Malaria has always been one of the major threats to public health in India, as it has been in many other tropical and subtropical countries. The effect of such a debilitating condition on the wellbeing and productivity of a population, already challenged by poverty, malnutrition, and other burdens is enormous. On account of the resurgence of the disease, malaria research has assumed a high degree of urgency. Research covering different aspects of the disease viz. operational aspects of malaria control, ecology, control of vectors, chemotherapy, drug resistance, serology, biochemical and immunological aspects is being carried out in India and also throughout the World (WHO, 1990; Siddiqui, 1991).

Inspite of enormous amount of literature available on malaria, very limited information exists on the physiological changes produced in the host by the malarial parasites. This deficiency can be ascribed primarily to the fact that biochemical studies on liver and serum of human subjects naturally infected with malaria are often complicated by the presence of concurrent infections and nutritional deficiencies (Desowitz, 1987).

The isolation and description of the rodent malaria parasites *Plasmodium berghei* by Vincke and Lips (1948) and subsequent studies on experimental transmission of this parasite have permitted the use of the laboratory mouse as a suitable model for experimental studies of mammalian malaria, under
controlled conditions Sadun (1965). In view of the above considerations, studies were set up to determine some of the detectable biochemical changes occurring in mice after infection with *P. berghei*.

Phenomenon of fatty degeneration in addition to centrilobular necrosis was mainly observed in liver during malarial infection, this was later confirmed by some biochemical studies. In our findings total liver fats were found to increase from 89 percent over control values at 60-70 percent parasitaemia. A similar pattern of increase in hepatic lipid content was also observed in rats, monkeys and humans infected with *P. berghei*, *P. knowlesi* and *P. falciparum*, respectively (Rao et al., 1969; Angus et al., 1971; Fletcher et al., 1987 and Bajpai and Dutta, 1990). Accumulation of lipid in liver of animal was also detected by electron microscopy (Fletcher et al., 1987) and by histochemistry (Angus et al., 1971 and Mercado and Von-Brand, 1958). We have also observed lipid deposition in centrilobular region of the liver lobule by histochemical technique. So increase in the level of total lipid in tissue mainly causes hyperlipidaemia at high degree of parasitaemia. There are several reasons for accumulation of fat in liver, which causes fatty liver in animal and human during malaria infection, for example liver plays a decisive role in metabolism and transport of lipids, as well, as in the maintenance of lipid levels in the
liver and in blood circulation.

The increase in the level of lipid in albino mice is probably due to a block in the release of hepatic triglycerides in plasma, or perhaps due to disturbed mitochondrial fatty acid oxidation. This last condition might be due to suppression of lipoprotein lipase activity (Hotez et al., 1984).

Malarial infection was also associated with decreased level of cholesterol content in liver. Seshadri et al. (1983) and Onongbu and Onyeneke (1983) have observed a significant depletion of liver cholesterol content in monkeys infected with P. falciparum. Similarly Rao et al. (1969), and Saxena et al. (1981) also reported decreased hepatic cholesterol content in mouse and rat liver. The present studies have shown that liver cholesterol content in albino mice was depleted significantly by (23.7%) at high degree of parasitaemia. Seshadri et al. (1983) have tried to understand the aetiology of reduction of hepatic cholesterol during P. vivax malarial infection. They have suggested that it might be due to an increased uptake by the infected erythrocytes at high parasitaemia. A similar reason might also be attributed to the decrease of liver cholesterol contents in the albino mice during the course of malarial infection.

Similarly fall in phospholipid levels in the liver
has been observed during the course of malarial infection in mice. Rao et al. (1967) have shown a significant increase in phospholipid levels in the spleen and liver of \textit{P. berghei} infected mice. They have however (Rao et al., 1969), further reported that phospholipid levels in the infected rat liver were decreased. Sharma et al. (1979) also reported decrease in phospholipid contents in infected mouse liver during \textit{P. berghei} infection. The phospholipids are essential for the maintenance of membrane structure, the extent of tissue damage with gradual rise in parasitaemia is thus reflected in decreased phospholipid contents.

