Malaria Research—What Next?

Vaccine development has hit some roadblocks, the disease is spreading, and funding agencies may be pulling out. The following three articles assess the quandaries for science and public policy

"IN THE HIGHlands of New Guinea, in one of the most primitive areas in the world, people are now asking for 'the shoots'—expecting a malaria vaccine," says Robert Gwadz, a medical entomologist who breeds mosquitoes for research. "Of course," he adds, "there is no malaria vaccine."

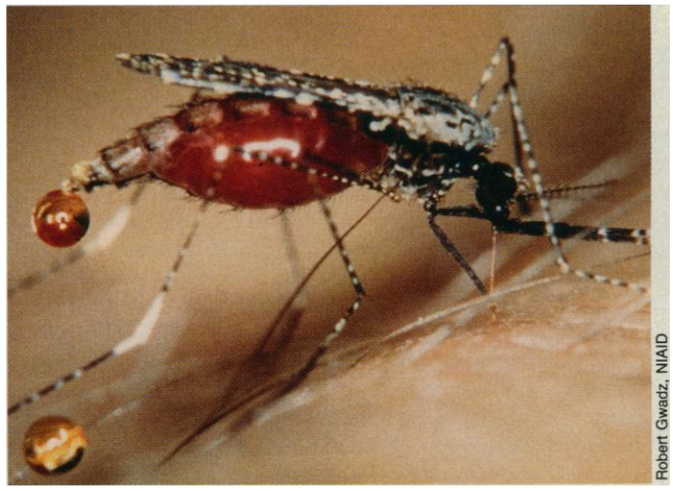
Even in the jungle, faith in medicine's ability to deliver a cure now runs ahead of reality, says Gwadz, who is on the tropical medicine staff at the U.S. National Institute of Allergy and Infectious Diseases.

The expectation of success also runs high in the developed world, where people tend to see the battle against malaria as a continuation of earlier campaigns against smallpox, polio, and yellow fever—all of which ended in victory. But malaria has followed a different course.

The big vaccine development effort in the United States hit some bumps in the road in the 1980s. The malaria parasite has turned out to be a wilier foe than many expected, so far defeating attempts to produce a vaccine, and the biggest U.S. research program has been racked by scandal. Funding agencies are wondering what to do next.

The disappointment is, if anything, deeper now because molecular biology promised the last good chance of defeating malaria in this century. A massive chemical assault on the disease-bearing mosquitoes faltered in the early 1960s and was formally abandoned by the World Health Organization (WHO) in 1969. During this time the parasite also developed resistance, in many areas of the world, to pharmaceutical drugs. Thus, when the genetic engineers offered to lend a hand in the 1980s, research agencies greeted them with an almost desperate embrace. But now the basic biologists, too, are showing the strains of battle, and the ultimate solution to malaria seems no nearer.

The letdown came late in the decade as clinical trials revealed that the current generation of vaccines are not effective. Researchers learned a great deal about the malaria parasite—a protozoan called *Plasmodium* that lives in the gut of mosquitoes and the human bloodstream—but they did not deliver a "product in a bottle," says Carlos Campbell, director of the malaria program at the U.S. Centers for Disease Control (CDC) in Atlanta. What they did produce were reams of data on the molecular structure of the parasite and its intricate relationship with the human immune system—data that are fascinating but also make the development of a vaccine seem much more complex than it once did.


Robert Gwadz, NIAID

The culprit. Anopheles dirus *caught in the act of drawing blood. This forest-dwelling mosquito is the main malaria vector in Southeast Asia.*

There are several promising approaches to a vaccine (see page 402), but apparently no consensus on which path is the best. This has made sponsoring agencies uncertain about which approach to fund in the future.

Laboratory directors have also become more cautious. Officials like Colonel Carter Diggs, the director of malaria research at the Agency for International Development (AID), and Louis Miller, director of the intramural program that includes Gwadz at the National Institutes of Health, hedge their bets. They advocate laying out a series of barricades, a sort of biological defense initiative. The candidate weapons on their list include partial remedies, new pharmaceuticals, new chemicals to attack the mosquitoes, pesticide-impregnated bed nets, and perhaps even the release of genetically engineered insects to disrupt the lives of wild mosquitoes (see page 400).

All these approaches may be valid, but as Louis Miller said in his president's address to a meeting of the Tropical Medicine Society in Hawaii last month, they need better coordination. He pointed out that research leaders at the National Institutes of Health (which spends about $7.5 million a year on malaria), at AID (which spends $8.5 million), and at the Department of Defense ($10 million) have "never met to discuss the common needs of the United States in this area." One reason, as Miller himself conceded, is that there may be "too much self-interest and parochialism," so that each group resents the other's suggestions. He also warned that "funding agencies are rethinking their involvement," a fact that he found baffling in light of his expectation that "problems with malaria will be getting worse in the years ahead."

