

Adenosine Triphosphate Level of *In Vitro* Erythrocyte — Free *P. falciparum*

Principal Investigators : Katchrinnee Pavanand, M.D.
 Douglas R. Stutz, MAJ, MSC

Assistant Investigators : Barnyen Permpanich, R.N.
 Nipon Chuanak

OBJECTIVE: To determine the ATP levels of erythrocyte — free *P. falciparum* during the course of maturation *in vitro*.

BACKGROUND: Extensive studies are underway by many investigators to explore the ATP Malaria Hypothesis¹⁻⁴. Many investigators have theorized that erythrocytic ATP levels of the host may play an important role in supporting the initial growth of the parasite, and that erythrocytes with low levels of ATP would be less viable and tend to rupture easier than erythrocytes with high ATP levels. In addition, a positive direct correlation of the erythrocytic ATP level with the severity of the primary clinical course of malaria is evident. Experiments in humans have been limited to the very early stage of infection to avoid undue risk to volunteers. Although supporting data may be obtained from experiments on rhesus monkeys, the effects of low ATP levels on inhibition of parasite development have not been elucidated. It has been suggested that in addition to the fact that red cells containing low ATP level may not survive to support the parasites, the initial ATP requirements of the parasite may be more than the cell can provide.

With the technique of *in vitro* culture of native *P. falciparum* developed here at this laboratory, the growth of parasites can be followed through maturity. The cell — free parasitic ATP level will be determined at different stages of development throughout the culture period and related to the morphology. The increase in parasitic ATP levels during the course of maturation *in vitro* will indicate the direct relationship between the intraerythrocytic parasites and their host cells.

The *in vitro* culture system of *P. falciparum* is the same as that previously described⁵ with the exception that ¹⁴C — isoleucine is not added to the culture media. The contents of several wells will be lysed by one of three methods being considered, under controlled conditions to prevent lysis of the parasites. Ammonium chloride lysis, selective pressure and immune lysis are three techniques to be attempted. The ATP levels of the freed parasites will be determined by the luciferase enzyme method as described by Stanley⁶.

PROGRESS: The luciferase — ATP measurement system is being standardized and selective lysis studies are underway. No conclusive data have been produced to date.

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