Malaria, which claims millions of lives every year is still a global health problem. In this country, more than 75 million positive cases for malaria were reported in 1953, when control measures were initiated. National Malaria Eradication Programme started in 1958 brought a spectacular reduction in the incidence of malaria and by 1965 no deaths due to malaria were reported from India. However, there has been resurgence of malaria and in 1977 about 5 million while in 1978 about 3.5 million malaria positive cases have been reported from India alone. Eradication of malaria thus, has now become more complex because of resistance of vectors to conventional insecticides and emergence of strains of *Plasmodium falciparum* resistant to 4-amino-quinolines and other conventional drugs.

In view of the inadequacy of the chemotherapeutic and insecticidal measures to control malaria, efforts have been intensified to develop a vaccine. Recently preliminary success has been achieved with the vaccination of a few owl monkeys, *Aotus trivirgatus*, against *P. falciparum* (Siddiqui, Taylor, Kan, Kramer, Richmond-Crum, Kotani, Shiba and Kusumoto, 1978), but it would take another 5-10 years to make a breakthrough in this area because it would involve the development of technology for large-scale preparation of purified antigen of different species of human malaria, free from host erythrocytes contamination.

The World Health Organization (WHO, 1977) have recently suggested the following guide-lines for basic research in the field of malaria.

Detailed biochemical and enzymological studies on the merozoites and other erythrocytic stages of malaria parasite and the erythrocyte membrane should be carried out. A knowledge of the enzymes of cell-free preparations of merozoites would help us to understand the role of merozoite enzymes in invasion process. Further, it would
also be necessary to develop simple and improved methods for obtaining merozoites in purified state free from host cell contamination as much as possible so that they could be used for biochemical characterization. Detailed study on the metabolism of malarial parasites and the enzymes (particularly the hydrolases) of the different stages of parasites would be valuable to study their role in intracellular digestion of haemoglobin by the parasite.

Methods should be developed for the subfractionation of membranes or organelle preparations of parasite and their biochemical characterization would be useful for understanding the enzyme make up of the parasite.

It is equally important to understand the mechanism of red cell invasion by the parasite as this approach would lead to the development of certain enzyme blockers or inhibitors that would ultimately prevent invasion of erythrocyte by the parasite and these studies might lead to the control of malaria. It would also be necessary to establish basic methodology for merozoite invasion into the erythrocyte in vitro so that the action of specific metabolic inhibitors, and other specific and non-specific enzyme inhibitors on the invasion process would be studied. In vitro invasion studies could also yield valuable information on the susceptibility of different hosts to *P. knowlesi* and this information might help in identifying new hosts for malaria parasites.

In view of the importance of above studies in understanding the host-parasite interaction in malaria, the present studies were initiated on the following aspects of *Plasmodium knowlesi* infection in rhesus monkeys which provides synchronized population of parasites in desired stage for biochemical analysis:
1. Methods have been standardized to produce synchronized parasite infection with *P. knowlesi* in rhesus monkey by controlling the photoperiodicity of the host.

2. Subcellular fractionation of the synchronized population of rings and schizonts has been undertaken and the purity of different fractions have been established by biochemical characterization of alkaline phosphatase (a marker for nuclear fraction), succinic dehydrogenase (mitochondrial fraction), acid phosphatase and acid protease (lysosomal fraction), glucose-6-phosphatase (microsomal fraction) and 5'-nucleotidase (membranes). It has been established that among the hydrolytic enzymes highest activity in the parasite was found with respect to acid protease and acid phosphatase both of which are lysosomal markers. It is important to mention here that ultrastructural studies on *P. knowlesi* so far have failed to identify lysosomes as such in the parasite.

3. A rapid and simple method has been developed for the production of partly purified preparation of merozoites of *P. knowlesi* from synchronized schizont population, which could be used for assay of hydrolytic enzymes of merozoites. So far no report on the enzymology of merozoites of *P. knowlesi* or any other malarial parasite is available but a knowledge of merozoite biochemistry would ultimately help to elucidate the mechanism of erythrocyte invasion by parasite. A suggestion in this direction was made earlier by Garnham, Bird and Baker (1960) and Peters (1969) that both sporozoite and merozoite might have an enzymatic mode of entry into the host cell.

4. Detailed biochemical studies have been carried out on the hydrolytic enzymes of the erythrocytic stages of the parasite (acid and alkaline proteinase, acid
phosphatase, acid and alkaline ribonuclease and acid and alkaline deoxyribonuclease). The enzymes of the host and parasite have been characterized to show that parasite possesses its own enzymatic machinery. The different properties like, hydrolysis of haemoglobin obtained from different sources, the effects of pH, time, temperature, stability, substrate specificity, substrate concentration, enzyme concentration, activators/inhibitors, antimalarial drugs and antibiotics, on these enzymes have been studied. In addition, partial purification of acid proteinase, alkaline proteinase and acid phosphatase of parasite with ammonium sulphate precipitation and DEAE - cellulose chromatography has been carried out to characterize various fractions.

5. **In vitro** invasion of rhesus and assamese monkey erythrocytes by *P. knowlesi* merozoites in the presence of homologous sera or sera obtained from other animals not susceptible to *P. knowlesi* has been studied. Also, the effects of specific and non-specific inhibitors of different enzymes and of metabolic pathways on the invasion and/or growth of parasite have been studied. These studies would throw some light on the mechanism of merozoite invasion, specificity of host erythrocytes and other factors involved in the invasion of erythrocytes by malaria parasite.