CHAPTER II

REVIEW OF LITERATURE
Documented malaria history began as early as in 1600 B.C. in ancient Egypt where the first description was found in the Edwin Smith Surgical Papyrus (Breasted, 1930). Thus, origin of malaria is perhaps related to origin of human beings. Hippocrates in 400 B.C. described malaria symptoms in the form of chills, periodic fever and even the fatal form of the disease which were perhaps caused by *P. falciparum*. Alfonse Laveran, a French army surgeon in Algiers, observed the parasite for the first time in 1880 in wet blood film of a soldier. Ronald Ross in 1898, described the complete sporogony of *P. relictum*, a bird malaria parasite, in mosquito *Culex pipiens fatigans*. At the same time the sporogony of *P. falciparum*, *P. vivax* and *P. malariae* in *Anopheles* mosquitoes was elucidated (Bastianelli and Bignami, 1899; Grassi et al., 1899). Raffaele (1934) discovered the exoerythrocytic schizogony of *P. elongatum* in tissue cells in the bone marrow of birds and later on the same cycle in *P. relictum* (Raffaele, 1936). End of the worldwar - II facilitated malaria research to a greater extent. Shortt and Garnham (1948) discovered pre-erythrocytic cycles in *P. cynomolgi* and *P. vivax* followed by in *P. falciparum* (Shortt et al., 1951). Thus, the complete life cycle of malaria parasite was finally established.

Malaria control measures were started with the discovery of the parasite. Gorgas in 1901, started a
programme for the control of Aedes and anopheline mosquitoes in Cuba (Wernsdorfer, 1980). Other control measures like drainage for mosquito control (Watson, 1935) and the use of pyrethrum (Chrysanthemum cinerariaefolium) powder as insecticide showed remarkable results in eliminating the mosquitoes. After World War II, DDT (1,1,1-trichloro-2,2-bis (parachlorophenyl) ethane) was used on large scale as insecticide although its insecticidal properties were discovered in 1939 (Muller, 1955). Soon after, other chlorinated hydrocarbons such as BHC (the - isomer of 1,2,3,4,5,6-hexachlorocyclohexane) and dieldrin (endo, exo- 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4,5,6,8,8-octahydro-1,4:5,8-dimethanonaphthalene) were also employed as insecticides.

In 1960, World Health Organization started a programme for the evaluation and testing of new insecticides. This programme resulted in the established use of Malathion, Propoxur, Fenitrothion, Primiphosmethyl, Londrin, Chlorophoxim, Mobam, Permethrin, Deltamethrin as imagocides and Fenthion, Temephos, Chloropyrifos, Jodphenphos, Methoprene, Diflubenzuron as larvicides (Davidson, 1982).

Chemotherapy of malaria

First chemotherapeutic agent introduced was quinine obtained from the cincona bark, a tree in Peru. History shows
the use of the drug in 17th century, first in Peru in 1638 and then in Spain in 1641 (Anonymous, 1927). However, quinine remained the only chemotherapeutic agent before 1930. Though the resistance against this drug was mentioned very early in the 20th century (Nocht and Werner, 1910), yet today it has an important role for the treatment of acute cases of malaria, particularly chloroquine-resistant *P. falciparum*, alone or in combination with other drugs.

The most widely used antimalarial is chloroquine and derivatives of 4-aminquinolines such as hydroxy-chloroquine and amodiaquine. Chloroquine was synthesized in 1934 but its antimalarial property was described after a decade (Butts, 1950; Jeffrey et al., 1954). Unfortunately, the report of chloroquine-resistant strains of *P. falciparum* from Columbia (Moore and Lanier, 1961) gave a severe blow to the use of this drug. Since then, it became a rapid expanding problem in most of the countries (Payne, 1987) particularly in Latin America, Asia (Clyde, 1974) and Africa (Campbell et al., 1979). Even reduced susceptibility of *P. vivax* to chloroquine has also been reported (Rieckmann et al., 1989). In laboratory, the resistance to chloroquine was demonstrated in different species of the parasite (Peters, 1970; 1974). Inspite of the widespread use of chloroquine and the existing complicacies of its resistance, the mode of action of chloroquine is not clear. Regarding the chloroquine action
various hypotheses have been postulated but without any conclusive proof (Banyal and Fitch, 1982; Yayon et al., 1984; Krogstad and Schlesinger, 1987; Zhang and Hempelmann, 1987).

