explored a number of possibilities within the context of the grand unified theories. Massless quarks might suddenly acquire a mass through a process known as spontaneous symmetry breaking, for example.) In any case, a low-density bubble forms and expands at virtually the speed of light. Hawking radiation enters from the surrounding de Sitter space and, during the next 10^{-42} second or so, converts to ordinary matter with a positive pressure. Human astronomers, denizens of the bubble some 10 billion or 20 billion years later, look back on it all and call it the Big Bang.

Where did all the matter and energy in the universe come from? It was Hawking radiation, says Gott. The 3 K cosmic microwave background radiation is a modern remnant. Why are the microwave background and the universe as a whole so well blended and uniform? Because in de Sitter space, says Gott, the Hawking radiation is naturally uniform.

Gott's bubbles form just like bubbles in a glass of beer—randomly. De Sitter space could easily hold an infinity of them. But alas, there is no way to communicate with our neighbors. Light would never make it from one bubble universe to the next. So how does Gott propose to test his model?

"We need to do more detailed quantum mechanical calculations of how bubbles behave in de Sitter space," he says. "If it becomes a nice, self-consistent theoretical framework, that would stand it in good stead.

"The most important observational test is the large-scale structure of the universe," he adds. "Galaxies and clusters never would have formed in a completely homogeneous universe. So there must have been some *in*homogeneities. But no one has ever been able to figure out where they came from. This theory might allow us to calculate the initial spectrum of random fluctuations, and see if those fluctuations could grow up into the large-scale structure we see today."—M. MITCHELL WALDROP

Leprosy Vaccine Trials to Begin Soon

Microorganisms isolated from armadillos are the basis of vaccine; investigators finally establish the disease in primates

Leprosy is a disease marked by myth and misinformation. The disease is commonly associated with the Bible, for example, but the mentions of leprosy in the Book of Leviticus and the Gospels may be mistranslations of the Hebrew and Greek terms for less serious forms of skin disease. It is widely believed that leprosy is highly infectious—and the term "leper" has come to mean an individual who is shunned by society—but in fact the disease is only mildly contagious and as much as 90 percent of the world's population may be immune. Individuals whose disease is controlled by drugs are completely noninfectious.

It is also believed that leprosy has been largely controlled. This may be true in the United States, where there are at most some 5000 cases, but it is not true in the rest of the world. There are about 12 million cases worldwide—3.5 million in India alone—and the prevalence in some small communities may be as high as 7 percent. Moreover, the problem is getting worse rather than better: the infective agent has begun to develop resistance to the most commonly used and most effective drug, dapsone.

But there is also a bright side. During the last decade, investigators have succeeded in infecting armadillos with leprosy, making significant quantities of the infective microorganism available for the first time. They are now using these microorganisms to produce a vaccine that is expected to undergo safety trials sometime this year. Some preliminary results from Venezuela suggest that this vaccine might even help people who already have the disease. Recently, furthermore, investigators have succeeded for the first time in infecting primates with leprosy, hoping to provide an animal model that more closely resembles the human disease. And finally, investigators are beginning clinical trials with combinations of drugs (see box) that promise to overcome drug resistance and that might even provide a cure.

> Production of the vaccine requires an extraordinary amount of cooperation among several research groups.

Leprosy is primarily a disease of the skin and peripheral nerves. It is characterized by a spectrum of severities ranging between two polar forms, tuberculoid and lepromatous. About 20 percent of leprosy victims develop the lepromatous form, which is characterized by skin lesions that appear over most of the body. The skin on the forehead and face thickens, with natural lines becoming exaggerated, and loss of facial hair can occur. Lepromatous patients are unable to produce a cell-mediated immune response to the microorganism for reasons that are not yet understood.

In the more common and less severe tuberculoid form, single skin lesions and loss of feeling in the involved areas are frequent early symptoms. Nerve involvement can also lead to damage to muscles and bones, and patients often inadvertently mutilate hands and feet because of the anaesthesia. These patients produce a partially effective cell-mediated immune response, but this weak response may also damage tissues. As many as 30 percent of all leprosy victims develop deformities.

Leprosy today is primarily a disease of the tropics; in the United States, it is restricted primarily to California, Texas, and Hawaii. The incidence in this country has more than tripled since 1965; many of the more than 225 new cases each year are discovered among immigrants from endemic areas. Investigators speculate that the higher incidence in developing countries may reflect poor sanitation. The disease is believed to be spread through nasal secretions.

