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Full Length Research Paper

Biochemical changes in the plasma of vervet monkeys (*Chlorocebus aethiops*) experimentally infected with *Trypanosoma brucei rhodesiense*

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Biochemical evaluation of plasma during disease conditions gives an indication of the functional status of the various body organs. Biochemical analysis of plasma from 32 vervet monkeys (*Chlorocebus aethiops*) infected with *Trypanosoma brucei rhodesiense* revealed at various stages of infection, dramatic increases in blood urea nitrogen, triglycerides, cholesterol and tissue enzymes including creatine kinase (CK), alkaline phosphatase (ALKP), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH). Serum levels of total protein however showed a decline one week after infection but returned to control levels while creatinine increased then declined to control levels. The changes in total proteins, enzyme levels and albumin indicate severe tissue damage, metabolic abnormality and the development of liver and kidney malfunctions similar to those reported in sleeping sickness patients. This demonstrates that the vervet monkey is a good model to study various aspects of the disease. The changes in total proteins, albumin and triglycerides could also have important implications on the pathogenesis of African trypanosomosis and the effectiveness of treatment.

Key words: Trypanosomes, biochemical changes, monkey model.

INTRODUCTION

Human African trypanosomosis (HAT) caused by *Trypanosoma brucei (T. b.) rhodesiense* is an emerging health problem (Kabayo, 2002; Waiswa et al., 2003; WHO, 2005). Unfortunately HAT is not given the attention it deserves in terms of drug development and also research in understanding the pathogenesis and pathology of the disease (Truc, 2003), with the aim to treatment and control of the disease.

Biochemical changes in humans and animals during trypanosomosis have been documented (Anosa, 1988a, b). It has been suggested that serum biochemistry might give an indication of the degree of damage to the host tissue as well as the severity of infection (Otesile et al., 1991; Cano et al., 2004). However, the HAT biochemical changes data available in trypanosome-infected humans is limited (Basson et al., 1977). The data is difficult to conclusively analyze since it is obtained from human studies that have not been conducted under experimentally defined conditions (Jenkins and Robertson, 1959a, b). This results from the fact that it is unethical to conduct experimental infection and carryout studies in human volunteers. This therefore means that the pathological studies can only be carried out using animal models (Farah et al., 2005).

Trypanosome-infected vervet monkey has been used as a model of HAT (Ndungu et al., 1994; Gichuki and Brun, 1999; Ngure et al., 2000). When vervet monkeys (*Chlorocebus aethiops*) are experimentally infected with *T. b. rhodesiense*, they develop both clinical and pathological changes that closely mimic the infection in hu-

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man. The infected monkeys develop an acute disease syndrome characterized by rapid onset of fever, enlargement of the spleen and lymph nodes, severe anaemia, rapid weight loss and death within 6 week (Schimidt and Sayer, 1982).

Although the biochemical changes have been used as indicators of tissue damage, specific organ functional defect and response to therapy during disease (Otesile et al., 1991; de Souza et al., 2000; Manga-Gozalez et al., 2004), they have not been exhaustively studied in the *T. b. rhodesiense* monkey model. Most of the studies in human trypanosomosis have been carried out using the chronic form of infection resulting from infection with *Trypanosoma brucei gambiense* (Abenga and Anosa, 2005; Awobode, 2006).

The present study investigates the biochemical changes in the plasma of vervet monkeys acutely infected with *T. b. rhodesiense* as indicators of tissue pathology and functional defects in organs.

MATERIALS AND METHODS

Animals

The 24 adult vervet monkeys, of both sexes and weighing between 3.5 and 4.5 kg used in this study were bought from the Institute of Primate Research (IPR) in Kenya, which maintains a breeding colony. They were initially housed in a quarantine facility for 90 days where they were screened for evidence of diseases, including zoonoses and trypanosomosis. During that time they became accustomed to handling and staying in individual cages. They were fed twice a day on commercial pellets, fresh fruits, and vegetables and water was provided *ad libitum*. Before the study, the animals were transferred to experimental wards where they were allowed to settle down for another 2 weeks before infection.

Research approval to carryout the present study was obtained from the Institutional Animal Care and Use Committee, and the animals used were cared for and used humanely throughout the study.

