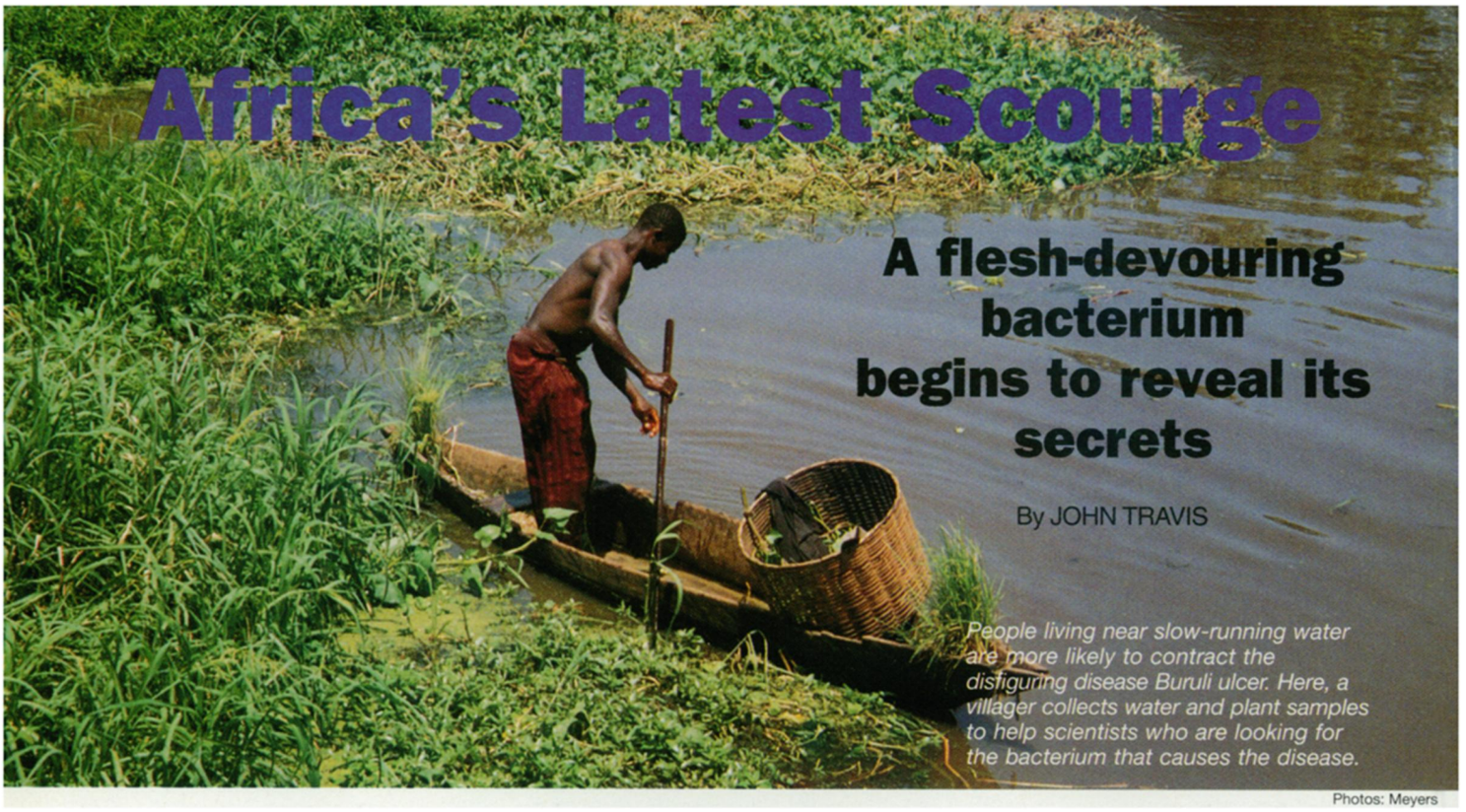
Africa's Latest Scourge

A flesh-devouring bacterium begins to reveal its secrets

By JOHN TRAVIS



People living near slow-running water are more likely to contract the disfiguring disease Buruli ulcer. Here, a villager collects water and plant samples to help scientists who are looking for the bacterium that causes the disease.

