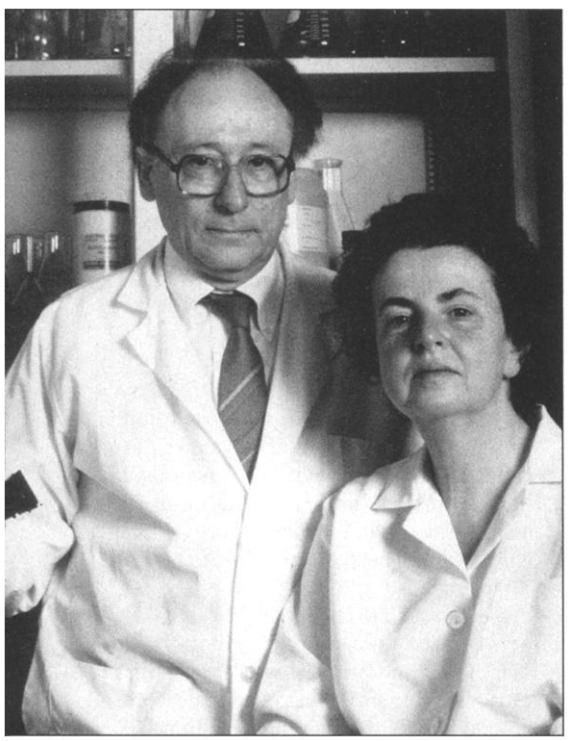
MALARIA
VACCINES

Searching for a Parasite's Weak Spot

Using a host of new technologies, vaccine developers are trying to target the parasite at every stage of its complex life cycle

"Optimism" is not a word usually associated with the pursuit of a malaria vaccine. "Frustration" is more appropriate. Over the years, researchers confronting the extraordinarily complex parasite have suffered a string of disappointments interspersed with some high-profile setbacks, as promising candidate vaccines have failed to perform up to expectations. The scientific obstacles are enormous: Compared to a virus, with its dozen or so genes and relatively monomaniacal approach to evading the human immune system, the malaria parasite has 14 chromosomes, perhaps 7000 genes, and a four-stage life cycle as it passes from humans to mosquitoes and back again. It is almost demonically efficient at evading the human immune response. Yet, something akin to legitimate optimism has been creeping into the field.



Pioneers. Victor and Ruth Nussenzweig, shown here in 1979, developed an early malaria vaccine.

A recent clinical trial in The Gambia demonstrated that a vaccine made from a single malaria recombinant protein can protect humans against the parasite—albeit for only a few months. Dozens of new vaccines are in the works, employing a host of technologies that promise to attack the parasite at every vulnerable point of its multistage life. And after years of subsistence funding, money is pouring into malaria vaccine research (see p. 428). The Bill & Melinda Gates Foundation plans to disburse $50 million through its new Malaria Vaccine Initiative, for instance, and at the U.S. National Institutes of Health (NIH) funding for overall malaria research has increased fivefold in the past decade and should exceed $50 million for 2001.

Researchers now predict that within 5 or 10 years they will have a successful vaccine that will actually save lives. But in malaria vaccine research, the concept of "success" comes with caveats. Few researchers expect that the first or even second generation of malaria vaccines will work like a polio or tetanus vaccine and protect the majority of those inoculated for a decade or longer. For example, the military, which has long taken a lead role in malaria vaccine development, is aiming for a vaccine that is 95% effective for 6 months, says Grey Heppner, chief of immunology at the Walter Reed Army Institute of Research in Silver Spring, Maryland. That would be enough to protect troops deployed for short periods in an endemic area.

Protecting the vast populations that live in malarial areas presents a much greater challenge. Ultimately, researchers hope to devise a vaccine that prevents death—which means targeting children under 5, who constitute 90% of malaria fatalities. The goal is to protect these children from the worst of the parasite's onslaught, although not necessarily from the disease itself. Then, the logic goes, the children will acquire their own natural immunity and, like adults in these regions, experience only mild symptoms from repeated infections. But that will likely require a vaccine that induces multiple immune responses against multiple stages of the parasite, says Steve Hoffman, head of the vaccine development program at the Naval Medical Research Center in Silver Spring, Maryland. "Nobody has ever made a vaccine like that before." Moreover, a truly global vaccine will have to handle not only *Plasmodium falciparum*—the most virulent parasite, against which most current research is directed—but *P. vivax* as well.

