DISCUSSION
5.1.1 LIGHT MICROSCOPY FINDINGS

Malaria in itself is a devastating human malaise, but cerebral malaria is its most perilous offspring. Cerebral malaria is one of the most serious complication of Plasmodium falciparum infection. It is defined as an acute infection with diffuse and symmetric encephalopathy (Oo et al., 1987). Twenty to fifty per cent of deaths from falciparum malaria are reported to be due to the involvement of central nervous system (Aikawa, 1988).

Cerebral involvement such as paralysis, ataxia, a comatose state, plugging of the capillaries and haemorrhages in the brain are clinical and histopathological features of this complication (Kamiyama et al., 1987). In the present study an attempt has been made to understand and comprehend this serious and often fatal complication of malaria. The extent of involvement of brain tissue during the course of a laboratory induced cerebral malaria was investigated in P. knowlesi infected rhesus monkeys. In this research, the use of non-human primates was found necessary for making meaningful comparisons because of their evolutionary kinship to man as manifested in anatomical/behavioural resemblances and other specific biomedical similarities. It is because of this close relationship that biomedical and behavioural studies of the non-human primates is able to provide important insights into parallel situations in man (WHO, 1988). Furthermore, our earlier studies have demonstrated
that *P. knowlesi* - rhesus monkey model provides an ideal system to investigate the extent of involvement of brain tissue during the course of cerebral malaria (Mahdi et al., 1987; Mahdi et al., 1988a; Mahdi et al., 1989a, Mahdi et al., 1989b).

Light microscopic studies revealed parasitic infiltration of virtually all the regions of the central nervous system. These studies demonstrated the typical features of cerebral malaria, such as brain capillary obstruction, adhesion of the infected red cells to the walls of the capillaries and small vessels, swelling of the vessel endothelium and plugging of the capillaries by parasitized and un-parasitized erythrocytes. In earlier investigations (Miller 1969 and Jervis et al., 1972) cerebral involvement was demonstrated only when parasitaemia reached around fifty per cent. We however found an involvement of all the parts of brain, even though the peak parasitaemia was only 4%. This latter finding is in agreement with the views of Bruce-Chwatt (1985) who observed that there is no direct relationship between the degree of parasitaemia and the occurrence of cerebral involvement.

An interesting finding of this investigation is the demonstration of malaria parasite in the capillary endothelial cells and their occasional presence in astrocytes (Mahdi et al., 1989a). This observation has not been made in any earlier, experimental study of cerebral malaria. In the present study on rhesus monkeys infected with
P. knowlesi, the deposition of dark-staining, minute bodies in the cytoplasm of glial cells and capillary endothelial cells is interpreted as malarial parasites on the basis of their striking similarity to various parasite stages. The presence of such microbodies within these cells is certainly a novel observation, although the precise mechanism of its entry into the cells which are not normally considered to be host cells for plasmodia is still obscure. Possibly, this may be due to the ingestion of infected red blood cells or to the multiplication of malaria parasites inside the cells. The latter occurrence is suggested by the presence of multiple parasites and the absence of degenerative changes. This hypothesis is also supported by the fact that only occasional astrocytes showed multiple parasites, whereas the neighbouring cells did not. Recently, Wickramasinghe et al. (1987) reported extravascular parasitized red cells in their studies with bone marrow of cerebral malaria patients. One of the explanations they gave for the extravascular parasitized red cells was the passage of red cells through endothelial cells.

5.1.2 EYE INVOLVEMENT

The involvement of eye and its adenexae has been previously described in cerebral malaria. The various conditions reported are keratitis, uveitis, retinitis pigmentosa, optic neuritis and ocular muscle paresis (Bywater, 1922). The finding of retinal haemorrhages and damage to the eye is a clear indication of the presence of
vascular lesions in the pathogenesis of cerebral malaria. Although, the precise pathogenic mechanism contributing to the initiation and the development of this disease still remains largely unknown. This investigation based on histopathological and electronmicroscopic studies provide further evidence of the eye damage in cerebral malaria. Literature survey revealed that some very early investigations are suggestive of an eye involvement in malarial infections (Bywater 1922; Smith 1906), but more recently this important aspect of cerebral malaria did not seem to have received the attention it deserved. Our present study demonstrates parasitic infiltration of virtually every part of the eye. This study confirms and further supports the earlier finding of retinal haemorrhages in malaria from some endemic areas (Looaressuwan et al., 1983). The above findings have shown that retinal haemorrhages are generally associated with several other manifestations of \textit{P. falciparum} infection, perhaps leading to the development of cerebral malaria at a later stage. A near complete occlusion of the blood vessels of retina by sequestrated parasitized erythrocytes in this study also supports the earlier finding of Yoeli (1976) who has shown occlusion of the brain capillaries. The infected erythrocytes appeared attached to the endothelial cells, although knob-like protrusions generally observed in \textit{P. falciparum} malaria were not visible in our electronmicrographs.

