after fertilization of the egg but before cleavage divisions have begun.

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*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**

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The high frequency of the genes for sickle hemoglobin, glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, and thalassemia in some populations may have resulted from biologic advantages conferred by the corresponding phenotype against falciparum malaria.[1-8] Falciparum (malignant tertian) malaria, which predominates in many tropical malarious areas of the world, is considered an extremely potent selective agent because of the substantial mortality associated with the disease, especially in infants and young children.

This report presents the results of studies on the relationship between the content of adenosine triphosphate (ATP) in the red cell and the rate of increase in parasitemia early during the clinical course of falciparum malaria in nonimmune volunteers. Initially, we became interested in this relationship as a result of the detection of differences in the distribution of values of erythrocytic ATP in the American Negro and American Caucasian populations. For example, 42 per cent of an American Negro sample of 449 individuals (320 males, 129 females) had erythrocytic ATP values below 3.0 μmoles per gram of hemoglobin, whereas only 16 per cent of a Caucasian sample of 402 individuals (247 males, 155 females) had

values below this level.[9] The gene pool of American Negroes, in contrast to that of American Caucasians, is to a large extent recently derived from an African Negro stock exposed to malaria for many generations. For this reason, certain differences in the genetic constitution of these two groups, particularly differences involving red cells (such as G-6-PD deficiency and sickle hemoglobin), may reflect a differential selective effect of malaria.

Interest in the relationship between erythrocytic ATP and falciparum malaria also stemmed from the suggestion of Neel[10] that a basis for the protection against malaria afforded by certain conditions that shorten the life span of the human red cell may be a shortened cycle of development of the parasite in the cell. Conditions associated with shortening of the life span of the human red cell may limit the ability of the cell to survive during the time normally required for completion of a full cycle of maturation of asexual erythrocytic forms of the parasite. Similar considerations with respect to ATP suggested that red cells having relatively low levels of ATP might be less capable of maintaining structural integrity during maturation of malaria parasites than red cells having relatively high levels of ATP.

Materials and Methods.—This study was conducted by measuring levels of ATP in red cells from 13 of 16 volunteers who had participated in a previously reported study of G-6-PD deficiency and falciparum malaria.[11] The volunteers were healthy American Negro men, inmates of Stateville Penitentiary, Joliet, Illinois; it was not possible to determine levels of ATP in red cells of three volunteers who had participated in the previous study and who had been transferred or released from the institution. All 13 volunteers from whom blood was obtained for assays of ATP had developed overt falciparum malaria during the previous study and sufficient time (more than 1 year) had elapsed, after radical cure of the infections had been effected, to allow complete hematologic recovery. Repeated assays of ATP in red cells of several individuals carried out over a long period of time (more than 2 years in some instances) indicated that levels of ATP in the red cells of a given individual remain relatively constant (small variations noted were within limits of experimental error of the assay). Assays of ATP were performed by the spectrophotometric method of Kornberg,[12, 13] in which hexokinase and G-6-PD are employed as coupling enzymes.

Details concerning the subjects, methods employed to obtain infections, and other methods of study have been described.[11] Summarized briefly, infections with the McLendon strain of *Plasmodium falciparum* were induced by the bites of 10 heavily infected mosquitoes, studies were carried out under carefully controlled conditions, parasite counts (asexual erythrocytic forms) were determined at frequent intervals, and antimalarial medication was administered (to terminate acute attacks of malaria) when parasitemia first exceeded 5000/cmm or when otherwise warranted clinically. None of the volunteers had sickle-cell trait. Seven of the volunteers from whom blood was obtained for assays of ATP were G-6-PD-deficient; the other six volunteers were not G-6-PD-deficient. In this report, results of assays of ATP in red cells of both G-6-PD-deficient subjects and subjects not G-6-PD-deficient are pooled; the previous study[11] disclosed that, under the particular experimental conditions employed, levels of parasitemia in G-6-PD-deficient subjects did not differ significantly from levels of parasitemia in subjects not G-6-PD-deficient; additional previous investigations[13] showed that levels of ATP in red cells of G-6-PD-deficient American Negro men were not significantly different from levels of ATP in red cells of nondeficient American Negro men.

The mean level of ATP in the red cells of American Negro men (320 subjects) as measured in our laboratory is 3.16 μmoles of ATP per gram of hemoglobin, with a standard deviation of 0.42 μmoles.[9] These values are in general comparable to those obtained by other workers using similar methods of assay,[14] although in most reports the racial origin of the subjects under study has not been published. The range of levels of ATP in the red cells of the 13 subjects reported here (2.72–4.11 μmoles per gram of hemoglobin) extended from a level approximately one standard deviation below the control mean to a level more than two standard deviations above the mean. Thus, the range of variation of levels of ATP in the red cells of the Negro population is reasonably well covered by the levels of ATP in the red cells of the 13 men comprising the present sample.