The causative agent, *Mycobacterium leprae*, is related to the mycobacterium that causes tuberculosis. One of its most unusual characteristics is an exceptional-

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ly long doubling period of between 11 and 13 days, compared to about 1 day for the tuberculosis mycobacterium and 20 minutes for *Escherichia coli*. At least in part because of this long doubling period, the incubation period in humans is from 3 to 6 years.

Despite the fact that *M. leprae* has been recognized for 109 years, scientists have been unable to grow it in culture for reasons that are not clear. Last November, J. Kazda of the Institute for Experimental Biology and Medicine in Borstel, West Germany, reported at the International Peat Symposium in Bemidji, Minnesota, that he had been able to grow the microorganism in a culture containing chemicals from peat moss. Other investigators in the past, however, have reported similar successes, only to find that their microorganisms were not *M. leprae*—which can be difficult to distinguish from other strains of mycobacteria. Scientists are thus anxiously awaiting confirmation of the identity of Kazda's bacillus.

For many years, the only source of *M. leprae* was biopsy tissue from leprosy victims. "This source began to dry up as more and more patients were treated with dapsone," says Waldemar F. Kirchheimer of the U.S. Public Health Service Hospital in Carville, Louisiana. The first "major breakthrough" occurred in 1960 when Charles C. Shepard of the U.S. Centers for Disease Control discovered that the bacillus will proliferate in the footpads of mice. The finding was not entirely fortuitous. Shepard—who is now considered the "grand old man" of the field—and others had reasoned that *M. leprae*'s affinity for skin, testicles, and peripheral nerves reflects the microorganism's need for temperatures slightly lower than normal body temperatures. The mouse footpad is such an environment, and the bacillus appears to be restricted to it. This discovery provided a stable, albeit modest, source of the organism, as well as providing a milieu in which to test the effects of potential drugs and vaccines.

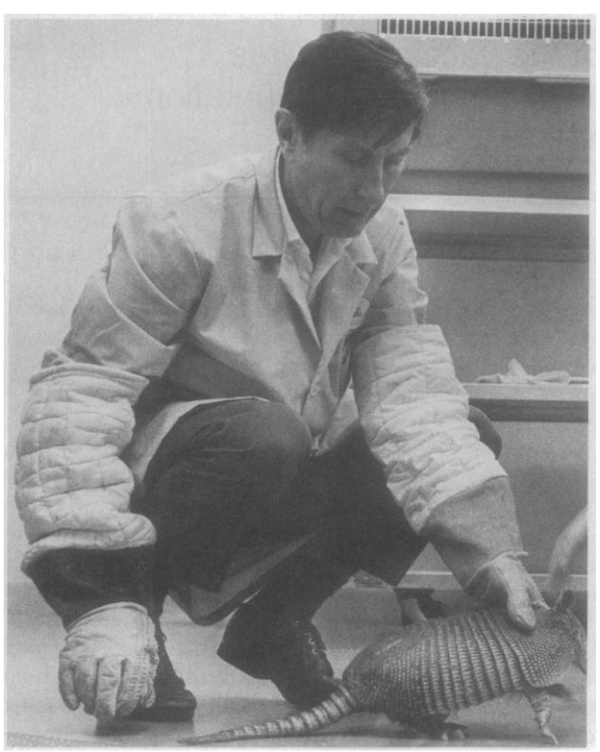
Some 9 years later, Kirchheimer and Eleanor E. Storrs, who was then at the Gulf South Research Institute but is now at the Florida Institute of Technology, collaborated to show that the native American nine-banded armadillo (*Dasypus novemcinctus*) could be infected with *M. leprae*. The armadillo is a burrowing, armor-plated mammal that is unusual in that it always produces four genetically identical offspring at each birth. Kirchheimer and Storrs chose it because its body temperature is 2° to 5°C lower than that of most other mammals. The infection is systemic, with an incubation period of a year or more, and the animals generally die of the disease. Perhaps most important, each 4.5-kilogram armadillo provides 150 to 250 grams of spleen and liver tissue containing as many as 10^{12} bacilli per gram, an ample quantity for both research and production of a potential vaccine.

Soon after the investigators had succeeded in establishing experimental leprosy infections in armadillos, Gerald P. Walsh, who is now at the Armed Forces Institute of Pathology, and his colleagues at Gulf South reported that they had found armadillos in the wild that also had the disease. Kirchheimer was unable to find such "naturally infected" animals for many years, perhaps because he was searching in different areas, and there was much debate about whether the animals had been infected in the wild or had escaped from the experimental facilities after infection. Only within the last year or so has it become generally agreed that the infection occurs naturally. There is, however, no evidence that the disease is transmitted from armadillos to humans.

