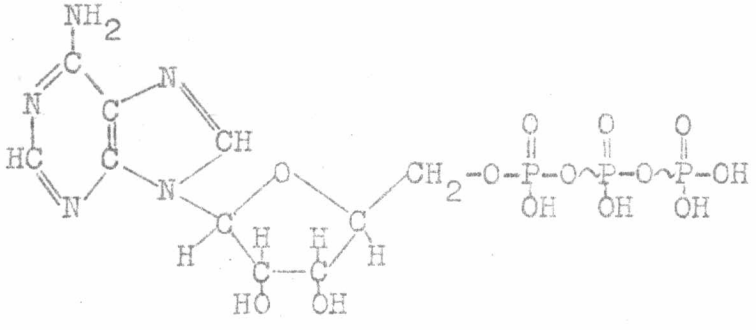
# CHAPTER IV

# DISCUSSION

Adenosine triphosphate (ATP) is a nucleotide, a molecule of which contains adenine, pentose and three molecules of phosphoric acid, its structure is:-



In the human red cell, ATP is essential for maintenance of cation balance and for the couple sodium-potassium active transport mechanism (Hoffman, 1962). Its major functions are glycolysis at the hexokinase and phospho-fructosekinase steps. ATP is also essential for maintenance of red cell shape and for normal in vivo survival of erythrocyte. If blood is stored for some time, it will have a significant decrease in ATP levels which correlates with its shorter survival in vivo (Akerblom et al., 1967). Determination of ATP content of

erythrocyte is also of considerable interest from the view point of hereditary abnormalities of red blood cell metabolism, such as pyruvate kinase deficiency and certain genetic disorders characterized by increased or decreased red blood cell ATP levels. Several methods for measurement of red blood cell ATP levels have been developed recently, these methods include:-

(1) Paper and column chromatography.

(2) Measuring glucose-6-phosphate formation in the hexokinase reaction with glucose-6-phosphate dehydrogenase (G-6-PD) and TPN.

(3) Employing the " backwards " glyceraldehyde phosphate dehydrogenase reaction to measure the oxidative of DPNH.

(4) The firefly technique.

Each of these techniques has been reported to give reproducible results with human blood, and adequate recovery of ATP added to blood have been acheived. The simplest and most reproducible of these methods is the firefly luciferase method (Beutler and Mathai, 1967).

In the present study, red blood cell ATP were determined by the luciferase enzyme using liquid scintillation counter following the method of Stanley and Williams (1969). It is a simple and rapid method which can determine ATP over a wide range of $10^{-9}$ to $10^{-12}$ mole and only 0.1 ml of whole blood was required. This method shows the excellent reproducibility and recoveries as shown in the present study (see Table 1. and 2.).

Red blood cell ATP levels could be altered by many factors. The results from the present study showed that the temperature, time factor and the extraction had the profound effects on the red blood cell ATP content. The ATP content decreased progessively to 42 % and 16 % of the original value when blood was stored at room temperature for 4 and 27 hours respectively. If blood was extracted first and kept in the refrigerator, the corresponding activity left was 73 % and 66 % respectively. These findings indicated that blood should be extracted immediately and should be assayed for ATP content as soon as possible.

Red blood cell ATP levels were determined in 20 normal monkeys. A mean value $\pm$ one standard deviation (99.02 $\pm$ 27.75

µM/100 ml RBC) in the present study was lower than the result (149.90 ± 21.76 µM/100 ml RBC) reported by Fletcher et al., (1970). Those authors used the luciferase enzyme and the spectrophotometer. It has been shown recently that the ATP content of red blood cell determined by the luciferase enzyme and the spectrophotometer was usually higher than that determined by the luciferase enzyme and the liquid scintillation counter (Beutler and Mathai, 1967).

There was a wide variation of the red blood cell ATP levels in rhesus monkeys infected with P. knowlesi malaria. Red blood cell ATP contents usually increased parallel with the rising of parasitaemia. This finding was in accordance with that of Fletcher et al., (1970) who reported that these ATP contents increased as the parasitaemia multiplied and the malaria changed from the ring form to the mature trophozoite.

The levels of ATP in red blood cells of various animal species infected with malaria have been studied extensively. These levels were found to fall during malarial infection in rats infected with P. berghei and P. vinckei (Brewer and Coan, 1969) and in ducks infected with P. lophurae (Trager, 1967). Using P. knowlesi infections in rhesus monkeys, Ball et al.,

(1948) showed that there was an increased high energy phosphate compounds in the erythrocytes. Trager (1950) showed that *in vivo* extracellular cultivation of *P. lophurae* required but was incapable of synthesizing ATP. More recently, Trager, (1963, 1967) suggested that the *P. lophurae* may utilize the energy-producing mechanisms of host erythrocytes. Many studies attempted to correlate the red blood cell ATP contents with the rate of the multiplication of parasitaemia. They had shown that the higher the red blood cell ATP contents the slower the number of parasitaemia in human infected with *P. falciparum* and rhesus monkeys infected with *P. cynomolgi* (Brewer and Powell, 1965; Eaton and Brewer, 1969).

As mentioned earlier, damage in severe forms of malaria is due to the obstruction of the flow of blood in vessels of various organs which has been demonstrated in the autopsy finding (Dudgeon and Clarke, 1917; Spitz, 1946). Many recently accumulated evidences suggested that the internal changes in red blood cell *per se* i.e., an increased viscosity of packed red blood cells suspensions and a loss of red blood cells filterability, may be responsible for the obstruction of the capillaries of the various organs in rhesus monkeys infected

with P. knowlesi and P. coatneyi malaria (Miller et al., 1971; Miller et al., 1972). The results in the present studies showed that there was an evidence of trapping of parasitized red blood cells in the brain of both normal and P. knowlesi-infected monkeys. However, the ATP contents of the parasitized red blood cells were found to be the same or higher than those of the normal red blood cells. Whether an increased viscosity of packed red blood cells suspension in vivo and a loss of red blood cells filterability were responsible for this trapping red blood cells are needed further investigations.