Fall 1980

Cultivating Killers: [Dr. William Trager]

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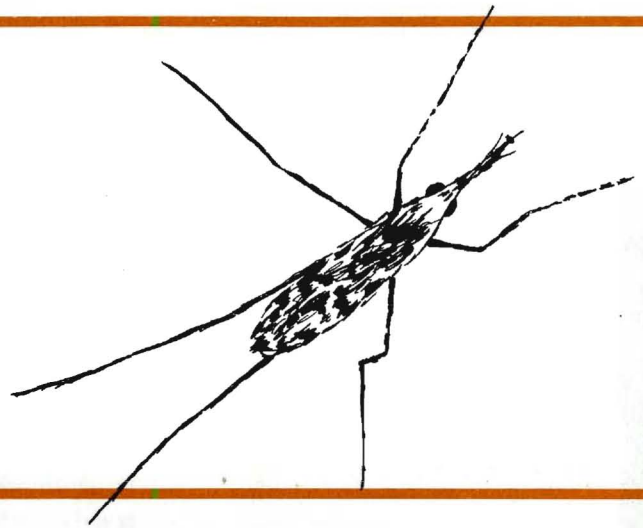


THE ROCKEFELLER UNIVERSITY

RESEARCH PROFILES

FALL 1980

Cultivating Killers

When William Trager was in high school in Newark, New Jersey, the caterpillars he collected sometimes hatched out parasitic flies instead of butterflies. "Another collector would have been disappointed, but I was fascinated," he recalls. "I guess I just always have liked parasites more than most people do."

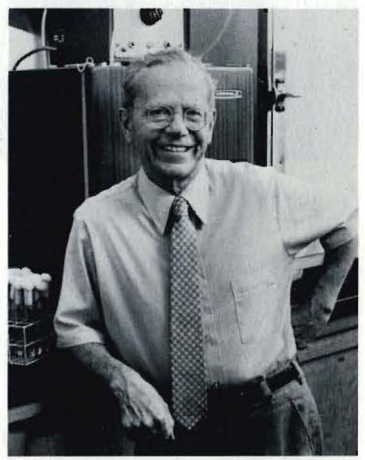
Professor Trager, head of the parasitology laboratory of The Rockefeller University, is one of the world's leading authorities on parasitic protozoa. In the ranks of these single-celled organisms, transmitted to host organisms by intermediary carriers such as insects, are the agents responsible for the largest number of debilitating or deadly diseases on earth, foremost among them malaria. Last spring, Dr. Trager flew to Geneva to accept the Samuel Taylor Darling medal of the World Health Organization for his contributions to malaria research. If a vaccine is developed for malaria, and he is confident one soon will be, his work will have been the catalyst.

Four years ago, Dr. Trager and an associate, Dr. James B. Jensen, reported the first continuous cultivation in a test tube of *Plasmodium falciparum,* the most lethal of the four species of malaria parasites that infect human beings. In the past, the lack of a means to cultivate the malaria parasite outside of a living host has been the major roadblock to making an effective vaccine. The achievement was likened to the discovery by Harvard researchers years ago that polio virus could be grown in tissue culture—the scientific breakthrough that led to a polio vaccine.

For basic scientists, asking questions about the processes of natural phenomena is the main job. When Dr. Trager first began studying malaria parasites, his object was not to find a vaccine, which was generally considered unfeasible.

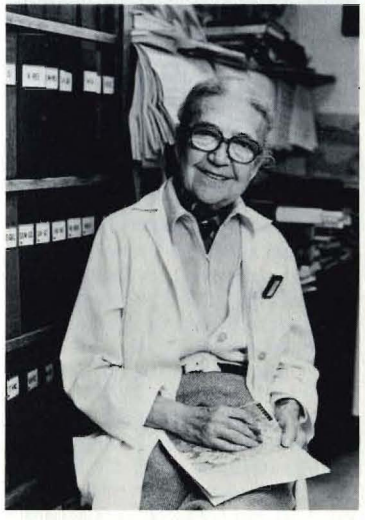
"My primary interest," he says, "was in the physiology and biochemistry of parasitism. I chose to work with malaria parasites, among others, because I thought they would provide good material for basic studies of the interactions between parasites and their hosts, something we still don't know a lot about. For example, the malaria parasite grows inside the red blood cell of its host. Why does it have to live in a red cell? What does it get out of the cell? How does it affect the cell? How does the parasite interfere with the host's development of immunity? How does the host manage to survive, when it does survive?"

A practical solution was assumed to have been found to malaria as a health problem in the years following World War II. Control of malaria mosquitoes was achieved in many areas through the use of powerful insecticides like DDT. In India, for example, malaria cases dropped from 100 million in 1952



Dr. Trager

In the laboratory: Doctors Robert T. Reese and Mary R. Motyl.