More significantly our results indicate a remarkable alterations in the rate of lipid peroxidation during chronic infection. The increase was observed to be of the order of (314\%) at 60-70 percent parasitaemia. Our results get support from the observations given by previous workers (Saxena et al., 1981; Chauhan et al., 1981). Who have also shown an increased level of lipid peroxidation in the liver of mice during \textit{P. berghei} infection. Enhancement in the rate of lipid peroxidation following malarial infection is similar to the observations of Bajpai and Dutta (1987). Recently a significant increase in the rate of lipid peroxidation in cerebellum and brain system of mouse following \textit{P. berghei} infection was shown by Mahdi et al. (1989). Chander and Kapoor (1990) also demonstrated
from their observations that during malarial infection increase in the rate of lipid peroxidation occurs in *Mastomys natalensis* infected with *Plasmodium berghei*. This raised levels of lipid peroxidation are perhaps available due to increased susceptibility of liver to an oxidative damage under the stress of a malarial infection (Sharma et al., 1979).

With the help of biochemical studies, only fatty infiltration in the liver was not observed but a state of hypoglycaemia was also seen (White et al. 1983). This have been due to an altered carbohydrate metabolism. During heavier infections, when liver is likely to be damaged more severely, there would be a state of hypoglycaemia, as has been observed in the present studies. The decrease in carbohydrate content was to be in the order of (78.76%). The reduction observed in carbohydrate content in the liver of albino mice is in agreement with the other reports given by several workers. Homewood and Neame (1980), Saxena et al. (1981) and Clark et al. (1987) have observed depletion in total carbohydrate content of liver in mouse and monkeys infected with *P. berghei* and *P. knowlesi*, respectively. Recently, Phillips et al. (1989) and Kawo et al. (1990) have also reported state of hypoglycaemia in humans during *P. falciparum* infection.

The cause of hypoglycaemia would appear to be due to rapid utilization of available sugar by growing parasites
and also an account of the inabilities of liver to store and produce sufficient amount of sugar. Saxena et al. (1981) and Seshadri et al. (1983) have also tried to explain the physiological cause of reduction of hepatic carbohydrate during malarial infection. They have found that the intraerythrocytic stages of parasite have no carbohydrate reserves and therefore, they obviously consume their nutritional share from host's carbohydrate reserve for their rapid growth and multiplication.

Depletion of total carbohydrate content of liver during malaria infection was also associated with rapid and significant decrease of glycogen and glucose content of liver. Present findings have shown significant depletion of glycogen content in liver of albino mice during P. berghei infection. Liver glycogen content was found to decrease by (84%) at acute infection. Fulton (1939) had examined this problem in P. knowlesi malaria in rhesus monkeys, and reported hypoglycaemia and depleted liver glycogen in the late stages of infection which was mainly due to large consumption of glucose by the parasite. Fletcher (1987) reported that hypoglycaemia is frequently associated with severe malaria at high parasitaemia. Earlier, Sadun et al. (1965) and Srivastava et al. (1984) have reported depletion of glycogen content in liver during malarial infection. Devakul and Maegraith (1958) have observed considerable decrease of liver glycogen in Macaca mulatta infected with
P. knowlesi. Chatterji and Gupta (1957) have noted similar finding in female rats infected with P. berghei.

However, the glucose contents were also estimated in liver in chronic infections. In our studies we have found depletion in glucose content in liver of mouse by (78.6%) at 60-70% parasitaemia. Fall in glucose content of liver was also observed by Saxena et al. (1981) in Mastomys natalenesis during P. berghei infection. Recently Paul et al. (1991) also reported hypoglycaemia in albino mice infected with P. berghei. Our results in mouse system were similar to those reported in human infections with P. falciparum. Possible mechanism for hypoglycaemia during malarial infection includes accelerated tissue metabolism and increased metabolic requirements of the parasite. Impaired hepatic gluconeogenesis with fatty infiltration causes hypoglycaemia (Filkins and Cornell, 1974). Increase in the concentration of lactic acid in the blood of host causes toxicity leading to tissue damage due to the utilization of glucose through glycolytic pathway by the parasites.

