Acute phase response in mammals is recognised as a normal homeostatic immediate physiological response to injury or infection (Stadnyk, et.al., 1991). Its primary role is to maintain the integrity of the tissue by restricting damage to the injured site. The acute phase response (APR) in inflammation may be divided into two phases: the local response, involving coagulation, kinin generation, phospholipid metabolism with vasodilation and cellular emigration, and the systemic response including fever, leukocytosis, changes in the concentrations of plasma heavy metals, and increased plasma levels of a number of hepatocyte derived proteins along with modifications to amino acid pools (Kushner, 1982; Koj, 1985; Gauldie (in press); Milanino et.al., 1986). Endocrine changes include increases in levels of circulatory cortisol, glucagon, catecholamines and thyroid hormones. The net sum of these acute changes is a general enhancement of body metabolism including increased protein catabolism, increased gluconeogenesis and a negative nitrogen balance. The experimental evidence adduced in the present study suggests generation of acute phase reaction in parasitic infections (viz. experimental malaria and experimental filariasis) as well.

**EXPERIMENTAL MALARIA:**

Malaria associated pathology is mainly due to three factors: high parasite density and consequently, massive amount of malarial pigment production, destruction of mature and immature
erythrocytes during the intra-erythrocytic development of the malarial infection; and hyperactivation of host-defense system. Hepatomegaly has been recognised as most common and specific symptom of malarial pathology in humans, primates and rodents. Most of the workers have reported accumulation of malarial pigment (haemozoin) within reticuloendothelial system (RES) of liver (Ash & Spitz, 1945; Jervis et al., 1968; Aikawa et al., 1980; Saxena et al., 1981). It is known that infection of P. falciparum in monkeys as well as P. berghei infection in mice and rats (Rao et al., 1969; Jervis et al., 1968, 1972; Saxena et al., 1981) leads to significant increase in wet weight of liver without significant change in its dry weight. Ramakrishna et al. (1950) and Rao et al. (1969) have suggested that increased liver weight associated with no change in dry weight was due to edema. In the present study, wet weight of liver was significantly increased by (54%; P < 0.001), (70%; P < 0.001) and (110%; P < 0.001) at 10 - 15%, 30 - 35% and 55 - 60% parasitaemia, respectively. At the same time however, it was found that the dry weight of liver registered a very modest increase of (3.6%; P < 0.02), (7%; P < 0.02) and (12%; P < 0.001) at 10 - 15%, 30 - 35% and 55 - 60% parasitaemia respectively. This modest increase in liver dry weight might be due to high level of lipid and malarial pigment accumulation in the liver. Increased wet weight of P. berghei infected liver of M. natalensis was observed to associate with loss of body weight of the host with rising parasitaemia. Similar diminution of host's body weight
has been reported earlier by Maegraith (1954) in rats infected with *P. yoelii* at the maximum parasitaemia. She suggested that the reduction in body weight was due to damage of healthy erythrocytes leading to insufficient O₂ supply to different organs and anemia. Loss of body weight may also be due to poor ingestion leading to semistarvation of the infected animals during the course of *P. berghei* erythrocytic infection.

