CHAPTER IV

DISCUSSION

Several methods for measurement of red cell ATP levels have been developed recently. The simplest and most reproducible of these methods is the firefly luciferase enzyme and liquid scintillation counter method. It is a rapid and economical method which can be used to determine ATP levels over a wide range of 10^{-9} to 10^{-12} mole. The reproducibility and the recovery of this method as shown in this study (Table 2 and Table 3) were quite satisfactory, especially in view of the minute quantities involved.

A number of conditions should be considered in the determination of the erythrocyte ATP levels. The results from the present study showed a rapid decline in red cell ATP levels when heparinized blood was stored at $4^{o}C$. These levels decreased progressively and reached 60 percent and 66 percent of their original values when the heparinized blood was stored at room temperature and at $4^{o}C$ for 4 hours. The corresponding figures for the extracted blood kept under these conditions were 72 and 84 percent respectively. It was, therefore necessary to precipitate and extract the heparinized blood immediately as changes occurred in the ATP levels if there was any delay even when the extracted blood was stored at $4^{o}C$.

The results further showed that the erythrocyte ATP content of the blood infected with P. falciparum malaria kept in the refrigerator, decreased much faster than that of the normal blood. This indicated that malarial parasites acted in someway to diminish the ATP in the infected blood.

RED CELL ATP LEVELS IN THAI BLOOD DONORS

The results in the present studies showed that red cell ATP levels in Thai blood donors determined by the firefly luciferase enzyme and liquid scintillation counter are in the same order of magnitude of values obtained by various different methods (Table 5). This result was also in accordance with the values obtained from the Thai blood donors reported previously (Areekul and Chantachum 1973).

RED CELL ATP LEVELS IN PATIENTS WITH P. falciparum MALARIA

The levels of ATP in red cells of various animal species infected with malaria have been studied extensively. These levels were found to fall during malarial infection, i.e., in rats infected with P. berghei and P. vinkei and in ducks infected with P. lophurae (Brewer and Coan, 1969; Trager, 1967). On the other hand, increased ATP level occurred in the erythrocytes of P. knowlesi infected monkeys (Eaton and Brewer, 1969; Ball et al. 1948; Dunn,1969;Fletcher et al. 1970, Areekul et al. 1974). The ATP content of

erytrocytes in patients with P. falciparum malaria in the present study showed no significant difference from that of the normal subjects. However, it must be pointed out that the parasitaemia in the present study was quite low, i.e. 12 per 1,000 red cells (range 1-105).

As shown in Fig. 7 the ATP content of the parasitized red cells decreased as the parasitaemia increased. It was therefore possible that the red cell ATP levels in patients with higher parasitaemia than in the present study may probably be lower than that of the normal subjects.

It is well established that ATP plays an important role in biological process starting with phosphorylation of glucose by hexokinase in the entire chain of glucose utilization. Malarial parasites also requires ATP for its survival, growth and multiplication. Findings of the parasitized red cells contained a higher concentration of ATP than the non-parasitized erythrocytes were in accordance with the previous results in monkeys and mice erythrocytes infected with P. knowlesi and P. berghei malaria respectively (Areekul and Thaijongrak, 1974; Ball et al., 1948; Fletcher et al., 1970). This may represent an accumulation rather than a synthesis of ATP. Since it has been shown that malarial parasites especially P. lophurae cultivated extracellularly required but was incapable of synthesis ATP (Trager, 1967). The parasites have to utilize the

energy-producing mechanism of the host erythrocyte especially P. berghei which was found to be dependent upon the host cell reserves to fulfil requirements of ATP (Nagarajan, 1968). There is evidence that mature mammalian erythrocytes of several species, including man, are incapable of de novo synthesis of adenine and it has been suggested that blood cells pick up adenine and other purine during circulation through the liver (Brewer and Coan, 1969). The adenine is then converted to adenosine by utilization of ribose provided by pentose phosphate pathway. This pathway has been shown to be particularly active in parasitized erythrocytes of several plasmodial infections (Herman and Herman, 1968).