Present findings also revealed decreased amount of total ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Quantitative estimation of total ribonucleic acid and deoxyribonucleic acid confirmed that liver RNA contents decreased by (29%) while DNA decreased by (79%) during severe
infection. The reduction in nucleic acid values as observed in the present studies has not been shown by Rao et al. (1969), who have observed a total increase in nucleic acid contents of liver in chicks during malaria infection. In addition, Pandey et al. (1986) have found no change in nucleic acid contents of liver in *P. knowlesi* infected monkeys. So it is difficult to ascertain the exact mechanism of reduction of nucleic acid contents of liver observed in our study. Kreier (1980) and Weber et al. (1988), have suggested that nucleic acids are made up of purine and pyrimidine nucleotides. In mammals, purines are both synthesized by denovo and obtained through salvage pathways while malaria parasites are incapable of synthesizing purine by denovo pathway as such and are totally dependent on salvage pathways. Absorption of nucleotides by the parasites from host under such conditions might be one of the reasons for reduction of nucleic acid contents in liver during malarial infection. Nucleic acid contents in infected liver were further assayed by histochemical staining techniques in present thesis.

Marked alteration in the rate of synthesis of protein has been reported in liver tissue of albino mice infected with *P. berghei*. In the present study, a significant fall in protein contents of liver was found at (60-70%) parasitaemia. This would suggest an appreciable alteration in the protein status of the organs of host. An extensive proteolysis makes readily available
a pool of free amino acids which are needed for rapid proliferation of parasites (Saxena et al., 1981, Fern et al., 1985). Another obvious reason for a decrease in the protein level is the depletion of RNA and DNA contents within the liver of infected animal. In fact, poor availability of nucleic acid could be responsible for lower levels of protein (Chander and Kapoor, 1990).

It is obvious that the interaction of malarial parasites and their hosts gives rise to certain somatic alterations, which may ultimately be reflected in biochemical changes in the body fluids. Therefore, correlation of clinical observations with multiple quantitative determination of the serum components may contribute to the diagnosis and prognosis of this infection, and may even permit an early assessment of the effect of various chemotherapeutic agents (Sadun 1966). The liver damage caused by invading parasites during acute malaria can be more conveniently demonstrated by means of several routine function tests. The enzymes generally used as indicators of liver dysfunction are, glutamic oxaloacetic transaminases (GOT), glutamine pyruvic transaminases (GPT), acid phosphatase and alkaline phosphatase. It has been observed that liver GPT decreased by (22%) and GOT by (42.5%) at 60 to 70 percent parasitaemia respectively. These results are in agreement with the earlier observations made by Lal and Hussain (1978), who
have also observed decreased levels of GPT and GOT activity in the liver of *P. berghei* infected albino mice, indicating a dysfunction of liver during infection. We have also observed marked alterations in the SGPT and SGOT levels in the serum of infected albino mice. SGPT and SGOT levels was increased by (188% ± 84%) respectively. Similar findings were also reported by Sadun et al. (1965), Lal and Hussain (1978) and Khanna et al. (1986) during malarial infection.

During malarial infection, an increase in the number of macrophages, lymphocytes and kupffer cells were also observed in the liver. It was confirmed by estimating the acid and alkaline phosphatase levels in the liver tissues. Biochemical estimations have shown a significant elevation of alkaline phosphatase activity. The elevation in liver alkaline phosphatase was of the order of (75%) at 60-70% parasitaemia. Increased levels of acid phosphatase activity in the liver was also observed in our studies. It was found to increase significantly by (90%) at high parasitaemia. Saxena et al. (1985) also reported similar findings in various animal models including mice, rats, and *Mastomys natalenesis* during *P. berghei* infection. Banyal et al. (1980) also reported acid and alkaline phosphatase activity in monkeys infected with *P. knowlesi*. They noted altered levels of these enzymes due to the membrane disruption of cytoplasmic organelles which ultimately leads to liver damage.
We have also estimated acid and alkaline phosphatase levels in the serum of infected and control animal groups. Acid phosphatase level in the serum was found to the order of (215%), while serum alkaline phosphatase level was elevated by (99%) during *P. berghei* infection.