Trypanosomes

The *T. b. rhodesiense* stabilate used was KETRI 2537, a derivative of EATRO 1989, which was isolated from a patient in Uganda by direct inoculation of blood and lymph node aspirate into a monkey (Fink and Schmidt, 1980) and later cryopreserved. Before being used to infect monkeys, the stabilate was passaged once in irradiated mice to raise the required number of trypanosomes for infection.

Blood sampling and analysis

The collection of blood and determination of the various plasma analyte levels using the Vettest 8008 analyzer (VeTest S. A., Switzerland) was as described by Ngure et al., (2000). Briefly, the animals were sedated by intramuscular injection with ketamine hydrochloride at a dose of 10 mg/kg body weight. Blood was collected from the inguinal vein and 2 ml immediately put into a tube containing heparin. In order to minimize the effects of exogenous lipids on plasma triglycerides and cholesterol, the animals stayed for at least six hours without food before sampling. Blood was centrifuged at 100 xg for 5 min, plasma was separated and stored at 20°C before analysis.

Experimental design

Twenty of the vervet monkeys in the current study were infected by one intravenous injection with approximately 10^4 trypanosomes in the inguinal vein. Before and during the course of the infection, daily clinical evaluation of each animal was done and the presence of trypanosomes determined from ear-prick blood. At weekly intervals the animals were anaesthetized, weighed, a detailed clinical examination carried out, and blood was collected. The blood was used for biochemical analysis. Four monkeys were kept as uninfected controls. When the animals were terminally sick at 6 weeks post-infection, they were euthanized and tissue samples collected for histopathological studies.

RESULTS

Clinical findings

The animals developed clinical signs of disease 5 to 7 days after infection, coinciding with the first wave of parasitaemia. These were characterized by dullness, reduced appetite, raised haircoat, periorbital oedema observed as swelling of the eyelids, enlargement of the spleen and superficial lymph nodes, progressive anaemia, rapid wasting and weight loss ranging from 0.5 to 1.2 kg. Trypanosomes appeared in the blood on day 5 of infection and the parasites persisted in circulation throughout the study.

Plasma biochemistry

Total protein and albumin concentration

The mean total protein concentration in uninfected animals ranged from 5.1 ± 0.5g/d1 (mean + 1SD) to 6.0 ± 0.5 g/dl while that of albumin was 3.1 ± 0.6 g/d1 to 3.7 ± 0.7g/dl and did not change significantly throughout the experimental period ($p \geq 0.05$) (Figure 1). Following infection there was no significant change in the levels of total proteins. Albumin concentration on the other hand significantly decreased rapidly from week two of infection reaching 1.8 ± 0.2 g/d1 ($p \leq 0.05$) by week 6 of infection when the experiment was terminated.

Triglyceride and cholesterol concentration

Triglyceride and cholesterol levels in blood are shown in Figure 2. The concentrations of both parameters showed marked animal-to-animal variations throughout the experimental period. The mean triglyceride concentration in uninfected animals ranged from 65.9 ± 9.5 mgd/1 to 93.5 ± 22.0 mg/d1 and did not differ significantly through out the experimental period ($p \leq 0.05$). After infection, the plasma triglyceride concentrations significantly increased rapidly from week one of infection to peak 358.3 ± 16.2 mg/d1) 21 days later. The significantly high triglyceride

