Relationship Between Erythrocytic Adenosine Triphosphate (ATP) Level and Human Malaria

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OBJECTIVE: To establish a quantitative assay of ATP in human erythrocytes for the determination of normal erythrocytic ATP levels in a Thai population and to determine its relationship to malaria infection.

BACKGROUND: 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 (1, 2). Comparative studies in American Negroes and Caucasians indicated the existence of different mean quantities of erythrocytic ATP between these two groups. Since the gene pool of the American Negro 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 caused by malaria. Further studies revealed that there is a strong positive correlation between the erythrocytic level of ATP and *P. falciparum* parasitemia (3). In human as well as simian infections, high ATP levels were directly associated with relatively high peak parasite counts (4). 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 is 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 erythrocytes with low ATP levels are less capable of maintaining their viability. This would result in the inability of the intraerythrocytic asexual parasites to develop completely, and the parasitized erythrocytes may rupture prematurely.

The purpose of this study is to investigate erythrocytic ATP levels in Thai populations continuously exposed to malaria infection, and to compare them with populations from nonendemic areas.

DESCRIPTION: A technique for quantitative assay of erythrocytic ATP utilizing a firefly luminescence method described by Stanley and Williams (5) was utilized. A calibration curve of ATP was 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 were counted in the liquid scintillation spectrometer.

In most experiments, heparinized blood was used for the quantitative assay. Immediately after venipuncture, the blood was precipitated with trichloroacetic acid and maintained at −70°C for assay of ATP. It was found that delaying precipitation of the blood resulted in a significant decrease in ATP levels (6). To prevent this loss, ACD solution at pH 5 was used as an anticoagulant (7) when immediate processing of blood specimens was not possible. One milliliter of blood was added to EDTA, mixed, and kept at 40°C (wet ice) for cyanmethemoglobin determination.

Populations from Bangkok and Lumpoon representing a nonendemic group were compared with an endemic group from Chonburi and Prachinburi. In addition to these two groups, another group of newborn infants from Women's Hospital in Bangkok was included in this study as a control.

RESULTS: As shown in Table 1 the mean erythrocytic ATP level of the nonendemic group was 3.78 micromoles/gram hemoglobin, and that of the endemic group was 3.47 micromoles/gram. In newborn infants, the ATP level was found to be higher than that of adults. This finding in newborn infants agreed with that reported by Gross et al (8), although the assay methods utilized were different. With the firefly luminescence technique, the results were found to be slightly higher than those produced by the hexokinase, G–6–PD technique. There was no significant variation of the mean levels of erythrocytic ATP in either group. Birthplaces and residential areas were used for comparison in this study; however, both groups shared a common gene pool. It appears that the quantitative level of erythrocytic ATP is under genetic control of a multifactorial type and this finding is more suggestive of a genetic control than an environmental control.

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.
2. Brewer, G.J. and Powell, R.D.: 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. 67:726–714, 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.E. 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.
6. Beutler, E. and Mathai, C.K.: A Comparison of Normal Red Cell ATP Levels as Measured by Firefly System and the Hexokinase System. Blood 30:311–320, 1967.
7. Beutler, E. and Duron, O.: Effect of pH on Preservation of Red Cell ATP. Transfusion 5:17–24, 1965.
8. Gross, L., Schroeder, E. and Brounstein, S.: Energy Metabolism in the Erythrocytes of Premature Infant Compared to Full Term Newborn Infants and Adults. Blood 21:755, 1963.

Table 1. A Comparison of Erythrocytic ATP Levels in Thai Populations Residing in Nonendemic and Endemic Malarial Areas

Subject Population	No. Case	Micromole ATP/gm Hb		Hb gm/100 ml.	
		Range	Mean	Range	Mean
Newborn					
Bangkok	120	3.2 – 7.3	4.83	11.2 – 18.5	14.96
Nonendemic Areas					
Bangkok	98	2.62 – 5.8	3.77 } 3.78	8.6 – 17.5	14.4
Lumpoon	84	2.66 – 5.0	3.79 }	7.6 – 17.3	13.7
Endemic Areas					
Chonburi	29	2.19 – 4.3	3.06 } 3.47	8.7 – 14.3	10.81
Prachinburi	198	2.91 – 5.2	3.89 }	6.6 – 17.3	13.64