There has been a phenomenal resurgence of malaria as all eradication programmes, based on vector control and chemotherapy measures, have proved to be of limited success (WHO, 1988; Prasad et al., 1990). This situation thus warrants alternative approaches for the control of this infection. In this connection serious efforts are being currently made in India and abroad to explore the possibility of developing vaccine (WHO Tec. Rep Ser. 1975; WHO, 1990; Siddiqui, 1991).

Considering the success of the various vaccine candidates, the use of a potent and safe adjuvant seems imperative for achieving an effective, protective immunity (Siddiqui, 1990). Besides several biochemical studies, we have also made some efforts to assess the role of the adjuvants in immunization and to assess biochemical and pathological events in an immunized host.

The aim of immunization of man and other animals by artificial means is mainly prophylactic against infections. Long lasting, effective immunity results from a controlled stimulation of the immune system by administration of harmless vaccines.
rather than from uncontrolled stimulation following a natural course of infection.

The most challenging aspect of malaria research is the isolation of pure parasite material from infected host erythrocytes. For the preparation of malaria parasite from infected blood, the removal of leucocyte cells is considered to be an important step, especially in rodent malaria where there is a large increase in the number of leucocyte during the course of infection (Hickman, 1969). The methods routinely used for the removal of leucocytes and buffy coat is by suspending the washed blood cells in several volumes of dextran solution, or by obtaining them as supernatant following sedimentation (Langer et al., 1967; Zuckerman et al., 1967). Sedimentation velocity centrifugation of washed blood cells on 10% dextran solution (Levy and Chow, 1973) or on Ficoll hypaque solution of density 1.08 gm/ml (Wallach and Conley, 1977) are some of the more commonly employed methods for obtaining the infected RBC free from leucocytes. These procedures are helpful in getting rid of only 75 percent of leucocytes. We used slow speed centrifugation in which about 85 percent of leucocytes were removed. Our results on leucocyte removal compare well with the previous study of Zuckerman et al. 1967. Most efficient method for removal of leucocyte is to pass the washed blood through a column of paper powder as reported by Homewood
and Neame (1976), or through a column packed with equal parts of ε-cellulose and microcrystalline cellulose (Beutler et al., 1976). We were able to remove 98.5 percent leucocytes by employing this method. Recently, Mons et al., (1988) demonstrated that the use of a commercially available cell select filter is more effective, rapid and a simpler technique than the use of cellulose powder.

After removal of leucocytes, the next step is lysis of parasitized erythrocytes. In the isolation of parasite, saponin lysis of *P. berghei* infected erythrocytes has provided a useful method for the recovery of intact parasites. The treatment with saponin results in 100 percent lysis of red blood cells causing the release of free parasites. Siddiqui et al. (1978 b) found that saponin is the most efficient non-ionic detergent for hemolysis, as also for removal of proteins and sialic acid from red cell membrane.

The released parasites can be further separated from erythrocyte contaminant by differential centrifugation, enzymatic digestion, free flow electrophoresis and density gradient centrifugation. But parasites can not be completely separated from erythrocyte fragments by using these procedures. In this study, we were able to obtained comparatively contaminant free *P. berghei* parasite material by means of Histopaque density gradient centrifugation and slow speed centrifugation. Purification
of parasite was further confirmed when Leishman stained smears of isolated preparation were microscopically observed.