Among 8-aminoquinolines, pamaquine and primaquine are considered as potent antimalarials as these eliminate gametocytes and the liver stages of the parasite (Muhlens, 1926; Wiselogle, 1946; Jeffrey et al., 1954; Schmidt and Coatney, 1955; Peters, 1970).

Pyrimethamine, a folate metabolism antagonist, has been used for the treatment of chloroquine-sensitive or resistant parasites. This is the only antimalarial whose mode of action is well elucidated as it is known to bind to dihydrofolate reductase (Ferone et al., 1969) and its resistance results due to altered enzyme (Cowman et al., 1988; Inselburg et al., 1988; Peterson et al., 1988; Snewin et al., 1989; Tanaka et al., 1990 a, b).

Drug combinations are very popularly used for the last 25 years particularly in drug resistant malarias. Malaprim, a combination of pyrimethamine and dapsone; fansidar, a combination of pyrimethamine and sulfadoxine have been used effectively against chloroquine-resistant P. falciparum (Pearlmann et al., 1977). The combination of quinine or chloroquine or amodiaquine with sulphonamides or sulphones or antifols were found to be very effective as compared to their individual effects (Sweeney and Strube,
Among quinoline methanols, mefloquine, a schizontocide, was found to be effective against chloroquine-sensitive, chloroquine-resistant and pyrimethamine-resistant strains of *P. falciparum* in owl monkey (Schmidt et al., 1978) and *P. vivax* (Dixon et al., 1985). However, resistance to mefloquine has also been reported (Karwacki et al., 1989; Kilimali et al., 1989).

Qinghaosu, extracted from *Artemisia annua*, a Chinese herb was found effective against *P. falciparum* and *P. vivax* (Qinghaosu Antimalarial Co-ordination Research Group, 1979; Peters, 1980) and asexual forms of the erythrocytic stages of chloroquine-resistant *P. falciparum* (Jiang et al., 1982; Klayman, 1985). Qinghaosu derivatives arteether, artesunate and artemether were tested to be efficient antimalarials (Waki et al., 1987; Gu et al., 1988; Idowu et al., 1989; Alin et al., 1990; Xuan et al., 1990).

**Immunity to malaria and malaria vaccine**

Immunity to malaria is species and partially strain specific (Jeffrey, 1966; Sadun et al., 1966). Humoral immunity in malaria was established with the passive transfer of serum (Cohen and McGregor, 1963). T-cell deprived mice vaccinated with sporozoites of *P. berghei* could not be protected when challenged with live sporozoites (Spitalny,
showing that humoral and cell-mediated immune responses play vital roles in malaria protection. Various studies have been undertaken to immunize animals with different species and stages of the parasite without much success. With the establishment of continuous in vitro culture of malaria parasite, *P. falciparum* (Trager and Jensen, 1976) and a breakthrough in the isolation of monoclonal antibodies (Köhler and Milstein, 1975), various antigens from different species and stages of the parasite have been isolated (Del Giudice et al., 1990; Gordon et al., 1990; Hollingdale et al., 1990; Holmquist et al., 1990; Kara et al., 1990; Rzepczyk et al., 1990; Tirawanchai and Sinden, 1990; Carlsson et al., 1991; Certa, 1991; Fenton et al., 1991; Jakobsen et al., 1991 a,b; Smythe et al., 1991).

The first attempt to vaccinate animals was undertaken in 1910 (Sergent and Sergent, 1910). Malaria vaccine research was accelerated in 1930s and the human immunization attempts were first conducted in 1936-1946 by using formalin treated blood stage parasites (Boyd and Kitchen, 1936; Heidelberger et al., 1946). Today, it is established that three stages, i.e., sporozoite, merozoite and other erythrocytic stages and gametocytes are the potential targets for a malaria vaccine. Monoclonal antibodies and recombinant DNA approach have made the malaria vaccine goal nearer.

