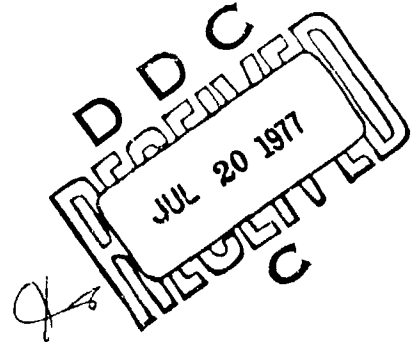


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with the Metabolism of the Host Red Cell"



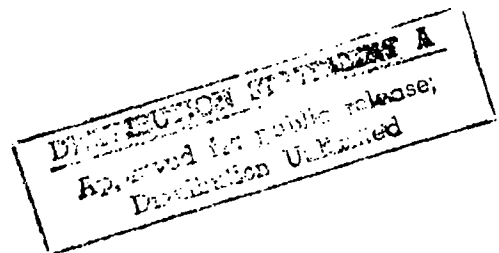
Final Technical Report

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(#20 continued) relationship of the severity of malaria in rhesus monkeys to their red cell ATP levels (Section D), and the pronounced decrease in red cell ATP levels that occurs under some conditions in rodent malarial (Section D). A related study to this general theme was the development of a treatment of malaria involving hyperoxia which was effective in suppressing P. vinckei in rats, but not P. berghei. X

The most important conclusions were:

- 1) Host red cell metabolism does influence severity of malaria, and natural selection has probably operated on metabolism in relation to malaria prevalence.
- 2) It is possible to maintain human red cells in good metabolic condition for 6 days at 37°C.
- 3) At least one type of animal malaria is sensitive to hyperoxia.

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Summary

The purpose of the project was to try to determine if variation in host red cell metabolism could influence malaria severity, and whether information obtained could be used in developing new treatments. During the tenure of this contract, a number of studies of the interaction of malaria and red cell metabolism were carried out. This included in vitro interactions in a cultivation system, discussed in Section A, the relationship of the red cell ATP levels of ethnic groups to natural selection by malaria, (Section B), the relationship of the severity of malaria in rhesus monkeys to their red cell ATP levels (Section D), and the pronounced decrease in red cell ATP levels that occurs under some conditions in rodent malaries (Section D). A related study to this general theme was the development of a treatment of malaria involving hyperoxia which was effective in suppressing P. vinckei in rats, but not P. berghei.

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Foreward

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences-National Research Council.

A. Malaria Cultivation System.

Progress was made in a malaria cultivation system using whole blood. Our approach to the cultivation problem is a departure from established techniques. First, it has been the philosophy of our approach that it is pointless to even introduce the parasite into the incubated blood until it was established that the red cells could be maintained in near normal metabolic state. Current knowledge indicates that the quality of the metabolic state of the incubated erythrocyte is best reflected by the level of ATP in the erythrocytes. ATP is an extremely important compound in red cell metabolism, representing the major source of high energy phosphate bonds. More to the point, it has been shown to be intimately correlated with the state of preservation of blood stored under blood-bank conditions.

Our approach to the problem of maintaining blood incubated at 37°C. in the best possible metabolic state has been to develop an apparatus which allows continuous dialysis of blood against large volumes of dialysing fluid. The desirability of maintaining continuous dialysis from the standpoint of erythrocyte metabolism is clear. Any sample of incubated erythrocytes which is not dialysed will, after a short period of time, accumulate lactic acid and the pH will drop to around 7.0. This is harmful from the standpoint of continued glycolysis and ATP levels in the red cell will decrease. It would be surprising if the malaria parasite were capable of vigorous and continuous growth in such a milieu. By continuous dialysis against a buffered dialysate a physiological pH and a physiological level of erythrocytic ATP is maintained. It is important that in maintaining circulation for purposes of dialysis that the red cells not be subjected to excessive trauma. Circulation was obtained by means of a gravity flow device which automatically alternated positions of two blood bags. Agents of any type, nutritional or pharmacologic, can be introduced into the dialysis fluid (or into the blood directly). Blood can be withdrawn from the circulation or new blood can be added to the circulation at any time. By modification of the dialysing fluid, with inclusion of 25 percent human plasma in the Krebs-Ringer-phosphate buffer, and by changing the dialysis fluid at 24 or 48 hour intervals, we were able to maintain normal or baseline levels of red cell ATP for up to 6 days. This was considerably beyond our original aspirations, and it is a significant contribution to the study of blood preservation, in and of itself.

B. Relationship Between Red Cell ATP Levels, Race, and Selection from Malaria.

1. U.S. Black and Caucasian populations.

We extended and confirmed our earlier observation that the American Black has considerably lower levels of erythrocytic ATP than the American Caucasian. Although the distributions of the two groups overlap, that of the Black is shifted downward with respect to that of the Caucasian. We also showed that erythrocytic ATP levels are genetically determined by means of family studies.