According to WHO, about 100 million people are clinically ill with malaria at any given time, and around 1 million—mostly very young children in Africa—die from it each year. The effects are so widespread in the tropics—ranging from lethargy to total incapacitation—that malaria has been blamed for impeding the development of entire nations.

The main strategy for fighting malaria through the 1960s was to use pesticides to kill mosquitoes that carry the parasite and to treat infected individuals with quinine-related drugs—mainly with chloroquine. However, the most lethal strain of the parasite, *P. falciparum*, is now genetically resistant to chloroquine. Health authorities are switching to a new compound, mefloquine, but already there are signs in Asia and Africa

that the parasite is resistant to this as well. It is only a matter of time before the resistance spreads, Campbell says, and "there aren't a lot of alternatives sitting around."

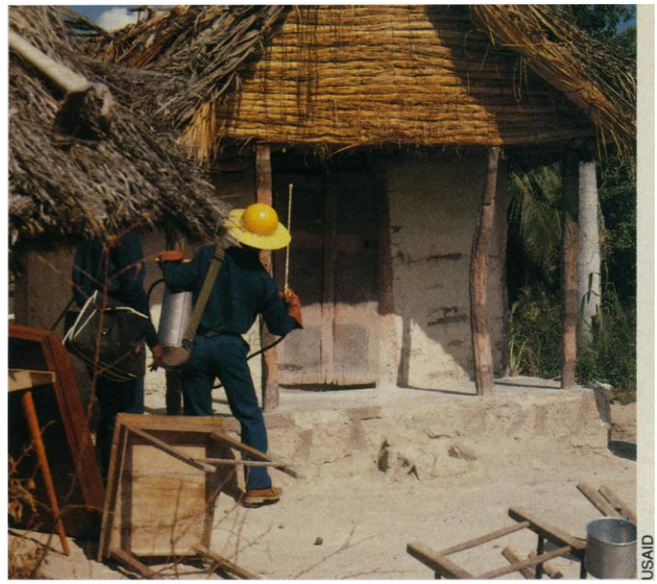
Although the chemical assault seems nearly spent now, it worked well in the beginning—deceptively so. As the WHO set itself the goal of eradicating malaria with the massive use of DDT in the late 1950s, many countries scaled back traditional mosquito control efforts, just at a time when resistance to pesticides and drugs began to appear. The reversal was striking. In Sri Lanka, for example, where only 17 cases of malaria were reported in 1963, an epidemic affecting millions erupted in 1968.

Today the mosquitoes and the four disease-causing strains of *Plasmodium* threaten to regain the ground they lost during the years of heavy pesticide use in the 1950s and 1960s. In fact, the situation may be as bad as it was two decades ago.

Carlos Campbell of the CDC says the big new issue is urban malaria. Disease-bearing mosquitoes are now appearing in cities such as Dar es Salaam and Kinshasa. In addition, environmental change seems to be stimulating the spread of malaria in Brazil, Indonesia, and Madagascar, to a "disastrous" extent, Campbell says. As jungles are cleared and forests burned, the local temperature may rise. Infected mosquitoes are moving into higher elevations while people with no natural immunity are moving into areas where the infected mosquitoes live. At present, for example, 90% of the malaria cases in the Western Hemisphere are reported in Brazil, where forest clearing proceeds on a vast scale.

Nor is the problem limited to the Third World. A "few dozen" local cases of malaria have appeared in a county near San Diego for 4 years in succession, says Campbell; the focus of infection seems to be migrants from Mexico or further south who first contracted the disease outside the United States.

In view of the need for immediate action, some public health officials are calling for modest, practical goals, such as educating



Not under control. *Spraying to kill anopheles mosquitoes in Haiti. In most parts of the world control measures have not succeeded.*

High-Tech and Low-Tech: Control Strategies Today

In 1957 the World Health Organization declared war on malaria. Armed with DDT to kill the mosquitoes and chloroquine, a cheap and effective drug, to cure the disease, armies of health workers around the world set to the task. At first all went well, but by the mid-1970s the disease had reappeared with a vengeance, largely because the disease-causing parasites had developed resistance to chloroquine (see story on page 399).

According to David Davidson, Jr., secretary of WHO's tropical disease program Scientific Steering Committee on Chemotherapy of Malaria, "there are very, very few areas where chloroquine is fully effective, as it was 25 years ago." For similar reasons insecticides are also less useful than they were a decade or two ago. Which leaves only the vaccine (see story on page 402).