Intravascular coagulation may be the primary cause of the retinopathy and the accompanying cerebral pathology. Mansen-Bahr
and Apted (1982) have suggested that stasis of the parasitized red cells may be responsible for such lesions in the eye. The capillary obstruction brought about by parasitic emboli has been shown to lead to permanent pigmentary macular changes. On the basis of our studies, we tend to suggest that retinal pathology may be the expression of an immunological intolerance/dysfunction (Mahdi et al., 1989b).

5.1.3 PATHOGENESIS OF CEREBRAL MALARIA

Sequestration of the infected erythrocytes, containing late developmental stages of the parasites in capillaries and venules of internal organs has been investigated (Poongponrath et al., 1988). Several electronmicroscopic observations have shown that older parasites are sequestrated in capillaries of brain, heart, intestine, placenta and other organs (Phillips and Gilles, 1988). Recently, Handunnetti et al. (1989) have suggested that parasite sequestration in the deep vasculature of the brain leads to tissue ischemia, which is responsible for the lethal character of *P. falciparum* infections. Erythrocytes infected with *P. falciparum*, *P. coatneyi* and *P. knowlesi* disappear from peripheral blood during the maturation of parasites from ring to schizont. This phenomenon, caused by attachment of these infected erythrocytes to vascular endothelium, has been termed deep vascular schizogony (Miller et al., 1971).
Although cerebral capillaries are described as the principal vessels occluded in fatal falciparum malaria, but in our study with *P. knowlesi*, the venules and veins were also found to contain many parasitized red cells. However, the distribution of parasites was irregular throughout the cerebral vessels. Venules and capillaries in one region of the cortex were completely filled with parasitized red cells, whereas an adjacent zone was virtually devoid of parasites. Similar observation was recorded by Clark and Tomlinson (1949) in autopsy studies of fatal *P. falciparum* infection in man.

Most of our light microscopic studies were confirmed by the electronmicroscopic observations. But we could not locate much of a parasitic infiltration of glial cells. In light microscopy too, we only occasionally found parasite deposits in the glial cells.

The knob-like protrusions which are responsible for attachment of *P. falciparum* infected erythrocytes to the endothelium were not present in the membranes of *P. knowlesi* parasitized erythrocytes. However, we found that the membranes of parasitized red cells conform directly to the endothelium and some of them attach to the extension of endothelial cells. A somewhat similar observation was earlier made by Poongponrath et al. (1985). But the "invagination of electron-dense membranes" as reported by Miller et al. (1971) was not revealed in our electronmicrographs. On the whole, the cerebral vascular involvement in *P. knowlesi* infected rhesus monkeys
appeared similar to that in fatal cases of \textit{P. falciparum} infections of man and again this latter finding is in concurrence with those of Miller et al. (1971).

The electronmicroscopic studies further revealed that in contrast to normal monkeys, the vessels of animals with cerebral malaria contained numerous macrophages. The cytoplasm of these macrophages contained phagocytosed parasitized erythrocytes and other inclusion bodies. Quite often, macrophages were found in intimate contact with and attached to the endothelium and in some instances the intervening cell membrane was not clearly identifiable indicating the possibility that exocytosis of lysosomal contents could have occurred. Rest and Wright (1979) reported this for the first time in \textit{P. berghei} infected hamsters. George et al. (1976) reported the presence of neutrophils in glomerular capillaries in immune complex nephritis associated with rodent malaria, but this has not been previously reported in \textit{P. knowlesi} - rhesus monkey model.

The relevance of any animal model lies in its resemblance to the human disease. Certain aspects of the histopathology of rhesus monkey model show similarity to that of humans. Bulging endothelium and monocytes within and around vessels are seen both in human cerebral malaria and as well as in the monkey model system. Intravascular monocytes attached to the endothelium and laden with malarial pigment, are difficult to distinguish from bulging
endothelium and parasitized erythrocytes packed into capillaries in human post-mortem brain sections (Rest, 1982).