A worthy foe

Vaccines against viruses work by stimulating the immune system with either an attenuated version of the virus or a piece of the virus. That primes the immune system to recognize the real thing when it comes along and eliminate it quickly from the bloodstream. "Those viruses or bacteria for which we do have vaccines look the same the whole time they are inside of you," notes Hoffman. This is not so for malaria, with its four different stages (see sidebar).

The cycle begins when an infected mosquito injects threadlike sporozoites into the bloodstream. The sporozoites travel to the liver, where they burrow into cells and multiply into tens of thousands of tiny round merozoites. Bursting out of the liver cells, the merozoites invade red blood cells; there, they multiply madly and cause the primary symptoms of the disease. Finally, some of the parasites enter a sexual stage and become gametocytes, which are taken back up into the mosquito, where they reproduce and the cycle begins anew.

These stages have evolved in part to avoid the two primary defenses of the human immune system: antibodies, which seek out and destroy invaders in body fluids, and killer, or cytotoxic, T cells, which attack infected cells. The sporozoites, for instance, don't remain in the bloodstream long enough to be hunted down by antibodies, and the merozoites don't stay in the liver long enough for killer T cells to mobilize and attack the infected cells. And once the merozoites move into red blood cells, they are safe from both arms of the immune system. Meanwhile, the parasite multiplies furiously at every step, so that even if an immune response is 99% efficient at wiping out the parasite at any one stage, there are likely to be enough parasites left to multiply and cause disease.

Over the half-century that researchers have struggled to create a malaria vaccine, they have based their belief that it can be done on two models. One is naturally acquired immunity: the fact that people in endemic areas who survive to adulthood no longer die of malaria and often don't show symptoms when they do get infected. When blood from these adults is transfused into children with high parasite loads in their bloodstream, the antibodies in the transfused blood dramatically reduce the children's load. The second is known as the attenuated sporozoite model. Since the 1940s, researchers have known that the sporozoites in an infected mosquito can be debilitated by zapping the mosquito with radiation. If enough of these attenuated sporozoites are then introduced into a human, they can protect against malarial challenge for up to 9 months. "The problem," says New York

CREDIT: COURTESY OF V. NUSSENZWEIG

Malaria Parasite Outwits The Immune System

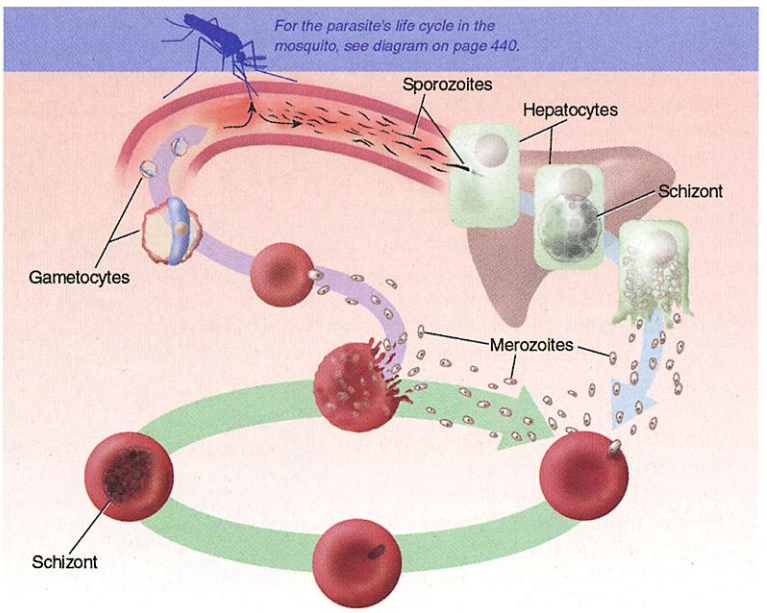
Given a few million years of evolutionary practice, the malaria parasite has become extraordinarily proficient at one task: evading the immune system of its human hosts. This it does in four distinct life stages.