Dr. Rudzinska

to 60 thousand in 1962. However, total eradication was never achieved and, in the decade that followed, malaria mosquitoes in large stretches of the tropical world began acquiring immunity to the insecticides. As spraying became less effective and more expensive, control programs were abandoned and malaria resurfaced as a major problem for global health. The world estimate of current cases is between 200 and 300 million. In Africa alone, at least one million children die of malaria every year. To compound the tragedy, malaria parasites in many areas have become resistant to present day antimalarial drugs.

One reason a successful malaria vaccine has never been developed is that a single exposure to malaria, unlike the case in some other diseases, does not produce long-term immunity. Recent findings indicate that it might be possible to induce longer immunity if the antigenic material—the substance produced by the parasite that triggers the body's immune response—could be administered in more concentrated amounts than are ordinarily produced in victims of the naturally-occurring disease. But what substance? Administered at what point in the disease cycle? In what dosage? These questions could not begin to be answered without a reliable method for cultivating the malaria parasites. As Dr. Trager succinctly states: "By the early 1970s, we knew we *had* to figure out how to grow the damned things."

Dr. Trager is a longtime master of laboratory culture techniques. More than 40 years ago, he achieved the first insect tissue culture, in which he grew viruses, and tackled another vexing problem: do insect-transmitted viruses multiply in the insect as well as the vertebrate host. To determine that they did meant getting absolutely uncontaminated mosquito tissue to culture, a considerable feat in the days before antibiotics. He has also worked extensively with trypanosome parasites. In 1974, he developed a new method for cultivating one of the principal agents of cattle trypanosomiasis, a disease that decimates food herds in Africa.

Because, in the past, he wanted mostly to study nutrition and physiology in malaria parasites, he worked at culturing them *outside* the red cells, which turned out to be an extremely difficult and tedious process. He did have some success with a species of bird malaria and, among his findings, he established the first direct evidence of the significance of nutritional factors in host susceptibility to the disease. Studies of human malaria, however, were limited for many years by the lack of a suitable experimental animal. Chimpanzees could be infected, but working with them was cumbersome and expensive. Then, in 1968, it was discovered that some strains of human falciparum malaria could be grown in a small South American monkey, thereby providing a more accessible source of infectious material. This greatly aided research, especially when vaccine development again came under consideration.

A REASONABLE IDEA

"When I began working to get an intracellular culture [a culture kept alive inside red cells but outside the host animal], I

was sure I'd have a long, hard road ahead," says Dr. Trager. To keep the parasites alive required determining and maintaining the right balance of red blood cells, serum, culture medium, carbon dioxide, oxygen, and temperature. "Quite a number of people had tried and failed, going back to around 1912 or so. But I thought I had a reasonable idea." Reasonable and, as it turned out, right.

As Dr. Trager describes it, his reasonable idea was based on "letting the red cells stay quiet instead of agitating them and having some way of providing them, at the same time, with a change in medium." The method he employed was to have a shallow stationary layer of red blood cells, within a laboratory vessel, covered by a shallow layer of medium that flowed slowly and continuously over the settled cells. The culture was maintained at a constant temperature and under an atmosphere containing fixed percentages of carbon dioxide and oxygen. Shortly after Dr. Trager got the first successful experiment going, James Jensen joined the lab and helped refine the technique. The cultures are easy to prepare with simple equipment, a real benefit in areas where trained technicians and research funds are in short supply, and Dr. Trager and his colleagues have traveled around the world teaching the method.

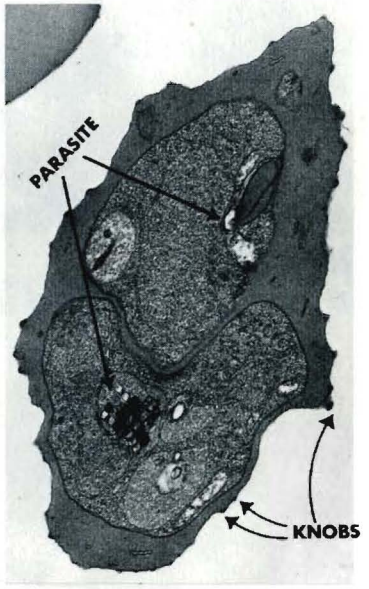
"I suppose you could say that we brought human malaria into the laboratory really for the first time," explains Dr. Trager. "What the cultivation has done is to permit the domestication of the parasite, so to speak. I won't say we've tamed it, not yet, but we do finally have it available. In the past, we could draw out some peripheral blood from infected animals and look at the early stages of the disease, but we had to kill the animals and remove the organs to get any picture of the later stages, and what we got was a very incomplete picture. Now we can look at the intracellular stages in action. We can begin to discern fine structures and see things we didn't know were there."