Synthetic peptides analogous to the circumsporozoite
(CS) protein containing species specific immunodominant epitopes were synthesized and dominant epitope of *P. falciparum* was found to be in synthetic dodecapeptide (NANP)$_3$ (Zavala *et al.*, 1985). (NANP)$_3$ conjugated with tetanus toxoid (TT) and used with aluminium hydroxide completely protected one out of three human individuals when challenged with live *P. falciparum* sporozoites (Herrington *et al.*, 1987). However, the survey conducted on human individuals did not show the protective effect of (NANP)$_3$-TT although the peptide was found to be immunogenic (Guiguemde *et al.*, 1990). Several malaria proteins, e.g., CSP, gp 195, Pf 155/RESA, GLURP have been shown to induce partial protection (Petersen *et al.*, 1990; Carlsson *et al.*, 1991). Various synthetic peptides analogous to asexual blood stages of *P. falciparum* for the immunization of human volunteers have been used by Patarroyo and his co-workers (Patarroyo *et al.*, 1987, 1988; Moreno and Patarroyo, 1989). Out of this a synthetic hybrid polymer SP (60) 30 has been used for human trials on large scale against *P. falciparum* in South America. Most of the studies on vaccination revealed that immune response was not sufficient for complete protection (Nussenzweig and Nussenzweig, 1990; Certa, 1991).

**Serological assays**

Serological assays have been used successfully to
detect the antimalarial antibodies for the last three decades.

The indirect haemagglutination (IHA) assay was first introduced by Desowitz and Stein (1962) for malaria. Since then, it has been used in serodiagnosis and seroepidemiology of malaria (Kagan, 1972, 1980; Ambroise-Thomas et al., 1974; Agarwal et al., 1981).

Indirect fluorescent antibody (IFA) test was described by Coone et al. in 1942. Brooke et al. (1959) applied IFA for malaria for the first time for the detection of P. berghei antibodies. Later on, protocols with relevant methodology were evaluated in a critical manner (Collins and Skinner, 1972; Ambroise-Thomas et al., 1974; Voller and Illeni, 1974).

Enzyme-linked immunosorbent assay (ELISA) was first developed by Engvall and Pearlmann (1972) and introduced for malaria by Voller et al. (1974). The assay is sensitive, specific and reproducible (Quakyi, 1980; Spencer et al., 1981; Wahlgren et al., 1983).

Radio-immuno-assay (RIA) was also used to detect antimalarial antibodies (Stutz et al., 1974; Avraham et al., 1983).

Biochemistry of malaria parasite

Biochemistry of malaria parasite has been reviewed
from time to time (McKee, 1951; Peters, 1969; Oelshlegel and Brewer, 1975; Sherman, 1979). One cannot be certain that biochemical study carried out on malaria parasite is solely of parasite origin unless vigorous efforts are made to make parasite preparations free of host erythrocyte materials, leucocytes and platelets. Various methods have been employed to isolate malaria parasite free from host cell contaminants by physical or chemical treatments (Kreier, 1977). However, the techniques employed to make the parasite cell-free should give a yield of parasites minimally contaminated with host components.

**Carbohydrate metabolism**

The beginning of biochemical investigations with malaria parasite involved studies of carbohydrates. Systematic studies on carbohydrate utilization was undertaken in 1930s and 1940s in different malaria parasite species. It was found that glucose and several other sugars as well as glycerol when added to parasite-infected erythrocytes disappeared rapidly with the increase in oxygen uptake (McKee et al., 1946; McKee, 1951; Honigberg, 1967; Homewood, 1977, 1978). The blood stages of malaria parasite do not store glycogen or polysaccharides (Dasgupta, 1960; Ciucu et al., 1963; Scheibel and Miller, 1969a; Homewood, 1978).

Glucose utilization by normal erythrocytes is very
little as compared to plasmodia-infected erythrocytes which consume glucose at a higher rate (Christophers and Fulton, 1938; Maier and Coggesshall, 1941; Wendel, 1943; Ball et al., 1948; Moulder, 1949; Warren and Manwell, 1954; Khabir and Manwell, 1955; Fulton and Spooner, 1956; Bowman et al., 1960; Scheibel and Miller, 1969a; Cenedella and Jarrell, 1970; Cenedella et al., 1970; Scheibel and Pflaum, 1970; Shakespeare and Trigg, 1973; Coombs and Gutteridge, 1975; Oelshlegel et al., 1975; Roth et al., 1988; Roth, 1990). This increase in the glucose consumption by infected erythrocytes was due to an alteration in the permeability of erythrocyte membranes after infection (Herman et al., 1966; Sherman and Tanigoshi, 1974; Neame and Homewood, 1975). It seems that in general, malaria parasites use mechanisms for the breakdown of glucose very similar to those used by a variety of other organisms.

