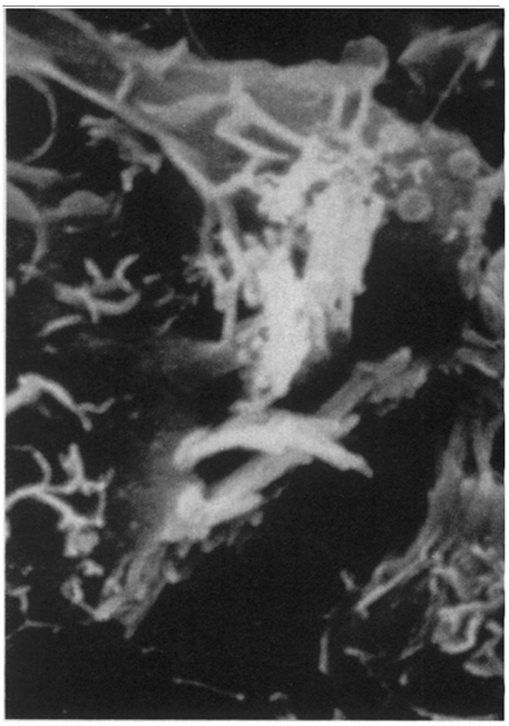
CAPTAIN of the MEN of DEATH

Scientists grapple with multidrug-resistant strains of tuberculosis

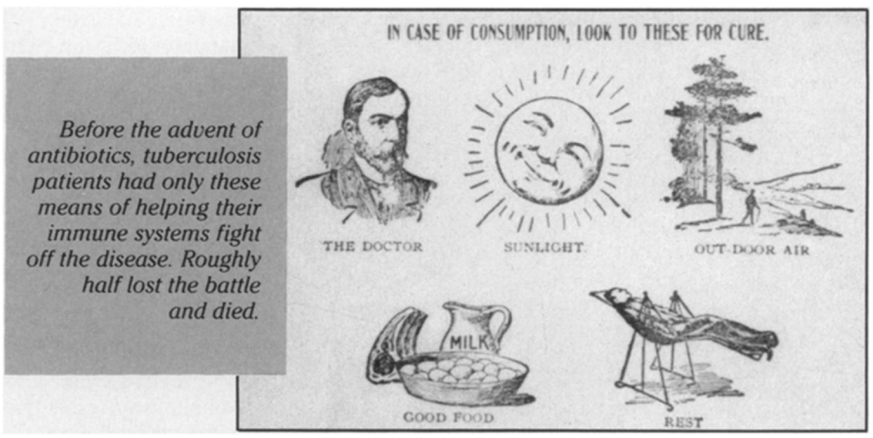


Jacobs

By CAROL EZZELL

The captain of all these men of death that came against him to take him away, was the Consumption, for it was that that brought him down to the grave.

— John Bunyan
The Life and Death of Mr. Badman (1680)



Before the advent of antibiotics, tuberculosis patients had only these means of helping their immune systems fight off the disease. Roughly half lost the battle and died.

Tuberculosis, the bacterial disease also known as consumption, had fully earned its rank as a leading cause of death by the time Bunyan wrote these lines in 17th-century England. The lung affliction was so prevalent, in fact, that it even figured in the art of the era, tragically curtailing the lives of the protagonists of a multitude of operas, plays, and poems. As recently as the turn of the century, tuberculosis was responsible for one-third of all deaths among U.S. adults between the ages of 20 and 45.

The advent of antibiotics in the 1940s curbed the sweep of tuberculosis within developed countries. Widespread testing for the infection and prompt antibiotic therapy reduced the prevalence of tuberculosis in the United States by an average of 6 percent each year between 1953 and 1985, from a total of nearly 85,000 cases to approximately 22,000.

Since 1985, however, this trend has reversed. U.S. physicians reported more than 26,000 cases in 1991, an overall increase of roughly 18 percent. While U.S. public health officials are concerned about this dramatic jump in and of itself, they are even more disturbed by an accompanying phenomenon — the emergence and spread of novel tuberculosis strains resistant to two or more of the drugs usually combined into a cocktail for curing the disease.

