WILLIAM TRAGER

1910-2005

A Biographical Memoir by
IRWIN W. SHERMAN
AND LARRY SIMPSON

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William Trager ~1950. Photograph courtesy Rockeferfeller Institute for Medical Research.

WILLIAM TRAGER

March 20, 1910—January 22, 2005

BY IRWIN W. SHERMAN AND LARRY SIMPSON

William Trager was born on March 20, 1910, in Newark, New Jersey. As a child he collected butterflies and caterpillars and sometimes, instead of the caterpillars metamorphosing into pupae, parasitic flies emerged.

"Another insect collector might have been disappointed," he recalled, "but I was fascinated." Indeed, this childhood interest in symbiosis (literally, organisms living together) would remain his life's work.

For more than 75 years, Trager's research centered on providing answers to three questions:

1. What factors does a host supply to its parasite?
2. Can these factors allow the parasite to go through complete development and reproduction in an environment outside of its natural host?
3. How do the parasite products affect the host?

Trager believed that answers to these questions would lead to new chemotherapeutic agents and protective vaccines, and that they would provide a molecular basis for understanding parasitism.

Trager received a bachelor of science degree from Rutgers University in 1930 and then went to Harvard University, where, he said, "I had the good fortune to meet Lemuel R. Cleveland and to become his first graduate student." Cleveland's laboratory had developed an improved medium to cultivate the human dysentery ameba *Entamoeba histolytica*, but the researchers were unable to remove the associated bacteria from culture. Cleveland recognized the importance of growing parasites free from other organisms—what we now call axenic cultivation—and he inculcated this goal in the mind of his young student.

More exciting, according to Trager, was Cleveland's discovery of a woodeating roach, *Cryptocercus punctulatus*, in which symbiotic flagellates were critical to the insect's ability to live on a diet of wood or cellulose. When he was able to establish cultures of the flagellates in an artificial medium, Trager provided conclusive evidence that the cellulose-digesting activity of the roaches was not due to their intestinal cells but to the symbiotic flagellates. He then went on to chemically characterize the flagellate cellulase.

For this work, Trager was awarded his PhD in 1933. He then joined the Department of Animal and Plant Pathology of the Rockefeller Institute for Medical Research in Princeton, New Jersey, working in the laboratory of Rudolf Glaser. Glaser had devised methods for culturing and purifying a number of parasites in axenic culture. Trager and Glaser were a perfect match. Both were original thinkers with facile imaginations, and they continually saw new possibilities in research.

As a fellow from 1933 until 1935, Trager developed the first successful insect tissue culture medium for growing the nuclear polyhedral virus (*Borrelina*), which causes a disease called grasseri in silkworms. This was no small feat, since Trager accomplished the culture before the advent of antibiotics. Following Glaser's success in culturing *Paramecium* free from other microbes (on which it fed) by using a medium that contained liver extract and heat-killed yeast, Trager was able to grow *Aedes aegypti* mosquito larvae that produced normal and sexually potent adults under sterile conditions in a similar medium. These experiments suggested that the mosquito larvae required at least two growth-promoting substances, and three years later Trager identified these as riboflavin and thiamin. This tour de force of axenic cultivation had a practical goal: to provide mosquitoes for studies of disease transmission. Indeed, a few years later Trager was able to demonstrate multiplication of the equine encephalitis virus in *Aedes aegypti* tissues *in vitro*.

Trager and Fred Bang, a pathobiologist, during their service in the Army Sanitary Corps. ~1944

In 1936 Trager married his high school sweetheart, Ida Susnow, and there followed the birth of three children: Leslie, Carolyn, and Lillian, who passed away November 10, 2006.

When the United States entered World War II and the military was faced with a limited supply of quinine to protect troops in the malaria-ridden South Pacific, there was a need for a better understanding of the physiology and biochemistry of the malaria parasite to determine the mode of action of alternative antimalarials and to discover new antimalarial drugs. Trager's earliest contributions to malaria came from his wartime experiences in New Guinea as a Captain in the US Army Sanitary Corps, where he was involved in the supervision of human trials with a new antimalarial, atabrine.

In 1950, the Department of Animal and Plant Pathology (including Trager) moved to the Rockefeller Institute for Medical Research in New York City, and Trager began to pursue systematic studies on the environmental conditions promoting the survival and development of malaria parasites *in vitro*.

Trager used to say, "You can't study something you can't grow." He was always interested in growing things. He believed that if you understood the nutritional needs of a parasite, you would be able to understand the molecular basis of parasitism. His approach was: once you got a culture established, you could start removing bits from it and in the process discover what is crucial in the medium for that parasite.