A very important host cell contaminant is the red blood cell material injected by growing parasites. The cell material is usually localized within the phagocyte (Diggs, 1966). Removal of this contaminant has always been a formidable task. The antigenic material obtained by ultrasonication of parasite (isolated on density gradient centrifugation on Histopaque), showed no host cell contaminant, in immunodiffusion and counter-immunoelectrophoresis, within the sensitivity limits of the tests used for checking such purity. These investigations were successful in obtaining the antigen preparation free from host erythrocyte contaminants. Immunological behaviour of antigen was also checked by employing some serological tests such as immunodiffusion and counterimmunoelectrophoresis.

The isolated antigen and homologus antisera in double immunodiffusion tests on an agarose gel gave at least three precipitin bands. At least one band out of these was quite diffused. This band appeared to be made up of more than one precipitin line. Diggs (1966) has carried out immunodiffusion studies on P. berghei antigen-antibodies system. He was also able to detect five precipitin lines in P. berghei antigen using rabbit antiserum. When mouse antisera were used, Seitz (1975) was able to identify four precipitin line. An apparent disparity
in the number of precipitin bands can be explained on the basis of inherent differences in the nature of antiserum, antigenic preparation and the numerous methods employed for extraction.

The antigenic material further, treated on polyacrylamide gel electrophoresis. This investigation also showed that host cell contaminants were not present in the antigenic preparation.

Fractionation of malaria parasite material was carried out for antigenic analysis. In an earlier study, Chavin (1966) used ion exchange chromatography for fractionation of soluble plasmodial extracts. But the results obtained were not fully satisfactory. D. Antonio et al. (1970) carried out fractionation of *P. berghei* by gel filtration on a Sephadex G-200 column. Fractionation of *P. knowlesi* antigen was carried out by using Sephadex G-200 column (D' Antonio, 1972) or Biogel A 1.5 (Simpson et al., 1974) but none of the above procedures was found very effective. Although some other workers (Hamberger and Zuckerman, 1976; and Grothaus and Kreier, 1980), who used preparative PAGE for fractionation of *P. berghei* antigen were not able to fully characterize the parasite material.

Electrophoretic analysis of immunoprecipitated and biosynthetically labelled malaria protein was achieved through sodium dodecyl poly acrylamide gel electrophoresis (Brown et al., 1982). SDS-PAGE technique has also been used by Grothaus et al. (1984) for characterization of *P. berghei* antigen. They
successfully identified some specific components which were important for the induction of immunity to infection. Wunderlich et al. (1987) have also characterized P. chabaudi parasite and its infected cell host host by employing this technique, and they obtained good results by using this method. We have also used SDS-PAGE for fractionation of soluble extracts of P. berghei. The P. berghei antigen resolved in the eleven protein components showing molecular weight in the range of Mr. 12,000 to 1,50,000 daltons (Sharma et al., 1990).

Reports from studies of several workers explained the efficacy of different immunomodulators to protect mice against lethal infections of P. berghei (Khullar and Sehgal 1990, Beuria et al., 1991). In the present studies attempts were made to protect mice against experimental infections of P. berghei following their immunization with purified antigen in combination with TDM (6'6'-Trehalose dimycolate). TDM had been discovered by Bloch (1950) as a glycolipid, secreted on the surface of mycobacterial cells and called cord "factor". Until recently, TDM was tested mainly in oil whereas aqueous suspension of TDM are now preferred, as being more acceptable, and nontoxic. TDM is often used as an adjuvant for natural vaccines, or more frequently for stimulating non specific host resistance leading to antibacterial, antiparasite, antiviral and antitumor activities in experimental models.
In our experiment, the mice which were inoculated with 500 ug TDM alone developed 100% protection against *P. berghei*. The enhancement of immunity was dependent upon the amount of TDM given to each mouse. One of the advantages of TDM is certainly the long duration of its action. The immunostimulation by TDM is supposed to increase gradually over the weeks. Mice were better protected against *Babesia microti* 7 weeks after an intravenous injection of 200 ug TDM in aqueous suspension, than after 3 or 5 weeks (Clark 1979). These workers have also reported that mice which were inoculated with 10 ug or 50 ug TDM, intravenously were not protected. Olds et al. (1980) also showed that number of *Schistosomulae* recovered from mice treated with 200 ug TDM was reduced, but an intravenous dose of 100 ug per mouse was not protective. TDM in a 1% squalane-in-water emulsion was also found to protect mice against *Toxoplasma gondii*, 4 weeks after intraperitoneal injection (Masihi and Colleagues, 1986). Lederer (1986) also obtained protection in mice by administration of TDM against *P. berghei*, *Toxoplasma gondii*, *Trypanozoma cruzi*, *T. musculi*, *Schistosoma mansoni* and *Mesocestoides corti*. They showed survival rate ranging from 40-100 per cent and the reduction in the infection rate from 30 to 100 percent. Recently, very good percent protection was observed in mice against *P. berghei* by administration of 500 ug TDM (Lederer, 1988). Our findings are supported from studies
of Lederer (1986, 1988) observations from our studies also showed that mice which were injected alone with TDM were negative for *P. berghei* specific humoral and cell mediated immunity. This shows the development of nonspecific immunity against *P. berghei* by administration of doses of 6-6' trehalose dimycolate. TDM has been shown to enhance non specific immunity against various infection.

