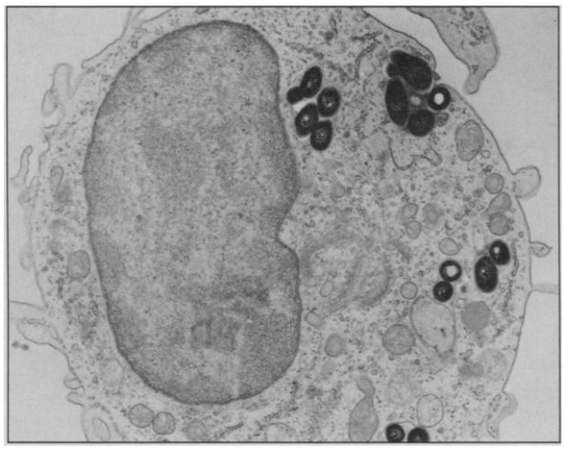
On the Track of "Killer" TB

The appearance of drug-resistant strains of the disease was predictable, but scientists are scrambling to understand how the bacillus works and to find weapons against it

WHEN A DRUG-RESISTANT STRAIN OF the tuberculosis bacillus began killing inmates in New York City prisons late last year, the small cadre of researchers who study the age-old scourge were not surprised. A major TB outbreak has been "just waiting to happen," says Barry Bloom, a Howard Hughes investigator at the Albert Einstein College of Medicine in New York. "This is a neglected disease that is now coming back to haunt us."

For years, Bloom and others note in frustration, U.S. health officials have treated TB as a disease that had been all but conquered. Research budgets shrank, the federal government stopped monitoring the appearance of drug-resistant strains, and in recent decades very few new drugs have been developed against the TB bacillus. Meanwhile, a deadly confluence of factors, including growing numbers of homeless and HIV-infected people, many of whom are immune suppressed and living in crowded conditions with poor access to health care, provided an ideal environment for the disease to make a comeback.



"Armor plated." *TB bacillus survives inside a macrophage. Researchers are trying to figure out how.*

McDONOUGH AND KRESS/ALBERT EINSTEIN COLLEGE OF MEDICINE

Now, like the sequel to an almost forgotten horror movie, tuberculosis is back with a vengeance: Drug-resistant strains of *Mycobacterium tuberculosis*, the highly contagious bacillus that causes TB, have been showing up in prisons and hospitals across the country and appear poised to spread to a broader population. Federal and state health agencies are consequently scrambling to remedy years of research neglect, and scientists are frantically trying to develop new diagnostic procedures, better drugs, and more effective vaccines. But researchers on the front lines say that inherent difficulties in working with TB's causative agent are hobbling their response to the crisis.

PACH BROS., N.Y.

Barry Bloom

Scientists confess they know frighteningly little about the molecular biology or genetics of *M. tuberculosis*. The bacterium differs radically from most other bacilli in its biochemistry, growth characteristics, and the nature of its protective cell wall—a prime target for antimicrobial drugs. Indeed, the mechanisms by which "front-line" TB drugs—isoniazid, rifampin, pyrazinamide, and ethambutol—kill the tubercle bacillus have remained essentially a mystery since these compounds were discovered decades ago. And even less is known about the means by which newly emerging strains of *M. tuberculosis* now manage to thrive in the presence of these once uniformly effective drugs.

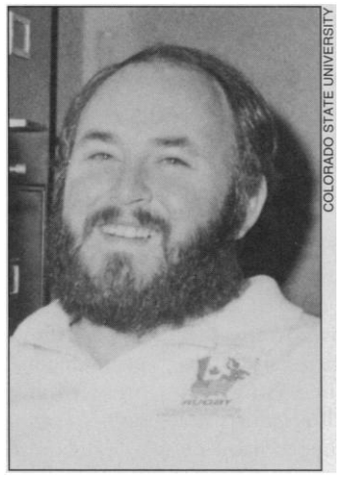
"We know very little about TB pathogenesis. And we know almost nothing about the kinds of immune response necessary to beat it," says Bloom. "Everybody said the battle was won," adds Michael Iseman, chief of the mycobacteriology disease service at the National Jewish Hospital in Denver, "so nobody really looked for a better cure for TB. Pharmaceutical firms didn't follow up after the first good drugs were marketed. Academic scientists largely bailed out because it wasn't a good avenue for them to launch their careers. It just wasn't a disease with sex appeal, and now we're in a catch-up game."