Or does it? In the face of failure to come up with a workable, effective vaccine, many malariologists are again looking to other control strategies, some very high-tech indeed, others resurrecting the simplest of methods.

John Playfair, professor of immunology at University College and Middlesex School of Medicine in London, is attacking the symptoms of malaria rather than the parasite itself. Although it is still not understood how the flood of merozoites produces the symptoms of malaria, Playfair points to a correlation between substances called cytokines (which include such things as tumor necrosis factor) and acute illness in malaria patients. In mice, at least, Playfair has shown that the release of cytokines does underlie development of severe symptoms.

Playfair's group and others have established that the burst of merozoites is accompanied by the release of antigens; those antigens are capable of stimulating cells in culture to release tumor necrosis factor. Playfair has made antibodies to the stimulator molecules, and he envisions—in the far-off future—a vaccine that does nothing to prevent transmission of malaria but protects young children from its deadly pathology.

"The best analogy," Playfair says, "is to antitetanus or diphtheria vaccines, which neutralize the toxins but have no effect on the bacteria that produce them, which are essentially harmless."

A different—but equally high-tech—tack is being taken by Robert Gwadz at the U.S. National Institutes of Health. Gwadz is attempting to genetically engineer mosquitoes that do not transmit malaria and use those strains to displace the ones that do.

Some strains of mosquito, we know, do not spread malaria in the wild. At every link in the chain that makes up the parasite's complex life cycle, Gwadz sees opportunities to "build better mosquitoes" by breaking those links, using suitable genetic engineering methods. His group has already inserted a foreign gene into mosquitoes, where it has so far remained stable for more than 50 generations.

The next step is to find the right weapons—perhaps some of the immune compounds newly discovered in other insects—and splice the genes for them into mosquitoes. Gwadz would then like to link expression of those genes to the expression of the genes that manufacture yolk for nourishing the female's eggs. The yolk genes are switched on after the female takes her blood meal; a killer compound switched on at the same time could destroy the parasites within the mosquito.

The way Gwadz tells it, swarms of the engineered nontransmitting mosquitoes would be released at opportune moments—occupying all available habitats and keeping out ordinary mosquitoes that are capable of spreading malaria. But Chris Dye, a researcher at the London School of Hygiene and Tropical Medicine who studies mosquito behavior, is skeptical. "This kind of optimism was around in the sixties and seventies, when people . . . were merely selecting refractory strains of mosquitoes. The results

Third World officials on how to use existing technology more effectively. This is the aim of a relatively new joint endeavor of the Rockefeller Foundation and WHO.

Likewise, says Jose Najera, director of WHO's division of tropical disease control, his agency long ago abandoned its more grandiose plans and now concentrates on training health-care workers to mitigate malaria's worst effects. At present, he says, "there are more countries where the situation is deteriorating than countries where it is improving." In what resembles a triage strategy, WHO recognizes that there are many places where broad control efforts cannot be sustained—perhaps because of local wars or strip-and-burn development. It focuses "in a selective manner" on building expertise in areas of high incidence, such as West Africa. One important goal, Najera says, is to dispel the notion that this is an outdated profession and to draw young people back into the field of mosquito and parasite control, which is now seriously short of expertise.

However, it would be a mistake to emphasize fieldwork at the expense of basic science, says Nina Agabian, a parasitologist with a joint appointment at the University of California at Berkeley and San Francisco. She also sits on AID's malaria peer-review panel. "There's a huge interest in the developing world in technology transfer," she says, "but you've got to have something in the pipeline" to deliver. Like many biologists, she is enthusiastic about extending the basic discoveries of the 1980s as a source of new technology for the future. This approach, she argues, will eventually yield the largest reward—perhaps a breakthrough in understanding the parasite's interactions with the human immune system.

The United States now spends about $26 million on malaria research—a "dismal" effort, considering the size of the problem, says Denis Prager, director of health programs at the John D. and Catherine T. MacArthur Foundation. This is one of the few private organizations that support tropical disease research, having spent $30 million over the past 5 years for studies on basic parasitology. Although MacArthur considered dropping out last year, it has made a pledge to continue, for now.

Several other international foundations make a significant contribution, as do the governments of Australia, Britain, France, and the Netherlands. But the global sum is still a tiny fraction of the amount the United States spends on, say, AIDS research alone.

