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Trager W & Jensen J B. Human malaria parasites in continuous culture. *Science* 193:673-5, 1976.
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Two methods were described for the continuous *in vitro* growth in human erythrocytes of the major human malarial parasite, *Plasmodium falciparum*. All stages of the erythrocytic cycle were present in the cultures, and infectivity to *Aotus* monkeys was demonstrated. This was the first report of continuous culture of any species of malarial parasite. [The *SCI*® indicates that this paper has been cited in over 850 publications.]

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The cultivation of parasitic organisms has always been one of my main interests. My first studies with avian malaria parasites were interrupted from 1943 to 1945, when I learned firsthand about human malaria while working in the Southwest Pacific as an officer in the Sanitary Corps of the US Army. On my return to The Rockefeller University I became interested in the possibility of extracellular culture of obligate intracellular protozoan parasites, and much of my research effort during the following 25 years was devoted to this goal. In experiments with the avian malaria parasite, *Plasmodium lophurae*, I obtained a limited extracellular development and showed that exogenous sources of ATP and coenzyme A were essential to it.[1-3]

Then, in 1975, the World Health Organization and the US Agency for International Development embarked on new programs aimed at developing a malaria vaccine. Cultivation of the parasites seemed essential, and I returned to the problem of intraerythrocytic development *in vitro*. In the meantime *P. falciparum* had been successfully transmitted to the South American owl monkey *Aotus* by W.A. Siddiqui,[4] providing a laboratory source of the parasites. Also, I had found in experiments with *P. coatneyi*, a parasite of the rhesus monkey with a biology very similar to that of *P. falciparum*, that the parasites developed better in a settled layer of red cells with a continuous slow flow of medium over it than under other conditions, such as the rocking flasks used in most earlier work.[5] I also found that for *P. coatneyi*, RPMI 1640 (a medium developed by G. Moore for human white cells) if supplemented with Hepes buffer and serum was better than any other medium previously tried. In addition, I had good reason to believe that a gas phase with about 5 percent CO_2 and with less oxygen than the 21 percent present in air at sea level would be favorable. I then put all of these factors together.

I inoculated a suspension of human AB erythrocytes with a small amount of falciparum-infected *Aotus* blood and placed it in two of the homemade flow vials, which provided for a flow of medium at 2 ml per hour over the settled cells and an atmosphere of 7 percent CO_2, 5 percent O_2, and a balance of N_2. The vials were sampled at one- or two-day intervals, and the excitement grew as I saw live parasites and evidence of multiplication. The cultures were diluted with fresh human red cells on the fourth day and then, as growth continued, every third or fourth day.

It was soon clear that continuous culture had been achieved, but the flow vials were not very convenient, and it was here that James B. Jensen made his significant contribution. He had just come to my laboratory as a postdoctoral fellow when I started the experiment with the flow vials that had such a successful outcome. In trying to simplify the method, we thought petri dishes with a daily manual provision of fresh medium would be worth trying. But how to control the gas phase? Although CO_2 incubators were already available, we did not have one, and, furthermore, incubators then on the market had no provision for maintaining a reduced oxygen tension. Fortunately, Jensen had done his graduate work with D.M. Hammond, who taught his students the use of candle jars for tissue cultures. We incubated our petri dish cultures in candle jars and found that we could get continuous propagation of *P. falciparum*.[6]

Once we knew how to do it, the methods for continuous culture of the erythrocytic stages of *P. falciparum* were so simple and flexible that they were soon taken up in laboratories all over the world. Cell biologists and molecular biologists found the parasites a fascinating material. Cultures of *P. falciparum* are now being used to study the mode of entry of the parasite into its host erythrocyte, its effects on the host cell, the mode of action of antimalarial drugs, and the nature of the parasite's resistance to chloroquine and other drugs. It is also used to screen for new drugs, to isolate and characterize strains and clones, to study the genetics of the parasite, and to identify immunogenic antigens for ultimate use in a vaccine.[7]

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2. ————. Malaria parasites (*Plasmodium lophurae*) developing extracellularly *in vitro*: incorporation of labeled precursors. *J. Protozoology* 18:392-9, 1971.
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4. **Geiman Q M, Siddiqui W A & Schnell I V.** Biological basis for susceptibility of *Aotus trivirgatus* to species of plasmodia from man. *Milit. Med.* 134:780-6, 1969.
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6. **Jensen J B & Trager W.** *Plasmodium falciparum* in culture: use of outdated erythrocytes and description of the candle jar method. *J. Parasitology* 63:883-6, 1977. (Cited 190 times.)
7. **Trager W.** The cultivation of *Plasmodium falciparum*: applications in basic and applied research on malaria. *Ann. Trop. Med. Parasitol.* (In press.)