ABSTRACT
Malaria in itself is a devastating human malaise, but cerebral malaria is its most perilous offspring. In the symptomatology and pathogenesis of malarial fevers of man, no other phenomenon appears more ominous than that of cerebral malaria. Its onset always carries grave implications and in most cases the condition is progressive and death is the outcome, unless prompt and adequate treatment is initiated.

In order to fully understand the pathogenesis and other aspects of the disease, it is necessary to develop a reliable laboratory model for the study of cerebral malaria. The need for these studies is as pressing today as it was in the past, particularly in view of the high mortality from the disease.

In the present study an attempt has been made to investigate and study some selected aspects of this serious and often fatal condition known as cerebral malaria. The extent of involvement of brain tissue during the course of a laboratory induced cerebral malaria was investigated in Plasmodium knowlesi infected rhesus monkeys. In spite of the prohibitive cost and other restrictions, the use of non-human primates was found essential for the proposed study of this nature. Because of their specific biochemical resemblances the use of these primates was found indispensible due to their evolutionary kinship to man, particularly when their anatomical and behavioural resemblances were taken as important parameters
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, viz: brain capillary obstruction, adhesion of the infected red blood cells to the wall of the capillaries and small vessels, swelling of the vessel endothelium and plugging of the capillaries by parasitized and un-parasitized erythrocytes. Furthermore, we found involvement of the brain tissue, despite the fact that the peak parasitaemia in the peripheral blood smears was only 4-5%. An interesting finding of this investigation is the demonstration of malaria parasite in the capillary endothelial cells and their occasional presence in astrocytes. Although cerebral capillaries are implicated as the principal vessels involved in fatal falciparum malaria, our studies revealed that venules and veins also contained many parasitized red blood 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 RBCs, whereas adjacent zone was virtually devoid of parasites.

Most of our studies in light microscopy were later confirmed by the electronmicroscopic observations, although electron microscopy failed to locate the parasitic infiltration of the glial cells. In light microscopy, only occasional parasite deposits were found. The knob-
like protrusions which are responsible for the 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 the parasitized red blood cells conform directly to the endothelium and some of them attach to the extension of the endothelial cells.

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 phagacytosed parasitized erythrocytes and other inclusion bodies. Quite often, macrophages were found in intimate contact with and attached to endothelium, but in some instances the intervening cell membrane was not quite identifiable, indicating the possibility that exocytosis of lysosomal contents could have occurred. Another interesting electronmicroscopic finding was the presence of platelets in the vicinity of macrophages and parasitized erythrocytes in the sections from choroid plexus. However, it was not possible to assign any significance for their presence. As we know that platelets along with fibrin are essential components of thrombi and we have found at some instances occlusion of blood vessels, therefore it may be possible that platelets have a certain role to play in this phenomenon. Further indepth investigations are certainly required before some conclusions can be drawn in this regard. In some monkeys the brain sections also revealed
lipofuscin pigment deposits.

We had also carried out light and electron microscopic studies from various regions of eye to find out its involvement during the course of cerebral malaria. The present study clearly demonstrated a parasitic infiltration of virtually every region of the eye. This study confirms and further supports our earlier finding of retinal haemorrhages. We have demonstrated a near complete occlusion of the blood vessels of retina by sequestrated parasitized erythrocytes. On the basis of our studies, we tend to suggest that retinal pathology may be the expression of an immunological intolerance/dysfunction.

Malarial infection in experimental animals is associated with various biochemical changes. There have been reports indicating alterations in the lipid metabolism in infected liver, spleen, kidney and brain in malaria infected animals. Lipids are essential components of cellular structures and are the major constituents of the brain. In order to investigate some biochemical parameters related to lipid metabolism, we also included P. berghei - mice model system in this study. The histopathological studies of the sections of infected mice brain revealed parasitic infiltration. The parasitic infiltration was not found as high as was observed in P. knowlesi - rhesus monkey model. The biochemical studies demonstrated a sizeable decrease in total lipids, phospholipids, cholesterol and ganglioside contents.
in the cerebellum, cerebrum and brain stem. Similarly, a remarkable increase in the rate of lipid peroxidation was observed in the cerebellum and brain stem of mouse brain following malarial infection. The increase in the rate of lipid peroxidation appears to contribute significantly to the reduction of the lipid levels in various areas of brain.

