ASSESSMENT OF THE ACUTE PHASE RESPONSE OF BABOON AND MOUSE MODELS TO *SCHISTOSOMA MANSONI* INFECTION

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A thesis submitted to the Graduate School in partial fulfilment of the requirements of the Degree of Master of Science in Biochemistry of Egerton University

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DECLARATION AND RECOMMENDATION

I hereby declare that this is my original work and has not been presented for any award in any institution or university.

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DEDICATION

For Caesar. You made it all possible.

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ACKNOWLEDGEMENT

This work is a testament to the Lord Almighty's infinite grace, love and faithfulness. He has been my all in all.

I would also like to thank everyone else who made the completion of this work possible:

First, to Dr. R.M. Ngure. Not only did you conceive this work, but you also nurtured it with true and tireless dedication. Your constant support saw me through the tough times.

To Dr. Dorcas Yole. For going the extra mile to make it better for me. Your strength and virtue will forever be an inspiration to me.

To the entire team at Institute of Primate Research. For all your technical help, your friendship and for holding the fort for me when I had to be away. Fred Maloba and Kiio Kithome; I could never have done it without you. Many thanks also to Sammy Kisara, Mary Nzuve, Fred Nyundo, and Richard Korir.

To my friends in the department of Biochemistry and Molecular Biology: Steve Gikuru, Nelson Wanyonyi, Francis Mwangi, Stella Bundotich, Judy Kavulani and Japheth Kakiya. And to those in the Chemistry Department who went out of their way to help me in the chemical analyses, especially Dr. Ouma Omollo, S.M. Kariuki and Bernard Onyiego.

Finally, I would like to thank my entire family for their support. And most especially, my dear husband, Dr. Caesar Mungatana.
For always believing in me, and for giving me the chance to carry out this study.

ABSTRACT

Schistosomiasis is a disease of grave concern through out the world because of its high morbidity and mortality rates. Success of treatment usually depends on the extent of damage already caused by the infection at the time of diagnosis. There is therefore great need for studies that may aid in the early diagnosis of the disease, before irreversible damage has already occurred, and in the effective management of treatment. During the acute phase response, which takes place at the very beginning of the inflammatory process, there are changes in concentrations of a large number of serum proteins and cations. These changes are attributed to the host response to tissue injury and are therefore useful indicators for the course and severity of a disease. In this Study, sequential serum specimens from baboon and mouse models infected with *S. mansoni* were collected throughout the course of acute infection and following treatment with praziquantel, a drug curative for *Schistosoma mansoni*. Sampling was also done in baboon models following post-treatment challenge by a second infection. In these specimens, haptoglobin was measured by ELISA, while albumin and the cations (iron, zinc and copper) were measured spectrophotometrically. In addition, the acute histopathology of the infected mouse models was studied for any correlations with changes in the serum proteins and cations. The results of the study showed that both mice and baboon models develop a similar acute phase response following infection with *S. mansoni* cercariae. In both models, there was an increase in haptoglobin and copper concentrations; and a decrease in albumin, iron and zinc concentrations following infection. Haptoglobin appeared to be the better indicator of early infection and residual tissue pathology. Similar changes, though notably less pronounced, were observed in the baboons that were challenged. The post-infection changes were seen to recover following curative treatment. This correlated well with reducing tissue damage and inflammation evidenced in the histopathological studies of treated mice. The findings of this study have enhanced the understanding of the acute phase response, with particular focus on the diagnostic and prognostic potential of haptoglobin, albumin and the serum cations, in *S. mansoni* infection. Further detailed studies involving a greater population of primate models, more frequent sampling times and a wider variety of analytes would be useful in validating such assays for use in human medicine.

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ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of variance
APP	Acute phase proteins
APR	Acute phase response
CNS	Central nervous system
Cp	Ceruloplasmin
CRP	C-reactive protein
ELISA	Enzyme linked immunosorbent assay
H & E	Hematoxylin and eosin
Hb	Haemoglobin
Hp	Haptoglobin
IL	Interleukin
SAA	Serum amyloid A
SAP	Serum amyloid P
SEA	Soluble egg antigen
SEM	Standard error of mean
SWAP	Soluble worm antigen preparation
WHO/TDR	UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases
TMB	Tetra methyl benzidine
WHO	World Health Organization
LPS	Lipo poly saccharide
TNFα	Tumor necrosis factor alpha

CHAPTER ONE

1.1 Introduction

Schistosomiasis, also known as bilharzia or snail fever, is a disease of humans caused by parasitic flatworms called schistosomes or blood flukes. Valuable information on human schistosomiasis may be obtained by studying the acute phase response (APR) of the disease using animal models.

