CHAPTER I

INTRODUCTION
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Malaria continues to remain a global health hazard inspite of the discovery of its causative organism, Plasmodium, more than a century ago. The mortality and morbidity inflicted by malaria is still a major problem of tropical and sub-tropical areas. There were 489 million clinical cases of malaria worldwide in 1986 with more than 2.3 million fatalities (Stürchler, 1989). In India, about 1.5 to 2 million annual incidences due to malaria have been reported during the last few years.

World Health Organization’s malaria eradication programme launched during 1956-1962 considerably reduced the malaria incidences. This was mainly due to the extensive use of insecticides to eliminate the vector. However, the high hopes of this programme were short-lived as the extensive insecticides sprays resulted in resistance of the mosquitoes to these chemicals. On the other hand, the emergence of drug resistant strains of malignant human malaria parasite during early 1960s gave a severe blow to this staggering programme.

In the absence of a suitable effective malaria vaccine and lesser advancement in the vector control research, chemotherapy still dominates the existing measures in combating this disease. The new chemotherapeutic agents developed are being used to treat the drug resistant cases.
However, the mystery of parasite is further complicated due to appearance of parasite resistant strains to these new compounds. For example, mefloquine is used in the treatment of chloroquine-resistant *P. falciparum* while mefloquine resistance has been observed in Thailand and Latin America.

Thus, it appears that sooner or later parasite will become resistant to the newly introduced chemotherapeutic agents also. This scenario is perhaps due to the incomplete knowledge about the parasite make up and the targeting sites of the available antimalarials. Folate antagonists, pyrimethamine and proguanil bind with higher affinity to parasite's dihydrofolate reductase (DHFR) as compared to the host enzyme. Except this, the mode of action of various other antimalarials has not been authentically established. This could be attributed to incomplete knowledge of the parasite specific metabolic pathways.

A number of malaria parasite antigens isolated by monoclonal antibodies and transformed through genetic engineering made malaria vaccine goal nearer. Certain enzymes, for example, aldolase induced immune response and exhibited protection. Similarly, certain biochemical parameters employed, elucidated the process of invasion of the host erythrocytes by the parasite.

Thus, it becomes imperative to undertake studies to reveal the biochemical make up of the parasite, active
targets of chemotherapeutic agents on the parasite, the sites easily accessible against which new compounds may be synthesized and the parasite proteins/enzymes generating protective immune responses.

Biochemistry of malaria parasite has been studied extensively. However, further research is still required to elucidate the various unresolved phenomena of the parasite. During asexual erythrocytic cycle, for its propagation malaria parasite obtains nourishment from the host’s blood. The major source of energy is sugars transported from the host. Glucose consumption is undertaken via the well known Embden-Meyerhoff pathway as the parasite is known to possess a strong battery of glycolytic enzymes. However, all the enzymes of this pathway have not been attributed to a single species. Some of the enzymes have been studied only in infected blood cells making the parasite preparations loaded with host contaminants. Further, some of the enzymes of this pathway have not been characterized for their biochemical properties.

Keeping in view the importance of glycolytic enzymes in the parasite metabolism, following studies were undertaken on rodent malaria parasite, P. berghei:

1. Glycolytic enzymes: hexokinase (HK), phosphofructokinase (PFK), aldolase (AL), enolase, pyruvate kinase (PK) and lactate dehydrogenase (LDH)
have been studied in the normal and *P. berghei*-infected erythrocytes and the presence of all the six enzymes have been established in the cell-free parasite.

2. The activity of HK, PFK, AL, PK and LDH was determined in the subcellular fractions of cell-free *P. berghei*.

3. Different kinetic properties of these enzymes like the effect of storage, temperature, pH, metal ions, inhibitors and substrate concentrations were studied in the cell-free *P. berghei*.

4. The effect of different antimalarials on the glycolytic enzymes has been evaluated.

5. HK, AL, PK and LDH were purified using sephadex G-200 column chromatography.

6. Purified HK, AL and LDH were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to determine their molecular weights.

7. The role of HK, AL and LDH in *in vivo* protection against malaria was evaluated by immunizing mice with the purified enzymes. The level of antimalarial antibodies was determined serologically by indirect haemagglutination (IHA) and indirect fluorescent antibody (IFA) assays.

8. *In vitro* inhibitory property of LDH antibodies was
determined by short-term *in vitro* culture of *P. berghei*.

9. Preliminary studies have been attempted on two enzymes of citric acid cycle: malate dehydrogenase and succinate dehydrogenase.