Hepatic pathology as a consequence of parasitaemia has further been revealed by biochemical studies. Lipid concentration in the liver of *M. natalensis* infected with *P. berghei* was found to be increased by 15%, 40% & 82% at 10 - 15%, 30 - 35% and 55 - 60% erythrocytic parasitaemia, respectively. An effort has been made in the past to understand the underlying causes for hyperlipidemic liver due to malarial parasitaemia. Liver plays a decisive role in the metabolism and transport of lipids, as well as in the maintenance of lipid levels in the liver and circulating blood. During an acute phase response, tumor necrosis factor has been proposed to mediate the hypertriglycerideremic response to infection by either increasing hepatic lipid synthesis or decreasing clearance of triglyceride rich particles through inhibition of lipoprotein lipases. Krauss, et al. (1990) demonstrated that within 90 min of administration of recombinant human TNF-α to rats, there was a rapid increase in plasma levels of very low density lipoproteins, apoprotein and lipid composition, as assessed by nondenaturing gradient gel electrophoresis. Their data suggested that the initial site of
TNS s metabolic effects is liver and the resulting increases in secretion and metabolic processing of VLDL may represent an early manifestation of the acute phase response. Another reason of lipid deposition in liver could be a depressed rate of mitochondrial fatty acid oxidation, leading in turn to excessive feedback inhibition of hepatic triglycerides. In this regard, the fact that the mitochondria of malaria infected liver suffer structural damage supports the above view (Fletcher & Maegraith, 1962; Maegraith, 1966). Malarial infection was also associated with decreased level of cholesterol content in liver. Seshadri et al. (1983) have observed a significant depletion of liver cholesterol content in monkeys infected with P. falciparum. The present study has shown that liver cholesterol content in M. natalensis was decreased significantly by (6%), (11%) and (21%) at 10 – 15%, 30 35% and 55 – 60% parasitaemia respectively. Seshadri et al. (1983) have tried to understand the aetiology of reduction of hepatic cholesterol during malarial infection. They have suggested that it might be due to an increased uptake of haemoglobin by the infected erythrocytes. We have not attempted to understand mechanism underlying decrease of liver cholesterol content in M. natalensis during the course of P. berghei erythrocytic parasitaemia.

Malarial infection was observed to induce alterations of not only lipid and cholesterol metabolism, but also of carbohydrate metabolism in mastomys liver. Acute inflammation, over a period
of 12 - 24 h has been reported to induce the liver to respond to the associated cytokine exposure by modulating its carbohydrate metabolism (Stadnyk et al., 1991). An increased glycogenolysis would lead to rapid and significant decrease of glycogen & total carbohydrate content of the liver. In the study presented here, total carbohydrate content was observed to decline by 16%, 47% and 77% at 10 - 15%, 30 - 35% and 55 - 60% parasitaemia, respectively. Several workers (Homewood, 1977; Homewood and Naeme, 1980; Saxena et al. 1981; Seshadri et al. 1983) have tried to suggest physiological basis of reduction of hepatic carbohydrate during plasmoduim infection. They surmise that since intra-erythrocytic stages of malarial parasites have no carbohydrate reserves (Sherman, 1983) and therefore they obviously derive and consume their nutritional share from the host's reserve carbohydrate for their rapid growth and multiplication (Mckee, 1951). It is well known that liver happens to be the chief store house for carbohydrates. The frequent use of host's carbohydrate reserves by the parasite ultimately results in the exhaustion of the same. Homewood and Naeme (1980) have further shown that the carbohydrate transport within malarial parasite, is however, increased during its erythrocytic infective phase. However, acute phase response augments this mobilisation of carbohydrate reserves to glucose which in turn is used by the parasite for its metabolism.

Decrease in hepatic glycogen content by 51%, 80% and 88% at 10-15%, 30-35% and 55-60% parasitaemia, observed in the present
study confirms earlier reports of Geofforion et.al. (1985) and Srivastava et.al. (1984) on depletion of host liver glycogen in mosaomys during erythrocytic P. berghei infection. Picture of glycogen depletion in liver due to malarial infection is known to be associated with mobilisation of glucose from host liver. In the present studies, decrease of liver glucose content was observed in M. natalensis during the course of P. berghei erythrocytic parasitaemia. It was decreased by (47%), (76%) and (81%) at 10 - 15%, 30 - 35% and 55 - 60% parasitaemia, respectively. It is known that glucose is very important for metabolism of plasmodium (Phillips, 1984). Mouse erythrocytes, infected with P. berghei are found to consume considerably more glucose, than the normal red blood cells (Bowmen et.al.1961). Glucose is readily metabolized by intraerythrocytic stages of P. berghei concomitant with the enhanced entry of non-metabolizable sugars into the parasitized erythrocytes (Homewood and Naeme, 1983). In other words, such an increased sugar influx is restricted to the parasitized red blood cells, and not found in the non-parasitized ones in the malaria infected animals. The influx takes place due to change in malaria infected cells, which may include both an increase in the simple diffusion component, as well as a modification in the carrier mediated portion of the entry processes. However, the details of such a possible mechanism are still unknown. Phillips (1984) suggested that in order to make the host’s glucose available to the parasite, it must pass through the red blood cell membranes and the membranes
of the parasitophorous vacuole. Such a transfer of glucose might be facilitated by increased permeability of parasitized erythrocytes membrane. It has been known that red blood cells and the malaria infected erythrocytes depend upon the metabolism of glucose to lactate by anaerobic glycolysis (Homewood & Naeme, 1983), such an increased metabolism of available glucose to lactate by the multiplying parasite, may have been the causative factor for hypoglycemia in the host.