Monocytes are attracted by various chemotoxins which include immune complexes (Allison and Houba, 1976), C5a and kinins (Snyderman and Mergenhagen, 1976). The implication of immune complexes in the pathogenesis of cerebral malaria is consistent with the observation that this may be prevented by neonatal thymectomy (Wright, 1968), antithymocyte serum (Wright et al., 1971) and with the complement changes noted in acute malaria (WHO, 1975).

Another interesting electronmicroscopic finding was the presence of platelets in the vicinity of macrophages and parasitized erythrocytes in sections from choroid plexus. Poongponrath et al. (1985) have also reported the presence of platelets in human cerebral malaria. From their studies they have concluded that the platelets attached to injured vascular endothelial cells may play a role in the obstruction of cerebrospinal fluid leading to cerebral oedema. Macpherson et al. (1985), however, failed to locate the presence of platelets in the cerebral vessels of \textit{P. falciparum} infected patients. French (1970) has concluded that the essential components of thrombi are platelets and fibrin.

In some animals lipofuscin pigment was detected in the brain with the electronmicroscopic studies. Gutierrez et al. (1976) have reported deposition of lipofuscin pigment in the liver of
P. falciparum infected Aotus monkeys. Similarly high concentrations of lipofuscin granules have been reported by Rosen et al. (1967) and by Brito et al. (1969) in patients with P. falciparum infection. But lipofuscin has also been considered as being part of normal ageing process of the cell (Glees and Hasan, 1976).

5.2 BIOCHEMICAL STUDIES

Host parasite relationship in malaria infection has been extensively studied (Von Brand, 1973). Malarial infection in experimental animals is associated with various biochemical changes. Fletcher and Maegraith (1972) have shown that metabolic activities of the host, as well as the parasite are significantly altered during the course of malarial infection. There are reports indicating alterations in lipid metabolism in infected liver, spleen, kidney and brain. The changes are initiated during the erythrocytic phase through mediators released in the host blood (Saxena et al., 1981). Lipids are essential components of cellular structures and are the major constituent of brain tissue. The lipid requirement for parasite growth inside the host erythrocyte is accompanied by exchange of lipids through plasma and body cells, causing severe derangements in lipid metabolism of the host (Majumdar et al., 1981). In recent years, however, workers have been engaged in assessing the possible role of highly reactive products of oxygen termed free radicals in the
pathogenesis of malaria and other parasitic infections (Clark et al., 1986). In addition to oxygen radicals, certain products of lipid peroxidation induced by the free radicals may be toxic to invading organisms (Gutteridge et al., 1982) and host cells (Morel et al., 1983). Descamps-Latscha et al. (1987) have reported that increased amounts of reactive oxygen species are produced during malarial infection and, that high levels seen in patients with severe form of the disease could contribute to pathological reactions. The study of Nnalue and Friedman (1988) suggested that singlet oxygen is the most effective neutrophil product killing or suppressing the growth of malaria parasite.

The results of this study carried out in P. berghei infected mouse confirmed infiltration of parasites in various regions of the brain. The study further demonstrated a sizeable decrease in total lipids, phospholipids, cholesterol and gangliosides in the cerebrum, cerebellum and brain stem. Similarly, a remarkable increase in the rate of lipid peroxidation was observed in the cerebellum and brain stem of the mouse following malarial infection (Mahdi et al., 1988c). Regional variation in the lipid contents could be explained by the fact that brain is a heterogeneous organ composed of many structural and functional components, having markedly different levels of functional and metabolic activity. The heterogeneous nature of the brain is of paramount importance in the evaluation and interpretation of biochemical findings (Hertz, 1969).
Biomembranes and sub-cellular organelles are the major sites of peroxidation damage (Tappel, 1970). Free radical mechanism leading to lipid peroxidation and degradation of the brain lipids with the loss of membrane integrity, are currently considered important contributing factors in the development of irreversible brain damage during ischaemic and other adverse conditions (Demopoulos et al., 1979). A close relationship exists between cellular injury, peroxidation of membrane lipids and oxidative damage to the cells (Sharma and Krishnamurti, 1976). Enhancement in the rate of lipid peroxidation following malarial infection is also corroborated by the observations of Bajpai and Dutta (1987). Saxena et al. (1981) have reported that in contrast to brain, significantly higher amounts of lipid peroxides are produced in liver, lung and spleen.