On a global scale, experts concede, the game was never close to being won. The so-called white plague consistently kills about 3 million individuals every year—more than any other single infectious disease. In the United States, however, incidence of the disease declined steadily after 1948—until the number of cases began to rise again in the late 1980s. Researchers say there is no evidence for increased mutation rates in *M. tuberculosis*, nor is increased virulence to blame for the recent fatal outbreaks of the disease. Rather, years of poor compliance among TB patients unwilling to take their medicine for the full 6 to 18 months needed to kill the bugs has led to the gradual development of strains that are now resistant to as many as nine of the 11 most commonly tested drugs (see box). And although a trend toward drug resistance has been obvious for some time, the severity of the crisis went largely unrecognized in recent years, in part because federal surveillance programs designed to track TB drug-resistance trends were eliminated in 1986 for budgetary reasons.

Now the handful of scientists who still work with *M. tuberculosis* are trying to make up for lost time, but they are up against a balky and wily foe. Most irritating to researchers is the bug's frustratingly slow growth rate in culture. While 8 hours is typically enough time to grow 10^8 colonies of *E. coli* in a culture dish, it takes a full 3 weeks to get that many *M. tuberculosis* colonies. It can take another 1 to 3 weeks to get a population big enough to perform a full panel of drug susceptibility studies, and an additional 3 to 4 weeks to get results from those tests. Equivalent studies with other bacteria can be accomplished in a matter of days.

In addition to slowing the pace of research, these glacial growth rates wreak havoc in clinical laboratories. Immune-suppressed individuals such as those with AIDS can get fulminant TB within weeks after exposure to the bacterium, and the disease

progresses much more rapidly in these individuals than it does in those with normal immune systems. "TB spreads like wildfire in AIDS patients," says William Jacobs of Einstein. "By the time we get culture results the patient may be dead."


COLORADO STATE UNIVERSITY

Ian Orme

Rapid and direct diagnostic tests of *M. tuberculosis* requiring little or no growth in culture are under development in several labs, but none has yet been adapted for clinical use. The polymerase chain reaction, for example, was recently shown to be a sensitive screen for *M. tuberculosis*. And researchers at the Becton Dickinson Research Center in Research Triangle Park, North Carolina, describe in the 1 January *Proceedings of the National Academy of Sciences* a related amplification technique that appears exquisitely sensitive and specific to tubercle DNA. But both methods require purified DNA and will need substantial refinements before they can be used widely for clinical diagnosis.

Meanwhile, immunologists and drug developers are investigating the bacterium's uniquely constructed cell wall to see how *M. tuberculosis* manages to survive in the lungs despite ingestion by peroxide-spewing macrophages. "These bugs are armor plated," says Ian Orme, an immunologist at Colorado State University in Fort Collins, noting that insights into the nature of that armor could lead to the identification of targets for new therapeutic compounds.

Orme is searching for an antigenic component of *M. tuberculosis* for incorporation into a recombinant vaccine. The current vaccine, known as BCG, is wildly unpredictable in effectiveness, protecting anywhere from zero to 80% of inoculated individuals. Recent studies by Orme appearing in this month's *Journal of Immunology* suggest that, contrary to popular belief, cell wall proteins may not be the right place to look for the most antigenic elements of *M. tuberculosis*. Orme has found that a protein secreted by the bacterium during the early stages of infection appears to activate white blood cells more effectively than do cell wall components; in the coming months he hopes to expand his immunization studies from cell cultures into mice.

As for new drug development, most researchers say they'd be more than happy at this point simply to understand how today's imperfect TB medicines work and then build on those findings to make a better product. Some people suggest that pyrazinamide blocks cell wall synthesis, "but it's really a lot of hand waving, and we certainly don't have the gene involved in resistance," says Jacobs. Similarly, scientists lack proof for the popular notion that rifampin interferes with RNA polymerase. And while there is biochemical evidence that isoniazid blocks the biosynthesis of mycolic acid, a component of the cell wall, genetic confirmation remains elusive. "So all together that's not a lot of help when it comes to understanding mechanisms of resistance or developing better drugs," Jacobs says.