Taking *Plasmodium falciparum*, the parasite that causes the most virulent strain of malaria, as an example, the cycle in humans begins when an infected *Anopheles* mosquito partakes of a blood meal and in the process dribbles her saliva, which contains thousands of threadlike sporozoites, into the bloodstream. Within 5 to 10 minutes, far too short a time for the immune system to muster an efficient antibody response, the sporozoites home in on the liver and infect liver cells. There, they are safe from antibodies but susceptible to killer T cells—if they stick around the 10 to 12 days necessary for these defending forces to mobilize. They don't. Instead, the sporozoites begin multiplying furiously. Each sporozoite forms a schizont, which contains some 30,000 round, compact merozoites. In less than a week, the schizonts rupture, killing the liver cells in the process and spilling millions of merozoites into the bloodstream. The merozoites quickly infect red blood cells—the one cell type in the body where they are safe from both antibody and killer T cell defenses.

In the red blood cells, these merozoites can go one of two ways. They can participate in a repeated process of amplification, fueled by the hemoglobin in the blood cell. Each merozoite forms another schizont, this time with 20 new merozoites in it. After 48 hours, the schizonts rupture and the cycle continues. The result is a 20-fold amplification of the parasite burden in the blood cells every 2 days.

Those merozoites that don't form schizonts in the red blood cells can develop into a sexual stage—becoming males and females, known as gametocytes—and reinfect mosquitoes. These are taken up into the mosquito's gut when it takes a blood meal from an infected person. In the gut, the female produces an oocyst—a cross between an egg sack and a cyst—out of which emerge new sporozoites. Over the next 2 weeks, the sporozoites infect the gut, the bloodstream, and finally the saliva glands of the mosquito, at which point she is prepped to reinfect humans.

People don't experience symptoms until the parasite invades the red blood cells. Inside the red blood cells, the parasite extrudes knobs onto the surface of the blood cells that cause them to stick to the lining of blood vessels and capillaries. The knobs, yet another evolutionary response to the human defense mechanisms, keep the infected blood cells from passing through the spleen, where they would be purged. By decreasing the rate of blood flow, they also result in one of the primary symptoms of malaria: severe anemia. Malaria's characteristic fever and chills arise when the schizonts rupture the red blood cells and in the process release not only merozoites but also a "malaria toxin." Moreover, as the parasites amplify in the bloodstream they can eventually infect two out of every three red blood cells. Explains Steve Hoffman, director of the malaria vaccine program at the Naval Medical Research Center in Silver Spring, Maryland: "Now you have, say, 60% fewer red blood cells and 60% less oxygen-carrying capacity, and you have severe anemia right then and there." **–G.T.**



The messenger. The female *Anopheles* transmits the parasite as she feeds.

CREDITS: (LET TO RIGHT) WHO/TDR; A. STONEBRAKER

University malaria vaccine researcher Elizabeth Nardin, "is that you have to expose the individual to the bites of hundreds or thousands of irradiated infected mosquitoes." The technique, needless to say, does not represent a viable vaccine strategy, but it has led researchers to study the attenuated parasites to find what in the sporozoite triggers the antibody response.

The initial breakthrough came in 1979, when vaccinologists Ruth and Victor Nussenzweig, a husband-and-wife team at New York University (NYU), identified the primary antibody target on the attenuated sporozoites. It is a protein that constitutes the bulk of the parasite's surface coating, now known as the circumsporozoite protein (CSP). Once John Dame and colleagues at NIH cloned the CSP gene in 1984, the race was on for a viable vaccine. One team from the Army, the Navy, and SmithKline Beecham Biologicals (SB Bio) of Belgium put the CSP gene into bacteria and generated a recombinant protein that they injected first into mice and then, in 1987, into six human volunteers. The Nussenzweigs and collaborators at NYU, the University of Maryland, and Hoffmann–La Roche synthesized a portion of the CSP protein and used that synthetic peptide as their vaccine, first successfully in mice and then in three human volunteers. "We both immunized at the same time and both got more or less the same results," says Hoffman, who was then a young member of the Army-Navy–SB Bio team. "We each got one individual protected. So we proved the principle, but the response was not as good as anyone wanted it to be, not nearly good enough for the field."

Among the volunteers who developed malaria were Hoffman and Rip Ballou, another young researcher on the Army-Navy–SB Bio team. The chilling experience—contracting the disease when they thought they were protected—taught them not only how nasty malaria is, they later said, but also just how difficult it is to beat.