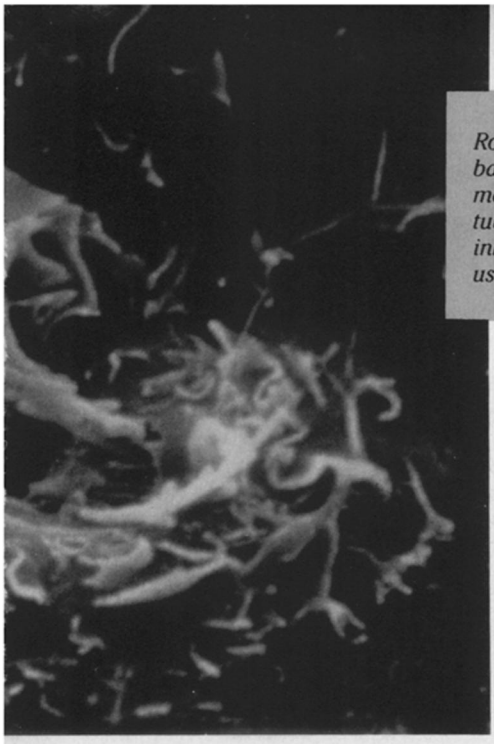
Such multidrug-resistant strains threaten to set therapy for the disease back more than 50 years, to a time when all that physicians could do for tuberculosis patients was counsel them to eat lots of nutritious food, rest, and take in plenty of sunshine and fresh air. "Multidrug-resistant TB is, in effect, pushing us back to pre-antibiotic days," former Secretary of Health and Human Services Louis W. Sullivan warned last November in an address to the World Congress on Tuberculosis in Bethesda, Md.

Researchers around the world are now carefully studying multidrug-resistant tuberculosis in order to devise new and faster tests for detecting resistant strains. Such tests would enable physicians to determine the combination of drugs with the highest likelihood of combating a particular patient's infection, thereby sparing the patient the necessity of taking a drug combo for weeks or months that in the end has little chance of eradicating his or her disease.

Scientists are also scrutinizing the genetics of the tuberculosis-causing microbe, *Mycobacterium tuberculosis,* looking for clues to how the bacterium evolves drug resistance. Understanding this resistance process should aid in the development of drugs to eradicate resistant strains.

So far, more than 36 states have reported cases of multidrug-resistant tuberculosis, with the highest incidences in the urban areas of New York, Florida, and New Jersey. According to statistics from the Centers for Disease Control and Prevention (CDC), one-third of all tuberculosis cases diagnosed in New York City in 1991 demonstrated resistance to one or more antibiotic drugs. These statistics are particularly frightening, health officials say, because even with treatment, between 40 percent and 60 percent of those who develop multidrug-resistant tuberculosis die — the same proportion of deaths that occurs among people with tuberculosis who never see a doctor.

Tuberculosis is spread through repeated exposure to airborne *M. tuberculosis,* usually from an infected person's cough. The main symptoms are fever, night sweats, weight loss, a racking cough, and spitting up blood. An estimated one-third of the world's population — including 15 million people in the United States — carry latent tuberculosis infection. However, only about 10 percent of these individuals ever develop active



Rod-shaped Mycobacterium smegmatis *bacilli swarm over white blood cells called macrophages. The related microbe that causes tuberculosis evades the immune system by infecting macrophages, the very cells that usually ingest and kill such bacteria.*

disease. Because the tuberculosis microbe grows very slowly – doubling only once every 24 hours, compared to once every 20 minutes for the common gut bacterium *Escherichia coli* – tuberculosis patients must continue to take antibiotic drugs for six to 12 months in order to eradicate all traces of their disease and insure against a relapse.

Multidrug-resistant strains of tuberculosis emerge in patients who stop taking their antibiotic medicines after a few weeks – when their worst symptoms subside – only to suffer a relapse and receive yet another course of antibiotics that they take for only a short period. An unpublished CDC study found that more than 20 percent of tuberculosis patients fail to complete their drug therapy.