The increase in the rate of lipid peroxidation appears to contribute significantly to the reduction of lipid levels in various areas of brain. Chander et al. (1987) have reported that host's lipid metabolism is severely affected during malarial infection. The results of the present study clearly indicate a significantly enhanced activity of endogenous lipids in malarial infection, which perhaps constitutes a major cause of reduction of brain lipids (Mahdi and Ahmad, 1989).

The killing of intracellular and extracellular pathogens by phagocytes is due in part to the production of oxygen radicals,
namely superoxide, hydrogen peroxide, hydroxyl radicals and possibly singlet molecular oxygen (Lockelay and Klebanoff, 1983; Docampo and Moreno, 1984). Protozoan parasites contain endogenous scavengers of oxygen-derived products that may be important in their defense against toxic oxygen radicals generated by phagocytes. The results of the present study showed significant alterations in hydroperoxide levels of mitochondria and microsomes of infected mouse brain specimens. There was a marked increase in lipid peroxide levels in brain microsomes, thus indicating damage of brain membranes by P. berghei infection.

Superoxide dismutase (SOD) activity has been reported from various parasitic microorganisms (Docampo and Moreno, 1984). The experiments carried out during the course of this study have demonstrated sizeable decrement in the SOD activity of P. berghei infected mouse brain as compared to un-infected control group, thus indicating low scavanging activity of free radicals. Arias and Walter (1988) have reported decrement of SOD activity in P. falciparum infected erythrocytes. Kulkarni et al. (1981b) have found to change in SOD activity in brain of rats infected with P. berghei. This may be due to non-involvement of brain in P. berghei - rat system, the model in which the study was carried out. However, Kulkarni et al. (1984) have reported diminution in the hepatic SOD activity in P. berghei infected mouse. Areekul et al. (1987) have found no change in SOD activity in P. berghei infected mouse spleen.
The SOD activity is said to be the natural defence mechanism against oxidative damage to the tissues (Fridovich, 1975). Low levels of this enzyme during malarial infection would clearly make the tissue all the more prone to oxidative damage. Areekul and Boonme (1985) reported diminished activity of another scavenging enzyme; catalase, in infected red blood cells of patients infected with *P. falciparum*, thus indicating that these cells are prone to damage of $H_2O_2$. Increasing evidence has been provided demonstrating the importance of oxidative stress in cellular immune mechanisms against *Plasmodium* parasites (Allison and Euguni, 1983).

5.3 MECHANISM PROPOSED FOR THE PATHOGENESIS OF CEREBRAL MALARIA

Although the symptoms of cerebral malaria are by now well documented (Daroff et al., 1987; Osuntokun, 1983) yet the underlying causes for this particular manifestation of the disease are not fully understood. Historically, cerebral malaria is an age old malady as it was first described at the turn of the century (Thumwood et al., 1988). Possible factors contributing to the development of cerebral malaria include: the blockage of cerebral vessels by parasitized red blood cells; deposition of immune complexes in brain capillaries; reduced humoral or cell-mediated immune responses; action of endotoxin and; action of tumor necrosis factor (Clark, 1987). Maegraith (1966) suggested that the loss of normal blood
brain barrier (increased permeability to albumin) initiates lesions and that obstruction of capillaries by infected red blood cells is secondary. Although Aikawa (1988) suggested that blockage of cerebral vessels is the major factor in the pathogenesis of cerebral malaria.

If the widely postulated explanation, the adhesion of knob-bearing parasitized RBCs, is to be accepted then we would expect cerebral malaria to develop routinely in every patient with falciparum infection. But apparently this is not true. Furthermore, such an approach has not yet been able to answer the question of malarial tolerance. The existence of a certain degree of tolerance is much in evidence (Kitchen, 1949; McGregor et al., 1956), from hyperendemic areas where children continuously exposed to malaria are often healthy, despite the high parasitaemia. On the other hand, there is no unequivocal evidence to suggest that plugging of capillaries is an essential part of the pathogenesis of human cerebral malaria (Thumwood et al., 1988). This aspect certainly needs further investigation; as factors other than adherence to endothelial cells by knob-bearing red cells will need to be identified for achieving a better understanding and comprehension of the disease as a whole.