In the life cycle of malaria parasites, forms called sporozoites, transmitted through the bite of the female Anopheles mosquito to the host, localize in the host's liver where they develop into merozoites. These break out of the liver cells and invade the red blood cells, where they keep dividing and infecting other blood cells, producing the symptoms of disease. Some merozoites go on to develop into gametocytes. If the host is bitten again by a mosquito at this stage, the gametocytes pass into the mosquito's stomach and produce new sporozoites that migrate to the mosquito's salivary glands and the cyle starts again.

One way of making a vaccine is to use whole organisms. This cultivation method has opened the way for harvesting merozoites in quantity, a prerequisite for producing a vaccine, and a merozoite vaccine has already been tried on monkeys with good results. To make a vaccine that is acceptable for human use, however, it is necessary to find a way of freeing the merozoites from the red cell material. This material can have extremely toxic side effects. It is also necessary to prolong immunity through the use of chemical boosters called adjuvants. Adjuvants are used in some vaccines for other diseases, but no safe malaria adjuvant has yet been discovered. Another possible approach is to find and use specific antigenic substances within the merozoite. Last spring, Dr. Araxie Kilejian of Dr. Trager's group, working with the culture method, reported that she had isolated a series of proteins among which may be the antigens that stimulate immunity.

THE KNOB STORY

The culture method has also figured in an ongoing mystery that Dr. Trager calls the knob story, which began in 1966 when he and Dr. Maria A. Rudzinska studied under the electron microscope some infectious malarial material he had brought back from Liberia. Dr. Rudzinska, a member of the Trager lab for 24 years, was one of the first researchers to apply electron microscopy to parasitology.



Electron-micrograph shows a human red blood cell infected with the malaria parasite Plasmodium falciparum. *The surface of the blood cell is distorted as a result of the infection and bears dense protrusions, the knobs.* X*13,000*

Dr. Kilejian

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"We found that the infected cells had little knoblike protrusions on their surface. We didn't know what to make of them. About the same time, we were studying a form of monkey malaria that behaves very much like human falciparum malaria and we found the knobs again, in cells with large parasites in them; when the parasites were small, at a younger stage of their development, the red cells had no knobs. We suggested that the knobs might be points at which infected cells attach themselves to the capillary endothelium, the membranous covering of the capillaries. That hunch later proved to be right. But that's where the story stood, until we got the parasites in test tubes.

"After we had been culturing a line of cells for about a year and a half, Dr. Susan Langreth, who was following and recording the culture with the electron microscope, noticed that the knobby cells had lost their knobs. Subsequently, the same thing has happened with every batch of cells after about the same amount of time. We don't know why. Are the knobless cells a mutant form? Are they the result of culture conditions? Are they still infective? Are they an attenuated line? An attenuated line might be used to produce a live vaccine.

"We won't be able to answer those questions until we can isolate and clone an absolutely pure knobless line, and that's presenting some tricky technical problems. Meanwhile, the knobs have helped Dr. Kilejian. She has found a protein in the knobby cells that is not present in the knobless ones and seems to be very similar to a protein she had previously isolated for duck malaria. If it's an immunogenic protein, then it might be a bet for a vaccine. A number of laboratories elsewhere have now taken up work on this knobless business."

A SPUR TO NEW RESEARCH

Beyond the search for a vaccine, the culture method has opened the way to other important work. As Dr. Trager points out, "It has proven to be the most efficient and economical tool for screening new antimalarial drugs and for learning how these drugs work. It has been the means through which we have learned how certain red cell abnormalities such as sickle-cell anemia confer relative resistance to falciparum malaria. With it, we are beginning to understand the factors that promote formation of gametocytes. Perhaps most exciting from a scientist's viewpoint, we are using cultures to study genetic variation; in other words, we're getting right into the parasite's DNA."

There is a renewed vigor in parasitology research, spurred largely by the urgency of the malaria crisis which has helped to focus the attention of the world health community on the magnitude of the overall problem in the Third World, where parasite-borne diseases take a staggering toll in human life and productivity. Malaria is the big emphasis, of course, but Dr. Trager's success with his culture method has had a stimulating effect in getting people to try again to culture other organisms. Within a year of his announcement, the same method with small modifications was used to make a vaccine against a widespread cattle disease caused by parasites that, like malaria, infect red blood cells.

Today, scientists from a variety of disciplines working with new tools and techniques, are looking at parasites from many view points. At The Rockefeller, for example, they are being studied in laboratories of cell biology, immunology, and medical biochemistry.

For Dr. Trager, this year celebrating his 70th birthday and his 47th year at the University, the goals remain essentially the same. "I was interested in physiology and biochemistry. That's what I've mostly studied and what I'm still studying. I remember once, years ago, having a chat with Dr. Gasser [Herbert Gasser, Nobel laureate and director of The Rockefeller from 1935 to 1953]. He was very interested in what I was trying to do then with parasite cultivation, but he wasn't worrying specifically about whether it would have practical results. I'm sure he thought it would be nice if it did, but the idea, really, was just to do good work." □