Within the spectrum of systemic reaction to inflammation, two physiological responses in particular are regarded as being associated with acute inflammation. The first involves the alteration of the temperature set point in the hypothalamus and the generation of the febrile response. The second response, on which this study was based, involves alterations in metabolism and gene regulation in the liver (Dinarello, 1984).

During the acute phase response, the biosynthetic profile of the liver is radically altered. Under normal circumstances, the liver synthesizes a characteristic range of serum proteins at steady state concentrations. Many of these proteins have important physiological functions and higher serum levels are produced during the acute phase response following inflammatory stimulus (Pannen & Robotham, 1995).

Among the acute phase proteins (APPs) that increase in serum concentration are the metal-binding proteins, haptoglobin (Hp) and ceruloplasmin (Cp). Albumin, which is the largest single serum protein fraction in the healthy individual, decreases in the acute phase. The systemic reaction also includes phenomena such as leukocytosis, and altered iron, zinc and copper concentrations in blood (Alsemgeest *et al,* 1994).

If the changes in APPs and serum cations during acute schistosomiasis of animal models are well characterized, they may be usefully extrapolated for clinical application as diagnostic indicators, prognostic indicators and for monitoring the course of infection and response to treatment in humans.

1.2 Statement of the Problem

Because of its high morbidity and mortality rates, schistosomiasis is a worldwide health concern. It is estimated that at least 200 million people are currently infected with

schistosomes and another 600 million are at risk of infection from the five species affecting man (WHO/TDR, 2002).

Drug treatment is still the principle method of control (WHO/TDR, 2002). However the degree of recovery from the infection depends on the extent of the damage caused by the infection. If extensive visceral fibrosis has already occurred, this cannot be reversed and there is permanent liver damage (Mohamed-Ali *et al,* 1999)

Since the liver is a vital organ in the body, it is of utmost importance that the disease be diagnosed in its early stages before irreversible damage occurs. Current methods of diagnosis are based on identification of scant eggs in the stool for *Schistosoma mansoni* and *Schistosoma japonicum* or in the urine for *Schistosoma haematobium.* Intradermal skin tests have also been used but only indicate positive after infections of 4-8 weeks (Elliot, 1996).

This study undertook to characterize various changes in serum analytes that occur in the course of the acute phase response of *Schistosoma mansoni* infection. This will be invaluable in helping to validate their use for detecting sub-clinical disease, determining disease severity and monitoring the response of the infection to treatment.

1.3 Objectives

1.3.1 General Objective

To study various aspects of the acute phase response of *Schistosoma mansoni* infection using well established baboon and mouse models.

1.3.2 Specific Objectives

- To measure albumin and haptoglobin in sequential serum samples collected from the animal models during the course of acute infection and following drug therapy.
- To measure iron, zinc and copper in sequential serum samples collected from the animal models during the course of acute infection and following drug therapy.
- To study the histopathological changes of infected animal models and relate them to changes in the mentioned serum proteins (albumin and haptoglobin) and cations (iron, zinc and copper).

- To evaluate the diagnostic and prognostic potential of the mentioned serum proteins and cations in acute *Schistosoma mansoni* infection.
- To compare trends in the mentioned serum proteins and cations during acute *Schistosoma mansoni* infection of the baboon and the mouse models.

1.4 Hypotheses

1.4.1 During the acute phase response following *Schistosoma mansoni* infection of the baboon and mouse models, there is a characteristic increase in serum copper & haptoglobin concentrations, and a depression in serum albumin, iron & zinc concentrations.

1.4.2 These changes reduce gradually following effective treatment and reach normal haematological values when tissue trauma and inflammation cease.

1.5 Justification of the Study

Schistosomiasis infects in excess of 200 million people and results in severe morbidity and mortality. It also causes considerable economic losses and frequently interferes with development projects, particularly water projects such as dams and irrigation schemes. It is therefore imperative that effective strategies for the control of transmission, morbidity and mortality be developed.

In April 2002, the WHO joint meeting of the expert committees on the control of schistosomiasis and soil transmitted helminthes made recommendations for research to address control needs. Among the recommendations made was the development of improved passive case detection and assessment of response to therapy.

This study examined the changes in serum concentrations of albumin, haptoglobin, iron, zinc and copper during the acute phase response (APR) of *Schistosoma mansoni* infection. This could help in defining their use as additional tools in early detection of the disease, even before the appearance of definitive signs and symptoms. Results of the study will also be useful in determining the potential of these acute phase reactants for assessing disease severity and tissue damage, and at the same time, monitoring the success of treatment. Measurement of these acute phase reactants may therefore provide a means to better the prognosis of the disease.