The killing of intracellular and extracellular pathogens by phagocytes is due in part to the production of oxygen radicals, namely superoxide, hydroxy radicals and possibly singlet molecular oxygen. In recent years, workers are engaged in assessing the possible role of these highly reactive products of oxygen termed free radicals in the pathogenesis of malaria and other parasitic infections. The results of 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. The experiments carried out during the course of this study have demonstrated sizeable decrease in the superoxide dismutase (SOD) activity of infected mouse brain compared to un-infected control group. The SOD activity is said to be the natural defence mechanism against oxidative damage to the tissues. Low levels of this enzyme during malarial infection would clearly make the tissue all the more prone to oxidative damage.
Numerous mechanisms have been postulated to account for the events leading to the development of cerebral malaria. None of the earlier hypothesis provided a clear picture. Based on our light and electron microscopy and some biochemical findings, and as also gleaned from the previous work, it could be concluded that the pathogenesis of cerebral malaria is a complicated process. Therefore, it is rather difficult to assign one single reason to describe its severity, on the contrary it is the outcome of an array of factors. On the basis of our studies and published findings we suggest that pathogenesis of cerebral malaria is the outcome of a triad, consisting of mechanical obstruction of the blood capillaries brought about by parasitized erythrocytes, combined with certain biochemical events (involvement of free radicals in particular) and the immunological dysfunction, all the three acting in concert.

To investigate the role of immunity in protection, or in minimizing the course of pathogenic events in cerebral malaria, rhesus monkeys were immunized against *Plasmodium knowlesi* whole antigen in combination with Muramyl dipeptide (MDP) as an adjuvant. The intracellular parasite, *P. knowlesi*, was isolated from the infected RBC by means of a multistep experimental protocol. The infected erythrocytes collected in an anticoagulant, ACD, were washed three times with chilled normal saline. For the removal of leukocytes, slow speed centrifugation was used. The washed erythrocytes were lysed by treatment with 0.2% saponin. Parasites were separated
from this lysate by density gradient centrifugation on histopaque. The purity of isolated parasite preparation was checked microscopically. For the preparation of antigen, the isolated parasites were disrupted by ultrasonication at 9 KHz for 20 min. The purity of the isolated \textit{P. knowlesi} antigen was checked by passing it through polyacrylamide gel electrophoresis. In PAGE the position of electrophoretically separated parasite proteins, along with host RBC proteins, showed that antigen preparation was free from host erythrocyte contaminants. Immunogenicity of the isolated antigen was checked by IHA against monkey sera and human immune sera obtained from falciparum malaria cases. The estimated ratio of protein : carbohydrate in the whole antigen sample was found as 18.7 : 1.

The animals were immunized in different batches by using \textit{P. knowlesi} antigen and the adjuvant. Indirect haemagglutination (IHA) and Enzyme Linked Immunosorbant Assay (ELISA) tests were used for the detection of humoral immune response. Highest reciprocal titre value of 512 was observed in the animals immunized by WAg-MDP by IHA and that of 1024 by ELISA. The antigen and control animal groups showed some slight antibody titres. Development of CMI in immunized monkeys was demonstrated by making use of DTH and LMIT. Skin reactions in animals sensitized with WAg-MDP combination showed a well developed zone of erythema with induration of 10.8 mm after 24 hrs. of intradermal injection. Animals immunized with WAg-MDP showed a maximum migration inhibition
of 43.8%. All the animals in experimental and control groups showed patent infection following challenge with live parasites. Monkeys receiving WAg-MDP combination showed survival rate of 50%. Animals belonging to the control groups showed 100% mortality.

Histopathological studies were carried out to detect the changes in the tissue architecture of the immunized and infected control animals. By and large, the normal tissue architecture was found to be intact in the liver, spleen, kidney and brain of the immunized animals compared to the infected control group. Sections from brain of infected control animals revealed that the blood vessels of cortex and medulla were completely plugged with parasitized erythrocytes. Parasitic infiltration was found in all the regions of the brain. However, there was no involvement of the brain tissue in the immunized animals following challenge. There was no parasitic infiltration even in the brains of immunized monkeys which died after the challenge. On the basis of our findings, it is suggested that whatever may have been the limitations in our vaccination attempts to prevent an infection, but it certainly provided a satisfactory protective cover as far as fatal involvement of the brain tissue is concerned.