

## CULTIVATING FRIENDS AND PARASITES\*

WILLIAM TRAGER

*Department of Parasitology, The Rockefeller University, New York, New York 10021*

Two years ago, when I had the privilege of giving the Craig lecture before this Society, my subject was the cultivation of parasites.<sup>1</sup> With the very similar title now, I cannot blame you if you begin to think I have gotten into a rut and can't get out. That's so likely to happen with advancing age. Actually, however, this time I will be telling more about people and places than about parasites. The parasites served to bring me to these people and places.

It was 3 years ago that it became clear that we had efficient, reproducible methods for continuous culture of the human malaria parasite *Plasmodium falciparum*.<sup>2</sup> *The Special Programme for Research and Training in Tropical Diseases of the World Health Organization then laid plans to spread this new technique to laboratories in countries where malaria is a major problem. I am happy to say that Dr. A. O. Lucas, Director of the Special Programme, is attending our meeting and that we will have the pleasure of hearing from him tomorrow at 11 in the morning.*

The first course on Cultivation and Biology of *Plasmodium falciparum* was scheduled to be given in spring of 1978 for the Southeast Asian Region. The Faculty of Sciences at Mahidol University in Bangkok, Thailand was chosen as the place. This was a very fortunate choice, as you will see. Dr. Walter Wernsdorfer of the Geneva Office of WHO and I organized the course. We arranged a schedule of lectures and seminars and of laboratory work. The last we considered especially important, since we wanted the course participants to start and maintain cultures of *P. falciparum* and to do some simple experiments with them. To accomplish this we needed a minimum time of 3 weeks. We found out what kinds of equipment the laboratory at Mahidol needed for the course—this ranged from incubators to candles, with desiccators to serve as candle jars, an important item.

The equipment was purchased by WHO and shipped in advance to Bangkok. When I arrived

a week before the start of the course, Dr. Wernsdorfer was already there and working on getting some of the crates of equipment out of customs. The last crate was obtained on the Saturday before the course opened and final preparations were made over the weekend. We found at Mahidol a most dedicated and capable group, from faculty to technicians to handymen. They had set aside and prepared for our use a seminar room and a very adequate laboratory equipped for the work of 4-5 pairs of students.

The local person who was in charge of the course was Dr. Chariya Brockelman of the Microbiology Department. Dr. Brockelman, a parasitologist who had worked mainly with helminths, had spent a few weeks at my laboratory during the previous fall and already knew the petri dish method. She had two very able young women graduate students as her assistants. They were Peerapan Tan-ariya and Rachana Santiyanont. They had that combination of ability and readiness to work hard that gets things done well. There were, furthermore, three faculty members of the Biochemistry Department, who were already interested in and working on biochemistry of malaria, who helped us with everything from giving a seminar to chauffeuring us around the wilds of Bangkok traffic. These were Drs. Bhinyo Panijpan, Praon Wilairat and Yongyuth Yuthavong. All of these people, and many others, became our friends.

My wife, Ida, was with me, as she was on the later courses, and we found ourselves being royally entertained, at people's homes as well as in restaurants. Thai food is very special and that alone would make a visit to Bangkok worthwhile. Unfortunately, I can't provide you with that, but I'd like to show a very small sample of the exotic and exciting sights of that remarkable city. (Pictures of the great temples, the gold Buddha, the floating market, must be seen in color; black-and-white could not do justice to them.)

Since the course, and actually as it turned out two successive courses that overlapped by 1 week, kept us busy, we did most of our sight-seeing on weekends and did not go far out from Bangkok. There was no need to. Often, the course participants went with us. We also could get well ac-

\* Presidential Address given before the 28th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Tucson, Arizona, 15 November 1979.

quainted with them because we all had lunch together as well as coffee breaks in the seminar room. Each weekday during the courses a delicious Thai lunch was set up as a buffet in the corridor outside the seminar room. Here and at the coffee breaks we could all discuss the course and other matters, even politics. It was fortunate that we could all communicate with each other in English. The participants of the first course were Drs. Phrem Bhatt from Bangalore, Chaudhury from Delhi, Im from South Korea, Joshi from Jodhpur, Keiltivati from Bangkok, Oo from Rangoon, Thaithong from Bangkok, and Tharavani from Bangkok. In the second course we had Drs. Sarikabuthi from Chieng-mai University in the north of Thailand, Sharma from Bombay, Wagh from Poona, Thomas from Kuala Lumpur, Kacharina from Bangkok, as well as Suzuki from Japan, Torby from Australia, and Billiault from France. It is gratifying that a number of these have written to me since the course ended and that several are now successfully working with cultures of *P. falciparum*.