Parasitaemia not only altered lipid and carbohydrate metabolism as discussed above, but also significantly changed protein metabolism of the host. Total protein content of liver in *M. natalensis* was observed to decrease by 13%, 26% and 40% at 10 - 15%, 30 - 35% and 55 - 60% parasitaemia, respectively. Besides the erythrocytic parasitaemia induced hepatic damage led to increase in level of serum transaminases. SGPT increased by 62%, 86% and 108% while SGOT increased by 21%, 66% and 86% at 10 - 15%, 30 - 35% and 55 - 60% parasitaemia, respectively. Similar findings have also been reported earlier by Sadun et al. (1965), (1983) during malarial infection. They have suggested that increased level of serum transaminases might be related with the lysis and destruction of healthy erythrocytes during intra-erythrocytic stages of malarial infection.

Serum picture of transaminases, discussed above was observed to correlate with changes in hepatic tissue transaminase (viz,
GPT and GOT), during malarial infection. Liver GPT decreased by (5%), (11%) and (16%) while GOT level decreased by (9%), (21%) and (32%) respectively at 10 - 15%, 30 - 35% and 55 - 60% parasitaemia. These results support the earlier findings made by Lal & Hussain (1978), who also observed decrease in liver GPT & GOT in mice during malarial infection. The significantly decreased levels of GPT and GOT activity found in liver of P. berghei infected M. natalensis, probably indicates dysfunction of liver during infection.

Our data on experimental malaria, demonstrating loss of body weight, increased protein catabolism, glycogenolysis, hepatomegaly and fatty infiltration in the infected liver at higher parasitaemia strongly suggest that malarial infection should elicit acute phase protein response. Experimental data on accumulation of acute phase proteins in the serum of M. natalensis following P. berghei infection demonstrated that indeed acute phase proteins (viz., α-2 macroglobulin and α-1 acid glycoprotein) are induced during malarial infection. However, the real inducers of acute phase proteins have been recognised to be IL-1, IL-6 and TNF arising from hyperactivated macrophages (Baumann, et al., 1990). It is now known beyond doubt that malarial fever and rigor is a result of this hyperimmune reaction and production of interleukin-1. Interleukin-1 has been known in the past to induce acute phase response in several mammalian species (Dinarello, et al., 1984). Miller et al. in 1951 showed that liver is the major organ for
the synthesis of acute phase proteins & therefore it was considered of interest to follow the synthesis of acute phase proteins by hepatocytes isolated from mastomys at > 40% *P. berghei* erythrocytic parasitaemia. Our in vitro study showed that acute phase proteins are indeed synthesized by hepatocytes at > 40% parasitaemia. The present study, thus demonstrates induction of acute phase response during experimental malaria. A study involving human volunteers infected with various Plasmodium spp showed significant increases in the plasma fraction (probably PI) (Klainer, et.al., 1968) and similar changes were detectable in naturally acquired disease (Klainer, et.al., 1969). It would appear to be manifestation of the parasitic infection induced acute phase reaction. It is now known that malarial infection not only affects the host liver in various ways but also induces an activation of its reticuloendothelial system (RES). Activation induces many changes in the cells, among which are: production of TNF, IL-1 and IL-6, the cytokines that exert multiple effects on the host. Of these effects, the most important is the elicitation of the hepatic acute phase response, which is accompanied by leukocytosis and the production of acute phase proteins. On the other hand, animals infected with *P. berghei* show suppression of the liver P-450 enzyme systems (Tripathi, et.al. unpublished) and reduced albumin levels (Tripathi, et.al. unpublished). While cytochrome P-450 levels were not measured during *P. berghei* infection, albumin synthesis decreased as was observed during serum analysis as well.
as during in vitro synthesis by hepatocytes. These two parameters could well be taken as negative acute phase markers since their levels decrease during *P. berghei* infection.