The end products of glucose utilization are different in different species of malaria parasite. The mammalian parasites incompletely oxidize glucose yielding organic acids, mainly lactic acid and pyruvic acid, whereas a significantly higher degree of oxidation occurs in bird parasites with the production of CO₂ as well as organic acids (Bovarnick et al., 1946; McKee, 1951; Bowman et al., 1960; Polet et al., 1969; Scheibel and Miller, 1969a; Sherman et al., 1969; Scheibel and Pflaum, 1970; Neame and Homewood,
Glycolysis

The consumption of glucose in malaria parasite occurs through glycolysis as all the species of malaria parasite possess a strong battery of glycolytic enzymes. However, all the enzymes of the pathway have not been attributed to a single species of *Plasmodium* in literature.

First enzyme of Embden-Meyerhoff pathway is hexokinase (HK). Primary indication of the presence of HK in malaria species, *P.gallinaceum* was shown by Speck and Evans (1945). However, as stated by investigators themselves no method was used to separate white cells. The increased activity of HK in *P.berghei*-infected erythrocytes was also reported (Fraser and Kermack, 1957) but again the contribution of host white cells was not known although the activity was found to be same in mature and immature red cells of mice. The presence of HK in different strains of rodent malaria parasites was demonstrated electrophoretically (Carter, 1973) but no controls for white cells or red cells were used. Evidence for the presence of HK in the cell-free parasite *P.berghei* was observed by Coleman *et al.* (1979). 4.5 fold increase in HK was shown in cell-free *P.knowlesi* as compared to normal monkey red cells (Saxena *et al.*, 1982). 25 fold increased activity of HK in *P.falciparum*-infected
erythrocytes over uninfected erythrocytes of the same age and from the same donor was demonstrated and kinetics showed that parasite HK had a lower affinity for glucose than the mammalian enzyme but the Km for ATP and the Vmax for both glucose and ATP for the two enzymes were similar (Roth, 1987).

Extreme elevation of HK (35 fold), phosphofructokinase (PFK) and pyruvate kinase (PK), the regulatory enzymes of glycolysis, in P. knowlesi-infected erythrocytes was demonstrated. In cell-free parasite the major portion (60-70%) of the total activity of all the three enzymes was associated with soluble cytosolic fraction (Sahni et al., 1988). Roth et al. (1988) and Roth (1990) also reported increase in the activity of HK, aldolase (AL), enolase and PK in P. falciparum-infected red cells as compared to the normal erythrocytes and observed electrophoretically distinct bands of enzyme activity.

Glucose phosphate isomerase (GPI), which converts glucose-6-phosphate into fructose-6-phosphate in the glycolytic pathway was demonstrated electrophoretically in rodent malaria parasite (Carter, 1970, 1973, 1978; Momen, 1979) and in P. falciparum (Carter and McGregor, 1973; Carter and Voller, 1973, 1975). Enzyme variants of GPI by isoelectric focusing and polyacrylamide gel electrophoresis were demonstrated in P. falciparum (Gardner et al., 1987).
Thaithong et al. (1989) demonstrated three variant forms of GPI, i.e., GPI-1,2 and 3 in a Thai isolate of *P.falciparum*. A gene coding for *P.falciparum* GPI was cloned and expressed in *Escherichia coli* and found to contain a 1773-base pair open reading frame (Kaslow and Hill, 1990).

Kruckeberg et al. (1981) had demonstrated increased activity of most of the glycolytic enzymes in *P. berghei* parasitized erythrocytes as compared to uninfected cells. However, it was not clear that this increased activity was solely due to parasite.

An acid-insensitive, electrophoretically separate and kinetically distinct form of PFK (which converts fructose-6-phosphate to fructose-1,6-diphosphate) was demonstrated in *P.berghei* (Sander et al., 1982). In cell-free *P.berghei* PFK was inhibited by ATP with the decrease in pH and F6P acted as a positive effector (Buckwitz et al., 1988). This enzyme showed allosteric properties similar to PFKs from different sources, i.e., activated by F6P and inhibited by ATP, but enzyme activity was only marginally increased by AMP, a potent activator of many PFKs while phosphoenolpyruvate in contrast to other PFKs, activated *P.berghei* PFK (Buckwitz et al., 1990a).