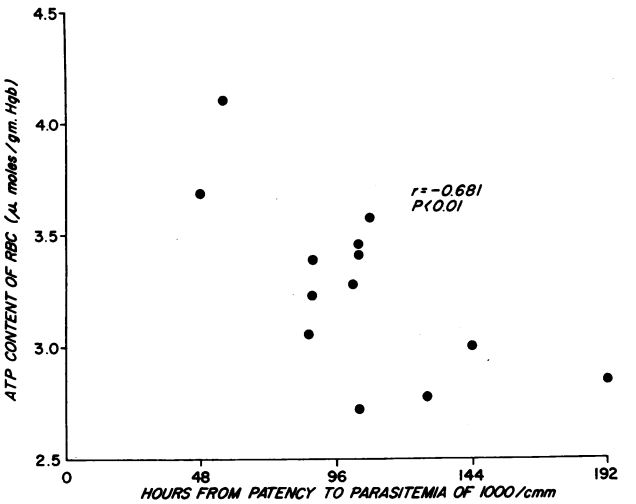

FIG. 1.—Correlation of erythrocytic ATP levels with rate of buildup of parasitemia in falciparum malaria. Patency refers to the time when parasites are first detected in the blood. Abbreviations: ATP, adenosine triphosphate; gm, gram; Hgb, hemoglobin; RBC, red blood cell; cmm, cubic millimeter.

Results.—Figure 1 shows the relationship between levels of ATP in the red cells and the number of hours elapsing from the onset of patency (the time at which parasitemia was first detected) until levels of parasitemia first exceeded 1000/cmm. A parasite count of 1000/cmm was selected as the end point for analysis because parasitemia in all volunteers studied reached this level before it was necessary to administer antimalarial medication to any of the volunteers. The correlation coefficient between these parameters, -0.68, is significantly different from zero at the 0.01 level of confidence.

The correlation coefficient is also significantly different from zero if lower levels of parasitemia are used for analysis (e.g., at 500/cmm, r is -0.66). At higher levels of parasitemia the sample size is reduced because of treatment of some of the volunteers with antimalarial medication, and the correlation coefficients are no longer significant (e.g., at 3000/cmm, r is -0.42). No relationship was apparent between length of prepatent period in the volunteers and the erythrocytic content of ATP.

Discussion.—These investigations indicate that, under the conditions of this study, a relationship obtained between the amount of ATP in erythrocytes and the rate of increase of parasitemia in the nonimmune Negro male volunteers infected with *P. falciparum*. The data suggest that the level of ATP in erythrocytes of the host may be an important factor that influences the clinical course of falciparum malaria. A retardation of the rate of increase of parasitemia associated with relatively low levels of erythrocytic ATP may, for example, allow more time for acquisition of immunity with a resultant lessening of both morbidity and mortality. Quantitative variation in levels of erythrocytic ATP (in the direction of lower levels) may constitute a system that confers a biologic advantage against falciparum malaria.

In contrast to the present studies on the relationship between erythrocytic ATP

and falciparum malaria, previous studies with nonimmune volunteers have not yielded convincing evidence to support the hypothesis that sickle-cell trait or G-6-PD deficiency confers an advantage against falciparum malaria.[11, 15, 16] This raises the possibility that the ATP system may exert effects quantitatively more marked than the effects of sickle-cell trait or G-6-PD deficiency in selective processes related to malaria. The system involving ATP may prove useful as a model for the study of selection in man. Genetic control of levels of erythrocytic ATP is likely multifactorial;[9] it should be informative to study, in appropriate populations, the interactions between the ATP system and systems under single factor control, such as G-6-PD deficiency and sickle hemoglobin.

One possible explanation for our findings is that red cells having a relative deficit of ATP may have a diminished ability to remain intact for the time normally required for maturation of asexual erythrocytic forms of the parasite. The level of ATP in red cells appears intimately related to the capacity of the cells to resist other types of stress. In accordance with the suggestion of Neel,[10] rupture of parasitized red cells before mature schizonts are formed, with premature release of parasites into the plasma, might lead to a retardation of rates of increase in parasitemia because of a lessened ability of the liberated parasites to survive or to initiate schizogony in other red cells. This hypothesis concerning the role of a "more fragile red cell" in malaria resistance has the advantage of providing a unifying explanation for the protection against malaria that may be conferred by rather diverse biochemical alterations of the erythrocyte. It is not possible, however, to exclude the operation, in some cases, of the other mechanisms, such as a failure of affected red cells to supply adequate amounts of one or more substances essential for normal development of the parasite.

The results of this study may have implications with respect both to therapy of malaria and to culture of malaria parasites in red cells *in vitro*. If the development of asexual erythrocytic forms of malaria parasites is in part dependent upon levels of ATP in red cells of the host, it is conceivable that efforts aimed at blocking or decreasing synthesis or regeneration of ATP in red cells of the host may afford a means of treatment of malaria. Considerable difficulty has been encountered in attempts to culture malaria parasites in red cells *in vitro;* culture of falciparum or other species of malaria parasites in red cells *in vitro* might be facilitated by the addition of agents such as those known to lead to improved maintenance or enchancement of levels of ATP in red cells stored *in vitro*.

Considerable additional information will be required to determine whether or not findings in accordance with those presented in this report can be obtained with other strains and species of malaria parasites and under other experimental conditions. It will be of interest to determine whether or not studies of other population groups currently or previously exposed to falciparum malaria will reveal distributional differences in levels of erythrocytic ATP similar to those detected in American Negroes. If the results reported here are to any extent generally applicable, variations in the genetic system controlling the level of erythrocytic ATP may have important implications for populations of many areas of the world.

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*INHIBITION OF DNA SYNTHESIS IN EHRLICH ASCITES CELLS BY ACTINOMYCIN D, I. DELAYED INHIBITION BY LOW DOSES**

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It is now generally accepted that a cell synthesizing DNA is a cell destined to divide in the very near future, a conclusion that is not invalidated by the few known exceptions.[1–3] Since cell division is temporally related to DNA replication, the factors that control the initiation of DNA biosynthesis are of particular interest to the