The steadiest international commitment has come from WHO, which spends roughly $5 million a year for fundamental research, according to Tore Godal, director of WHO's Tropical Disease Research program. The agency tries to support ideas that are not well funded by governments or industry, Godal says. These include an attempt to develop a "transmission blocking" vaccine that could knock down rates of incidence but would not directly benefit recipients. WHO also has drafted some academic centers to serve in a role normally played by drug companies—testing new ideas for chemotherapeutic agents and developing them into practical medicines. Because industry is not interested, Godal says, "we find that we increasingly have to focus on a few areas" of high-risk research.

USAID

"Dismal": *Denis Prager on U.S. funding of basic research in malaria.*

Indeed, it was academic science in Europe and America that offered the best chance of progress during the 1980s as the situation worsened in the Third World. Molecular biologists reasoned that they could use their new tools—monoclonal antibodies and gene splicing—to create a vaccine based on the structure of the surface coat of the sporozoite, which is the form of the parasite injected by mosquitos into the blood.

Funding agencies poured millions of dollars into a high-profile crusade to do precisely this. Researchers and pharmaceutical companies joined the excitement, signing agreements, issuing press releases, and injecting test sporozoite vaccines into primates and humans.

When the new ideas were tried in clinics, however, the parasite-host interaction proved more complex than expected, and the strong immunological response that appeared in animals did not show up in humans. Today, research on this technology continues as before, making steady scientific progress, but it has not yet come near the goal of providing a vaccine. The experience has been disappointing, although many parasitologists say it was not the researchers who created the impression that success was just around the corner, but agency directors seeking to justify budgets.

"The reason the vaccine work on malaria has been a disappointment is that we got our expectations too high," says Prager. "And the reason we got our expectations too high, in my view, was that AID [the biggest backer of vaccine research] tried to promise too much too quickly and they tried to get publicity for the early research before it was appropriate." He adds that it is slightly reminiscent of "the cold fusion business: if you hold press conferences and draw attention to very early preliminary results, the likelihood in this field is that you're going to be disappointed, because this is an unbelievably complex area."

For those who made promises, however, like AID, the disappointment hit hard. Not only did the vaccine program fail to produce a clear success, but top management at AID succumbed to internecine personal battles, prompting Congress to order an investigation by the General Accounting Office.

The report, issued in October 1989, urged AID to overhaul the program and introduce a more rigorous system of peer review, among other things. Since then, AID has gone through several programmatic upheavals, canceled a few longstanding grants, appointed new reviewers, and provided evidence for indictments against a leading malaria researcher, Wasim Siddiqui, and the program's former chief technical officer, James Erickson, alleging that they misappropriated funds. AID and

were always failures. . . ."

If high-tech solutions fail, then, as Bill Collins of CDC in Atlanta says, it's "back to the turn of the century and bed nets."

But bed nets, like malaria itself, are staging a comeback—with a new wrinkle. Several groups are experimenting with bed nets impregnated with insecticides. The big advantage of the treated nets is that because they kill the mosquitoes rather than just keeping them out, they work even when they have some holes or when an arm or a leg touches the net.

In China, 2 million impregnated nets have proved "extremely effective," according to Chris Curtis of the London School of Hygiene and Tropical Medicine. A trial in The Gambia that had been planned to run 2 years was halted after 1 year because the impregnated bed nets were so effective "it was unethical to continue," says Curtis. And his own research in Tanzania indicates villagers are happy to reimpregnate their own nets every 6 months.

These three control strategies are but a few of those that researchers are currently considering. Since a malaria vaccine has taken on something of the character of a mirage—vanishing as it is approached—control strategies can be expected to assume even greater significance in the next few years. ■ **J.C.**

the other U.S. sponsors of basic malaria research also asked the Institute of Medicine to conduct a comprehensive review of the field and to submit recommendations in 1992 to guide future research. The chairman has not been named, but the staff study director has been selected. He is Stanley Oaks, formerly of the Department of Defense Medical Research and Development Command.

Several researchers are wondering whether all this turmoil means that AID's basic research program is about to end. Diggs, the current director, says there is no plan to retreat and that funding is expected to continue at around $8.5 million a year. But his boss, Bradshaw Langmaid, acting assistant administrator for science and technology at AID, concedes that everything depends on the will of Congress. Langmaid also explains that as a "service agency" AID always has a difficult time justifying a program of pure research if it is seen as requiring sacrifices in other, direct forms of assistance. But, he adds, "Malaria is a forgotten problem for much of the research establishment," and the agency intends to continue its support, if only because no one else seems ready to take on the job.