Clark et al. (1986) have suggested that cerebral malaria may be caused by increased vascular permeability due to endothelial damage, which may itself be caused by active oxygen metabolites
such as hydrogen peroxide, superoxide anion and hydroxyl radical. Such metabolites can be released by neutrophils and by activated macrophages (Badwey and Karnovsky, 1980). We have demonstrated the presence of macrophages in monkeys having cerebral malaria.

As an inevitable consequence of aerobic metabolism, the oxygen radicals are also generated inside leukocytes, enabling them to kill phagocytosed microorganisms (Babior et al., 1973). Unfortunately, such leukocytes from their outer membrane also secrete $O_2^-$ along with other mediators into the surrounding tissue (Nathan and Root, 1977). The main source of oxidant stress, in vivo, is the presence of phagocytic leukocytes, polymorphonuclear neutrophils and macrophages (Nnalue and Friedman, 1988). During phagocytosis, or, when the plasma membranes of these cells are appropriately perturbed they undergo a respiratory burst and produce substantial quantities of superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen. These reactive oxygen intermediates can oxidize proteins (Broe et al., 1981), and membrane lipids (Mead, 1976), and can damage DNA (Cathecart et al., 1984) in nearby cells. This indiscriminate and self-inflicted process contributes to the tissue damage of inflammation (Freeman and Crapo, 1982), and clearly has the capacity to cause tissue damage in parasite induced disease.

Oxygen radicals being very transient species merely set in motion a destructive chain reaction. When lipids react with
free radicals they undergo a series of molecular rearrangement termed peroxidation and form a series of oxidation derivatives, including lipid peroxides, lipid hydroperoxides and aldehydes (Esterbauer, 1982). In our studies we have shown enhancement in the rate of lipid peroxidation and also a marked increase in the levels of lipid peroxides and hydroperoxides (Mahdi et al., 1988d). Rest (1983) postulated that monocyte-derived mediators (monokines), particularly arachidonic acid metabolites are the key constituents of pathogenesis. It has been observed that monocytes which metabolise arachidonic acid become vacuolated (Bruce et al., 1979) and produce free oxygen radicals (Kuehl and Egan, 1980) when they phagacytose particles (Johnson et al., 1976). These free oxygen radicals could be responsible for the endothelial cell damage (Kontos et al., 1980) with haemorrhages occurring in areas where the endothelium is most susceptible to oxygen toxicity.

Based on our studies of light and electronmicroscopy, as also on the basis of biochemical findings and the evidences available from the previous work, only one thing could be concluded that the pathogenesis of cerebral malaria is a complicated process. Looking at all the possible factors, it is rather difficult to assign one single reason to describe its various manifestations, as it definitely appears to be an outcome of an array of factors. On the basis of our studies and published findings (Mahdi et al., 1987, Mahdi
et al., 1988b; Mahdi et al., 1988c; Mahdi et al., 1988d; Mahdi et al., 1989a, Mahdi et al., 1989b; Mahdi and Ahmad, 1989) we suggest that the pathogenesis of cerebral malaria is the outcome of a typical triad constituted by: the mechanical obstruction of blood capillaries with parasitized RBCs; the biochemical events particularly the involvement of free radicals and; the immunological dysfunction; all acting in concert.

5.4.1 IMMUNIZATION OF RHESUS MONKEYS USING MDP AS AN ADJUVANT: PROTECTION STUDIES

Workers in the last 40 years have variously attempted to immunize monkeys against malaria using blood stages of parasites. An obvious advantage for investigators in these experiments is that blood stage parasites produce infections that are biologically similar to human malarial infection (Coatney et al., 1971). Schimidt-Ullrich et al. (1982) have shown that *P. falciparum* schizonts and *P. knowlesi* merozoites share some common antigens. The recent malaria vaccination studies in our laboratory by Khanna (1988) have yielded some encouraging results. Keeping the above studies in mind we immunized rhesus monkeys against *P. knowlesi* using whole antigen in combination with Muramyl dipeptide (MDP) as an adjuvant. These studies were carried out to check the degree of protection, if any, as also to check the availability of any possible minimizing factors in the
course of pathogenic events in cerebral malaria.