Photos: Meyers

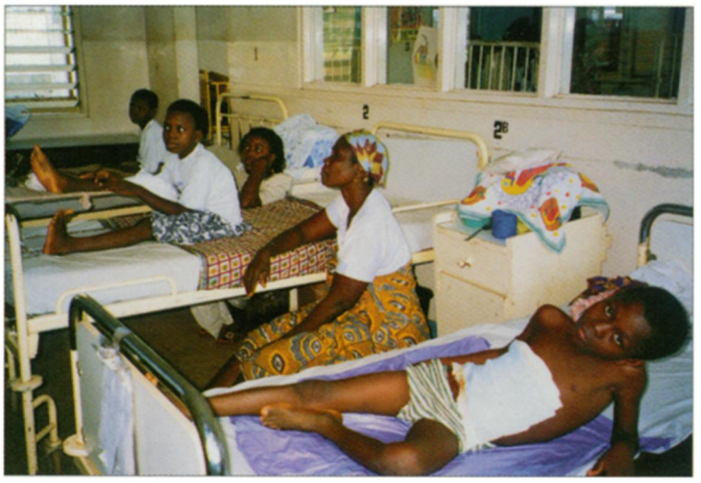
Last month, when he visited a small hospital in rural Ghana, Wayne M. Meyers found that 58 of its 100 beds held people afflicted with a disfiguring infectious disease of the skin and underlying tissue. More than half of the people with the illness, Buruli ulcer, were children, and most were under the age of 10.

To Meyers, a microbiologist at the Armed Forces Institute of Pathology (AFIP) in Washington, D.C., the hospital visit offered disturbing evidence that the illness is on the rise in Africa.

"It's a major health problem. Some of these patients will have to stay in the hospital for many months," says Meyers, who first encountered Buruli ulcer while investigating leprosy in Africa in the 1960s.

Caused by a bacterium known as *Mycobacterium ulcerans,* the disease starts as a painless, though sometimes itchy, swelling in the skin. From this marble-like nodule, concealed destruction spreads for days or weeks. A toxin secreted by the proliferating microbe ravages the subcutaneous fat layer, destroying so much tissue that the skin above finally dies and sloughs away. A lesion may spread over the full chest or back or an entire limb. Occasionally, an infection chews its way to, and even through, bone.

There is no proven drug treatment for Buruli ulcer, so physicians can only limit the bacterium's spread by surgically excising the dead tissue and covering the wounds with skin grafts. With this crude strategy, physicians often must amputate an infected limb or cut out an eye. Even though most of the massive skin ulcers eventually heal on their own, the scarring can limit muscle and joint movement, leaving patients with permanent disabilities.



Children with Buruli ulcer fill a rural hospital in west Africa. One child (foreground) just had dead tissue on his belly excised.

Over the past century, cases of Buruli ulcer have appeared in 25 countries around the world. Africa, and most recently the western part of the continent, has borne the brunt of the disease. The Ivory Coast, Benin, and Ghana now record thousands of cases each year.