Findings of a reverse relationship between the ATP levels of parasitized red cells and parasitaemia in the present study conforms well with a report that the level of ATP may influence the rate of increase of parasitaemia in the erythrocyte (Brewer and Powell, 1965). These authors had also suggested that a low level of ATP in the host red cells may be a factor limiting the development of P.falciparum trophozoites and that the parasites were in fact dependent on the host as a source of ATP (Brewer and Powell, 1965).

The result in the present study showed that non-parasitized red cells from patients had lower ATP content than the normal blood. This finding was in accordance with results obtained

in patients with P. falciparum malaria (Brewer and Coan, 1969). The ATP depletion in non-parasitized red cells has also been suggested to be an additional factor in producing anaemia out of proportion to the number of cells infected (Brewer and Coad, 1969).

RED CELL ATP LEVELS IN THE NORMAL AND P. knowlesi INFECTED MONKEYS.

The mean value of red cell ATP levels in 20 normal monkeys (99.02 μM/100 ml RBC) in the present study was slightly lower than a mean figure of 149.90 μM/100 ml RBC obtained by Fletcher et al. (1970). There was no significant difference between the mean value of the normal group and those of the monkeys during the infective stage and convalescence.

Increased erythrocyte ATP levels in monkeys infected with P. knowlesi has been reported by Ball et al., (1948), Dunn (1969), Eaton and Brewer (1969) and Fletcher et al. (1970). Fletcher et al. further showed that the increase in the ATP levels was mainly associated with the growth and development of the parasites and also suggested that segmentation of the parasite was accompanied by decrease in ATP levels. ATP was needed not only by the malarial parasites to supply energy and nucleotides for the variety of synthesizing process taking place in a rapidly growing

and dividing parasite but also for maintaining the structural and functional intregrity of the erythrocyte host (Fletcher et al., 1970).

RED CELL ATP LEVELS IN THE NORMAL AND P. berghei-INFECTED MICE

A mean value for red cell ATP levels in 21 normal mice was found to be 105.94 µM/100 ml RBC. This figure was in accordance with these quoted for human and monkeys red cells in this paper.

In the present study there was a rise in red cell ATP levels in mice infected with P. berghei (Table 11, Fig. 11), and these levels showed no correlation with the proportion of red cells infected. It was probable that younger red cells, which were preferentially infected by P. berghei had somewhat higher ATP levels than the older red cells. However, the effect of age on levels of ATP in red cells in the present study was not sufficiently pronounced to explain this result. Furthermore, Brewer (1969) concluded from the available evidence that there was no difference in ATP content of young and old cells in normal human erythrocyte populations. Finding of increased erythrocyte ATP levels in P. berghei-infected mice was in accordance with results reported earlier in P. knowlesi-infected monkeys (Ball et al., 1948; Dunn, 1969; Eaton and

Brewer, 1969; Fletcher et al., 1970). However, these levels were found to fall during malarial infection in rats infected with P. berghei and P. vinckei and in ducks infected with P. lophurae (Brewer and Coan, 1969; Trager, 1967).

Findings that the increase in erythrocyte ATP levels in P. berghei infected mice occurred mostly in the parasitized erythrocytes (Table 12) was in accordance with the results reported previously by many workers. It has been shown that monkey erythrocytes parasitized by P.knowlesi contained a somewhat higher concentration of ATP than unparasitized (Ball et al., 1948). Fletcher et al. (1970) found that although increase in ATP levels took place mainly in erythrocytes of P.knowlesi-infected monkeys as a result of the metabolic activities of the parasites., smaller but appreciable increases also occurred in the non-parasitized cells as well. Since malarial parasites require ATP for survival, growth and multiplication, and it could possibly not synthesize ATP due to a lack of at least one enzyme for the formation of this substance (Trager, 1950, 1963 and 1967). The malarial parasites may therefore have to utilize the energy-producing mechanisms of the host erythrocyte, especially P. berghei which was found to be dependent upon the host cell reserves to fulfil ATP requirement (Nagarajan, 1968). Finding of the increment of ATP levels in the parasitized erythrocytes probably indicated that these cells

were better able than non-parasitized red cells to pick up purine nucleotides needed for ATP synthesis.

Considering that the ATP content was higher in red cells of P. berghei-infected mice than in those of normal mice, the hypothesis that the rigidity of red cells in this species of malaria might be due to ATP depletion was not supported.