In fact, genetic studies of *M. tuberculosis* have become possible only recently. Only 3 years ago researchers achieved the first stable insertion of plasmids into the bacillus, allowing geneticists to begin directed mutation experiments. And even when a gene has been successfully transfected, the bacteria's slow growth means scientists have to wait a long time to find out exactly what

Drug Resistance and Sanataria

Drug-resistant tuberculosis isn't a new phenomenon. By the late 1940s, only a few years after the introduction of the first effective TB drug, streptomycin, strains resistant to that compound emerged. Before long, clinicians realized that TB could easily develop resistance to a single drug and often to two, but that a three-pronged attack tended to be effective. Taking this insight one step further, the Centers for Disease Control (CDC) recommended last month that TB patients be given an expanded cocktail of four drugs immediately upon diagnosis.

Recently, however, the emergence of *M. tuberculosis* strains resistant to almost every available TB drug has sparked renewed interest in the reestablishment of sanataria or hospitals with isolated TB wards to attack the root of the problem: patients not taking their full course of drugs. Although patients can feel well within 2 to 3 months, it can take 6 to 18 months before all the TB bacilli in an infected person are killed off.

"In the past, as long as patients were in hospitals and sanataria, there was very little problem with compliance or major outbreaks of drug-resistant strains," says Dixie E. Snider Jr., head of CDC's division of tuberculosis elimination. "But with the move toward out-patient treatment and self-administration, we started getting problems." The increasing incidence of TB in the homeless, substance abusers, and prisoners has exacerbated the crisis. "These are people for whom taking their TB pills is not the highest priority," Snider says.

Most states have laws allowing individuals with active TB or other contagious diseases to be "detained," usually in hospitals, to ensure they take their medications until they are noninfectious. In New York City, 40 such detention orders were promulgated in 1991, compared to 11 in 1990, according to city health department officials.

AP/WIDEWORLD

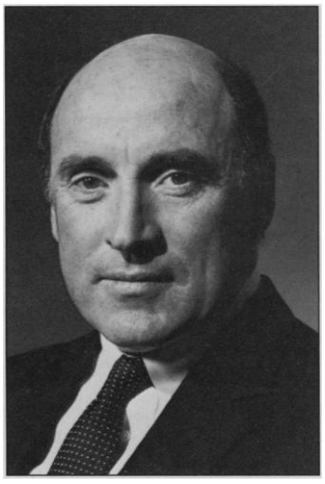
Shades of the past. *The first "cure cottage" for TB, in Saranac Lake, N.Y.*

But TB patients can be legally detained only until tests indicate a lack of *M. tuberculosis* in their sputum, even though the organism at that point generally remains alive in the lungs. Since many patients discontinue their medication after release, the disease frequently reemerges—often in a form resistant to the drugs to which it has been exposed. Thus funding for improved outreach programs is critically needed, health officials say, to prevent the spread of TB to the general population from the immune-suppressed and indigent population now most at risk.

"The scary thing is that TB is the disease that's going to be transmitted from HIV positives to non-HIV-infected people," says William Jacobs, a microbiologist and immunologist at Einstein. "There is very good cause to be alarmed." ■ R.W.

they've created.

Still, in a few labs, results are trickling in. Iseman says his colleagues at Jewish Hospital are closing in on a gene that encodes resistance to isoniazid. And Aimee Stanley, a doctoral student at Colorado State University, recently identified two regions of mycobacterium DNA that confer resistance to ethambutol. The region she is focusing on is large, however—about 22.6 kilobases—and it will take a lot of work to fill in the details. "Mycobacterium molecular biology is still in its childhood, if not in its infancy," Stanley says.



Michael Iseman

The lack of good animal models is also hampering TB research. Guinea pigs are used in some studies but are more resistant to the disease than researchers would like. And while mice are reasonably good models for studies of TB immunity, their failure to develop classic lung lesions compromises their value for pathogenicity studies, notes Arthur Dannenberg, who studies TB pathogenesis at Johns Hopkins. TB-susceptible and TB-resistant strains of rabbits have been bred, but the relevance of those models to human tuberculosis remains controversial.

TB researchers also note that for the sake of safety, most laboratory studies have used the "Erdman" strain of *M. tuberculosis*, a drug-sensitive strain that poses little risk to researchers. But increasingly it seems that if scientists are to get the upper hand on the current epidemic, they will have to work with the more dangerous, drug-resistant strains that are causing the problem. Such studies, in which animals inhale aerosolized drug-resistant mycobacterium, require so-called BL-3 laboratories designed to prevent release of highly infectious agents. Now it looks as though the researchers themselves will be facing significant new risks.