Despite their success with armadillos, scientists still hoped to produce the disease in primates, since they are more closely related to humans. Isolated cases of apparent leprosy in primates were observed, but investigators were unable to transmit the disease to other animals. Then, in 1979, William E. Greer and his colleagues at Gulf South recognized leprosy in an adult female mangabey monkey that had been imported from Central Africa for unrelated studies. The monkey was turned over to the Delta Regional Primate Research Center for study by a team headed by Peter J. Gerone of the center and Wayne M. Meyers of the Armed Forces Institute of Pathology. The animal soon began to develop paralytic lesions—"an exciting development," Meyers says, because it had never been observed in animals before. In fact, the animal became so sick that it had to be rescued with dapsone and other drugs.

The group reported recently that they were able to transmit the disease from the monkey to armadillos, as well as to two other mangabeys. In addition, two more mangabeys developed leprosy after being inoculated with *M. leprae* from armadillos. The team is now making plans for a much larger study, in which the Yerkes Regional Primate Research Center will also participate, to study the transmission and immunology of leprosy among mangabeys. In particular, they hope that comparative studies of mangabeys and other primates will give some clues about why most humans are not susceptible to leprosy.

The ability to grow *M. leprae* in armadillos gave new impetus to attempts to develop a vaccine for leprosy. Previously, most work involved studies of immunization with related mycobacteria in



Emory University

New impetus

The discovery that the leprosy bacillus would grow in armadillos gave new impetus to attempts to produce a vaccine. The heavy gloves worn by Charles C. Shepard are to protect the investigators from bites and scratches.

hopes that they would share enough antigens to provide protection. The greatest experience has been with bacillus Calmette-Guerin (BCG), an attenuated or weakened variant of *Mycobacterium bovis*, which causes bovine tuberculosis. Shepard has shown that vaccination with BCG will prevent proliferation of *M. leprae* in mouse footpads, but results in humans have been equivocal. A large-scale vaccination with BCG in Uganda reduced the expected incidence of leprosy by 80 percent, while a similar trial in Burma reduced it by only 20 percent. Meanwhile, Shepard has shown that vac-

The Main Line of Defense Slips a Little

The front line in the war against leprosy is manned by drugs, particularly dapsone (4,4′-diaminodiphenyl sulfone or DDS), an inexpensive drug that has revolutionized the care of leprosy. First introduced in the 1940's, dapsone kills *Mycobacterium leprae* only very slowly. Treatment halts the course of the disease, however, and renders the patients noninfectious so they may be treated at home rather than in stigmatized leprosariums. Lepromatous patients may need to take the drug for the rest of their lives. Most who stop taking it eventually have a relapse, even after 20 years or more of therapy. The cost per patient is only about $5 per year, but India alone uses 50 tons of dapsone every year at a cost of $20 million.

But the front line is not holding firm. Resistance to dapsone has been observed at least since 1964, and the incidence is increasing. At a World Health Organization (WHO) conference in Rangoon, Burma, last November, investigators from WHO's THELEP (Chemotherapy of Leprosy) Program reported preliminary results from the first major studies of dapsone resistance. They found that secondary resistance (resistance among patients who have been receiving the drug regularly) had remained about the same, with rates varying from 3.6 percent in Shanghai to 6.4 percent in South India. But the incidence of primary resistance to dapsone (resistance among patients who had never received chemotherapy) had risen alarmingly, reaching 18 percent in Chingleput, South India, and 40 percent in Bamako, Mali.

There are, of course, other drugs that are also effective against leprosy. The two most important are rifampin and clofazimine, but they also must be administered for years and they cost 100 to 300 times as much as dapsone. Moreover, they have more severe side effects than dapsone, and strains of *M. leprae* resistant to them have been observed. The approach that must now be tried, says Charles C. Shepard of the U.S. Centers for Disease Control, is the use of combinations of these drugs.

THELEP is now sponsoring three sets of trials of drug combinations. In the first trials, now under way in India and Mali, combinations of the three drugs are being given to lepromatous patients who have never been treated for the disease. In the second set, also under way in India, intensive therapy with combinations is being given to lepromatous patients who have already been treated with dapsone. The therapy will be continued for as long as 3 years, then halted; the investigators hope to achieve a relapse rate of less than 1 percent per year after therapy is stopped, about the same rate that is now achieved with tuberculosis. In the third set of trials, to be begun soon in a site that is yet to be determined, intensive treatment will be given to tuberculoid patients who have never received therapy, again with the intention of stopping after 2 or 3 years.