**EXPERIMENTAL FILARIAISIS:**

*A. viteae* infection in mastomys is an example of murine asymptomatic filariasis with distinct prepatent, patent and latent stages, simulating human filarial infection. When sera from all the three stages (i.e., prepatent, patent and latent stage) of *A. viteae* infection were evaluated by crossed immunoelectrophoresis and SDS-PAGE, acute phase proteins started to appear during prepatent stage, while patent stage infection showed peak levels of acute phase reactants. During latent stage the level of acute phase reactants declined. Demonstration of acute phase response during *A. viteae* infection suggests induction of IL-1 and IL-6 molecules. IL-6 molecules are believed to be produced by macrophages and a class of Th-2 cells following T-cell stimulation by the antigen presenting cell (Decker, 1990; Mosmann et al. 1986; Cherwinski et al. 1987). IL-6 would thereby qualify as mediator of inflammation when acute phase proteins are the markers. This was further confirmed by in vitro studies where synthesis of acute phase proteins by hepatocytes was followed by autoradiography during patent stage of *A. viteae* infection. The results demonstrated induction of two major acute phase proteins, alpha-2 macroglobulin and alpha-1 acid glycoprotein. On the other hand cytochrome P-450 and other
drug metabolizing enzymes were assayed to monitor the effect of *A. viteae* infection on their activities. It was observed that cyt P-450 levels decreased by (18%), (22%) and (37%) respectively in prepatent, patent and latent stages of infection while arylhydrocarbon hydroxylase and glutathione s-transferase did not show significant changes. Similar observations have recently been reported by Stadnyk and Gauldie (1991) who have used two natural nematode parasites of rodents, *Nippostrongylus brasiliensis* and *Trichinella spiralis* for their studies. They reported that animals infected with *N. brasiliensis* show suppression of the liver P450 enzyme system (Tekwani, et.al. 1987), a biphasic reduction in the host's appetite coincident with lung and intestinal pathology (Ovington, et al. 1985) and reduced albumin levels (Ash, et al. 1985). Induction of acute phase response leads to several fold increase in synthesis of what are called positive acute phase proteins. However, synthesis of certain other proteins like cyt P-450, albumin etc., whose synthesis declines during acute phase response have been recognised as negative acute phase markers. It would therefore, appear that acute phase response is indeed induced in mammals during parasitic infections.

It would appear that acute phase response arises from the hyperactivated macrophages due to stimulated immune system. Now interleukins or cytokines are recognised as the soluble mediators of this response (Baumann et al., 1990). To demonstrate that the parasite stimulated host lymphoid system stimulates the acute
phase protein synthesis in liver, it was considered of interest to investigate the effect of sera (from the parasite infected malaria and filaria and conditioned media from Con A stimulated splenocytes) on primary hepatocyte culture.

IN VITRO MODEL FOR MONITORING ACUTE PHASE RESPONSE:

A major aspect of the current research on the hepatic acute phase response focuses on the cellular and molecular mechanism by which the expression of acute phase proteins is regulated in liver cells. Most studies in this direction rely on tissue culture experiments, since regulatory processes can be assessed under more defined conditions in vitro than in vivo. In search of a suitable experimental tissue culture system, many investigators have selected cultures of adult hepatocytes. Advantages are the nontransformed phenotype of the cells and the ease by which a large number of differentiated cells can be isolated and maintained in culture. Specific culture conditions have been devised that better preserve the adult phenotypes in long term cultures (Enat, et.al. 1984; Fraslin, et.al. 1985; Grieninger, et.al. 1978 and Isom, et.al. 1985). In the study presented here, efforts were made to develop a suitable model for studying the differentiated functions of hepatocytes in vitro on the one hand and then to follow the effect of parasitic infections on acute phase protein response on the other. During the course of the study, several differentiated functions of hepatocytes viz, albumin synthesis, cytochrome P-450, oxygen
uptake etc., were studied under defined culture conditions.