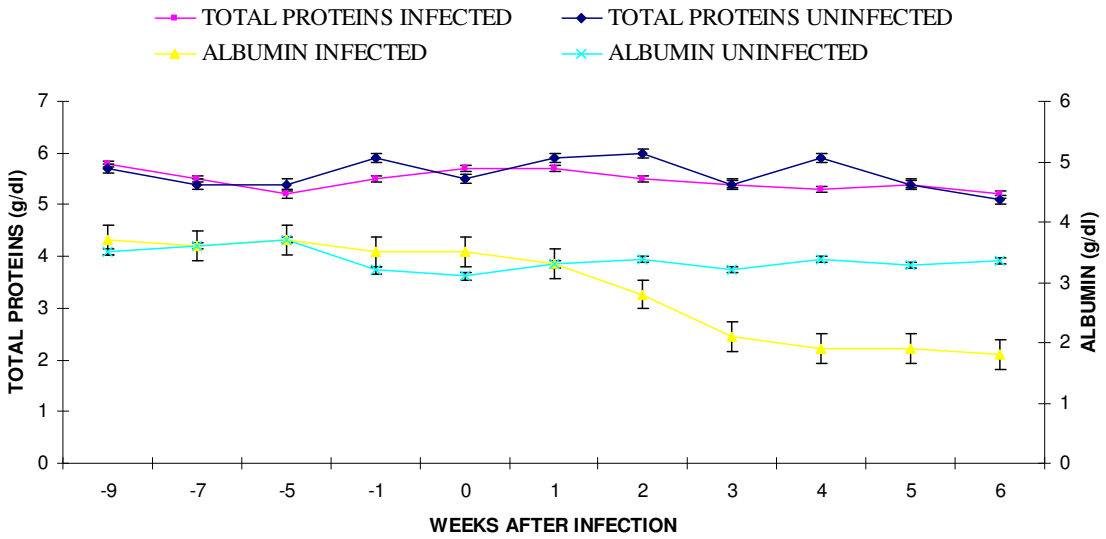

Figure 1. Changes in plasma total proteins (g/dl) and albumin (g/dl) levels in *Chlorocebus aethiops* (vervet monkeys) infected with *Trypanosoma b. rhodesiense.*

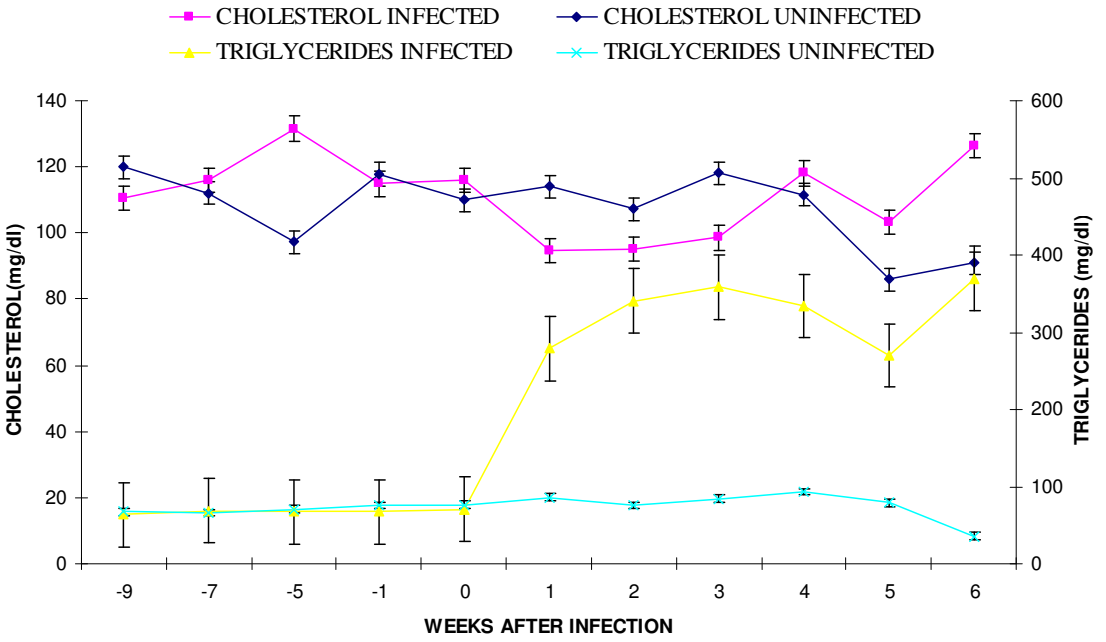

Figure 2. Changes in plasma cholesterol (mg/dl) and triglycerides (mg/dl) levels in *Chlorocebus aethiops* (vervet monkeys) infected with *Trypanosoma b. rhodesiense*.

concentration persisted throughout the experimental period ($p \leq 0.05$). The cholesterol level in uninfected animals ranged from 86 ± 14.7 mgd1^{-1} to 118.2 ± 9.6 mg/dl and did not change significantly during the experimental period ($p \leq 0.05$). Infection caused a transient fall in cholesterol from week one of infection and continued for 3 weeks post-infection. However, on the fourth week of infection the cholesterol concentration significantly and persistently increased till termination of the experiment six weeks post infection ($p \leq 0.05$).