The extract of *P. gallinaceum* was able to split fructose-1,6-diphosphate to triose phosphate thus showed the presence of AL but these extracts were contaminated with host
AL was also demonstrated in *P. knowlesi* (Saxena et al., 1982) and *P. falciparum* (Roth et al., 1988). Gene coding for *P. falciparum* AL in 14 parasite isolates from three continents was found to be highly conserved. This 41 kD blood stage antigen (p41) induced immunity to malaria in monkeys (Certa et al., 1988). Dobeli et al. (1990) demonstrated *P. falciparum* AL expression in its tetrameric form in *E. coli* and constants Vmax and Km of the recombinant enzyme correspond to the parasite derived AL. Antibodies raised against the recombinant *P. falciparum* AL in rabbit inhibited the natural AL but no cross reaction with human AL was detected. Knapp et al. (1990) observed that the genomic clone isolated for *P. falciparum* AL was interrupted by one intron which divided the coding region into two exons. The first one codes for initiation methionine while the second one encodes for the residual 368 aminoacids of the protein. The protein was expressed in *E. coli* in enzymatically active form. Antisera cross reacted with aldolases of different species correlating the strong conservation of this enzyme during evolution. Srivastava et al. (1990) also showed the specificity and inhibitory activity of antibodies raised against *P. falciparum* AL. For the synthesis of AL protein, UAG, a chain termination codon was recognised as the start signal because *P. falciparum* lacks AUG initiation codon for translation (Ghersa et al., 1988).
The study on P. knowlesi and P. berghei-infected red cells demonstrated that malaria parasite introduced a PK isozyme into their host red cells in an amount sufficient to alter red cell glycolysis and the gel electrophoresis pattern showed a new band of PK isozyme in infected cells (Oelshlegel et al., 1975). Trager (1967) also reported PK and phosphoglycerate kinase (PGK) in the avian malaria parasite, P. lophurae-infected red cells. Recently, gene encoding for 3-PGK of P. falciparum was studied for primary sequence analysis (Hicks et al., 1991).

Although the whole sequence of glycolytic enzymes was not reported in a single malaria parasite species but lactate dehydrogenase (LDH) activity was shown in almost all the malaria parasite species. As early as 1945, Speck and Evans showed the presence of LDH in P. gallinaceum extracts but the extract was contaminated with host material (Speck and Evans, 1945). Later, parasite specific LDH activity was reported from P. berghei, P. knowlesi and P. lophurae (Broun, 1961; Sherman, 1961, 1962). Carter (1973) showed that LDHs from different strains of P. vinckei had different electrophoretic mobilities. The similar method was used for the detection of LDH in two different strains of P. falciparum (Carter and Voller, 1973; Carter and Walliker, 1977). LDH was also shown to be present in P. falciparum by the detection of
separate isoenzymes in different strains of the parasite from Africa (Carter and McGregor, 1973; Carter and Voller, 1975). Carter (1978) and Momen (1979) also demonstrated LDH in rodent malaria parasites. Electrophoretic separation of LDH of *P. knowlesi* showed that there were three isozymic bands of LDH in normal red cells while in cell-free parasite there was a single band of high intensity (Saxena et al., 1982). These workers also showed 2 fold increase in LDH activity in cell-free *P. knowlesi* as compared to normal red cells.

Phisphumvidhi and Langer (1969) described some of the properties of LDH of *P. berghei* and its host red cells. Both LDHs were stereospecific for L(+) -lactic acid and no significant difference existed between the host cell and the parasite LDH in Km values for lactic acid and nicotinamide adenine dinucleotide (NAD, oxidized and reduced). However, the value for pyruvate with the parasite LDH was 1/36th that of host cell. Saxena et al. (1986) reported that *P. knowlesi* LDH consists of four identical subunits of 31 kD and the enzyme resembled rabbit muscle (M₄) LDH in its electrophoretic mobility. Purified LDH was inhibited completely with 100 umole p-chloromercuric benzoate (pCMB), Ag⁺ and Hg⁺ and such inhibition can be reversed by β-mercaptoethanol or L-cysteine. Oxalic acid was shown to be competitive inhibitor of pyruvate. Vander Jagt et al. (1981) partially purified and characterized LDH from *P. falciparum*. 
Gene encoding for LDH was demonstrated by Simmons et al. (1985). A monoclonal antibody (Mc Ab 7.2) has been shown to bind \textit{P. falciparum} LDH with a subunit molecular mass of 35 kD. Low titre and non-precipitating antibodies were also raised against \textit{P. falciparum} isozyme bands (Pasha et al., 1987). Polyclonal antibodies raised against \textit{P. knowlesi} (H-strain) schizonts showed reactivity with different strains (\textit{P. cynomolgi}, \textit{P. berghei}, \textit{P. yoelii}, \textit{P. falciparum} and \textit{P. vivax}) of malaria parasites but did not react with three isoenzymic forms of mammalian LDH (A^4, B^4 and C^4) as well as with LDH from some protozoans and helminths (Kaushal et al., 1988).

