foyers with showers. The entire working space is sealed and a negative air flow keeps microscopic organisms from escaping. "But needless to say, mice are not microorganisms," says Martin. Adds Dinah Singer, chairman of the NIH biosafety committee that approved Martin's work: "It is a truism of nature that mice escape."

With such a possibility in mind, Martin constructed a mouse jail that should contain a mouse possessing even the most extraordinary tenacity and skill. First of all, the mice are in cages within a sealed glass hood called a glove box. There are three ways out of a glove box. First, a mouse could try to swim for it through a "dunk tank" filled with 12 inches of Clorox bleach. Second, the mouse could try to survive in the double-doored autoclave which automatically sterilizes the debris removed from the cages. Since the autoclave operates by scalding with pressurized steam, chances for survival would be minimal. Finally, a mouse could chew its way through one of the thick black rubber



Malcolm Martin

Work on transgenic mice involves extraordinary containment procedures, but "at this point, they're perfectly normal-looking mice."

gloves that researchers use to handle the animals within the glove boxes. Martin says this risk is real enough. Fortunately, the mouse would still be a long way from freedom. "He'd have to get through five locked doors," says Martin. An additional boundary was constructed before the innermost door so that a researcher entering the room could see if there was a mouse poised to run. As a final precaution, the floor is dotted with mouse traps.

Rifkin is not overly impressed. "I don't care if they do have moats with Clorox and mousetraps," says Rifkin. There are still terrorists, for example. Martin says all the locks were changed prior to the experiment and that admittance is limited to his staff. Rifkin counters: "The point is that there are no protocols for dealing with this kind of research. Just because they're doing it right at NIH doesn't mean they'll do it right in some other lab at some other place."

William Gartland, chief of the Office of Recombinant DNA Activities at NIH, says that there are rules for working with transgenic animals. According to NIH's Guidelines for Research Involving Recombinant DNA Molecules, a sort of bible that contains the federal commandments regarding work with recombinant genetic material, if less than two-thirds of a viral genome is transferred into any nonhuman vertebrate, it may be propagated in a BL1 facility. But for experiments like Martin's, where the whole genome of a human pathogen is transferred, the appropriate containment level is decided by an Institutional Biosafety Committee such as Singer's.

Singer confirms that there are no established protocols for work with transgenic mice with HIV. From the beginning, Martin had stated that he would do the experiment in a BL4 laboratory, but Singer stresses that there are no federal regulations that forced Martin to do so. In fact, Singer's committee was not even certain that it had the authority to review Martin's experiment. "We knew we were setting precedent and so we thought very, very carefully about this experiment," says Singer. "We agreed that AIDS was enough of a medical emergency that it was worth trying the experiment. If it works, it would be of such value. But there are risks."

If there are inherent risks with Martin's experimental mice, Richard Axel at Columbia is not sure what risks are associated with his modified cell lines. In 1986, Axel and colleagues Steven McDougal at the Centers for Disease Control in Atlanta and Robin Weiss at Chester Beatty Laboratories in London introduced a gene called CD4 into a cell line called "HeLa," an epithelial cell line derived from a cervical carcinoma of a Baltimore woman named Henrietta Lacks who died in 1951. The CD4 gene codes for a receptor on the cell's surface that is also called CD4 (or T4), the site of binding for HIV infection in human T lymphocytes and certain macrophages. Axel and his co-workers got HeLa cells to express the receptor. Then they successfully infected the transformed cells with HIV.

In Rifkin's suit, the science of Axel's work is somewhat muddled. The legal document keeps referring to "T4 receptor cells on the surface of white blood corpuscle cells." Rifkin apparently is confusing proteins with whole cells. The suit states that researchers have placed HIV "into an extraordinarily virulent and infectious line of cancer cells known as HeLa cells . . . thus increasing the virus' host range and potentially leading to the further hazardous dissemination of the AIDS virus genome." HeLa cells were, and perhaps still are, a notorious contaminant of other cell lines. Because of this, Rifkin maintains that Axel's HeLa cells could contaminate other cell lines and that laboratory workers around the world could be unwittingly exposed to the AIDS virus.