2. Sardinian Studies.

With the collaboration of Dr. Siniscalco of the University of Leiden, we conducted a survey of 250 individuals of the Sardinian population. We found

that the levels of ATP in the Sardinian population as a whole are appreciably lower than the U.S. Caucasian and are about equal to that of the U.S. Black. This supports the hypothesis that in a malarious area, selection operates to lower levels of erythrocytic ATP.

One of the more exciting aspects of the Sardinian study was our confirmation of an initial report by Prins et al. that individuals with thalassemia trait have significantly lower levels of ATP per red cell than individuals who do not have thalassemia trait. We confirmed this in Sardinia and it raises the interesting possibility that the protective effect of thalassemia may be due to a major genetic modifying influence on levels of ATP.

C. Susceptibility of Genetically Different Types of Sheep to Anaplasmosis Ovis, an Intra Red Cell Parasite.

Background. Most mammalian erythrocytes are high in potassium content and low in sodium. Some mammals, such as the dog and cat, are exceptions to this general rule and have low potassium and high sodium levels in their red cells. A few species, including domestic sheep are polymorphic for erythrocyte potassium. Evans has shown that a single gene, two allele system is responsible for the polymorphism in sheep with almost complete dominance of the low potassium gene.

The primary cause of the different electrolyte compositions of the two types of sheep red cells is not yet known with certainty. Potassium levels in the red cell are maintained through the hydrolysis of ATP by sodium-potassium dependent ATPase. Tosteson has studied this enzyme in the sheep erythrocyte and his results indicate that the high potassium red cell has a four fold greater ATPase activity than the low potassium red cell. Although differences in ATPase activity may account for the differences in potassium content in the two types of sheep red cells, variation in quantitative level of ATP, the other important factor in cation transport, could also be playing an important role. If variations in ATP levels in the two kinds of potassium cells were present, it seemed possible that the selective agent maintaining the polymorphism might be an intraerythrocytic parasite (Eaton, J. Unpublished). In suggesting this possibility, we drew an analogy to the relationship in man between malaria and ATP levels.

Anaplasmosis ovis, an intraerythrocytic parasite disease of sheep and cattle, seemed a likely candidate (Eaton, unpublished). It is generally held that the distribution of the low potassium gene in sheep is predominantly lowland. This coincides with the distribution of anaplasmosis. From the relationships, we developed the hypothesis that the low potassium type sheep erythrocyte is more resistant to anaplasmosis than the high potassium cell.

Our first study consisted of comparing the ATP levels in the high and low potassium sheep erythrocytes. We demonstrated that the high potassium red cell has a mean level of ATP thirty one percent greater than the low potassium red cell. These differences were highly significant. The work until this point supported the hypothesis that the low potassium red cell might be more resistant to anaplasmosis since the differences in ATP in the Black were also in the lower direction.

In collaboration with John Eaton, a graduate student in our laboratory, and Dr. Clifford Beck and Dr. David Clark of Michigan State University, we infected four high potassium and four low potassium sheep with a culture of anaplasmosis ovis. Two, each of the high potassium and low potassium sheep were of a Dorset-Suffolk cross, and two, each, of the high potassium and low potassium sheep were Tunis crosses.

We detected no difference in the rate of parasite growth, the maximum levels of parasitemia, changes in temperature, changes in body weight, degree of anemia, or any other characteristic between two potassium types. We did detect however, a very significant effect of breed on the anaplasma infection. The Dorset-Suffolk crosses were highly susceptible to the disease, while the Tunis crosses were relatively resistant. Such a breed effect had not been previously reported for this infection. The cause of the resistance at this time is unknown (Eaton, unpublished).

D. Interaction of Red Cell ATP Levels and Severity of Malaria.

1. In studies of P. cynomolgi infections in rhesus monkeys, we showed that the higher the levels of red cell ATP in the animals, the higher the peak parasite count. This relationship was highly statistically significant.

2. In studies of rodent malaras, we established that under certain conditions the red cell ATP levels decreased markedly during infection. This effect went well beyond the infected cells, indicating perhaps that the infected were obtaining purine precursors at an increased rate, depleting ATP levels of uninfected cells. This is one possible mechanism for blackwater fever and the tendency of hemolysis to be more severe than simply destruction of infected cells.

E. The Treatment of Malaria with Hyperoxia.

We obtained reproducible and marked results with 3 atmospheres (absolute) of compressed air in the treatment of P. vinckei in rats. This level of hyperoxia did not have much of an effect on P. berghei in rats. One hundred percent oxygen at atmospheric pressure showed some effect in P. berghei, but the results were somewhat variable. The explanation for this was the occurrence of pulmonary damage in many rats from 100% oxygen after 36-48 hours. Thus, variable oxygen tension was achieved in the blood of the rats according to whether or not they had suffered pulmonary damage.

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