\textbf{Citric acid cycle}

Till today, the presence of a functional citric acid cycle in plasmodia remains a mystery. The cristate mitochondria in avian malaria parasites provided certain clues for the presence of the enzymes of the cycle (Aikawa, 1966; Aikawa et al., 1966). In the mammalian parasites the mitochondria are acristate (Howells, 1970). Recently, the mitochondria in \textit{P. yoelii} of rodents were found to be cristate whereas acristate mitochondria were shown in \textit{P. falciparum} by electron micrographs of saponin-released malaria trophozoites of highly purified mitochondrial fractions isolated from the intraerythrocytic stages (Fry and
Beesley, 1991). However, in most mitochondria the enzymes of the citric acid cycle are not a part of the structure of cristae. Thus, the presence of cristae does not prove the presence of a functioning cycle.

Malate dehydrogenase (MDH) was the only enzyme to be demonstrated with some degree of certainty in *P. lophurae*, *P. berghei* and primate malaria (Sherman, 1966; Carter, 1970, 1973; Carter and Voller, 1973). *P. lophurae* MDH appeared to be extramitochondrial (i.e., soluble) and may play a role in reoxidation of reduced NAD for glycolysis (Sherman, 1966) and serve in a capacity similar to that of LDH. Lang-Unnasch (1992) purified *P. falciparum* MDH from asexual erythrocytic stages. The parasite MDH has an apparent subunit molecular weight of 32 kD and enzyme was shown to be cytoplasmic. Cytochemically, succinic dehydrogenase (SDH) was demonstrated in cristate mitochondria but not in acristate mitochondria of *P. brasilianum* (Sterling et al., 1972) and the enzyme could not be detected in the erythrocytic stages of *P. berghei* (Nagarajan, 1968a; Howells, 1970). However, the presence of SDH was observed in erythrocytic stages of chloroquine-resistant *P. berghei* (Howells et al., 1970).

Isocitrate dehydrogenase, specific for the rodent malaria parasite *P. berghei* could not be demonstrated (Howells and Maxwell, 1973a,b). A nicotinamide adenine dinucleotide phosphate (NADP)-specific isocitrate
dehydrogenase with a molecular mass of 80 kD and pH optimum 7.5 has been purified from *P. falciparum* by HPLC. 20% to 60% of NADPH was produced by this enzyme depending upon the developmental stage of the parasite (Vander Jagt et al., 1989).

The general conclusion that can be drawn from the literature is that there is very little convincing proof for the presence of citric acid cycle in primate and avian malaria parasites while rodent malaria parasites do not contain a functioning cycle.

**Pentose phosphate pathway**

Bowman et al. (1961) studied the oxidation rates of 1-^{14}C and 6-^{14}C labelled glucose in *P. berghei* and a less active pentose phosphate pathway was noticed. Later, the presence of glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGD), transketolase and ribose phosphate isomerase were demonstrated in these cells (Langer et al., 1967). However, the pentose phosphate pathway was found to be low in different species of malaria parasite (Bryant et al., 1964; Herman et al., 1966; Scheibel and Miller, 1969a,b; Cenedella and Jarrell, 1970; Shakespeare and Trigg, 1973). The G6PDH activity attributed to *P. berghei* by Carter (1973) might have originated from host cells. G6PDH activity could not be detected in the cell-free *P. falciparum*
(Theakston et al., 1976), *P. knowlesi* (Fletcher and Maegraith, 1962; Theakston and Fletcher, 1971), *P. berghei* and *P. gallinaceum* (Theakston and Fletcher, 1971). However, an increased activity of G6PDH and 6PGD was observed in monkey erythrocytes infected with *P. knowlesi* (Fletcher and Maegraith, 1962). Later, G6PDH was not detected using biochemical methods by Fletcher et al. (1977) and Momen (1979). Hempelmann and Wilson (1981) indicated electrophoretically the existence of G6PDH in *P. knowlesi* and *P. falciparum* but not in *P. chabaudi*. Indications have been given that *P. falciparum* can adapt to G6PDH deficient host cells (Luzzatto et al., 1983; Usanga and Luzzatto, 1985). This can only be possible by the induction of parasite derived enzyme. A slow moving G6PDH found in parasitized G6PDH deficient red cells was the induced parasite G6PDH (Yashida and Roth, 1987). The slow movement of *P. falciparum* G6PDH on polyacrylamide gels was due to its large size (M₁ Ca. 450,000) and the activity was less than 10% of normal red cells (Ling and Wilson, 1988). *P. berghei* G6PDH was purified (along with the host enzyme) by affinity chromatography with 2151-ADP-sepharose 4B and it was shown that malarial G6PDH significantly differed from that of red cells in its electrophoretic properties (Buckwitz et al., 1990b). The subunit molecular weight of parasite specific enzyme was 55 kD in contrast to 59 kD of erythrocytes. The polyclonal
antibodies raised against rat erythrocytes G6PDH showed no cross reactivity with the enzyme from *P. berghei*. *P. falciparum* G6PDH was also purified and the parasite enzyme showed distinctive biochemical properties as compared to normal red cells (Kurdi - Haider and Luzzatto, 1990).