To assess survival, he used the highly susceptible and readily available bird malaria, *Plasmodium lophurae*. Early *in vitro* experiments with *P. lophurae*-infected red blood cells provided evidence for a favoring effect of a balanced, high-potassium salt solution, a low oxygen tension, concentrated red cell extract, glucose, serum, chick embryo extract, and a low concentration of liver extract. Trager extended parasite survival to between ten and sixteen days at 40–41^{0}C by the addition of calcium pantothenate, as well as fresh red blood cells.

During this same period (1943–1947) he was able to show that biotin and a fat-soluble factor influenced chick susceptibility to *P. lophurae*. He also found that the euglobulin fraction from adult chickens, when injected into young chicks or ducklings, lessened the number of parasites in the blood, indicating that there were immune factors present in adult chicken plasma.

To directly address the physiology of the intracellular malaria parasite, Trager undertook the axenic cultivation of *P. lophurae* outside of the red blood cell. After four years of painstaking effort, he published a series of lengthy and detailed papers on the favoring effect of ATP, coenzyme A, malate, pyruvate, reduced glutathione, and red cell extract on the parasite's extracellular asexual stages. He then went on to show a favorable effect of folinic acid and pantothenate on parasites free

Rudzinska's first micrographs clearly showed that malaria parasites ingest the contents of the host red cell by phagotrophy with the formation of food vacuoles, and within the food vacuoles the hemoglobin is digested and malaria pigment (hemozoin) is formed.

of the confines of the red blood cell, and he described the antimalarial action *in vitro* of antipantothenates.

Trager postulated that within the red blood cell, *P. lophurae* required para-aminobenzoic acid and folic acid, whereas the extracellular *P. lophurae* required folinic acid or some other coenzyme form of folic acid. His idea was supported by studies from other laboratories in which the antimalarial pyrimethamine was shown to interfere with the formation of folinic acid, and studies showing that para-aminobenzoic acid and folic acid enhanced the intracellular growth of the human malaria parasite *P. falciparum*. These results on the different roles of para-aminobenzoic acid and folinic acid were analogous to what he had previously found for pantothenate and coenzyme A: that pantothenate, the precursor of coenzyme A, favored parasite growth within the red blood cell, while coenzyme A favored extracellular growth. Trager then showed that all the enzymes in the biosynthetic pathway to coenzyme A were present in the duckling red cells, but absent from erythrocyte-free *P. lophurae*.

In the mid-1950s, Trager was having lunch with Maria Rudzinska, who was then just beginning to learn the techniques of electron microscopy in the laboratory of George Palade and Keith Porter. As was usual when Trager was present, the conversation turned to malaria parasites. He said, "I often wonder how these parasites enter a red blood cell without disrupting it." Very soon they realized that electron microscopy might provide the answers. Rudzinska and Trager decided to collaborate, and soon they had an NIH grant to study the fine structure of *P. lophurae*.

Rudzinska's first micrographs clearly showed that malaria parasites ingest the contents of the host red cell by phagotrophy with the formation of food vacuoles, and within the food vacuoles the hemoglobin is digested and malaria pigment (hemozoin) is formed. In pioneering work they described the mitochondrion, the parasitophorous vacuole membrane, and the nucleus of *P. lophurae*. In addition, collaborating with Phyllis Bradbury, they described the ultrastructure of *P. falciparum* and identified "knobs" on the surface of the infected erythrocyte that contribute to sequestration. Rudzinska and Trager then extended these fine-structure studies to another blood dwelling parasite, *Babesia*, in which they discovered sexual stages and a unique arrowhead-like organelle for penetration of the tick peritrophic membrane by the zygote, enabling them to describe the mechanism of its invasion into red blood cells.