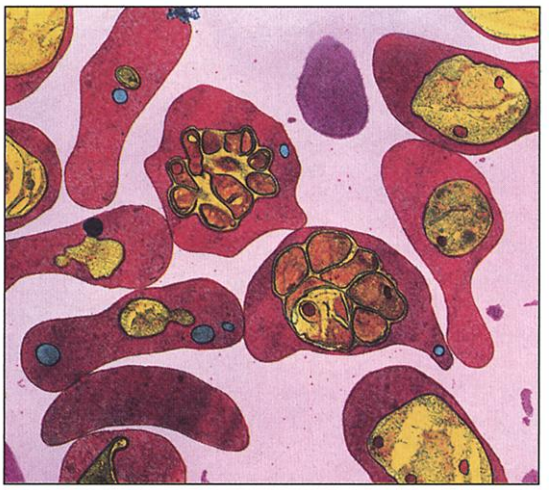
"That vaccine worked by making antibodies that prevent the sporozoite from getting into the liver," says Hoffman—a strategy that made a lot of sense. The problem was that "if just one of those sporozoites gets through, you have 30,000 merozoites 1 week later. Then you lose the battle, because the immune response you generate to fight the sporozoite doesn't do anything to those 30,000 merozoites. It doesn't even see them."

Since then, the two groups have been working methodically to boost the antibody response generated by their vaccines. The Army-Navy–SB Bio team, for instance, tested two dozen vaccines between 1986 and 1996, when their perseverance paid off. They inoculated seven subjects with a modified version of the CSP vaccine—known as RTS,S—and six were protected against a malaria challenge 3 weeks later. This was followed with an RTS,S field trial in 306 adult volunteers, organized by the British Medical Research Council in The Gambia. The results "were unprecedented," says Joe Cohen of SB Bio. In the first 2 months, the vaccinated group experienced 71% fewer clinical episodes of malaria than a control group. Over the next few years, the researchers plan to continually boost the immune response generated by the vaccine while testing the current version in progressively younger volunteers. Eventually, if the vaccine remains safe and effective, they hope to test it in children between ages 1 and 5. "We feel there is good chance this vaccine will behave as well if not better in children," says Cohen. "Of course we won't know until we actually evaluate it."

Strength in numbers

Despite the success so far of RTS,S, few researchers believe that it—or, for that matter, any vaccine made from a single malaria antigen—will be sufficient to control malaria in the field. That will require deploying not just one weapon but the full armamentarium. Since that realization has sunk in, the intense competition of the early days has evolved into a sprawling collaboration, as researchers explore the potential of various antigens—some 40 are now under study—from the different stages of the parasite's life cycle. They hope to elicit both antibodies and killer T cells and to attack the parasite wherever it makes itself visible.

One promising strategy is to ambush the parasite at another weak point: when it leaves the liver, but before it invades the red blood cells, where it becomes effectively unreachable. This could be done by targeting the surface proteins of the merozoite. A successful antibody attack against these proteins might prevent the merozoite from penetrating the red blood cells, says Heppner of Walter Reed, which in turn should prevent the cyclic amplification that leads to the clinical disease. "If we can find a vaccine that can block invasion of merozoites into red cells," he says, "then we [should be able to] completely suppress the infection or suppress it to a trivial level." Numerous research teams are trying to do just that.



The enemy within. The malaria parasite infects red blood cells, where it multiplies out of reach of the immune system.

In an effort to prevent transmission of the disease, NIH scientists have embarked on a novel strategy to create a vaccine that would muster antibodies against the reproductive stages of the parasite that appear only when it's in the mosquito. In its sexual stage, the parasite normally lurks in red blood cells until it detects a drop in temperature that suggests it is now safely in a mosquito's gut. Then it leaves its hiding place, reproduces, and starts the process of infecting the mosquito. Using a Trojan horse approach, Louis Miller and his colleagues are trying to create antibodies that can be smuggled into the mosquito's gut with the mosquito's blood meal. When the parasites break out of the blood cells, the antibody will be there to take them on. "Once the antibodies are taken up along with the parasite," says Miller, "they will block the fertilization of the male and female sexual stages and, if that doesn't work, block the zygote produced from burrowing its way through the stomach wall and infecting the mosquito. This mosquito then can't transmit the parasites back to other people." Miller and his colleagues think such a transmission-blocking vaccine could be used in combination with a more traditional vaccine and preventive methods such as impregnated bed nets to help eliminate malaria from an endemic region—provided that transmission rates in that area are not tremendously high. So far, the NIH team has demonstrated that the transmission-blocking vaccine works in mice and rabbits; it hopes to begin human trials by next fall.

