

Continuous *in vitro* Cultivation of *P. falciparum*
and Malaria Antigen Isolation

Principal Investigator : Katchrinnee Pavanand, M.D.

Associate Investigators : Barnyen Permpanich
Somchit Tulyayon
Prasit Sookto
Niphon Chuanak

OBJECTIVE : To maintain strains of *P. falciparum* recovered from human subjects in a continuous *in vitro* culture system for large scale production of parasites.

BACKGROUND : Different techniques for *in vitro* culture of *P. falciparum* have been described (1-4). The parasites obtained were found to be infective in subsequent *in vivo* and *in vitro* experiments but the quantity produced was not sufficient for antigen preparation. Recently Trager (5) has described a continuous culture system utilizing a slow continuous flow of culture medium over a thin layer of parasitized erythrocytes. Jensen (6) has simplified the technique for propagating the viable parasites using petri dishes inside a candle jar. The system lends itself to many useful investigations including the possible preparation of a protective vaccine.

METHODS : A continuous *in vitro* culture system for *P. falciparum* was set up as described by Jensen. Infected blood specimens from malaria patient were suspended in RPMI 1640 medium supplemented with HEPES buffer and human AB serum. Target erythrocytes from individual AB blood donors were used for subcultures when parasitemias in original cultures reached about 5-6%. Aliquots of the highly parasitized cell suspension were preserved with glycerine - manitol at -70°C (7) for subsequent experiments. Different cryoprotective agents will be tested to determine the best cryopreservation method for these parasites. Further investigations are being conducted to clarify the question of the possibility of replacement of human serum in culture medium, the improvement of synchronicity of the asexual erythrocytic cycle, and infectivity after cryo-storage.

RESULTS : A strain of *P. falciparum* from Sriracha, Cholburi Province, has been established in a continuous *in vitro* culture system. This system allows an increase in parasitemia of 20 fold after 96 hours of incubation. Although individual non immune AB blood donors were screened for optimum support of *in vitro* parasite growth, variations in parasite increase were observed among different donors. The use of a pooled AB serum will be attempted in subsequent cultures when adequate numbers of suitable donors are obtained in an attempt to eliminate individual differences.

Investigations are being conducted in the following areas :

1. The possibility of replacement of human serum with a more easily obtained substance in the culture medium. In the established technique, it is

necessary to supplement the RPMI 1640 medium with 10% human AB serum to support *in vitro* development of *P. falciparum*. Preliminary results suggested that half the AB serum required may be replaced with inactivated fetal calf serum.

2. Attempts to isolate different stages of the parasite, to improve the synchronicity of the asexual erythrocytic cycle during *in vitro* culture, by gelatin floatation were not successful. Separation of parasites by Ficoll gradient centrifugation is being evaluated.

3. Different batches of specimens frozen at -70°C using glycerol as the cryoprotective agent were tested for *in vitro* infectivity. The greatest loss of intact parasitized cells up to 50% was found in a specimen stored up to 60 days. Although the viability of preserved parasites in regards to the *in vitro* infectivity was restored, this method appeared to be unsatisfactory. Attempts will be made on other cryoprotective agents available. Storage in liquid nitrogen (-195°C) appears to be more conducive to the maintenance of intact parasitized cells. This is a preliminary report.

REFERENCES :

1. Diggs, C.L. *et al.* Penetration of Human Fetal Erythrocytes by *Plasmodium falciparum*. J. Parasit. 57:187-188, 1971.
2. Phillips, R.S. *et al.* Culture of *Plasmodium falciparum* *In Vitro* : A Subculture Technique Used for Demonstrating Antiplasmodial Activity in Serum from Some Gambians, Resident in an Endemic Malarious Area. Parasit. 65: 525-535, 1972.
3. Mitchell, G.H. *et al.* The Effect of Human Immune IgG on the *In Vitro* Development of *Plasmodium falciparum*. Parasit. 72:149-162, 1976.
4. Triggs, P.I. Invasion of Erythrocytes by *Plasmodium falciparum* *In Vitro* Parasit. 71:433-436, 1975.
5. Trager, W. and Jensen, J.B. Human Malaria Parasites in Continuous Culture. Science 193:673-675, 1976.
6. Jensen, J.B. and Trager, W. *Plasmodium falciparum* in Culture : Use of Outdated Erythrocytes and Description of the Candle Jar Method. J. Parasit. 63(5):883-886, 1977.
7. Diggs, C.L. *et al.* Protein Synthesis *In Vitro* by Cryopreserved *Plasmodium falciparum*. Am. J. Trop. Med. Hyg. 24(5):760-765, 1975.