That is extremely unlikely, counters Axel. He points out that there is a world of difference between the words "contamination" and "infection." Axel also argues that his transformed HeLa cells generally "do not do well" and adds that "the cell lines with virus are at a gross selective disadvantage." Axel notes that these cell lines cannot grow outside of tissue culture and that laboratory workers should be well aware of whether the cells they're working with have CD4 surface receptors or not. As for the value of his experiments, Axel says that it was precisely these kinds of experiments that helped identify CD4 as a receptor for the AIDS virus. Also the work led to the synthesis of soluble CD4 protein which is a potential inhibitor of viral infection in humans. This in turn could lead to new drugs to block or slow infection by HIV.

In addition to seeking to enjoin the experiments of Axel and Martin, Rifkin's suit attacks oncogene research in general. Rifkin is concerned about "the new generation of cloning vectors." According to Rifkin, these vectors could introduce oncogenes not only into laboratory strains of bacteria, but into the wild strains of *Escherichia coli* naturally residing in the human digestive tract. "Such a person's digestive tract would then become a kind of factory, continuously producing oncogene proteins," the suit states.

Several researchers who work with oncogenes dismissed the possibility of such an occurrence as "unreasonable." "After a decade of working with these materials there is no indication that this is a dangerous undertaking," says Robert Weinberg of the Whitehead Institute in Cambridge, Massachusetts. "Even if an oncogene got into the bacteria that reside in your gut and began producing oncogene proteins, so what?" asks Phillip Sharp, director of the Center for Cancer Research at the Massachusetts Institute of Technology. Sharp says: "Oncogenetic proteins produced by bacteria and exposed to mammalian cell surfaces do not cause tumors. That is a nonconcern and you can quote me."

Both Weinberg and Sharp stress that one oncogene alone is not sufficient to turn normal cells into cancerous ones. There are a number of factors at work in cancer, says Weinberg, such as other genes, carcinogens, or viral infection.

Rifkin prefers not to get too bogged down in scientific debate. "The whole point of the suit is get NIH to do an environmental impact statement. Then, we can ask all the questions we want. The public can comment. We can see whether these new technologies require more or less safeguards," says Rifkin.

The last such environmental impact statement was completed by NIH in 1977 and examined the first Guidelines issued in 1976. Rifkin believes that the original environmental impact statement is sadly out of date. "When they did the original impact statement they'd never heard of the word AIDS," says Rifkin. "We think it's time to stop and reevaluate what has happened during the past 10 years." Rifkin insists that "it is not enough for a bunch of scientists to tell us everything is okay. By law, the process has to be a public one."

The law Rifkin refers to is the 1982 National Environmental Policy Act, which requires a federal agency to prepare a supplemental environmental impact statement when "there are significant new circumstances or information relevant to environmental concerns. . . ." Says Rifkin: "You can't tell me that somebody from NIH is going to stand up in court and tell the judge that there haven't been significant changes in the last 10 years."

Someone might. Robert Lanman, NIH's legal adviser, says that his office is working on a response to Rifkin's suit. Gartland says that although NIH has not done an environmental impact statement since 1977, the agency has produced about a half dozen "environmental assessments," a much less formal process that does not involve lengthy public comment.

