

A Quantitative Study on the Level of Adenosine Triphosphate in  
Human Erythrocytes by Luciferase Enzyme

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

Associate Investigator :                Vicharn Panich, M.D.<sup>1</sup>

**OBJECTIVE:** To establish a quantitative assay of ATP in human erythrocytes for determination of normal erythrocytic ATP levels in a Thai population and their relationship to malaria infections.

**DESCRIPTION:** It is known that there is considerable variation in the levels of erythrocytic ATP between individuals in a population, and that this level is constant in healthy individuals<sup>1, 2</sup>. Comparative studies in American Negroes and Caucasians indicated the existence of different mean quantities of erythrocytic ATP between these two groups<sup>3</sup>. Since the gene pool of the American Negroes is derived from an African Negro stock exposed to malaria for many generations, the lower mean levels of ATP in this group suggests selection pressure for malaria. Further studies revealed that there is a strong positive correlation between the erythrocytic level of ATP and *P. falciparum* parasitemia<sup>3</sup>. In human as well as simian infections, high ATP levels were directly associated with relatively high peak parasite counts<sup>4</sup>. It has been suggested that the protective mechanism against malaria infection may result from the following:

a. Erythrocytic ATP levels of the host play an important role in supporting the initial increase of parasitemia. With the lower level of ATP, a retardation of the primary increase in parasitemia will be seen, resulting in a less severe clinical course of infection.

b. The role of ATP in maintaining metabolism and viability of living cells indicates that red cells with low ATP levels would be less capable of maintaining their viability. This will, in turn, result in inability of the intraerythrocytic asexual parasites to develop through completion and rupturing of the parasitized erythrocytes may occur prematurely.

It is our aim to investigate erythrocytic ATP levels in Thai populations continuously exposed to malaria infection and to compare them with populations from non-endemic areas. Results obtained may reveal a significant protective mechanism against *P. falciparum* infection.

A technique for quantitative assay of erythrocytic ATP utilizing a firefly luminescence method described by Stanley and Williams<sup>5</sup> is being established. A standard curve of ATP is obtained by adding an aliquot of fresh extract of desiccated firefly lanterns to various known concentrations of ATP in phosphate buffer, pH 7.4. The resulting light pulses are counted in the liquid scintillation spectrometer.

**PROGRESS:** A comparative study utilizing fresh and frozen (in liquid nitrogen) blood specimens is in progress. Although the firefly luminescence method assay of ATP has advantages over other techniques described, a limitation of this procedure is that blood specimens must be processed soon after collection to prevent a reduction of ATP levels. Modifications of the method of red cell lysate preparation are being developed. It is anticipated that results obtained in these experiments will provide a means for measurement of ATP in erythrocytes collected from field surveys.

**REFERENCES:**

1. Brewer, G.J., and Powell, R.D.: A study of the relationship between the content of adenosine triphosphate in human red cells and the course of falciparum malaria: A new system that may confer protection against malaria. Proc. U.S. Nat. Acad. Sci. 54: 741—745, 1965.

---

<sup>1</sup> Division of Hematology, Department of Medicine, Siriraj Hospital.

2. Brewer, G.J., and Powell, R.D.: The adenosine triphosphate content of glucose-6-phosphate dehydrogenase-deficient and normal erythrocytes, including studies of a glucose-6-phosphate dehydrogenase-deficient man with "elevated erythrocytic ATP." *J. Lab. Clin. Med.* 67: 726-741, 1966.
3. Brewer, G.J.: Genetic and population studies of human erythrocyte adenosine triphosphate (ATP). *J. Lab. Clin. Med.* 66: 858-859, 1965.
4. Eaton, J.W., and Brewer, G.J.: Red cell ATP and malaria infection. *Nature* 222: 389-390, 1969.
5. Stanley, P.E., and Williams, S.G.: Use of the liquid scintillation spectrometer for determining adenosine triphosphate by the luciferase enzyme. *Anal. Biochem.* 29: 381-392, 1969.