"Short, inadequate courses of drug treatment, due primarily to the loss of patients to follow-up, who then pass their resistant microbe to others, seems to be the major culprit in the increasing frequency of multidrug-resistant strains in New York City," observes David E. Rogers of Cornell University Medical College there. "Many of these patients so distrust the system that medications may be viewed as simply another means of putting them down," he says. "This raises the chances that after a month or two of therapy, or when symptoms of the disease are lessened, the patient will quit the medications and break contact with the health care system."

Rogers explains that one in every 1 million tuberculosis microbes carries a natural resistance to isoniazid, the most important antituberculosis drug, and one in every 100 million such microbes is resistant to rifampin, another first-line antituberculosis medication. Sporadic antibiotic treatment simply kills off the microbes that lack resistance, enriching the population of resistant ones so that they eventually overwhelm the ability of the body's immune system to keep them in check. Because each site of tuberculosis infection in a patient's lung contains between 10 million and 100 million microbes, Rogers says, the process can happen in a matter of months.

Ninety percent of all multidrug-resistant tuberculosis cases recorded so far have occurred among individuals infected with HIV, the virus that causes AIDS. Some HIV-infected individuals abuse intravenous drugs, reside in cramped shelters for the homeless, and fail to take their medicines regularly.

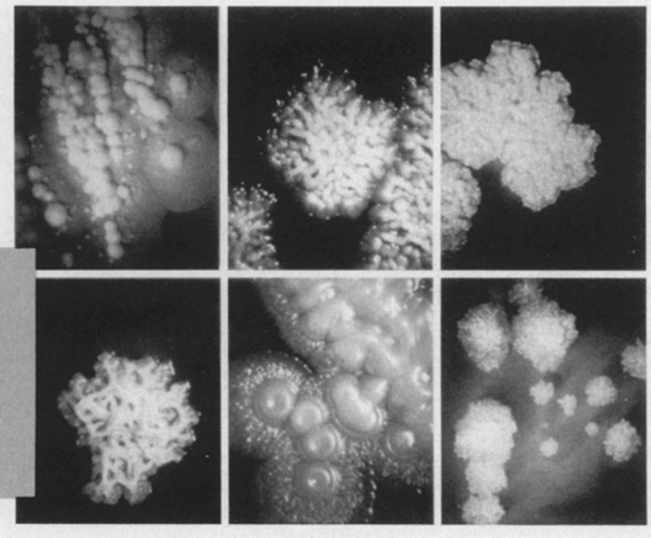
A team of researchers in Europe has recently discovered that *M. tuberculosis* does not actively *acquire* resistance to isoniazid – as many other types of bacteria acquire resistance to various antibiotics – but instead *loses* its susceptibility to isoniazid. In the Aug. 13 NATURE, Stewart Cole of the Pasteur Institute in Paris and his colleagues report that *M. tuberculosis* evades isoniazid's effects by dropping the gene encoding the enzyme that renders the bacterium vulnerable to the drug.

To uncover this gene, Cole and his coworkers chopped up DNA isolated from an isoniazid-susceptible strain of tuberculosis and spliced each of the resulting DNA fragments into colonies of a strain resistant to the drug. By observing which of the colonies failed to grow on culture media containing isoniazid, Cole's group determined which DNA fragment contained the so-called "vulnerability" gene.

Next, Cole's team determined that the gene directs the production of an enzyme called catalase-peroxidase, which other scientists had previously found in reduced amounts within isoniazid-resistant strains of tuberculosis. They were able to confirm that isoniazid susceptibility results from the catalase-peroxidase gene by finding that resistant strains isolated from two patients lacked the gene.