TDM can also induce local immunity against an airborne, tuberculosis infection. Pimm et al. (1979) also demonstrated that intraperitoneal injection of TDM causes suppression of an ascitic rat tumor. Similar antitumor activity of TDM in mice has also been reported by Sakurai et al. (1988). Yarkoni et al. (1977) showed that after intraperitoneal administration of TDM into mouse, phagocytosis of *L. monocystogenes* by peritoneal macrophages increased. On the basis of the facts, Yarkoni et al. (1977) concluded that TDM activates macrophages. Similarly, Kierszenbaum (1984) observed macrophages activation, a few days after intraperitoneal injection of TDM in mouse. These macrophages produced large quantities of $\text{H}_2\text{O}_2$ following triggering by phorbol myristate acetate (Lepoivre and Colleagues, 1982). On the basis of various reports there are sufficient evidences to believe that non specific protection, in mouse, following TDM administration, is mainly achieved due to macrophage activation.
A more specific type of protection was also clearly noticeable in our animals which were immunized with antigen TDM mixture. The animals which were immunized with antigen-TDM combination showed a very high level of circulating *P. berghei* specific antibodies as detected by ID, IHA and ELISA tests. Animals which were immunized with antigen alone were not able to generate such a high level of antibodies. Kumar and Ahmad (1984) in their experiments on immunizations with Ag plus TDM combination showed more protection compared to animals immunized with antigen alone. Sharma et al. (1985) also reported that animals which were immunized with amoeba-TDM combination had higher titres for amoeba specific antibodies. The level of antibodies was comparatively lower in animals injected with amoeba antigen alone. Lederer (1986) also demonstrated that administration of Ag in combination with TDM in mice gave better response against *P. berghei* infection. Recently, in our laboratory, Pathak (1987) had carried out immunization studies in golden hamsters by inoculating purified *Leishmania* antigen with TDM. The above study showed that animals which were immunized with Ag-TDM combination showed better protection compared to hamsters immunized with Ag alone.

The cell mediated immune response was generated only in animals treated with *P. berghei* antigen in combination with TDM. When antigen-TDM combination inoculated mice were checked
for delayed type hypersensitivity reaction, the infiltration of mononuclear cells was fairly evident. The polymorphonuclear leucocytes played an important role as was evident by leucocyte migration inhibition tests in our studies. Kumar and Ahmad (1984) also reported the development of cell mediated immunity in mice immunized with P. _berghei_ antigen-TDM combination. Sharma et al. (1985) observed a strong CMI response in hamsters sensitized by an amoeba - TDM combination. No CMI response was reported in animals immunized with antigen alone. In our studies, no CMI response was observed in TDM and saline injected animals also. These mice failed to generate any humoral and cellular immune responses. But the animals which were injected with Ag-TDM combination showed well defined humoral as well as cell-mediated immune responses which were mainly responsible for 100 percent protection in mice against P. _berghei_ infection.