Oxygen uptake measurements were carried out to determine the integrity of the hepatocyte plasma membrane on the one hand and to monitor the effects of antioxidants on survival of hepatocytes under the normoxic conditions in primary culture on the other. It was observed that after 7 days culture in synthetic medium plus DMSO and Se, substantial number of hepatocytes remained viable and consumed measurable oxygen. The antioxidant properties of DMSO may be explained by describing it as an oxygen radical sink, while Se has proven antioxidant properties and so the protection afforded by its addition to medium is suggestive of role of antioxidants in hepatoprotective function. Similarly supplementation of the culture medium with DMSO had dramatic effects on the synthesis of albumin and maintenance of cytochrome P-450 in vitro. While addition of DMSO not only increased albumin synthesis but also albumin secretion by the cells, removal of cysteine from the medium and addition of DMSO supplemented synthetic medium protected the cells from the loss of cytochrome P-450 content for upto 7 days. The mechanism of action of DMSO is not very well understood but several theories have been proposed. Because DMSO penetrates biological membranes (Jacob, et.al. 1964), the drug may function as a carrier for specific nutrients and hormones. DMSO also alters the structure of proteins and nucleic acids and as such, may directly alter gene expression. In its ability to maintain differentiation of normal adult hepatocytes, DMSO may act directly by controlling
expression of differentiated liver specific gene products or indirectly by altering hepatocyte responsiveness to other components such as hormones or nutrients.

Although it is possible to maintain hepatocytes in differentiated state in a medium supplemented with hormones growth factors and DMSO in short term cultures, further biochemical analysis is required to determine the normality of these hepatocytes with regard to other liver specific functions, such as hormonal induction of specific enzymes, maintenance of cytochrome P-450 and ability to activate chemical carcinogens. During an acute phase response, however, as optimal regulation of acute phase protein genes has to be achieved within 24 h, the deterioration of the cell phenotype noted after days in culture are less problematic (Koj, et.al. 1984). Indeed experiments, on primary mouse and rat hepatocytes, for instance, have demonstrated that prominent stimulation of a variety of acute phase proteins can be accomplished in short-term cultures. However, even in those experiments, concurrent loss in the expression of proteins other than the measured plasma proteins does occur, which in turn might influence the regulation of acute phase proteins (Aiello, et.al.1988; Clayton, et.al.1983; Jefferson, et.al.1984). In the study presented here, primary hepatocyte cultures were used as a model to follow the synthesis of acute phase proteins in vitro. Culture supernatants from splenocytes (prepared during patent stage of *A. viteae* infection) when added to hepatocytes monolayers led to increased synthesis
of acute phase proteins and depressed level of albumin. Similarly supernatants from *P. berghei* infected splenocytes enhanced synthesis of acute phase proteins by hepatocytes. Similar results have been obtained by Koj et al. (1984) who have used different cytokine preparations from human monocytes to follow the synthesis of acute phase proteins by hepatocytes. They showed that cytokines consistently increased the synthesis of \( \alpha_1 \) macroglobulin and fibrinogen and depressed that of albumin.

The findings presented here therefore provide some evidence that acute phase proteins are indeed synthesized by hepatocytes during *P. berghei* and *A. viteae* infections.

Questions regarding mechanisms involved in the acute phase response remain to be answered. Among these is the question of how acute phase states not involving lipopolysaccaride are mediated. Bacterial infections are only one of a large number of possible stimuli, suggesting that other endogenous monocyte stimulators must exist. Investigations on the molecular basis of the acute phase response have only begun to elucidate a myriad of fascinating intercellular regulatory events. The fascinating problem of why some proteins behave as strong acute phase reactants in animals and not in man, or vice versa, needs immediate attention. A better knowledge of the pathophysiological mechanisms of the acute phase response will improve the treatment of inflammatory diseases in man.