Cole and his colleagues suggest that isoniazid itself has no antituberculosis activity but requires the catalase-peroxidase enzyme to convert it into active form. The catalase-peroxidase gene does not have to be completely deleted to confer isoniazid resistance: Cole says that scientists in his laboratory have now isolated resistant strains that carry nonfunctional, mutant forms of the gene. However, he and his colleagues are still not sure how the active form of isoniazid kills *M. tuberculosis.*

Physicians rely upon several decades-old methods to diagnose tuberculosis – namely, recognizing the symptoms, spotting characteristic lung "cavities" on a chest X-ray, and analyzing a patient's sputum for rod-shaped microbes that fail to lose a red stain when treated with an acid solution. The widely used tuberculin skin test – which involves injecting a purified protein derivative of the tuberculosis microbe under an individual's skin and wait-



Jacobs/© HHMI RESEARCH IN PROGRESS 1991

Because the bacterium that causes tuberculosis, Mycobacterium tuberculosis, *grows very slowly and poses a health threat to laboratory workers, many tuberculosis researchers conduct their experiments with safer, faster-growing mycobacteria, such as the ones depicted at right growing in colonies.* Mycobacterium smegmatis *(top center panel, top right panel, and bottom left panel) most closely resembles the tuberculosis microbe.*

ing to see whether a red welt emerges – only identifies people who have been exposed to the infection, not necessarily those with active disease.

Only 61 percent of all tuberculosis isolates taken from patients are tested for susceptibility to the seven commonly used antituberculosis drugs, according to Dixie E. Snider Jr. and William L. Roper of the CDC in Atlanta. Currently, drug susceptibility is tested by growing cultures of the tuberculosis microbe – a process that can take several weeks – and exposing the cultures to various antibiotics to see which ones kill them and which ones don't, which can take several more weeks. Because of the time lag, "Patients with unrecognized drug-resistant disease may be treated with ineffective regimens and thus continue to transmit infection," Snider and Roper caution in the March 5, 1992 NEW ENGLAND JOURNAL OF MEDICINE.

Diagnosing multidrug-resistant tuberculosis is a particular problem among HIV-infected patients, who face a mortality rate from tuberculosis of between 70 and 90 percent, usually within one to four months of developing symptoms. Unlike most tuberculosis patients not infected with HIV, those with the virus usually develop *M. tuberculosis* infections outside the lungs, in lymph nodes and other organs. Ironically, HIV-infected tuberculosis patients often test negative on the tuberculin skin test because their weakened immune systems cannot produce the telltale red welt that signals *M. tuberculosis* infection.

For these reasons, tuberculosis researchers seek to develop faster and more specific tests for the disease – especially for detecting multidrug-resistant cases. At the World Congress on Tuberculosis, William R. Jacobs Jr. of the Howard Hughes Medical Institute at Albert Einstein College of Medicine in New York City described what he calls a "turn-off-the-light assay" for diagnosing drug resistance in sputum samples taken from patients. The test "reduces a three-week culture wait to a matter of hours," says Jacobs. This would allow physicians to tailor a regimen of tuberculosis drug therapy to the particular type of microbe afflicting each of their patients.

The test uses luciferase, the enzyme responsible for a firefly's glimmer. Jacobs and his colleagues spliced the gene encoding luciferase into a virus-like particle that selectively infects Bacillus Calmette-Guerin (BCG), a microbe that is related to *M. tuberculosis* but easier to work with in the laboratory. The genetically engineered particle, called a phasmid, caused cultures of BCG to glow.

By observing the cultures' response to various antituberculosis drugs, Jacobs' team found that they could easily determine each culture's susceptibility to the drug. If a particular drug darkened a culture, it signaled that the culture was susceptible to that drug. But if the culture continued to glow, it indicated the presence of drug-resistant microbes.

Jacobs and his colleagues are now testing whether the approach works in *M. tuberculosis*. "If it does, it could revolutionize testing for tuberculosis-drug resistance," he predicts.

To build upon successes like the luciferase test, the U.S. government is beginning to put more money and other resources into tuberculosis research and prevention. The Public Health Service (PHS) has developed a national plan for combating multidrug-resistant tuberculosis that includes improving methods of detecting and containing drug-resistant outbreaks of the disease and fostering the discovery of new antituberculosis medicines and ways of motivating patients to complete drug therapy. To help implement the plan, Congress tripled the PHS's 1992 appropriation for tuberculosis.

Through these and other measures, public health officials hope to reduce once again the prevalence of tuberculosis in the United States and eradicate multidrug-resistant strains. "There is no question that we have a large problem on our hands," says Cornell's Rogers. However, he asserts, "It is a containable and manageable one if we roll up our sleeves and get on with it." □