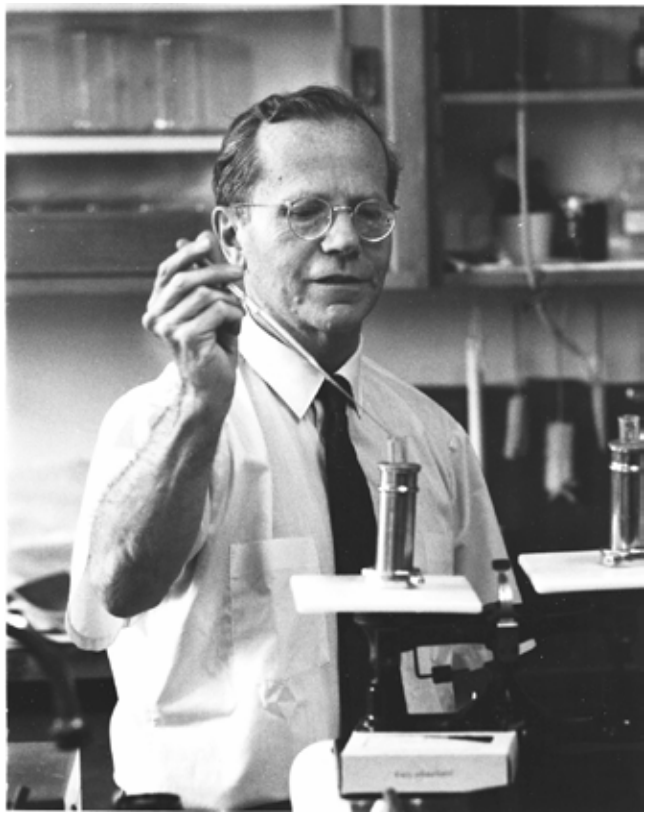
Up until 1960, serious reservations for developing a malaria vaccine for humans had been raised on clinical, logistical, and economic considerations. Because immunity to malaria in humans develops slowly and incompletely, the assumption was that vaccination would not improve on the immunity developed by repeated severe infections. And some contended that a malaria vaccine would not only be costly to develop, but also would serve

only as an adjunct to the inexpensive and effective insecticides and antimalarials.

Full of hubris, the 8th World Health Organization Assembly meeting in Geneva, in 1955, endorsed a policy of global eradication of malaria with reliance on chloroquine treatment and DDT spraying. However, by the 1960s many parts of the world where eradication had once seemed possible were experiencing resurgence of malaria, due to mosquito resistance to insecticides and resistance of the malarial parasite to the once effective chloroquine. It became apparent that the Global Eradication of Malaria Program was a failure and eradication could not be achieved. As a consequence, there was renewed interest in a malaria vaccine.

From 1941 onward, Trager had maintained *P. lophurae*-infected red cells *in vitro* using a rocker-dilution method developed at Harvard University during World War II that had been used to cultivate the monkey malaria parasite *P. knowlesi*. The system consisted of red cells suspended in nutrient medium, which was gently rocked to simulate blood flow and gassed with humidified 5 percent carbon dioxide and 95 percent air. Under these conditions, *P. lophurae* growth was less than optimal, and reinvasion rates were so low that continuous culture could not be achieved.

During the 1950s and 1960s, Trager attempted unsuccessfully to cultivate *P. falciparum* using the



Trager "at the bench" in his laboratory in Theobald Smith Hall, Rockefeller Institute. ~1960

rocker-dilution method. In the fall of 1975, he returned to the problem of cultivation of *P. falciparum*. Unable to obtain *Aotus* monkeys (which are susceptible to *P. falciparum*), he did the next best thing and worked with a species of monkey malaria, *P. coatneyi*, that closely resembled *P. falciparum* but would produce heavy infections in

the readily available rhesus monkeys. He tried commercially available tissue culture media using the rocker-dilution method, but again, continuous cultivation could not be achieved.

Trager then decided to abandon the rocker-dilution method and substitute a flow vial system in which the culture medium would move gently over a settled layer of infected red cells. His reasoning was that since *P. falciparum* (and *P. coatneyi*) spend most of their 48-hour development cycle attached to the post-capillary venules, agitation of the infected red cells might be detrimental to parasite growth and merozoite invasion.