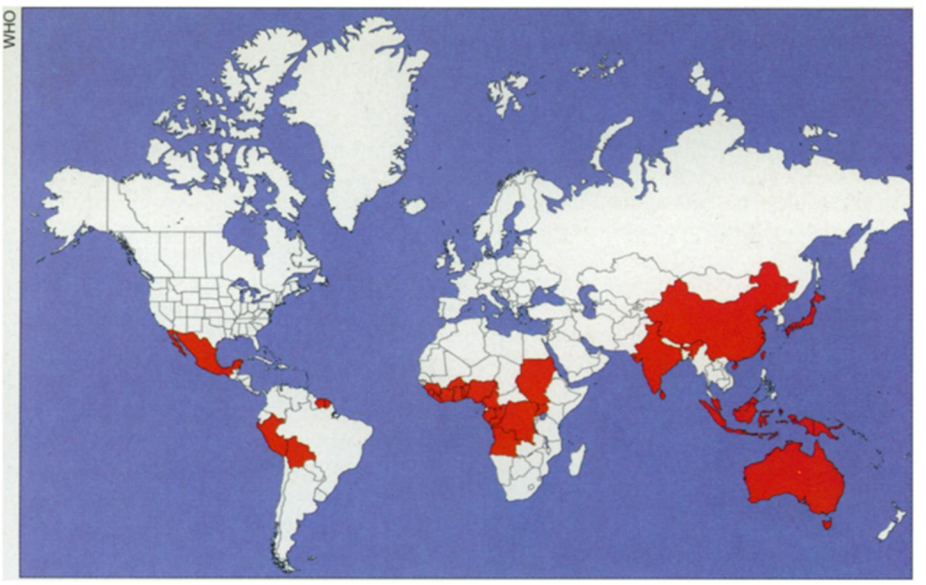
Scientists haven't pinned down the number of people infected with the microbe, but *M. ulcerans* is "probably one of the real emerging pathogens in the world," says Jordan W. Tappero of the Centers for Disease Control and Prevention (CDC) in Atlanta.

That's a troubling thought to scientists since they know little about the microbe. "Everything about [Buruli ulcer], from the bacteria to its products to the disease, is just really mysterious," says Pamela L. Small of the National Institute of Allergy and Infectious Disease's Rocky Mountain Laboratories in Hamilton, Mont.

Yet, investigators have made some inroads. Small and her colleagues, for example, recently identified an unusual toxin made by the bacterium. That finding, and several others, give investigators renewed hope that a therapy or a vaccine for the illness is on the horizon. "A little bit of effort in this area might have far-reaching returns in terms of treatment and prevention," says Tappero.

The microbe responsible for Buruli ulcer has two notorious relatives—the germs that cause tuberculosis and leprosy. All three belong to a group called mycobacteria. Unlike the other two, however, *M. ulcerans* generally doesn't live inside the cells of its host but dwells in the material surrounding cells.

M. ulcerans may someday prove as



Countries (red) where Buruli ulcer is found.

troublesome as the tuberculosis and leprosy mycobacteria, according to the World Health Organization (WHO). It estimates that the incidence of Buruli ulcer in Africa will surpass that of leprosy. In some regions, Buruli ulcer already poses more of a health care problem than tuberculosis.

In 1997, WHO responded to this emerging threat with the Global Buruli Ulcer Initiative, a program dedicated to investigating and eradicating the gruesome disease. The following year, the presidents of the Ivory Coast, Benin, and Ghana attended an international conference on Buruli ulcer and pledged to coordinate their efforts to battle it.

"There are so many simple questions that could be answered with a small amount of money," says Small, a member of WHO's Buruli ulcer scientific task force.

With their discovery of a toxic molecule made by *M. ulcerans,* Small and her colleagues resolved one basic question. In the late 1960s, scientists hypothesized that the microbe secreted a toxin that diffuses beyond the site of infection. Examination of tissue killed by the bacterium had prompted this speculation. "The damage extends far beyond the location of the organisms," notes Meyers.

Investigators at AFIP confirmed the presence of a toxin when they demonstrated that the secretions of *M. ulcerans* triggered cell death identical to that caused by the microbe itself. Yet Meyers and other investigators never could identify and purify the destructive element within those secretions.

Small's team had similar difficulty until one of the group, Kathleen M. George, recently wondered if *M. ulcerans'* toxin might be a fatty molecule, a lipid, rather than a protein like other bacterial toxins. By mixing the secretions of the mycobacterium with chloroform and methanol, the investigators eliminated proteins from the mixture, but not lipids. The resulting concoction still killed mouse skin cells.

"That was the key experiment," says Small.