Not surprisingly, many of the scientists who have been arguing for years for increased TB funding express some anger that it has come to this. "First the government cuts back its funding for TB research for decades, then it wants us to grow 15 liters of drug-resistant TB and spray a fine mist in a roomful of mice," says Orme of Colorado State, home to one of the few BL-3 tuberculosis labs in the country. "To tell you the truth, we're fairly nervous about doing experiments on aerosolized multiple drug resistant strains." Orme says his lab just received a brand new aerosol machine with a huge, 10-inch rubber gasket that should guarantee no leakage and no accidental exposure. But reassuring as that gasket is, he says, "I think we're going to put lead weights on top" of it.

Expanding on Orme's frustration, Bloom points to the federal government's growing failure to care for the nation's poor and disenfranchised, noting that at this point it will take more than rubber gaskets and lead weights to put a lid on the new TB. "I see this epidemic as a major indictment of the country's health care infrastructure," he says angrily. "Why is it that the United States deals with health problems only when there is a crisis?" ■ RICK WEISS

Rick Weiss is a science writer based in New York City.

Panel Swims Against the Tide in Wetlands Policy

Both ecologically and politically, 1991 was a bad year for the nation's wetlands. Not only did these vital ecological systems continue to lose ground to bulldozers and the effluvia of urban civilization, but President Bush—having once pledged a "no net loss" wetlands management policy—proposed a new federal definition of wetlands that could, in one bureaucratic stroke, reduce areas under federal environmental protection by as much as 50%. In this charged atmosphere, you might expect the National Research Council (NRC) to produce a report decrying hasty action and calling for more study. But the NRC's Committee on Restoration of Aquatic Ecosystems has issued a far more challenging call*—it urges the federal government to begin an expansive new program aimed not merely at preserving existing wetlands but at reclaiming aquatic ecosystems that have already been damaged through pollution or development.

Strictly speaking, the panel's focus extends beyond wetlands to aquatic ecosystems such as streams, rivers, and lakes. As environmental buffers, these systems recycle nutrients, purify water, reduce the risk of floods, and shelter a wide variety of animal and plant species. But the report notes that the United States has lost nearly 117 million acres of wetlands alone since the 1780s.

The consequent degradation of wildlife habitats, higher levels of water pollution, and greater flood hazards demand a "comprehensive and aggressive" restoration effort, the report states. To set priorities, such a program will require a "triage" that focuses attention on systems that will be lost without intervention. Once these are stabilized, restorationists can turn to other degraded systems that require extensive work. Committee chairman John Cairns Jr., an ecotoxicologist at Virginia Polytechnic Institute, says the project should aim for a net increase of 10 million wetlands acres, restoration of 400,000 miles of streams and rivers (or approximately 12% of the total) by 2010, and restoration of 1 million acres of lakes by 2000. The committee did not estimate the cost of this effort, however.

The panel also provided no guidance on how to reach these ambitious goals beyond urging state and federal agencies to develop detailed plans. According to its report, the panel saw its role less as an architect of the restoration effort than as a herald announcing the feasibility of "repairing" damaged ecosystems to a close approximation of their original state. Unfortunately, as the panel acknowledges in several case studies included in the report, such efforts are likely to fall short of full restoration. One such study, for instance, notes that while a $10-billion phosphorus control effort in Lake Michigan succeeded in restoring water quality, it came too late to prevent the loss of 15 million tons of the lake's silica. As a result, the lake's original complement of phytoplankton "cannot be restored."

Committee members seem to have appreciated the irony of then Vice President Bush's 1988 call to environmental activism by reproducing it in their report: "It is not enough merely to halt the damage we've done. Our natural heritage must be recovered and restored." Cairns is in full agreement, adding that delay will merely increase cleanup costs. "Doing it now will be a lot more cost effective than postponing it even a decade or two," he says. "You only have to look at Eastern Europe and Russia to see the horror story of postponing environmental restoration." ■ DAVID P. HAMILTON

*"Restoration of Aquatic Ecosystems: Science, Technology, and Public Policy," Committee on Restoration of Aquatic Ecosystems, National Research Council, November 1991.