In the first course at Bangkok, Dr. Brockelman started a new culture line from a patient with falciparum malaria in a Bangkok hospital. This line, since designated as FCM-1, was used for the laboratory work for both courses and grew very nicely. It was used to test drug sensitivity and found to be resistant in vitro to chloroquine at 0.1  $\mu\text{g}/\text{ml}$ , the highest concentration tested. It has since been shown to be a chloroquine-resistant strain, not surprising in Southeast Asia where most of the falciparum malaria is now chloroquine-resistant.

This matter of chloroquine resistance took us on our longest trip out from Bangkok, a 3-hour drive to the Malaria Center for Region 1 in a town called Phra Phuttabat. Here is based the very able Director of Malaria Control for Thailand, Dr. Surin Pinichpongse. Phra Phuttabat is in a region with plenty of malaria, despite continuing vigorous efforts at control. At the time of my visit Drs. Wernsdorfer, Rieckmann, and Lopez-Antunano were there trying out Dr. Rieckmann's in vitro micromethod,<sup>3</sup> based on the culture method, for assessing chloroquine resistance. The method worked well, and as I remember, all nine samples tested were chloroquine resistant. Phra Phuttabat is itself a most interesting place. The name means Buddha's footprint and there is a famous temple within which one can see enshrined a large elongate depression said to be the giant footprint of Buddha.

The third WHO course on Cultivation and Biology of *P. falciparum* was given at the University of Ibadan in Nigeria at the same time I was in Thailand. This was for the African region and was given by my colleagues Dr. J. Jensen and R. Reese with Dr. P. Trigg representing W.H.O. Some time perhaps they will tell you about that.

In the fourth and fifth courses I was again personally involved. The fourth, for the American region, was given this past June at the Gorgas Memorial Laboratory. Since I was not able to stay for the full time of the course, I had with me two experienced people from my laboratory, Dr. L. W. Scheibel and Ms. Marika Tershakovec. Dr. Lopez-Antunano of PAHO and WHO, stationed at the Gorgas, was responsible for local organization, with the strong support of Dr. A. S. Benenson, Director of the Gorgas Laboratory, and Dr. R. Rossan. Once again, we had the most wholehearted cooperation and excellent advance preparations. This time, since falciparum malaria is not readily available in Panama City, I had brought with me frozen cultures of my reliable African strain FCR-3/Gambia. We started these a few days before the course was to begin, but they did not grow well. Fortunately, Dr. Rossan had the Uganda Palo-Alto strain of falciparum going in *Aotus* monkeys. This strain has been put in culture by Siddiqui (personal communication). Using rabbit serum, as a serum compatible with both human and *Aotus* erythrocytes, we and the course participants did get initial cultures. But again, they did not grow very well. This was most disappointing. The students assured us they were nevertheless learning a lot, but Bill Scheibel and Marika Tershakovec and I spent many frustrating hours trying to find out what was wrong. After I had left, Dr. Scheibel showed that there must have been something toxic either in the redistilled water or more likely on the glassware, which looked very clean. By borrowing both water and glassware from a tissue culture laboratory in the Canal Zone (Middle America Research Unit Branch of the Gorgas Laboratory), he succeeded in rescuing declining cultures of the Uganda Palo Alto line started from *Aotus*. I understand the cultures at the Gorgas are now thriving, as are cultures since begun in their home labs by several of the participants. The participants in this course were Drs. Ceneviva and Magalhães from São Paulo, de Monsalve from Bogotá, Pividal from Havana, Malagon from Mexico City, Edrissian from Teheran, and Allred from Riverside, Cali-

fornia. Again, the course did not permit long trips. Perhaps our most interesting excursion was a Sunday outing to the San Blas Islands where the Cunha Indian women make their traditional molas and pose for the tourists.