Mix and match

In numerous labs, researchers are now looking for the right mixture of antigens, or a "gemische," in the words of Gina Rabinovich, director of the Gates Foundation's Malaria Vaccine Initiative, that could constitute the ultimate vaccine. "You [would] have some liver-stage antigens, some blood-stage antigens, eventually maybe a transmission-blocking component, and maybe something specifically against *Plasmodium vivax*, for a world where you have transmission of both [*P. vivax* and *P. falciparum*]. This would be the combination to end all combinations, although it makes all the issues in the creation or production of any other vaccine look simple."

This is where a new technology known as DNA vaccines might make a big difference. These are stretches of "naked" DNA containing genes for viral proteins, which are expressed when the DNA is taken up by muscle and other cells in the body. DNA vaccines have a few enormous advantages and one potential—and equally enormous—disadvantage. The latter is that nobody knows whether DNA vaccines can induce a sufficient immune response to protect a human against any disease, let alone malaria. As for the advantages, DNA vaccines are relatively easy to make compared to a synthetic or recombinant protein (*Science*, 5 December 1997, p. 1711). Moreover, DNA vaccines, unlike a recombinant or synthetic peptide vaccine, call into action the killer T cells, which destroy infected cells. And that would provide researchers with a long-sought weapon: a way to target infected liver cells, which so far have evaded attack.

Adrian Hill and colleagues at Oxford University have created a DNA vaccine with genes coding for antigenic parts of six different malaria proteins, while Hoffman's team has cocktails with the genes from five, eight, and even 15 different antigens in the same vaccine. In animal studies, these cocktails have raised both antibody and killer T cell responses to attack the malarial parasite in the bloodstream as well as in liver cells. Both teams are also betting on a technique known as a prime boost, in which a DNA vaccine is used to ready the immune system and then a second vaccine—either a recombinant protein or a recombinant virus—is used to enhance the immune response. On 18 September, Hill and colleagues began a field trial in The Gambia of their six-part DNA vaccine, followed by an attenuated version of the vaccinia virus that eliminated

smallpox, modified to express the same malarial components as their DNA vaccine.

As science advances, the lingering pessimism in the field arises more from the financial constraints of developing a malaria vaccine. According to a recent NIH report, it takes more than a quarter of a billion dollars and a dozen years to take a vaccine from research through licensing in industry. Much of that money is spent on large-scale clinical trials, which are still a distant dream of malaria vaccine researchers.

"Within 5 years there will be a number of vaccines out there being tested for efficacy," predicts NIH's Miller. "Within 10 years, some will be ready for a company to try to get them licensed." The ultimate challenge, says Miller, may be convincing a company to take a chance on a vaccine for a market that is enormous but is also hopelessly impoverished.

–GARY TAUBES

MALARIA

▶ DRUGS

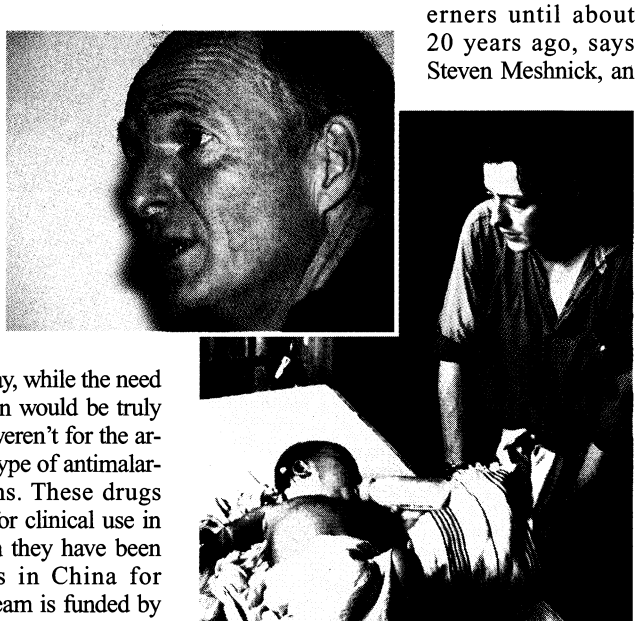
Reinventing an Ancient Cure for Malaria

As drug resistance renders cheap antimalarials ineffective, a promising candidate has emerged from an overlooked source

Nowhere on Earth is malaria more threatening than in northwestern Thailand. Here, the deadliest form of the parasite, *Plasmodium falciparum*, has been toughened by decades of exposure to antimalarial drugs—conditions that promote the survival of drug-resistant strains. In this caldron, says researcher Nicholas White of Bangkok's Mahidol University, a "nightmare scenario" is brewing: Local parasites are becoming resistant to every cheap drug that works. The old standby, chloroquine, "is gone, just about everywhere," agrees Pierro Olliaro of the World Health Organization (WHO), and resistance to newer drugs is emerging.