Rifkin has successfully used the National Environmental Policy Act before. In 1986, he sued the Department of Defense, which is currently in the throes of preparing an environmental impact statement covering research at all government and contract laboratories doing work for the Biological Warfare Defense Program, which is studying such deadly subjects as yellow fever, anthrax, and botulism. As part of the settlement, Rifkin gave up his request that the research be enjoined; the military agreed to do the environmental impact statement. Rifkin and his attorneys seem prepared to discuss a similar deal with NIH. Whether officials at NIH want to listen is another matter, though it would be an interesting conversation. ■ **WILLIAM BOOTH**

Fat Survey Trimmed in Lean Budget

Body fat provides a biological record brimming with information about people's exposure to chemicals. So for more than two decades, the Environmental Protection Agency (EPA) has tested fat tissue from people across the nation to track the fate of toxic substances. The findings from these surveys have flagged potential public health problems, prompted the agency to ban or restrict the use of certain hazardous chemicals, including DDT, dioxin, and PCBs, and indicated whether tighter regulations are indeed working.

But EPA, whose budget has become ever leaner under the Reagan Administration, is proposing to trim away the $1.2-million monitoring program, known as the National Human Adipose Tissue Survey. Advocates of the survey assert that without the program, health officials and policy-makers will be significantly handicapped in regulating toxic chemicals.

EPA is currently apportioning money appropriated by Congress for fiscal year 1988 and plans to phase the fat survey to keep within its funds. The agency is on the verge of approving its final budget. The program's proponents are hoping that at this late date Congress will appropriate new money to bail it out and give it permanent authorization.

Martin Halper, director of the exposure evaluation division in the office of toxic substances, supports the survey but says that budgetary constraints have forced him to eliminate it together with other programs not mandated by Congress. In 1981, the annual budget for the exposure division was $41 million, but since then it has been steadily cut. This year, the division's budget is $17 million. "The Adipose Tissue Survey was the only single nonmandated program that was left," Halper says. "There wasn't much choice but to cut it because there was no money. It's hard to get blood from stone."

There is no other comparable program in the public or private sector to monitor human exposure to toxic chemicals, EPA officials and others outside the agency say. In the program, a network of pathologists and medical examiners across the country collect fat tissue in a statistically designed survey from individuals who died in accidents and who have undergone elective surgery. The samples are then analyzed by gas chromatography and mass spectrometry, which provides precise fingerprints of chemicals accumulating in humans. About 10,000 tissue specimens—some dating back to the early 1970s—are archived in Kansas City for research, and the mass spectral information is stored on tape at the National Bureau of Standards.

Richard Thomas, director of the National Academy of Sciences' committee on toxicology, says that the fat tissue survey "is an important program because it gives a way to judge whether levels of toxic substances are increasing or decreasing in humans and, in the end, whether regulations are having an effect, especially with chemicals such as dioxin." Morton Lippmann, chairman of the EPA science advisory board subcommittee on indoor air quality and professor at New York University, says, "Early detection is important for these chemicals." The survey "is an extremely valuable resource."

Ellen Silbergeld of the Environmental Defense Fund and others argue that the survey findings also help to determine which chemicals are potential problems. Tens of thousands of chemicals in commerce are still untested for their potential health hazards, according to a 1984 report by the National Academy of Sciences. The EPA survey "is a good way to prioritize" worrisome chemicals, Silbergeld says.

Thomas and others say that the survey findings also provide direct information about human exposure to toxic chemicals and, by doing so, help to validate experimental models used by researchers to predict exposure and health risks to people.

The survey results were instrumental in spurring EPA to regulate PCBs, says Joseph Breen, program manager of the survey. In the early 1970s, 85% of the American population had detectable levels of PCBs or polycholorinated biphenyls in their bodies, according to EPA estimates based on the survey. By the mid-1970s, the percentage shot up to 100%, spurring the agency to impose strict regulations on the toxic chemical. By 1983, the survey showed that fewer people, especially children, had detectable levels, which was strong evidence that the PCB rules were working.

EPA's fiscal 1988 budget has enough money to keep the refrigerators in Kansas City with the tissue samples plugged in and the tapes maintained at the National Bureau of Standards, but no money for additional surveying and chemical analyses. Halper of EPA says, "This is the kind of program that needs guaranteed funding. As a discretionary program, it will always be vulnerable to cuts." ■ **MARJORIE SUN**