The scientists eventually purified a yellow waxy substance, which they called mycolactone. In test-tube experiments, it prompts skin cells to become rounder and eventually die. Moreover, when injected into guinea pigs, mycolactone mimics infection by *M. ulcerans.*

"We can take this molecule, put it into an animal, and get both the cell death and the immunosuppression that's characteristic of the disease," says Small, who described her team's work at the American Society for Microbiology meeting in Chicago last month. The scientists also had reported results in the Feb. 5 SCIENCE.

Disruption of the normal immune response to a bacterial infection is a hallmark of Buruli ulcer. When tissue starts to die from an infection, white blood cells called neutrophils normally rush in, generating the pus typical of many wounds. In Buruli ulcer, however, neutrophils rarely enter the dying tissue, giving the ulcers an unusual "clean" look, says Small. Mycolactone has a similar suppressive effect on the immune cells.

When the researchers examined the structure of mycolactone, they found that it actually belongs to a family of lipidlike molecules called polyketides. Other polyketides include well-known antibiotics, such as erythromycin, and drugs that suppress the immune system, such as rapamycin.

Small's group speculates that *M. ulcerans* employs its toxin to ward off predators in its natural habitat. Soil bacteria frequently synthesize polyketides for such a purpose.

Small predicts that the study of *M. ulcerans* and mycolactone will also provide insight into diseases caused by other mycobacteria. While those responsible for

tuberculosis and leprosy don't seem to make toxins, they do have the cellular machinery needed to synthesize polyketides, she notes.

The discovery of mycolactone is "an absolute breakthrough," comments C. Harold King of Emory University School of Medicine in Atlanta, who has also been trying to isolate *M. ulcerans'* toxin. King hasn't stopped his search, however. He continues to believe that *M. ulcerans* may have additional protein toxins or proteins that aid mycolactone's destructiveness.

Small doesn't dismiss that possibility. She notes, however, that spontaneously arising strains of the mycobacterium that don't make mycolactone produce a harmless infection that the immune system quickly controls. These strains offer encouragement that an antitoxin treatment could slow the progression of Buruli ulcer. While an antitoxin would not kill *M. ulcerans,* it might give the immune system a fighting chance to repel the invader, says Small.

Developing an antitoxin may not prove simple. Antitoxins usually consist of antibodies made by injecting a toxin into animals and allowing their immune system to respond. Polyketides, however, don't generate a strong immune response. Consequently, scientists will try to subtly modify mycolactone to make it more provocative to the immune system.

An antitoxin would make a welcome addition to the bare arsenal currently available to physicians faced with Buruli ulcer. Although a vaccine would offer the most cost-effective solution, medical researchers have made little progress in that direction. Some evidence suggests that the BCG vaccine, which many people receive to protect against tuberculosis, confers partial immunity to Buruli ulcer. This protection lasts only 6 months, however. Scientists don't yet know if booster doses would extend that period.

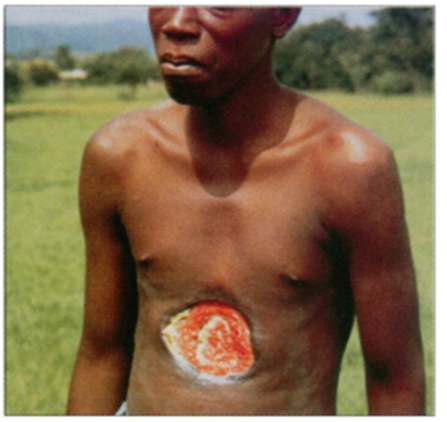
In terms of drug treatments, there has been no well-designed study to test the value of antibiotics. Some physicians have reported success with various drugs, but others have found that the same drugs bring no improvement. "There's a tremendous amount of soft data on this [issue]," says Small.

The futility of some past antibiotic use may reflect the fact that Africans with Buruli ulcer generally do not visit physicians until far into the disease's progression. "Treatment with one or two antibiotics on people with football-sized ulcers doesn't work very well. It's too late; the skin is already gone," notes Tappero.