**Oxygen utilization**

*P. lophurae, P. knowlesi* and *P. falciparum* utilizes oxygen in *in vitro* intraerythrocytic development (Scheibel et al., 1979). Cytochrome oxidase activity was demonstrated in platelets-free preparations of *P. knowlesi, P. berghei, P. cynomolgi* and *P. falciparum* (Scheibel and Miller, 1969a,b). Electron microscopy revealed that the activity was associated with both cristate and acristate mitochondria of mammalian and avian parasites (Theakston et al., 1969; Platzer, 1977). Cytochrome oxidase itself does not establish the presence of a functional electron transport chain. Gutteridge et al. (1979) suggested that the utilization of oxygen in plasmodia may be coupled to the *de novo* synthesis of pyrimidines.

**Carbon dioxide fixation**

CO₂ fixing enzymes, phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxykinase have been identified in *P. berghei* (Forrester and Siu, 1971; McDaniel and Siu, 1972). Although the enzyme has not been confirmed in
mammalian cells and other species of malaria parasite but C\textsuperscript{14} was found to incorporate into organic acids and amino acids when radioactive sodium bicarbonate was added to cell suspensions of P. knowlesi, P. berghei and P. lophurae-infected cells (Nagarajan, 1968b; Sherman and Ting, 1968; Sherman, 1977; Homewood, 1978). Thus, it is believed that malaria parasite has the property to fix carbon dioxide.

**Lipids**

Total lipid content was higher in P. knowlesi, P. berghei and P. lophurae-infected erythrocytes as compared to the normal erythrocytes and about 80% of the fatty acids of parasite total lipid was unsaturated (Ball et al., 1948; Cenedella et al., 1969; Rao et al., 1970; Angus et al., 1972; Beach et al., 1977; Holz et al., 1977). Phospholipids were also found to be in greater proportion in P. knowlesi as compared to the red cells (Rock, 1971a,b; Angus et al., 1972). Although the two major phospholipids phosphatidylethanolamine and phosphatidylcholine were present in both red cells and P. knowlesi but phosphatidylserine was observed to be absent in P. knowlesi and P. berghei (Rock et al., 1971; Dezeeuw et al., 1972; Beckwith et al., 1975). The major difference in neutral lipids was in cholestrol level (Rock, 1971a,b). P. knowlesi parasitized red cells contained 15-20% cholestrol (of total lipids) whereas 30%
cholesterol was found in normal erythrocytes (Angus et al., 1972).

Among the fatty acids, palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) were the commonest fatty acids of the total lipids of free parasites, parasitized cells and red cell membranes (Rock, 1971a,b; Angus et al., 1972). Palmitic and oleic acids (saturated fatty acids) increased after infection. Stearic acid declined in P. knowlesi and P. berghei (Ginger, 1967; Beach et al., 1977). However, an increased stearic acid content was found in P. lophurae-infected cells (Beach et al., 1977; Holz et al., 1977).

Recently, Vial et al. (1990) reviewed lipids in malaria-infected erythrocytes and explained that there was a considerable increase in phospholipids, neutral lipids and fatty acids but not cholesterol in infected cells. Phospholipids and fatty acids were found to be different in composition and distribution in the infected cell membranes. Parasite induction of new lipid molecules in the host remained inexplicable because normal red blood cells do not possess the lipid biosynthesis activity.