Blood urea nitrogen and creatinine concentration

The concentrations of BUN and creatinine are shown in Figure 3. The BUN and creatinine in uninfected monkeys ranged from 6.7 ± 1.1 to 11.5 ± 1.9 mg/dl and 0.4 ± 0.1 to

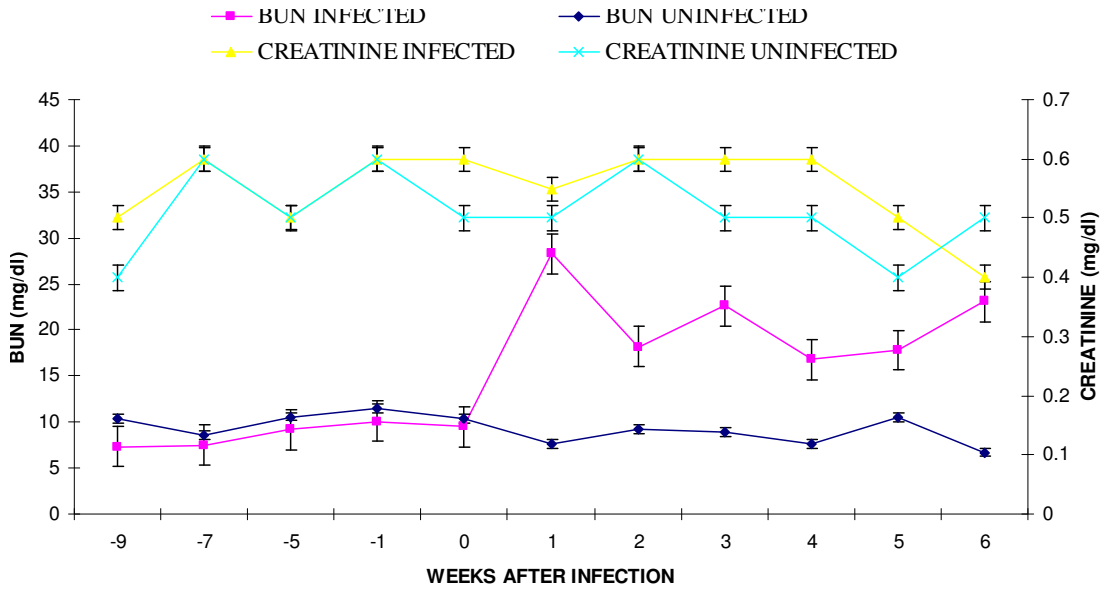

Figure 3. Changes in plasma blood urea nitrogen (BUN) (mg/dl) and creatinine (mg/dl) in *Chlorocebus aethiops* (vervet monkeys) infected with *Trypanosoma b. rhodesiense*.