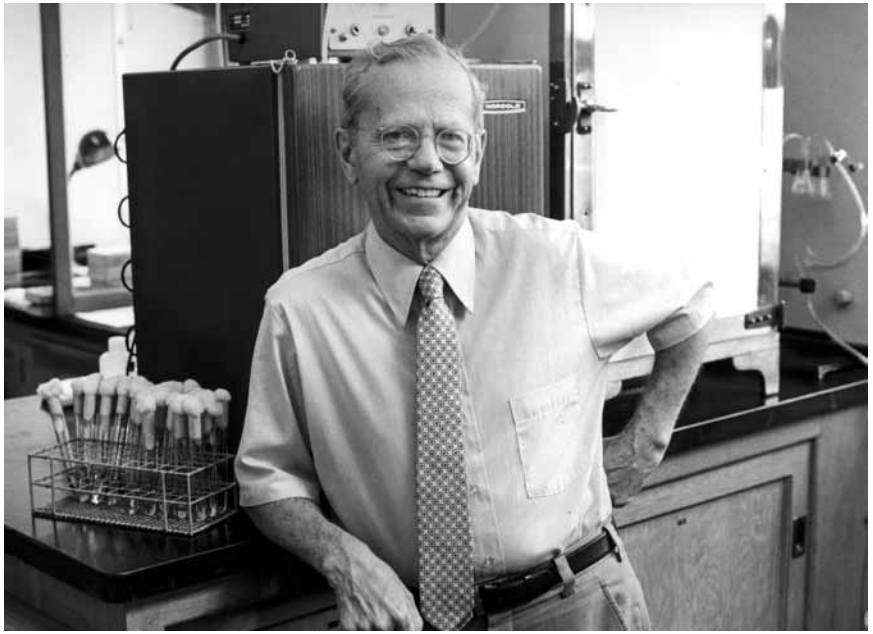
At this time, James B. Jensen, a postdoctoral fellow who was experienced in the cultivation of intracellular parasites, joined Trager's lab. Trager was able to obtain *Aotus* monkeys and during February 1976 they tested the suitability of commercial media with red cells removed from an *Aotus* monkey infected with the FVO strain of *P. falciparum*. The method involved washing the infected *Aotus* red cells, diluting them with human AB red blood cells in 15 percent human serum, and placing them in flow vials with RPMI 1640 medium. They also changed the gas mixture to one that was high in carbon dioxide and low in oxygen (7 percent CO_2 + 5 percent O_2 + 88 percent N_2). Under these conditions, and with a settled layer of red blood cells in the flow vials, it was possible to maintain the parasites for twenty-four days by adding fresh uninfected red cells every three or four days. They had achieved the continuous culture of human-infective *P. falciparum* in red blood cells for the first time. Trager's success in the cultivation of *P. falciparum* is an affirmation of Pasteur's aphorism, "in the field of observation, chance only favors prepared minds."

Jensen then had the idea to simplify the system by using an old microbiological candle-jar method to generate a CO_2-rich atmosphere. He put some Petri dish cultures of *P. falciparum*-infected red blood cells inside a large glass desiccator with a lighted candle and after the flame went out, he closed the stopcock. The candle jar was incubated at 37°C for several days. When Trager was shown the Giemsa-stained slides, he was convinced that Jensen was on to something. Then, in the summer of 1976, Milton Friedman, a graduate student in the Trager lab working in the west African country of The Gambia, arranged for a sample of human blood infected with *P. falciparum* to be sent to New York. This was diluted with RPMI 1640, placed into a candle jar, and incubated. The line grew very well (much better than the FVO strain of *P. falciparum*) and became the FCR-3 strain.

In the years after 1976, Trager and Jensen traveled the world instructing others in the continuous cultivation of *P. falciparum*. The impact of continuous cultivation of *P. falciparum* was phenomenal. It spawned a renaissance of research on the immunology, cell biology,

and molecular biology of this parasite. Since the 1976 publication of this monumental work, there has been an explosion of information about falciparum malaria. The readily available, laboratory-tamed *P. falciparum* initiated much new research on the genetics of red cell susceptibility, parasite antigens, differentiation into gametocytes, determination of the number of chromosomes and sequence of the genome, and testing for drug sensitivity. Trager also developed several well-characterized clones of *P. falciparum* and found that gametocyte formation would occur *in vitro* in some of these clones. The successful *in vitro* culture of *P. falciparum* has been and continues to be the basis for development of a protective malaria vaccine.

Trager was also interested in the kinetoplastid protozoal parasites. Stimulated by his friendship with Leslie Stauber, a leader in the field of pathogenic *Leishmania* and faculty member at Rutgers University, Trager showed that intracellular amastigotes of *Leishmania donovani* could be cultured extracellularly at 37°C for several days. This led to the development of continuous culture systems for *L. donovani* amastigotes in other labs. Trager then studied the African trypanosome *Trypanosoma vivax* during a very fruitful sabbatical year at the West African Institute for Trypanosomiasis Research in Nigeria. There he was able to establish tsetse fly tissue cultures for the first time and use these to culture all the life cycle stages of *T. vivax*. This was a remarkable achievement and it was the stimulus for a great deal of research on the African trypanosomes.



Trager in his laboratory. ~1988

In 1957, probably due to the influence of the *Crithidia* expert Seymour Hutner at Pace University, Trager became interested in developing a defined medium for the continuous culture of a kinetoplastid protist, the lizard parasite *Leishmania tarentolae*. He succeeded in developing a defined medium containing salts, glucose, seventeen amino acids, purines and pyrimidines, and B group vitamins. Using this culture medium, Trager showed that the cells required both folic acid and an unconjugated pteridine, biopterin. He then investigated the riboflavin requirement and found that the

concentration of riboflavin selectively affected the length of the flagellum.