1.6 Scope and Limitations

The study was carried out using two schistosome animal models: mice and baboons. Mice were used for preliminary studies because of their relative availability and low-cost of maintenance. They also allow frequent sampling because of the relative ease with which the sampling procedure (heart puncture) is carried out. However, there are significant differences between human and murine schistosomiasis. The direct extrapolation of results obtained in the mice experiments to human applications can therefore be hindered unless they are reflected in the primate experiments.

Baboons are the most commonly used non-human primate models in schistosomiasis (Nyindo *et al,* 1999). Results obtained from baboon experiments are subsequently closely reflective of the disease process in humans. However, baboons are very costly and not as readily available, and therefore the study only utilised small sampling populations of five baboons per each of the two baboon experiments. Sampling times were also further apart than in the mice experiments because of the ethical implications of administering anaesthetics to the baboons during sampling. Serial histopathological examination of the tissues was only possible in the mice experiments because mice could be sacrificed at each sampling point. Baboons on the other hand were only sacrificed at the end of the experiments.

The study only investigated the acute response of two proteins and three cations. A more comprehensive study would involve studying a larger number of acute phase proteins and serum ions during acute schistosomiasis, and using a larger number of animals. However, due to the constraints of time and money, this was not possible.

CHAPTER TWO

LITERATURE REVIEW

2.1 Schistosomiasis

Schistosomiasis is a disease of humans caused by parasitic flatworms called schistosomes, or blood flukes. There are 3 main species of schistosomes infecting humans: *Schistosoma mansoni, Schistosoma japonicum* and *Schistosoma haematobium.*

Table 1: Major Species of schistosomes causing human disease (Lucey, 1993)

S. mansoni	- Found in Africa and South America - Located in mesenteric veins draining colon of host - Snail hosts are *Biomphalaria* species including *pfeifferi* and g*labrata*
S. haematobium	- Found in Africa - Located in veins draining the urinary bladder plexus of host - Snail hosts are *Bulinus* species including *physopsis*
S. japonicum	- Found in South East Asia - Located in mesenteric veins draining ileum of host - Snail hosts are *Onchomelania* species

2.1.1 Classification

The three major species of *Schistosoma* parasitic to man, *S. mansoni, S. haematobium and S. japonicum,* all belong to the family *Schistosomatidae.* These are relatively unusual Digeneans, as they have separate sexes, and adult forms are located within the circulatory system. The cercariae are also directly infective to the definitive hosts, including humans. They fall under the subfamily *Schistosomatinae* and the genus *Schistosoma* (Rollinson & Simpson, 1998).

2.1.2 Epidemiology

It is estimated that 200 million people are currently infected with schistosomiasis and another 600 million are at risk of infection (WHO/TDR, 2002). Schistosomiasis is one of the most widespread human parasitic infections only second to malaria in socio-economic and public health importance in tropical and subtropical areas (WHO, 1988). It is endemic in 74 tropical developing countries, in whose rural areas it constitutes the most prevalent water-borne disease (WHO, 1985). In Kenya, *Schistosoma mansoni* is found in isolated foci in Machakos, Kitui, Muranga, Kirinyaga, and Kiambu and along the shores of Lake Victoria and the Tana and Athi rivers (Highton, 1974).

Schistosomiasis is a complex of parasitic infections acquired by man in a wide variety of freshwater habitats and transmitted by specific aquatic or amphibious snails. The distribution of these host snails defines the geographical range of endemic schistosomiasis. People acquire the infection by entering freshwater in which infected snails have shed schistosome larvae (cercariae) that can actively penetrate human skin. The cycle is completed when infected people pass eggs of the parasite in faeces or urine and these eggs reach the water to hatch and liberate a second larval type, the miracidium. The miracidium is capable of finding and penetrating vector snails, where it completes its development into the infective larvae. Thus, the schistosomes' lifecycle and transmission is tied to snail vectors, human water contact and unsanitary conditions (Damian *et al,* 1976).

2.1.3 Pathology

The pathology associated with schistosome infection arises primarily from the schistosome eggs, both those in the intestine and those swept back by the blood to the liver, and on occasion to other tissues such as the brain and lungs. At least 50% of the eggs laid fail to reach the exterior. Single sex infections, which have no egg production, cause little pathology (Sadun *et al,* 1970). Clinical signs of the disease take on different phases, mainly the acute and the chronic.