**Utilization of amino acids**

Plasmodia obtain amino acids for protein synthesis in the following ways: (i) Malarial parasites fix carbon
dioxide and synthesize the amino acids alanine, aspartic acid and glutamic acid (Sherman and Ting, 1966, 1968; Polet et al., 1969); (ii) P. knowlesi-infected erythrocytes accumulate isoleucine, methionine, leucine, cysteine and histidine present in the plasma or host cell (Sherman, 1977); (iii) Intraerythrocytic parasite digests 25% to 75% of the host cell haemoglobin (Sherman, 1979). During this proteolysis of haemoglobin there is a release of amino nitrogen and the deposition of insoluble heme (hemozoin).

**Proteolytic enzymes**

Malaria parasite degrades the host haemoglobin to obtain sufficient amino acids for the synthesis of protein. In malaria, golden brown-black pigment (hemozoin) accumulated after degradation of haemoglobin was shown to be the coupling of ferriprotoporphyrin with denatured polypeptides or protein (Honigberg, 1967; Homewood, 1978).

Cook et al. (1961) demonstrated the activity of acid and alkaline proteases in P. knowlesi and P. berghei. The activity of acid proteases in P. berghei - infected erythrocytes was 5-10 times higher than the normal erythrocytes (Levy and Chou, 1973) and P. knowlesi possessed 3-5 times higher activity of acid and alkaline proteases in cell-free parasite as compared to ghosts and 30-50 times more than that of hemolysate (Banyal et al., 1982). Levy et al.
(1974) demonstrated an acid protease in *P. knowlesi* and *P. falciparum*. A cathepsin-D like protease from avian malaria parasite, *P. lophurae*, was purified and characterized by Sherman and Tanigoshi (1983) while an acid peptidase, a neutral aminopeptidase and an alkaline endopeptidase in *P. falciparum* were confirmed by DEAE - cellulose chromatography, gel filtration on sephadex G-200 and isoelectric focusing (Gyang et al., 1982). An aminopeptidase from *P. falciparum* and an acid proteinase of *P. berghei* were also purified (Vander Jagt et al., 1984 ; Sato et al., 1987). *P. falciparum* enzyme was inhibited by bestatin and phosphoramidon but not by leupeptin, pepstatin and chymostatin whereas *P. berghei* acid protease was found to be inhibited by pepstatin. Bernard et al. (1987) identified a neutral endopeptidase from *P. berghei* and *P. chabaudi*.

The study conducted on an aminopeptidase and acid protease from chloroquine-resistant and chloroquine-sensitive *P. falciparum* showed no difference in the properties of these enzymes in two types of parasites (Vander Jagt et al., 1987). However, an early study showed 700-800% greater protease activity in chloroquine-resistant parasite as compared to chloroquine-sensitive *P. berghei* (Mahoney and Eaton, 1981) which may be due to pigment interference in protein determination. Acidic protease of 37 kD obtained from cytosoluble extract from *P. berghei* and *P. falciparum* was strongly inhibited by HgCl$_2$, ZnCl$_2$, chymostatin, leupeptin
and aprotinin (Deguercy et al., 1990). Schrevel et al. (1990) reviewed the proteases in malaria parasite-infected red cells and concluded that specific proteinases were identified using fluorogenic peptidyl-AEC substrates and by SDS-PAGE which are involved in the merozoite reinvasion into the red cells. Parasite cytoskeleton could be dissolved by 37 kD proteinase found to cleave spectrin. The structural inhibitor analogous to the Val-Leu-Gly-Lys (or Arg) P. falciparum neutral protease substrate appeared to stop the invasion process by merozoite.

Purines and pyrimidines

It has been confirmed that plasmodia do not synthesize purine ring *de novo* and the necessary purines have to be obtained by salvage pathway mechanism (Konigk, 1977; Sherman, 1977). In contrast malaria parasite has the capacity for the *de novo* synthesis of pyrimidines (Walsh and Sherman, 1968; Guttridge and Trigg, 1970). Gero and O’Sullivan (1990) reviewed purines and pyrimidines in malaria parasite and demonstrated that the parasite synthesizes enzymes for purine salvage, pyrimidine bio-synthesis pathway *de novo* and for folate cycle. Plasmodia also alter the erythrocyte membrane for the transport of purines. Some of the enzymes of these pathways were purified and found to be homologous to the human enzymes but differ in physicochemical and kinetic properties.