The most delicate aspect of malaria research is the isolation of pure parasite material from host erythrocytes. The major obstacle encountered in this work is usually the removal of leukocytes and host RBC contaminants. The methods commonly used for the removal of leukocytes and buffy coat are by suspending the washed cells in several volumes of dextran solution or obtaining them as supernatant on sedimentation (Langer et al., 1967; Zuckerman et al., 1967). These procedures are usually helpful in removing only 75% leukocytes. We used slow speed centrifugation in which about 85% leukocytes were removed. Our results on leukocyte separation compared favorably with previous study of Zuckerman et al. (1967). The most efficient method for the removal of leukocytes is to pass the washed blood either through a column of paper powder (Homewood and Naeme, 1976), or through a column packed with equal parts of alpha cellulose and microcrystalline cellulose (Beutler et al. 1976). Recently, Mons et al. (1988) demonstrated that the use of commercially available cell select leukocyte filters is more effective, rapid and simple method for the removal of WBCs than the use of cellulose powder.

We employed saponin to induce lysis of red blood cells. The treatment resulted in 100% lysis of red blood cells, causing the release of free parasites. Christopher and Fulton (1939) for the first time used saponin for lysing erythrocytes. It has been
reported earlier that the parasites released by saponin lysis are free from host cell material (Jerusalem and Eling, 1969). Siddiqui et al. (1978b) found saponin as most efficient non-ionic detergent for haemolysis, as also for the removal of proteins and sialic acids from red blood cell membranes.

The released parasites can be further separated from erythrocyte contaminants by differential centrifugation, enzymatic digestion, free flow electrophoresis and density gradient centrifugation. By means of histopaque density gradient, we were able to obtain a comparatively pure preparation of *P. knowlesi*. A very important host contaminant is the red blood cell material ingested by the growing parasites. Removal of this contaminant has always been a formidable task. The antigenic material obtained following ultrasonication of parasites was treated on poly-acryl-amide gel electrophoresis. These results also showed that host cell contaminants were not present in the antigenic preparations.

In the present studies, we used whole *P. knowlesi* antigen for immunizing rhesus monkeys. The antigenic preparations were mixed with MDP and given twice, intramuscularly, at an interval of three weeks according to Siddiqui et al. (1979). Monkeys receiving WAg-MDP showed a survival rate of 50%, with a prepatent period of 12 days. Animals belonging to antigen, adjuvant and saline control groups showed 100% mortality. Average peak parasitaemia in the
animals immunized with WAg-MDP preparation was found to be 10.5% only. These animals showed the presence of circulating antibodies, although the cell mediated immune responses observed in the animals were of lower order. Khanna (1988) reported that cell mediated immune responses observed in the animals immunized by MDP were significantly lower compared to those obtained in animals immunized by FCA (Freund's Complete Adjuvant). A synthetic derivative of MDP (nor-MDP) given in mineral oil, has proved only partially effective as an adjuvant for merozoite vaccination of Macaca mulatta against P. knowlesi (Mitchell et al., 1979). The MDP, whether used as such, or emulsified in oil, was effective in mouse system (Khullar et al., 1985). Siddiqui et al. (1977) demonstrated that P. falciparum morozoite antigen emulsified with FCA was able to induce complete protection of vaccinated monkeys. Siddiqui et al. (1979) demonstrated that stearoyl MDP (6-0-stearoyl-N-acetyl muramyl-L-alanyl-D-isoglutamine) could replace FCA for effective immunization of owl monkeys against infection with P. falciparum.

Results of the two human trials on the safety and efficacy of vaccines against P. falciparum sporozoites have been reported recently (Ballou et al., 1987; Herrington et al., 1987). Both these vaccines were prepared from the immunodominant epitope of a circumsporozoite protein, the main component of sporozoite surface coat (Nussenzweig and Nussenzweig, 1985). Young et al. (1985) and Ballou et al. (1987) have used alum hydroxide as an adjuvant
that the endothelial cells lining the liver sinusoids are also phagocytic
in nature and they can transform into Kupffer cells (Aikawa et
al., 1968). Macrophages containing pigment enter the sinusoids
with the portal blood. In _P. falciparum_ infections of human subjects
and experimental monkeys (Jervis et al., 1972; Gutierrez et al., 1976),
large number of parasitized cells are found attached to the endothelial
cells. The increased number of cells in the sinusoids causes sluggish
circulation in the organ, with resultant congestion and central
necrosis due to portal hypertension (Skirrow et al., 1964).

Light microscopy of the kidney sections revealed completely
atrophic tubules at some places, while in other areas cloudy swelling
of the epithelial lining cells and haemoglobin granules in the proximal
tubules were observed (Rosen et al., 1968; Boonpuckanavig et al.
1973). In our studies, kidney sections from infected control animals
showed large pigmented glomeruli while immunized animals showed
no change at all.