Meanwhile, investigators are looking at existing drugs and new chemicals in an attempt to find inexpensive drugs that might be better than rifampin and clofazimine. But this process is hindered by the inability of investigators to grow *M. leprae* in culture. In a typical drug-screening program, a few milligrams of a potential drug can be tested for activity against the cultured microorganism in a few days. To study activity against *M. leprae*, however, the chemicals must be administered to mice for at least 2 months and a minimal study for a new drug requires at least 10 grams of material—a large amount for many newly synthesized chemicals.

One way to get around this impasse is to screen the chemicals against related mycobacteria. One bacillus that has been useful for this purpose is *Mycobacterium lufu*, a nonpathogenic microorganism from Africa that can be cultured and that is very sensitive to dapsone—suggesting that its drug receptors are similar to those of *M. leprae*. Many chemicals have been found that have some activity against *M. lufu*, says Shepard, and investigators are looking at synthesized derivatives that might be more active. All potential drugs must eventually be tested in mice. Some of the more promising are sulfones and cephalosporin derivatives, he adds, "but there are really no red hot candidates right now."

Other investigators are trying a different approach. K. P. Prabhakaran of the U.S. Public Health Service Hospital in Carville, Louisiana, for example, is studying the metabolism of *M. leprae* in the hope of eventually defining an appropriate culture medium. Meanwhile, he has found an enzyme, diphenol oxidase, that the bacillus uses to oxidize phenolic compounds. This enzyme is inhibited by sodium diethyldithiocarbamate, a chelating agent that is now used in the therapy of Wilson's disease. Prabhakaran and Waldemar F. Kirchheimer of the hospital have shown that the drug inhibits the growth of *M. leprae* on mouse footpads, and they plan to conduct trials in armadillos soon. Kirchheimer views their results as "very promising," and notes that the drug is already approved for use in humans and would not have to undergo safety trials if it is effective in armadillos.

Other investigators are searching for ways to speed up the identification of resistant microorganisms. This is now done in mice, a process that takes 9 to 12 months. Arvind Dhople of the Florida Institute of Technology has developed a new technique that measures the adenosine triphosphate (ATP) content of *M. leprae* to find out whether they are viable after drug treatment. He uses a firefly bioluminescence technique to measure ATP in a test that costs $2 to $3 and takes about an hour. Repetitive tests on bacilli isolated from a patient will show in as little as 3 months whether the organisms are losing ATP, a sign that the drugs are working, or whether ATP levels remain constant, an indication of drug resistance. WHO is now funding tests of the technique on patients in Argentina, Brazil, Surinam, and India.—T.H.M.

cination of mice with 18 other strains of mycobacteria provides no protection.

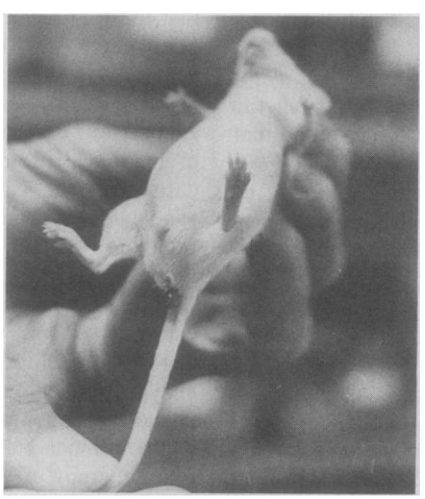
The greatest hope thus lies in immunization with *M. leprae* itself. One of the crucial early discoveries, made by Shepard, was that heat-killed *M. leprae* are more immunogenic than the live bacilli—a surprising finding in that other mycobacteria, such as BCG, lose their immunogenicity when they die. Shepard demonstrated that immunization of mice with killed bacilli will prevent growth of bacilli in the footpads, and he and Maurice J. Lefford of the Trudeau Institute in Saranac Lake, New York, have independently shown that these bacilli provoke the delayed hypersensitivity reaction characteristic of successful vaccination. Barry R. Bloom of the Albert Einstein College of Medicine and Richard J. W. Rees of the Medical Research Council in London have also demonstrated delayed hypersensitivity in guinea pigs—even though the animals are not susceptible to infection. And Kirchheimer, using killed bacilli, has successfully immunized armadillos.