Early diagnosis of an *M. ulcerans* infection is therefore crucial, especially since scientists suspect that the microbe may sometimes reside in the body for months or years before it starts to eat away tissue. King and his colleagues have identified antibodies targeting *M. ulcerans* in patient blood samples and developed an assay for the microbe. They've tested it on stored blood samples and plan to conduct African field trials. Among other issues, the investigators need to confirm that their assay can distinguish between infections of *M. ulcerans* and other mycobacteria.

A blood test revealing past or current infections with *M. ulcerans* may settle pressing epidemiological questions. For example, how much of the African population has been exposed to the microbe?

"It might be that a large portion is infected, but only a small percentage go on to develop disease, perhaps months to years later," says Tappero. He notes that a similar situation occurs in tuberculosis and leprosy.



A medium-size lesion on a man with Buruli ulcer.

"We think the disease is only the tip of the iceberg," agrees Paul Johnson of the Monash Medical Center in Clayton, Australia, another member of the WHO task force on Buruli ulcer.

Australian researchers have long been acquainted with Buruli ulcer. Although Buruli ulcer has most likely existed in Africa for more than a century, *M. ulcerans* was first isolated by Australian scientists in 1948. Physicians in that country know the condition as Bairnsdale ulcer, after the town that suffered a small outbreak in the 1930s and 1940s. The moniker Buruli ulcer arose in the 1960s, when a large number of cases struck the Buruli region of Uganda.

Johnson and his colleagues still puzzle over why a predominantly African disease flares up on occasion in Australia. "It's a very strange thing that this tropical disease appears in a temperate country," he says.

That oddity highlights crucial areas of ignorance concerning *M. ulcerans.* No one knows where the microbe usually lives or how it gets transmitted to people. "We just haven't found where the bug is replicating in the environment. It's really maddening," says Small.

For several decades, scientists have tried to grow *M. ulcerans* from soil, water, and plant samples taken from infected regions. They've had no success, perhaps because it's a slow-growing organism that other microbes crowd out in a laboratory dish.

The one consistent environmental association with the disease has been stagnant, recently dammed, or slow-running water. Most cases in Africa arise in rural villages near swamps or wetlands, and outbreaks have occurred after flooding.

In 1997, Johnson and his colleagues offered the strongest evidence linking *M. ulcerans* and water. The disease hit a small town on an island near Melbourne. By mapping the location of each of the nearly 30 cases, the scientists concluded that the mycobacteria were somehow moving into people from a local golf course.

Johnson and his colleagues had just developed an assay that could screen environmental samples for a DNA sequence unique to the microbe. When they tested water from the golf course's irrigation system and a swamp on the course, the researchers detected the mycobacterium's telltale genetic tag.

If stagnant or slow-running water provides *M. ulcerans* its home, what then accounts for the recent rise in Africa of Buruli ulcer? "Our speculation is that the reemergence of this disease may be caused by deforestation and a resulting increase in flooding," says Meyers.

How people contract Buruli ulcer still remains unexplained, making it difficult for physicians to recommend ways to avoid the disease. Anecdotal evidence indicates that openings in the skin—from gunshot wounds, needle injections, or even splinters—increase a person's risk of getting Buruli ulcer. *M. ulcerans* can't by itself penetrate the skin, researchers therefore have concluded.

Insects may also help the bacteria get under a person's skin, according to a report in the March 20 LANCET. Certain water bugs living on plant roots in endemic areas of Benin and Ghana test positive for *M. ulcerans* DNA and appear to carry the mycobacteria, say Meyers, long-time Buruli ulcer researcher Françoise Portaels of the Institute of Tropical Medicine in Antwerp, Belgium, and their colleagues.

These water insects might transmit the microbes. "They do bite people and can fly some distances," notes Meyers.

"It's an exciting hypothesis that needs to be pursued," says Tappero.

Still, unlike mosquitoes, which are reservoirs for malaria-causing parasites, the water bugs aren't considered the natural host for the mycobacteria. The scientists suspect the insects accidentally pick up the bacteria, perhaps by eating other creatures infected with *M. ulcerans.*

The search for the lair of this elusive and dangerous bacterium continues. □