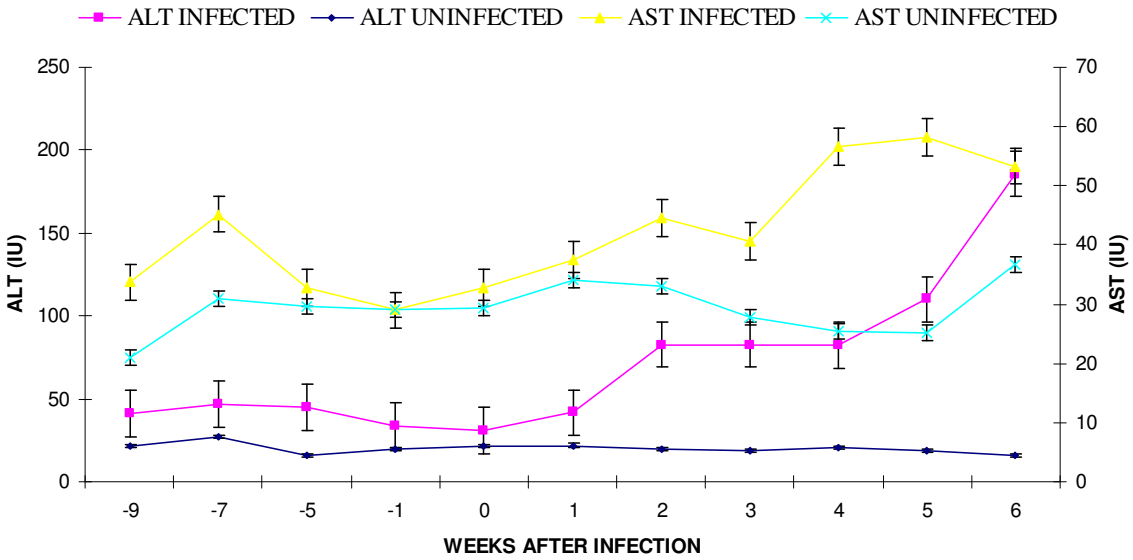

Figure 4. Changes in plasma alanine amino transferase (ALT) (IU) and aspartate aminotrasferase (AST) (IU) levels in *Chlorocebus aethiops* (vervet monkeys) infected with *Trypanosoma b. rhodesiense*.

0.6 ± 0.9 mg/dl, respectively. Values of both parameters in the uninfected monkeys did not vary significantly during the experimental period. However after infection, although there were fluctuations of BUN concentrations, the levels increased significantly starting first week of infection and persistently remained elevated throughout the infection ($p \leq 0.05$). However, the concentrations of creatinine increased significantly during the first week of infection ($p \leq 0.05$) but these levels thereafter declined to pre-infection levels where they were maintained during the remaining experimental period.

Tissue enzyme concentration

There was observed individual variation of enzyme levels in each animal and also between the different tissue enzymes. The tissue enzyme concentrations in uninfected animals did not vary significantly during the infection ($p \leq 0.05$). All the tissue enzymes analyzed showed significant increases in plasma concentrations in infected

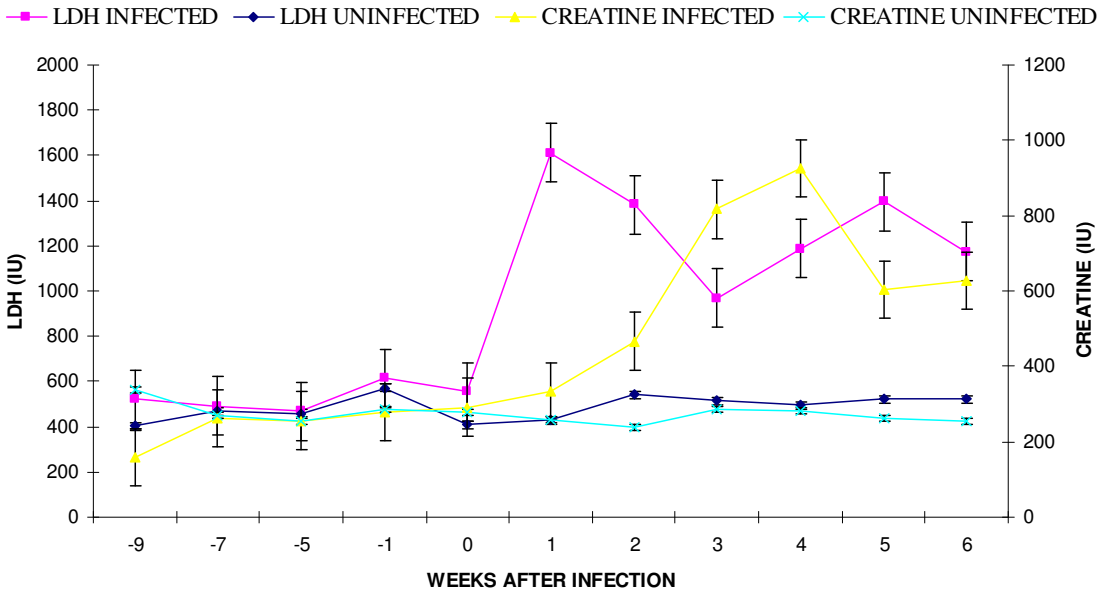

Figure 5. Changes in plasma lactate dehydrogenase (LDH) (IU) and creatine kinase (IU) levels in *Chlorocebus aethiops* (vervet monkeys) infected with *Trypanosoma b. rhodesiense*

animals with progress of the disease. The degree of increase was however dependent on the specific tissue enzyme. The aminotransferases (AST and ALT) plasma concentrations increased significantly from week 2 of infection ($p \leq 0.05$) (Figure 4). However the increase in ALT was more marked compared to AST.