Later this summer Drs. Ceneviva and Magalhães helped Dr. J. Jensen give a course in Brazil, so the method continues to spread. Perhaps its most interesting recent spread has been to the People's Republic of China. This story began in the spring of 1977 when my wife and I were lucky enough to be included in a Rockefeller University group that spent 18 days in China, with visits to five cities. I am very glad that Dr. Jing-Bo Jiang whom I met on that visit, and a colleague, Dr. Chen, both from Sun Yat Sen University, are here to attend this meeting. In Shanghai I gave a talk on the culture method, then only a year old. Within a year I had communications from Chinese workers who were successfully duplicating the results, first with the monkey malaria *P. knowlesi* and then with *P. falciparum*. At the same time requests had gone from the Ministry of Health in Peking to W.H.O. for a course on cultivation in China, and to me to take two Chinese scientists for a 2-week period in my laboratory with the understanding that they would assist in the course. This all worked out. Last February Drs. Guo Sheng-qi and Gao Min-xin arrived. Both were from the Institute of Biological Products, Dr. Guo from Shanghai and Dr. Gao from Peking. Both became thoroughly acquainted with our culture methods, so that when I arrived in Shanghai last August 17 they were both there and had going excellent cultures of two lines of *P. falciparum*. These had been started from two patients on Hainan Island, where falciparum malaria is still very prevalent. Here for the first time English was not adequate for communication. I had with me my colleague Dr. K. P. Chang who speaks fluent Chinese and would give laboratory instructions directly without need for an interpreter.

The course, the fifth to be given, and the first ever to be jointly sponsored by WHO and the Ministry of Health of the People's Republic of China, was housed at the Institute of Parasitic Diseases in Shanghai. Here we had superb facilities, including four laminar flow hoods for the use of the eight students. We again had the strong support of Dr. Mao Shu-pi, Director of the Institute. Dr. Mao had been at the N.I.H. years ago and speaks excellent English, as does his wife who is an English teacher. Incidentally, he had the fine

idea of putting my wife to work two mornings a week doing an English conversation class with a dozen of his faculty. She enjoyed this, especially since it gave her a chance to get to know people better. Also helping me with the malaria course were Dr. Wang Xiu-Feng from the Ministry of Health in Peking, Wang Li-do and Yi Sen-hai from Shanghai, who coordinated everything. I must give special mention to Dr. Liu Er-xiang from the Institute of Basic Medicine, Chinese Academy of Medical Sciences, Peking. Dr. Liu served as interpreter for the lectures and seminars. Without any text, he listened to a few sentences from me, then translated them into Chinese. Similarly, he translated questions from the Chinese into English for my benefit and that of Dr. Peter Trigg, who represented WHO.

The eight participants in the course were all medically trained. Three were from Shanghai, Dr. Li De-min from the Institute of Biological Products, Dr. Liu De-quen from the Institute of Parasitic Diseases and Dr. Zhoue Yuan-Chang from the Second Military Medical College; one from Peking, Dr. Fan Rugong of the Institute of Fundamental Medicine; two from Canton, Dr. Xi Yuan-ling, the one woman, who was from Guangdong Provincial College of Advanced Training for Medical Cadres, and Dr. Li Za-quan from the College of Traditional Medicine; Dr. Sun Hui-yuan from the Chengdu Institute of Biological Products, and Dr. Cheng Jin-fu from the Fujian Medical College.

The course started in the usual way with me giving a general introductory lecture open to the interested public. This was attended by about 100 people. The later seminars, mostly by me but several by Dr. Trigg and a few by Chinese workers on malaria, were usually attended by about 20 people from some of the scientific institutions in Shanghai in addition to the eight participants. The laboratory had to be limited to the eight participants. We often had discussions in the lab, with Dr. Chang as interpreter (Fig. 1).

The laboratory work went smoothly. We used one of the two culture lines being maintained by Drs. Guo and Gao. The participants soon had their own cultures going and used them for several successful experiments. Here, for example, are the results obtained with chloroquine (Table 1).

