

In vitro Gametogony of Plasmodium falciparum

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OBJECTIVE : To establish an **in vitro** technique for the production and maintenance of sexual erythrocytic forms of **Plasmodium falciparum**.

BACKGROUND : Despite extensive studies of gametocytes the gametogony of plasmodial species is still not fully known. To date no conclusion has been made on the origin of gametocytes regarding whether they arise from special merozoites or if a particular stimuli is required to trigger their development.

In human infections mature gametocytes appear in peripheral circulation, with the developing stages being confined to the bone marrow. Only during severe infections do immature forms appear in peripheral smears. Although these sexual parasites do not cause clinical symptoms, their existence could prevent the eradication of malaria in a particular area. Some antimalarials utilized in treatment affect the crescent wave, where gametocyte numbers are not suppressed during treatment but are increased (1). Gametocidal effects of various drugs therefore need to be tested. An **in vitro** test system would greatly facilitate determination of these effects.

PROGRESS : In previous studies a technique for the **in vitro** culture of erythrocytic asexual **P. falciparum** parasites was developed (2). The culture technique proved to be well suited for studies of plasmodial response to different antimalarials both morphologically and radiochemically (3). Studies of sexual parasites **in vitro** are now being performed utilizing a modification of this technique. During the culture period, nutrients for the growing parasites are replenished and their metabolites are removed at various intervals. Parasites have been maintained in culture up to 228 hours using this procedure. Bacterial contamination has prevented a longer culture period. Infected blood specimens for culture were obtained from patients attending the Somdej Sri Racha Hospital, Cholburi, Thailand. Pretreatment samples were collected randomly. In all cases, a control culture was initiated within a few hours and aliquots of blood were frozen for subsequent studies (4).

The development of gametocytes **in vitro** was successful in cultures of blood from four different patients. None of these infected blood specimens exhibited the sexual form of the parasite upon initial examination. Gametocytes were detected after 48 hours incubation in one patient, while the remainder were observed at 65, 132 and 138 hours incubation respectively. The gametocytes progressed to more mature stages during the culture period. The development of gametocytes **in vitro** was confirmed by the observation of two immature gametocytes in one erythrocyte. This form of parasitized red cell normally occurs in the bone marrow or spleen. In the same smear, a number of erythrocytes with multiple infection of trophozoites were seen indicating an active production of new bloods in the culture.

Intact gametocytes varied from 1.4% to 5.4% of asexual parasites (counted against 10,000 r.b.c.). A small number of mature gametocytes were detected after 204 hours incubation. Mature female gametocytes were long and thin, but not typically curved. The nuclear chromatin appeared as a dense, deeply stained mass located in the center, but not obscured by the pigment rodlets. Mature

males were short with round ends, and pink cytoplasm. The chromatin was diffused, pale staining and somewhat obscured by the pigment granules.

Late gametocytes were numerous with the females appearing in various shapes. Those most often seen were spindle shaped and pointed while a small number were elliptical. The chromatin appeared as a deeply stained mass, centrally located or displaced to one side. The pigment was rod shaped and was seen clumped near the chromatin mass.

The number of late male gametocytes was very small when compared to the female population. The morphology was similar to that described in the fully matured males except that they were more rounded than spindle shaped. In both populations the female/male gametocyte ratio was approximately 3:1.

The morphology of both mature gametocytes varied from the typical crescent shapes found in peripheral blood smears. These alterations may be the result of the unnatural conditions produced in the *in vitro* culture system. On only one occasion did it appear that the gametocytes were in the exflagellation stage. The appearance of these gametocytes was similar in that there was only one long flagellum extending from a single pole of the gametocyte. It has been confirmed that under unnatural conditions the exflagellation process results in such appearance (5).

In addition, a number of gametocytes with a pink staining mass extruding from the body of the gametocytes were seen. A similar observation of this form of gametocyte was first described in 1960. It was confirmed that this appearance was confined to *P. falciparum* (6).

These observations prompted efforts to study the infectivity of these gametocytes. A number of clean *Anopheles balabacensis* mosquitoes were fed on cultures of *P. falciparum* at varying intervals. Satisfactory numbers of mosquitoes were fully engorged after each feeding, but no oöcyst development was detected seven days later.

Gametocytes also developed in subsequent cultures made utilizing frozen blood specimens from two patients. This compared favorably with cultures of freshly drawn blood from the same patients. No oöcysts developed in mosquitoes fed on these cultures.

It is significant that the sexual cycle can be initiated *in vitro* with blood containing asexual parasites. The observation of gametocyte production and exflagellation indicates that this culture system possibly provides the proper conditions for the generation, growth, and maintenance through maturity, of the sexual forms of the parasite. Failure to produce oöcysts in mosquitoes may be due to various factors. Gametocyte population may be too low, or they may not be infective at the time of feeding. The *in vitro* environment may not provide the conditions necessary to produce gametocytes which are infective. Efforts to improve the existing culture technique are currently being pursued in this laboratory.

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