The concentrations of LDH and CK are shown in Figure 5. LDH increased significantly from week 1 and remained persistently elevated during the period of infection ($p \leq 0.05$). CK concentrations on the other hand gradually increased from week 1 of infection to reach peak levels in week 4 of infection. This was followed by a slight decrease that was still significantly higher than the controls. The CK levels were however significantly elevated in all infected animals throughout the infection ($p \leq 0.05$).

The concentrations of ALKP are shown in Figure 6. The concentrations in ALKP in the infected animals increased significantly starting one week after infection and remained elevated throughout the experimental period ($p \leq 0.05$).

DISCUSSION

These studies demonstrate that infection of vervet monkeys with *T. b. rhodesiense*, results in an acute disease syndrome accompanied by a wide range of biochemical changes in plasma, including increases in plasma levels of blood urea nitrogen, triglycerides, cholesterol and tissue enzyme, and decreases in the levels of albumin. Creatinine showed an increase and then returned to control levels while total proteins decreased then reverted to control levels. Although the route of infection by intravenous injection differs from the scenario in man where infection is by tsetse fly infection, the Biochemical changes were similar.

The elevation in triglycerides is in agreement with that observed in rabbits infected with *T. b. gambiense* (Diehl and Risby, 1974) and humans infected with *T. b. rhodesiense* (Huet et al., 1990). However, the hypercholesterolaemia observed in this study, with the exception of an in initial decrease, is in contrast to a hypocholesterolaemia reported in humans infected with *T. b. gambiense* (Huet et al., 1990; Awobode, 2006). Inconsistent changes in cholesterol levels following trypanosome infection have been observed as a rise in rabbits inoculated with *T. brucei* (Goodwin and Guy, 1973), a slight decrease in rats infected with *T. b. rhodesiense* (Dixon, 1967), and a decrease in ruminants infected with *Trypanosoma congolense* and *Trypanosoma vivax* (Roberts, 1975). Changes in cholesterol therefore appear to depend on the infecting trypanosome species and the host. The increase in plasma triglyceride concentrations is similar to the hypertriglyceridaemia observed in rabbits infected with *T. b. brucei* (Rouzer and Cerami, 1980) and humans infect-ed with *T. b. gambiense* (Huet et al., 1990). The increase in triglycerides is due to marked inhibition of the adipose tissue enzyme lipoprotein lipase (LPL) responsible for clearing lipids from plasma, a process that is markedly inhibited by tumor necrosis factor (TNF) (Beutler and Cerami, 1988). Marked increase in pro-inflammatory cytokines including TNF has been observed in the plasma of *T. b. rhodesiense*-infected vervet monkeys (Maina et al., 2004) and could be responsible for the observed changes. The changes in total proteins and albumin concentrations in this study are consistent with observations in sleeping sickness patients who develop severe hypoalbuminaemia and/or