Group 1 had a problem with the serum they used and had to repeat the experiment under somewhat different conditions, so their results are not included in the table. You will note that in-



FIGURE 1. A laboratory discussion in Shanghai. Dr. Chang is in the foreground. In the back, facing the camera, are (from left to right) Drs. Kuo, Trager, and Mao. The eight course participants form a semicircle. Note on the left the improvised continuous flow set-up which worked quite nicely. Also note the large fan. The laboratory was not air-conditioned but the fan could be run much of the time, since we had laminar flow hoods for the sterile work. Shanghai is hot in August and early September.

TABLE 1

Results of course experiment on the effect of chloroquine on growth of *P. falciparum*, Line FCC-2

Group	Dish no.	Avg % parasites before drug on day		Chloroquine added days 2 and 3 at concentrations	Avg % parasites on day 4
		0	2		
2	1-3	0.6	2.5	1.0 $\mu\text{M}$	0.9
	4-6	0.6	3.1	0.33	1.0
	7-9	0.6	2.6	0.11	2.6
	10-12	0.6	3.5	0—Control	2.6
3	1-3	0.5	2.4	1.0 $\mu\text{M}$	0.5
	4-6	0.5	2.6	0.33	0.4
	7-9	0.5	2.6	0.11	3.9
	10-12	0.5	2.5	0—Control	3.2
4	1-3	0.5	0.9	1.0 $\mu\text{M}$	1.0
	4-6	0.5	1.1	0.33	1.0
	7-9	0.5	1.1	0.11	0.8
	10-12	0.5	1.2	0—Control	7.5
Dr. Gao	1-3	0.3	1.6	1.0 $\mu\text{M}$	0.01
	4-6	0.3	1.5	0.33	0.06
	7-9	0.3	1.9	0.11	0.3
	10-12	0.3	1.8	0—Control	5.0

hibition was evident at 0.11  $\mu\text{M}$  in two of the four sets of cultures but was complete in all at 0.33  $\mu\text{M}$  (about 0.1  $\mu\text{g/ml}$ ) or above. This strain is definitely a chloroquine-sensitive strain even though it came from Hainan Island where chloroquine resistance has been reported. It is worth adding that Dr. Peter Trigg used the same culture line in a 1-week course on the Rieckmann *in vitro* micromethod for testing chloroquine resistance and found the same result. His course was given in Shanghai, the week after mine, to other participants, and again under WHO auspices.

In Shanghai we lived in a hotel, as we had in Bangkok and in Panama, and again the hotel was within reasonable walking distance of the laboratory. This time I had the most difficulty persuading my hosts that I would not get lost or run down or collapse from exhaustion if I walked instead of being driven. The first couple of times I had to sneak out quietly in advance, but when they found that nothing untoward happened to me, they agreed to let me make the 15 minute

walk whenever I wanted. It's interesting to compare the walks in the three cities. That in Panama was certainly the quietest and most beautiful. Bangkok was very noisy from all the crowded motor traffic. Shanghai was noisy too from all the honking buses and taxis—they blow their horns repeatedly to keep the hordes of bicyclists and pedestrians out of their way.

In Shanghai, as in the other places, we could not take time to go far. But there was much to see in the city and we did take a weekend trip by train to Hangchow and a Sunday trip to Soochow, both renowned for their beautiful gardens. Dr. Mao kindly went with us on some of our trips and we had the good fortune to be entertained by him and Mrs. Mao at their home. Of course, we also enjoyed several of those excellent banquets for which the Chinese are famous. Here our problem was how to eat all of the good food provided.

Cultures of *P. falciparum* are now being actively investigated not only in several laboratories

in the United States and Europe but also in Thailand, in Panama, Brazil, and Colombia, in India, in China, and very likely in other countries as well. It will be interesting and exciting to see where the next major advance in the battle against malaria comes from. However it happens, I am sure the friends I have made in these countries will have contributed to it.

Finally, let me again thank all of you for the honor you conferred on me by electing me to the presidency of the Society.

#### REFERENCES

1. Trager, W., 1978. Cultivation of parasites in vitro. *Am. J. Trop. Med. Hyg.*, 27: 216-222.
2. Trager, W., and Jensen, J. B., 1976. Human malaria parasites in continuous culture. *Science*, 193: 673-675.
3. Rieckmann, R. H., Sax, L. J., Campbell, G. H., and Mrema, J. E., 1978. Drug sensitivity of *Plasmodium falciparum*. An in-vitro microtechnique. *Lancet*, 1: 22-23.