Meanwhile, Phillip Draper of the National Institute for Medical Research in Mill Hill, England, has developed a process for purifying the microorganisms. Plans are now being made for trials of a killed-bacillus vaccine under the sponsorship of the IMMLEP (Immunization Against Leprosy) Program of the World Health Organization (WHO).

The production of the vaccine is "a curious situation" because it requires an extraordinary amount of cooperation, says Bloom, who is chairman of the steering committee. The armadillos are grown in the southeastern United States by Kirchheimer, Storrs, Shepard, and others. Harvested tissues are flown to London for purification by Draper. Samples of the harvested bacilli are then sent to Thomas Buchanan at the U.S. Public Health Service Hospital in Seattle and Morton Harboe of the Institute for Experimental Medical Research in Oslo, Norway, for identification of the antigens. Samples are also sent to Shepard and Bloom to test whether they are capable of provoking an immune response in mice and guinea pigs, and to Kirchheimer for vaccination of armadillos.

In this manner, the investigators have been preparing a bank of purified bacilli for future research purposes. Clinical trials, however, will require purification of the bacilli in a laboratory licensed to produce vaccine for use on humans. The Wellcome Company of England is therefore using Draper's procedure to prepare a batch of bacilli for the first safety trials, which are scheduled to begin later this year. These studies are designed to show how large a dose of vaccine is necessary to produce an appropriate immune response without significant side effects. They will be conducted on healthy volunteers in Europe and America in deference to Third World sensitivities about serving as guinea pigs for experimental drugs and vaccines. The studies in Europe will focus on individuals who have previously been vaccinated with BCG (as protection from tuberculosis), while those in the United States will focus on individuals who have not. Once the appropriate dose has been determined, a second group of individuals will be vaccinated and monitored for as long as 10 years to ensure that immunization lasts long enough to be effective.

Trials of therapeutic efficacy should begin in another 3 to 5 years, and will obviously have to be conducted in developing countries, but it is not yet clear where such trials will be held and on what populations. The problems involved are formidable. Household and work contacts of lepromatous patients have about six times the normal risk of contracting leprosy, but that risk is still very small. In most tropical countries, the incidence of leprosy is only about 0.5 case per 1000 individuals per year, and at least four out of every five are the tuberculoid type. This means, says Bloom, that it may be necessary to vaccinate 1000 people to see a diminution of one detectable case of lepromatous disease per decade. Even in the areas where the incidence is as high as 5 cases per 1000, effective clinical trials will require vaccination of more than 600,000 people.



Emory University

The first breakthrough

The mouse footpad was the first place the leprosy bacillus was grown.

Furthermore, because the incubation period of the disease is so long, the vaccinated population will have to be studied for 10 to 15 years before the results can be evaluated. Says Bloom: "There is basically no precedent for using a vaccine against a disease of such long duration and low prevalence." An additional problem is that some of the vaccinated population will be harboring the bacilli at the time of vaccination. "One can almost certainly expect a cry from the public health and administrative authorities that the vaccine is causing disease," he adds. Despite these problems, WHO remains firmly committed to use of a vaccine.

Preliminary evidence that this approach should be useful in humans has been provided by Jacinto Convit and his colleagues at the Dermatological Institute in Caracas, Venezuela. Because Convit is not working with WHO funds, he is not restrained by its strictures regarding safety trials. He has adapted Draper's technique for purifying *M. leprae*, combined the microorganism with BCG, and given repeated inoculations to patients with lepromatous leprosy. His results in several hundred patients suggest that the vaccination restores cell-mediated immunity: Within 18 months, bacilli were cleared from the patients' bodies and the progress of the disease was halted. If existing disease can be stopped in this manner, prophylaxis should be even more effective. The IMMLEP group will almost certainly try to repeat Convit's work once the safety trials have been completed.

Not everyone agrees that vaccination is the appropriate approach. Kirchheimer, for one, argues that vaccination would be "fantastically expensive." Fewer than 25 percent of leprosy cases throughout the world now receive drugs because of lack of money, lack of personnel, and the stigma associated with the disease, he argues. The problems with the vaccine would be even worse. The immune defect of lepromatous patients also weighs against use of a vaccine, he contends. He and some others argue that the disease could be eradicated just as effectively and more cheaply if all patients were treated with drugs.

Bloom, though, thinks the problem of increased resistance to drugs militates in favor of vaccination: "Two months ago [before the results of a drug resistance survey were revealed], I thought vaccination would be a useful adjunct to chemotherapy. Now I think it's a bloody necessity."—THOMAS H. MAUGH II