Flush with new funds and aiming at targets now being provided by genome sequencers, researchers are trying to concoct the next generation of antimalarials (see p. 439 and *Science*, 17 March, p. 1956). But those drugs are a decade away, while the need today is urgent. The situation would be truly desperate, White says, if it weren't for the arrival in the 1990s of a new type of antimalarial from Asia: artemisinins. These drugs haven't yet been approved for clinical use in Western countries, although they have been used as herbal remedies in China for 2000 years. White, whose team is funded by Britain's Wellcome Trust and WHO, is championing one member of this family, a water-soluble form called artesunate. He thinks it may be the most potent new weapon against malaria in decades. It could also be a lifesaver for children in remote villages.

Abundant clinical data show that artesunate knocks down the number of parasites in the blood faster than any other drug does, according to Steve Hoffman of the U.S. Naval Medical Research Center in Silver Spring, Maryland. If given early, it can stop an infection from progressing to a deadly coma. Yet it must be used with care, says White, partly because of concerns about neurotoxic effects but mainly to avoid promoting drug resistance. The past approach, using malaria drugs one at a time and replacing them as they toppled "like dominoes," only encouraged resistance. So White argues that artesunate must be deployed in combination with other drugs to hit the parasite with a complex challenge.



Ground zero. Nicholas White (*inset*) and François Nosten are conducting trials in Thailand.

CREDITS: (TOP TO BOTTOM) COURTESY OF P. PRAKONGPAN; WELLCOME TRUST UNITS, SOUTHEAST ASIA

Artemisinins were unknown to Westerners until about 20 years ago, says Steven Meshnick, an epidemiologist at the University of Michigan, Ann Arbor. But 2 millennia earlier, Chinese herbalists noted that fevers could be treated with a tea based on the flowering plant qinghao (*Artemisia annua*). An ether extract of qinghao, qinghao-su, gained scientific prestige in China during the 1960s, when a search for organic drugs revealed that it was effective against the mouse form of malaria. Chinese researchers developed new drugs and tested them on thousands of patients, publishing medical reports in English in the early 1980s.

One of the first Westerners to pounce was Dan Klayman, a U.S. Army malaria researcher, now deceased. Unable to obtain samples of qinghao from China, Klayman eventually found some *Artemisia annua*, a weed called sweet wormwood, growing near his lab in Washington, D.C., and cultivated it. (Klayman's review of the Chinese clinical work and his lab's work on qinghao's chemistry appeared in *Science*, 31 May 1985, p. 1049.) In the 1980s, both Army- and WHO-sponsored researchers began testing an oil-based version for intramuscular injection to treat severe malaria. It was a stable formula, but it was sometimes poorly absorbed, and in animal studies it injured the brainstem at high doses. White thinks it was a mistake not to move quickly to other formulations.

Meanwhile, Chinese research in the 1970s and 1980s suggested that the water-based artesunate formulation given by tablet or needle worked well. China now manufactures artesunate, and White estimates that more than 1 million people have used it safely in Asia. What's more, says White, recent trials in Thailand suggest that a combination of artesunate and mefloquine is rapidly absorbed and immediately bioavailable and is seemingly "a better drug."

But artesunate got trapped in a regulatory cul-de-sac. Although Chinese researchers had published safety and efficacy data, Western authorities looked askance at their research methods. WHO, for instance, declined to launch major clinical trials of artesunate in the 1980s, after some of its advisers expressed concerns about neurotoxic effects seen in animal studies. White suspects the biggest roadblock was that artesunate "came from the wrong place." It didn't have the right regulatory "credentials ... which people seem to regard almost as religious edicts."

In 1994, as mefloquine resistance spread through Thailand, WHO decided to support trials of artesunate with mefloquine as the last line of defense. White and co-authors reported in the 22 July issue of *The Lancet* that this oral therapy yielded efficacy of "nearly 100%." Today, WHO, the Wellcome Trust,