There are several other adjuvants which provide good protection in mouse infected with P. _berghei_. Use of glucan as an adjuvant in combination with antigen gave 100 percent protection through development of well defined cellular and humoral immune responses (Kumar and Ahmad, 1985. Maheshwari and Siddiqui, 1989 and Maheshwari and Choudari, 1990). Glucan was also shown to enhance non-specific resistance in experimental animals against various infections but to lesser extent as compared to TDM.
There are numerous reports in the literature demonstrating the protection in mice following the administration of offered Bacillus Calmette Guerin (B.C.G.). It has been successfully used as an adjuvant against large number of infections. Thus BCG has been shown to protect mice against P. berghei (Parashar et al., 1982). Since BCG alone could not afford 100 percent protection in animals, we used TDM in our studies for achieving better protection. TDM seems to enhances host resistance nonspecifically by providing adequate protection to host.

Histopathological studies of various tissues were further carried out to confirm the degree of protection in experimental animals against P. berghei infection. We observed that rodent malaria parasite P. berghei used in this study adversely affects the host vital organs like liver, spleen and kidney, while tissue sections from immunized animal showed no change. Histopathological findings from immunized animals showed no tissue lesions, indicating a normal tissue structure.

Since ages, malaria is a major cause of renal complications (Thayer, 1898). It is reported that malaria impairs the renal function in rodents also (Miller et al. 1967). The histopathological changes recorded in appropriately infected were similar to human malaria (Blainey et al., 1960). Light
microscopy of kidney sections revealed haemoglobin granules in proximal tubules (Boonpucknavig et al., 1973). Tubular damage observed in this study was comparable to glomerulonephritis reported in *P. falciparum* infection (Iseki et al., 1990). In our study we have also observed large and pigmented glomeruli while immunized animals showed no change at all.

As part of the host defence reaction, the proliferative changes in spleen are commonly observed in all human subjects, primates and rodent malaria (Kreier, 1980; Aikawa et al., 1980). The hyperplasia of white and red pulp as a sign of host defence reaction was also observed. White pulp hyperplasia was seen due to the proliferation of endothelial cells, macrophages and lymphoid elements. Neutrophil infiltration was abundant especially in the area of necrosis (Taliferro and Mulligan, 1937). Pigment was seen in macrophages, polymorphonuclear leucocytes and parasitized red blood cells. However the section of spleen showed that the white and red pulp were free from pigment deposition in immunized animal.

Hepatomegaly is a very common feature of malaria pathology in humans (Ash and Spitz, 1945), primates (Boonpucknavig et al., 1984) and rodents (Jervis et al., 1968, Bajpai and Dutta, 1987). Malaria parasites metabolize haemoglobin and produce hemozoin pigment, while another type of pigment,
hemosiderin, is produced by lysis of red blood cells. The excessive deposition of these pigment gave dark brown colouration of liver as observed in this study also (Ash and Spitz, 1945; Jervis et al., 1968; Aikawa et al., 1980). Necrosis in centrilobular region of liver is well established in humans (Desowitz, 1967) and monkeys (Rigdon and Thomas, 1942) and in rodent malaria (Jervis et al., 1968). In the present study centrilobular necrosis has also been observed in heavier infections (60-70%). Parasitized and nonparasitized red cells and other infiltrating cells viz. polymorphonuclear cells and lymphocytic cells, were seen in dilated sinusoids of human cases (Rosen et al., 1967) and in primates (Jervis et al., 1968; Gutierrez et al., 1976). Kupffer cells of liver increased tremendously in numbers and became hypertrophied with emulsified malaria pigment and other phagocytosed substances (Taliaferro and Mulligan, 1937; Brito et al., 1962; Aikawa et al., 1980). Liver pathology and histological changes similar to those reported in primate models have also been observed in this study. Necrosis may be caused by obstruction of free flow of blood through the liver sinusoids. Congestion may be a primary factor responsible for the necrosis and such centrilobular degenerative changes may be due to anoxia (Rappaport, 1963). Our observations also showed that infiltrating cells and kupffer cells occupied the whole of sinusoidal spaces and reduced the blood flow, thus causing anoxia which may have
a damaging effect on the liver parenchyma which ultimately causes tissue necrosis as observed during *P. berghei* infection. Such profound biochemical alterations in the liver were also mainly responsible for tissue damage. This was already confirmed quantitatively (by biochemical estimation) and qualitatively (by some histochemical) studies.