2.1.3.1 Acute Schistosomiasis

The initial stage of the acute phase is often known as Katayama fever. It is normally found in young children/young adults or foreigners with no previous exposure to the disease (Butterworth *et al,* 1989). The acute reaction is in response to the sudden high level of antigen exposure and is usually associated with the onset of egg deposition. Clinical symptoms consist of skin-rashes, asthma-like episodes, daily fever, malaise, diarrhoea, swollen lymphnodes and aching joints, in addition to a number of other unspecific symptoms. Pathologically, it presents in the liver as florid granuloma with a rich infiltration of eosinophils. Frequently, heavy infections can lead to fibrotic chronic schistosomiasis or the death of the patient (Dasilva & Carillo, 1992).

2.1.3.2 Chronic Schistosomiasis

In chronic schistosomiasis, the patient experiences diarrhoea and fever. In children the infection can depress their growth rate. The infection also leads to enlargement of the liver and spleen. Fibrosis of the liver can result in portal hypertension, hepatosplenomegaly, ascites and oesophageal varices leading to fatal haematemesis (Cheever, 1968).

Schistosomiasis is regarded as severe when there is granuloma formation, mainly in the liver, as often happens in the acute phase. After egg deposition, which occurs primarily in the periportal area, the egg, becomes surrounded by a dense infiltrate composed mainly of lymphocytes, macrophages and a variable number of eosinophils, held together in an extra-cellular matrix. Granuloma formation is mediated by the T-cells (Goes *et al,* 1994).

Eggs measure up to 70μm in width and therefore cannot transverse the capillary beds as the blood flows through the liver. Granuloma size and cell composition vary depending on the schistosome species, host species, intensity and duration of the infection. The tissue location can also have an impact on granuloma size. The principle factor however is how immunosuppressive the host is to the schistosome egg antigen (Dessein *et al,* 1988). Although some of the tissue response is due to the physical presence and damage by the egg,

the majority of the pathology is due to the host response to the soluble egg antigens (SEA) which are released through sub- microscopic egg shell pores (Goes *et al,* 1994).

LiVolsi *et al,* (1989) described the pathology and clinical features of schistosomiasis in humans as follows: Ova deposition results in obliterative fibrosis of portal vein branches and portal tract fibrosis. This, in turn, causes prominent portal space enlargement, described macroscopically as "pipestem" (Symmer's) fibrosis. Liver function remains normal until late in the disease. Common findings include splenomegally with hypersplenism and pancytopenia.

2.1.4 Diagnosis

The most obvious method of diagnosis is the identification of eggs in the stool for *Schistosoma mansoni* and *Schistosoma japonicum*, and urine for *Schistosoma haematobium*. For these types of diagnosis, a simple faecal smear is often inadequate; therefore, some type of egg concentration technique is required. If no eggs are found, but the outward symptoms are suggestive of schistosomiasis, then rectal, liver or bladder biopsies may be necessary (Ripert, 2000).

An intradermal skin test using schistosome antigen has also been found to be a fairly sensitive immunological test. But this test will not show positive until the patient has been infected for 4-8 weeks. In other instances, the circum-oval precipitation test has also been found to be fairly sensitive and is particularly useful for determining success of treatment, because the test becomes negative after all the eggs in the tissues have been killed. The test depends on observing the formation of a precipitate around isolated schistosome eggs in the presence of sera from infected individuals (Elliot, 1996).

2.1.5 Treatment and Control

Drug treatment is still the principal method of control and the drug of choice is Praziquantel (WHO/TDR, 2002). Praziquantel works by causing severe spasms and paralysis of worm's muscles. It incites damage to the parasite's skin internally such that the parasite disintegrates and is removed by the host's immune system (Ripert, 2000). At the molecular level, it seems that the contraction of the schistosome musculature is due to an interference with inorganic ion transport mechanisms, resulting in a decreased influx of K^+,

but an increase in influx of Ca^{2+} and Na^{+} into the worms. The precise molecular sequence of events is still not understood and remains under investigation (Sturrock *et al,* 1993).

The degree of recovery from the infection, however, depends on the extent of the damage caused by the infection. If extensive fibrosis has already occurred, this cannot be reversed and therefore there is permanent liver damage (Mohamed-Ali *et al,* 1999). In addition, the efficacy of praziquantel seems to depend on the patient's prior exposure to the disease. Recently, it has been found that certain populations not previously exposed to schistosomiasis, respond poorly to praziquantel treatment as their immune systems are not primed. It seems that the mode of action of praziquantel is such that to be fully effective it requires the participation of the patient's immune system (Ripert, 2000).

In mice and primates infected with *S. mansoni* and given curative doses of praziquantel, a striking feature observed was the rapidity of resolution of cellular reactions and fibrosis in tissues containing eggs (Webbe *et al*, 1981).