Trager became interested in the unusual mitochondrial DNA of the kinetoplastids known as the kinetoplast. He found that the dye acriflavin produced akinetoplastic (i.e., lacked visible kinetoplast DNA) *L. tarentolae* in defined medium culture, but only at high levels of riboflavin. These cells could not be subcultured, but they provided a chance to study the fine-structure morphology of the kinetoplast. Trager and Rudzinska in 1964 showed that the mitochondrial cristae were selectively affected and the dense fibrillar material in the mitochondrion became diffuse and eventually disappeared. Since the mitochondrial membranes were maintained in these cells, they introduced the term "dyskinetoplastic" to replace "akinetoplastic." His was one of the first fine-structure investigations of this organelle, and it illustrated the breadth of Trager's scientific interests.

In 1979 Trager returned to the termite protozoa, on which he had worked during his PhD thesis research. Trager and Michael Yamin, his graduate student, demonstrated that the cellulase activity of *Tritrichomonas termopsidis* was not dependent on symbiotic bacteria, either extracellular or intracellular. They were also able to uncover the nutritional role of these protozoa in the life of the termite.

Bill and Ida Trager. ~1980.

During his lifetime, Trager received many awards and honors, including honorary degrees from Rockefeller University (1987) and Rutgers University (1965), the Manson Medal of the Royal Society of Tropical Medicine and Hygiene (1986), the Leuckart Medal (1982), the S.T. Darling Medal of the World Health Organization (1980), the Augustine Le Prince medal of the American Society for Tropical Medicine and Hygiene (1991), and Thailand's Prince Mahidol Award (1994–1995). In 1973 he was elected to the National Academy of Sciences (USA) and from 1973 to 1974 he

Trager was the exemplar of a bench scientist. He worked in his Rockefeller laboratory on a daily basis for almost seven decades. With his own hands he prepared reagents, set up cultures, and meticulously recorded his observations and conclusions. The impeccable thoroughness with which he pursued his science resulted in the high degree of reproducibility and reliability that are characteristic of his work.

was a Guggenheim Fellow. Trager was president of the Society of Protozoologists from 1960 to 1961, president of the American Society of Parasitologists from 1973 to 1974, and president of the American Society of Tropical Medicine and Hygiene from 1978 to 1979. He served as editor of the *Journal of Protozoology* from 1953 until 1965.

Trager's contributions to malaria and kinetoplastids are represented by more than two hundred research publications and two books, *Living Together: the Biology of Animal Parasitism* (New York: Plenum Press, 1986) and *Symbiosis* (New York: Van Nostrand Rheinhold, 1970). A representative sample of his publications is shown in the selected bibliography that follows.

However, he did much more than that. He served as the guiding light for a large number of malariologists including (alphabetically) Peter Bennett, V. Bhasin, Phyllis Bradbury, Chariya Brockelman, Fred Brohn, Phuc Nguyen Dinh, Milton Friedman, Gokal Gill, Fred Gyang, Robert Herman, Michael Hollingdale, James Jensen, George Jackson, Araxie Kilejian, Susan Langreth, Norbert Lanners, Barclay McGhee, Mary Motyl, Julie Olsen, Margaret Perkins, Edward Platzer, Robert Reese, William Scheibel, Irwin Sherman, Wassim Siddiqui, Ira Singer, Harold Stanley, Ann Vezza, and Jonathan Williams.

Trager sparked interest in kinetoplastids in his graduate students and postdoctoral researchers, including (alphabetically) Andrew Balber, K.P. Chang, Alan Clarkson, Philip D'Alesandro, Dennis Dwyer, Jan Keithly, Stuart Krassner, Curtis Patton, Miki Rifkin, Larry Simpson, Phyllis Straus, and Rolf Steiger. Others in his laboratory who worked on symbionts, parasites, and insects include (alphabetically): Dickson Despommier, Wallace Fish, Dunne Fong, Abraham Held, Steve and Marlene Karakashian, Joseph Peleg, Earl Weidner, and Michael Yamin.

Trager was the exemplar of a bench scientist. He worked in his Rockefeller laboratory on a daily basis for almost seven decades. With his own hands he prepared reagents, set up cultures, and meticulously recorded his observations and conclusions. The impeccable thoroughness with which he pursued his science resulted in the high degree of reproducibility and reliability that are characteristic of his work.

Through it all, Trager was always modest and generous in the exchange of ideas and methods. Each of us (I.S. and L.S.) was made to feel as if we were his colleagues. His support extended from the very first day we entered his laboratory until the time of his death in 2005.

Trager encouraged students and postdoctoral fellows to seek out independent positions and expected that we would carry with us that which we learned at Rockefeller. However, most of us felt that we never really left the Trager lab, for his support and high standards were always with us. He enriched our lives beyond measure.

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