We have carried out histopathological studies in immunized animals also by light microscopy. Sections from immunized animal showed that the tissue architecture was found more or less normal and free from pigment deposition.

After completion of immunization, we have assayed some biochemical parameters in the serum of immunized animals in order to confirm whether immunization of animals, could be, if any help in minimizing the biochemical alterations or not. The enzymes generally used as indicators of liver function tests such as SGPT, SGOT, acid phosphatase and alkaline phosphatases were estimated. During infection, the levels of these enzymes were found increased (Lal and Hussain, 1978), but in immunized animals, SGPT and SGOT levels returned to normal. Khanna et al. (1986) also reported similar findings in their rhesus monkeys. When animals were fully recovered from infection, the activities of both, the enzymes, acid and alkaline phosphatase were found normal.
The results of this study suggest that *P. berghei* infection is of a synchronous nature and can be completely fatal to experimental animals.

Malarial infection causes various structural and functional alterations in different organs of host viz. liver, spleen and kidney. Our results conclude that hepatotoxicity assumes special significance in experimental infection of rodents. The above findings is supported by the fact that liver act as the primary organ for host's homeostasis, and as such various chemical constituents and enzymes of liver including, total lipid, phospholipid, cholesterol, lipid peroxidation, protein, DNA, RNA, liver transaminases and liver phosphatases are invariably altered, significantly. Histochemical studies were also helpful in confirming that massive biochemical changes take place during malarial infection. Similarly, due to liver dysfunction, changes were also observed in serum transaminase and serum phosphatase contents.

Immunization studies were helpful in checking the degree of protection in animals against the infection. Several methods were used for the isolation of *P. berghei* antigen in pure form. These results are largely based on the various methods which were employed for obtaining antigen preparations in this laboratory. During these investigations, we found that histopaque density gradient centrifugation procedure can be
usefully employed for obtaining comparatively pure antigen preparation. Similarly, sodium do-decyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method was found useful for characterization of *P. berghei* antigen. The antigen extracts thus obtained were found immunologically active. Successful immunization of albino mice against *P. berghei* antigen and TDM was carried out. The inoculation of *P. berghei* antigen alone was capable of generating only a weak humoral and cellular response in immunized animals. Our results indicate that *P. berghei* antigen must be used in combination with a potent adjuvant for obtaining better results. The animals immunized with Ag-TDM combination showed good humoral and cellular immune responses following challenge with, *P. berghei* parasites, showing 100 percent protection. In our investigations, injection of TDM alone also showed 100 percent protection. Therefore TDM also provides non-specific resistance to the host as evident from our observations.

The most striking feature in our study was that at high parasitaemia, contents of serum phosphatase and serum transaminase were altered due to liver dysfunction. But in immunized animals these values were near normal. These results further indicate a good correlation between host's resistance to infection and the availability of these enzymes.
The malarial infection adversely affected the host's vital organs such as liver, spleen and kidney. Pathological lesions in the mouse liver infected with *P. berghei* were identical to those observed in primates, human malaria due to *P. knowlesi* and *P. falciparum* infection. Whereas histopathological studies in immunized, protected animals showed normal tissue architecture, free from pigment deposition.

In brief, the various experiments performed during this work showed that infected animals showed massive biochemical alterations in tissue and serum. Similar tissue alteration were also observed in histopathological examination of the infected liver. Such alterations were not seen when similar examination were carried out in immunized animals.

It can be concluded from the results of various experiments that immunization studies might be helpful in minimizing the pathophysiological changes in animals infected with *P. berghei*. 