Spleen sections showed hyperplasia and hyperaemia in the
white and red pulp. The sinusoids were dilated with numerous infected
red blood cells. There was a marked congestion of splenic sinusoids
and enormous amount of pigment was found in the swollen macrophages.
Neutrophil infiltration was abundant, especially in the necrotic
areas (Taliaferro and Mulligan, 1937). However, sections from
immunized animals showed less amount of pigment deposits and
hyperplastic white pulp.
in their sporozoite vaccination studies. Both candidate vaccines have been shown to be well-tolerated with no serious, local or systemic effect.

A number of candidate antigens are being considered for development of vaccines against asexual blood stages of malaria parasite. The principal targets for vaccines include antigens on the surface of merozoites or mature schizonts, merozoite-antigens released during red cell reinvasion, and parasite-antigens on the surface of infected cells. The genes encoding several of these antigens have been cloned and investigated in primate models as possible candidate vaccines. Recently, Siddiqui et al. (1987) have shown that complete protection of Aotus monkeys against \textit{P. falciparum} malaria could be achieved using merozoite surface coat precursor protein, gp-195, derived immunogens. More recently, Chang et al. (1988) have evaluated the possible need for a multivalent gp-195 vaccine to achieve clinical immunity in a susceptible population.

Presently, human trials are being conducted in Columbia of two polymeric synthetic hybrid molecules based on synthetic peptides corresponding to merozoite-specific proteins (Patarroyo et al., 1988). Results from these initial human trials of a malaria vaccine have been both encouraging and disappointing at the same time (Cattani, 1989). Vaccine candidates have not been as immunogenic as was initially expected. The strategy of targeting a single
synthetic peptide of fusion protein as a vaccine has not been as effective as the use of irradiated whole sporozoites in man (Clyde et al., 1975) and animal models as well (Vanderberg et al., 1969). It would appear even less likely that a single blood stage antigen will form the basis for an effective vaccine, given the antigenic diversity that exists among malaria parasites in nature. The role of cell mediated immunity also appears essential for effective protection, indicating that the requirement for T-cell epitopes in a vaccine may always be related to enhancement of immunogenicity, as also to the protection against vaccine-induced variation as reported for an antigen of *P. knowlesi* (Klotz et al, 1987).

5.4.2 IMMUNOPATHOLOGY

The light microscopic studies of the sections from immunized animals in the present investigation revealed normal tissue architecture. The central vein and sinusoids of liver from un-immunized monkeys were dilated and showed presence of pigment. In immunized monkeys the central vein, though found dilated, was devoid of any pigment. No pigment could be seen in the sinusoids and Kupffer cells which were found compressed. In un-immunized animals, the phagocytic Kupffer cells of the sinusoids were found swollen and contained pigment deposits. It seems that during an early infection, the perilobular Kupffer cells start to hypertrophy and active phagacytosis of pigment and infected erythrocytes occurs. It is generally accepted
Sections from brain of infected control animals revealed that the blood vessels of cortex and medulla were completely plugged with parasitized RBCs (Yoeli and Hargreaves, 1974; Mahdi et al., 1989a). Parasitic infiltration was observed in all the regions of the brain (Mahdi and Ahmad, 1989). Noticeably, there were few structural changes in the central nervous system (Gutierrez et al. 1976). The small-sized blood vessels and capillary endothelium showed slight proliferation, while the parasitized erythrocytes were found attached to the endothelium (Mahdi et al., 1989b). This particular finding is however in contrast to the results reported by Gutierrez et al. (1976). Recently, Thumwood et al. (1988) have reported breakdown of blood-brain barrier in murine cerebral malaria. In our studies of the sections of brain from immunized animals we found un-parasitized erythrocytes in the blood vessels of cortex and white matter. There was no involvement of brain tissue in the immunized animals following challenge. Neither there was any parasitic infiltration observed in the brain of immunized monkeys dying after the challenge. We carried out such immunization experiments in order to assess the candidate vaccines presently in use mainly for checking their efficacy in reducing blood parasitaemia as also their possible role in increasing or decreasing the process of sequestration of infected erythrocytes to brain (Aikawa, 1988). On the basis of our findings, it could be concluded that whatsoever might be the limitations of a malaria vaccine in safeguarding a
malaria infection, it does afford considerable protection against cerebral malaria by inhibiting a full fledged involvement of the brain tissue.