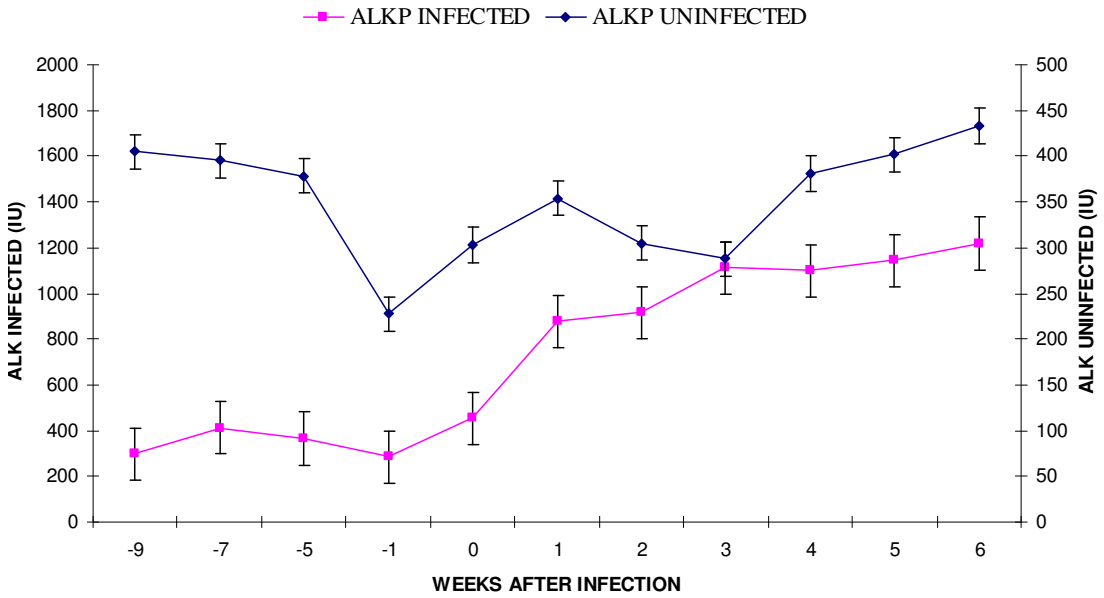

Figure 6. Changes in plasma levels of alkaline phosphatase (ALKP) (IU) levels in *Chlorocebus aethiops* (vervet monkeys) infected with *Trypanosoma b. rhodesiense*

hypoproteinaemia (Jenkin and Robertson, 1959a; Abenga and Anosa, 2005; Awobode, 2006). The cause of the decrease in albumin is difficult to elucidate. Albumin is a negative acute phase protein during trypanosomosis (Karori et al., 2008). Its decrease could result from reduced synthesis in the liver as part of the acute phase response, loss through the kidney and intestine or increased utilization by the trypanosomes as a nutrient, since they require it for optimal survival (Coopens et al., 1987). Thus since infection of monkeys resulted in high parasitaemia, increased utilization by parasites probably contributed to the decrease in albumin. It is unlikely that a defective hepatic failure in replacement of the albumin was the only cause. This is due to the long plasma half-life of albumin of 26 days, which could have resulted in a slower depletion of albumin in such a case. Certain trypanocidal drugs such as suramin, being polyanions, have a high binding capacity for lipoproteins and albumin (Muller and Wollert, 1976). The binding of suramin to albumin enables it to remain in plasma of patients for a number of weeks following a single intravenous admini-stration (Goodman and Gilman, 1970). Thus a decrease in albumin concentration in the blood of infected patients and monkeys could affect the pharmacokinetics of such drugs, affecting their availability to trypanosomes and leading to therapeutic failure.

BUN is a by-product of protein catabolism while creatinine is released during muscle metabolism. Increased BUN and creatinine levels are consistent with results from infection of monkeys infected with *T. b. rhodesiense* (Sadun et al., 1973) and human infected with *T. b. gambiense* (Awobode, 2006). BUN and creatinine are products cleared from the body through the kidneys and as such their measurement during disease are good indicators of renal function (Ramakrishnan et al., 1995). The causes of elevated BUN levels include kidney disease such as glomerulonephritis and excessive protein catabolism and febrile conditions. Fever and glomerulonephritis are common features of trypanosomosis and presumably act together to elevate BUN. Similar defects in renal function during trypanosomosis have been observed in man (Basson et al., 1977). Indeed, gross and histological changes affecting kidneys have been demonstrated in trypanosome-infected dogs (Murray et al., 1975) and humans (Anosa, 1988a, b), which could explain the observed changes in kidney function in the present study. Tissue enzymes are found in the cytoplasm or mitochondria and are released into circulation due to changes in cell membrane permeability or frank necrosis (De Souza et al., 2000). The rise in tissue enzymes observed in plasma from the monkeys in this study is consistent with those observed in trypanosome-infected humans (Basson et al., 1977; Anosa 1988a, b; Awobode, 2006).

This increased plasma tissue enzyme may be related to organ damage, particularly the liver, muscles and kidney reported to occur during trypanosomosis (Anosa, 1988a, b). Monitoring changes in serum tissue enzymes has been of diagnostic value in judging the extent of tissue injury in pathological condition in animals (Manga-Gonzalez et al., 2004) and humans (Adams et al., 1991; De Souza et al., 2000; Rastogi, 2006).

The pathological changes in skeletal muscle, cardiac muscle and liver among other organs, could explain the increased enzyme concentrations in plasma of infected monkeys.

The present study has demonstrated that vervet monkeys infected with *T. b. rhodesiense* undergo biochemical changes similar to man, and thus can be used to study aspects of trypanosome infection in man. The biochemical changes that have been reported could have a significant effect on the efficacy of treatment with drugs that are bound to plasma albumin.

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