It would be fatuous to claim that the prospects for a "cure" are better in this decade than they were in the last. But researchers argue strongly for extending basic malaria studies on grounds that they are not only interesting biologically but because they remain the most cost-effective way to invest antimalaria funds. William Trager, a Rockefeller University scientist who with James Jensen discovered a method for raising *Plasmodium* in the laboratory, says that in spite of the setbacks in the 1980s, "very interesting progress has been made" in molecular studies of malaria, and "there are many reasons to be optimistic, even about the synthetic vaccines." Agabian, likewise, argues that scientists until now have used the tools of molecular biology in a superficial way, to apply old paradigms of immunology to the complex relationship between humans and *Plasmodium*. Real progress will come, she thinks, when researchers develop entirely new concepts in immunology arising from current work with *Plasmodium* and other protozoa.

Some agencies are finding it difficult to justify an open-ended commitment to basic research now that it is clear that genetic engineering will not deliver any quick or universal remedy for malaria. However, as Miller said recently, "Where there are no known solutions or only partial or expensive control measures, research, slow and unpredictable as it may be, is the only hope."

■ **ELIOT MARSHALL**

Malaria Vaccines: The Failed Promise

"1990—TROPICAL SCOURGE CONQUERED. The first human malaria vaccine reaches clinical use. Developed at New York University, the preventative is made from a gene-cloned protein from the cell surface of the malaria parasite. It is effective against all four species that infect human beings."

Or so the editors of *Omni* magazine predicted in 1985. Time's up. But a vaccine against malaria seems no nearer now than it did then. Why not?

The answer lies in the complex cycle that connects the parasite, called *Plasmodium*, the many species of anopheline mosquitoes in which it mates, and the humans who are bitten by those mosquitoes. The parasite has shown a surprising immunologic variability, and vaccine strategies that once seemed straightforward have proven frustratingly ineffective in recent years.

The life cycle of *Plasmodium* includes three main stages: the sporozoite, the merozoite and the gametocyte. When an infected mosquito bites, it injects thousands of sporozoites into the blood. Carried to the liver, they take up residence in liver cells. There they multiply, each forming hundreds of merozoites. Ultimately the packed liver cells burst, releasing merozoites into the blood stream.

Within seconds, each merozoite invades a red blood cell. Again, massive proliferation ensues, continuing until the red cells burst and release more merozoites. It is the simultaneous bursting of waves of infected red cells that leads—by mechanisms still not fully understood—to the chills and fever of malaria.

Sometimes after a merozoite infects a red blood cell, it develops not into more merozoites but into male and female gametocytes, which constitute the parasite's sexual stage. When a mosquito bites an infected person, it may suck up some gametocytes, which emerge from their red cells in the mosquito's stomach. There they find each other and mate, yielding an egg that eventually releases thousands of sporozoites to initiate the cycle again.

The presence of three tempting targets—sporozoite, merozoite, and gametocyte—once seemed to make the task of developing a malaria vaccine easier. And in 1985, when the savants at *Omni* were summing up the future, there was reason for optimism. The first *Plasmodium* gene had recently been cloned: the gene that codes for the circumsporozoite (CS) protein, which surrounds the infective sporozoite. It had been known since the 1940s that chickens could be protected by infecting them with sporozoites weakened by radiation; similar data were obtained for mice in the 1960s and humans soon after.

With those findings—and the CS gene in hand—it seemed a vaccine might be right around the corner. And prototype vaccines were right around the corner: the first trials took place just 3 years after the gene was cloned.

Unfortunately, vaccines based on a genetically engineered version of the CS protein were disappointing. In two trials of different vaccines only one of nine subjects was protected—and even that case is suspect. Andy Waters, a molecular biologist in the malaria section of the laboratory for parasitic diseases of the National Institute of Allergy and Infectious Diseases (NIAID), says the one lucky volunteer suffered an allergic reaction to the vaccine itself. The parasites were killed by the allergic reaction, Waters said; "it was nothing to do with immunization per se."

The reason for this failure may be that *Plasmodium* and the human immune system have been playing hide-and-seek for countless generations. In playing that evolutionary game, the sporozoite has acquired the capacity to change its CS protein coat in myriad ways, offering a bewildering variety of identities that enable at least a few sporozoites to evade immune surveillance. Hence a vaccine engineered against a particular CS protein is useless against the rest—and the infection takes hold. Even a "cocktail" of CS proteins is unlikely to be effective, because the parasite may quickly evolve new variants in its proteins.

Fortunately, researchers were also taking aim at the other targets. Target number two was the merozoite, the stage that multiplies asexually in the blood. The merozoite, however, is a fleeting target. At large in the blood for only a few seconds, it is—like the sporozoite—extremely variable immunologically. Therefore many malariologists were surprised in 1987 when Manuel Patarroyo and his colleagues at the National University of Colombia announced that they had suc-