

Studies on In vitro Erythrocyte Penetration by P. falciparum

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OBJECTIVE: To explore the possibility of in vitro penetration by P. falciparum in a marked recipient cell system and to study the effect of serum from patients with a history of recurrent malaria on the in vitro penetration.

DESCRIPTION: The system for detection of penetration in vitro involves the inclusion of fetal erythrocytes in the culture. These cells can later be distinguished from adult cells by differential elution of hemoglobin. The presence of a parasite within a fetal cell is evidence of penetration in vitro. The culture media and technic have been described in a previous annual report (1970) and by publication (Diggs et al, 1971).

PROGRESS: Previous reports describe studies in which some degree of inhibition of fetal cell reinvasion by P. falciparum was found when serum from immune subjects was introduced into the culture media. Also, the inhibition of reinvasion by whole Ig globulins (half saturated $(\text{NH}_4)_2\text{SO}_4$ fraction) from 28 immune individuals has been comparably studied and reported. A complement dependent growth requirement for the in vitro cultivation of P. falciparum has been detected. It has become obvious that collection of P. falciparum infected blood from patients is seasonally dependent, yet a continual requirement for this complicated experimental study. Thus, an attempt has been made to preserve and store parasitized erythrocytes. The goal is to devise a technic for retaining the viability of intact parasites for long term studies. Initially, heparinized blood from patients was incubated with 50% glucose (1:20) and stored in liquid nitrogen as blood-sand pellets. The results were not satisfactory due to complete hemolysis of red cells upon thawing. To overcome this problem, dimethyl sulfoxide was introduced as a protective agent against freezing damage to the parasitized cells. Aliquots of infected blood with different concentrations of DMSO were stored in liquid nitrogen preceded with a stepwise freezing down before reaching liquid nitrogen temperature. At different intervals, aliquots of the frozen blood were thawed in a "thawing-out" solution at 42°C and the degree of red cell hemolysis was determined. The washed intact parasitized cells were then cultured, and the parasites grew and invasion of fetal erythrocytes occurred. Improvement has been made to achieve the least hemolysis upon thawing. This technic has permitted the collecting of P. falciparum infected blood from malaria patients in different locations in Thailand for comparative studies. In addition, storage of P. berghei, P. coatneyi, P. cynomolgi and P. knowlesi has been similarly studied. The recovery upon thawing in each instance has been satisfactory and patent infections were established in clean recipient animals. The determination of maximum storage of infected blood while retaining viability is in progress. At this reporting, maximum storage for P. falciparum has been 150 days, and for P. berghei 64 days.

SUMMARY: Observations of the interaction of P. falciparum and convalescent sera in vitro have continued. Improvement in the freezing and recovery of intact viable parasites has been achieved.

PUBLICATIONS:

Diggs, C.L., Pavanand, K., Permpanich, B., Numsuwankijkul, V., Haupt, R., and Chuanak, N. 1971. Penetration of Human Fetal Erythrocytes by Plasmodium falciparum in vitro. J. Parasitology 57, 187.