Other methods of control, other than with drug therapy, which have been tried, include drainage of marsh areas where snails breed, improved sanitation, education, the use of molluscicides and introduction of bio-control agents such as predatory snails. However, these methods, though helpful to a slight extent, have not produced satisfactory results. In addition, there is no effective vaccine as yet (Chitsulo, 2000).

2.1.6 The Baboon and the Mouse as Models for Human Schistosomiasis

Valuable information on human schistosomiasis has been provided using primate models (Johansen *et al,* 2000). Damian *et al,* (1992) found that the baboon reproduces the acute schistosomiasis syndrome synchronously and repetitively. The baboon is in fact the most frequently used non-human primate in schistosomiasis research for a variety of reasons, as described in a review by Nyindo & Farah (1999). Baboons acquire natural infections (Fenwick, 1969), develop disease and acquire protective immunity similar to that in man (Nyindo & Farah, 1999). Moreover, the baboon is not an endangered species, being widely distributed in Eastern Africa where it is regarded as a pest by farming communities. Extensive studies have been conducted on the parasitological, pathological and immunological responses of baboons to experimental schistosome infections (Farah *et al,* 1997; Muller-Graf *et al,* 1997; Farah *et al,* 2000b; Cheever *et al,* 2002).

The other commonly used animal models for the study of schistosomiasis are the laboratory mice. Their popularity is attributed to their availability, fecundity, low cost and susceptibility to experimental infection (Farah, 2000). They also permit more frequent bleeding intervals, since at each sampling point, the mouse has to be sacrificed, as opposed to repeated anaesthetizing in baboons. This is very useful when close sampling intervals are required, as in this study. Laboratory maintenance of mice is also far much cheaper than baboons. This makes them the models of choice for preliminary studies, which are then extended to primate models before they can be tried in humans.

2.2 The Acute Phase Response

The term 'acute phase response', (APR), refers to the inflammatory response of the host occurring shortly after any tissue injury (Kushner, 1982; Dinarello, 1984; Baumann & Gauldie, 1994; Raynes, 1994; Pannen & Robotham, 1995; Koj, 1996). The APR is non-specific by nature: the origin of the injury can be infective, immunologic, neoplastic, traumatic, or parasitic (Kushner & Mackiewicz, 1987; Stadnyk & Gauldie, 1991). In its narrow sense, the term APR refers to the changes in concentrations of a large number of serum proteins that are associated with the host response. These changes are predominantly the result of alterations in the pattern of protein synthesis in the liver (Pannen & Robotham, 1995).

The magnitude and type of the acute phase protein response is dependent on the host and on the stimulating factor. Bacterial infections and other infections where tissue invasion is involved are amongst the most potent stimulators (Whicher & Dieppe, 1985). The purpose of the APR is to prevent further injury to the organ, to isolate and destroy the infective organism, to remove the harmful molecules and debris, and to activate the repair processes that are necessary to return the organ to its normal function (Dinarello, 1984; Baumann & Gauldie, 1994). From a teleological point of view, APR is a primitive event that helps to permit the survival and maintain physiological homeostasis during the period following injury (Dinarello, 1984).

An acute phase response detected by an increase in the serum concentration of acute phase proteins, has been documented following several parasitic infections in mice including *Trypanosoma cruzi (*Scharfstein *et al,* 1982; Araujo-Jorge *et al,* 1992; Luz & Araujo-Jorge,

1994), *Trypanosoma brucei brucei* (Pluschke *et al,* 1986; Shapiro & Black, 1992; Ngure *et al*, 1997), *Schistosoma mansoni* (Pepys *et al,* 1979), and *Nippostrogylus brasiliensis* (LaMontagne *et al,* 1984).

The APR is part of the non-specific immune response, and its various components are relatively consistent despite the large variety of conditions that induce it. The APR is later followed by the specific immune response, which in contrast is selective (Hirvonen, 2000).

2.2.1 Production of APPs

Acute phase proteins (APPs) are a large and varied group of glycoproteins in the serum, which are unrelated to immunoglobulin. They are synthesized mainly by liver parenchymal cells and released into the bloodstream in response to a variety of stressors such as local inflammation, bacterial & parasitic infection, endotoxin injection, neoplasia and thermal or mechanical injury, as part of the acute phase of inflammatory reaction.

Inflammatory mediators regulate the synthesis and release of serum APP from liver. These mediators fall into four major categories; interleukin-6-type cytokines, interleukin-1-type cytokines, glucorticoids and growth factors. Cytokines mainly stimulate the APP gene expression while glucorticoids and growth factors function more as modulators of cytokine action (Baumann & Gauldie, 1994).

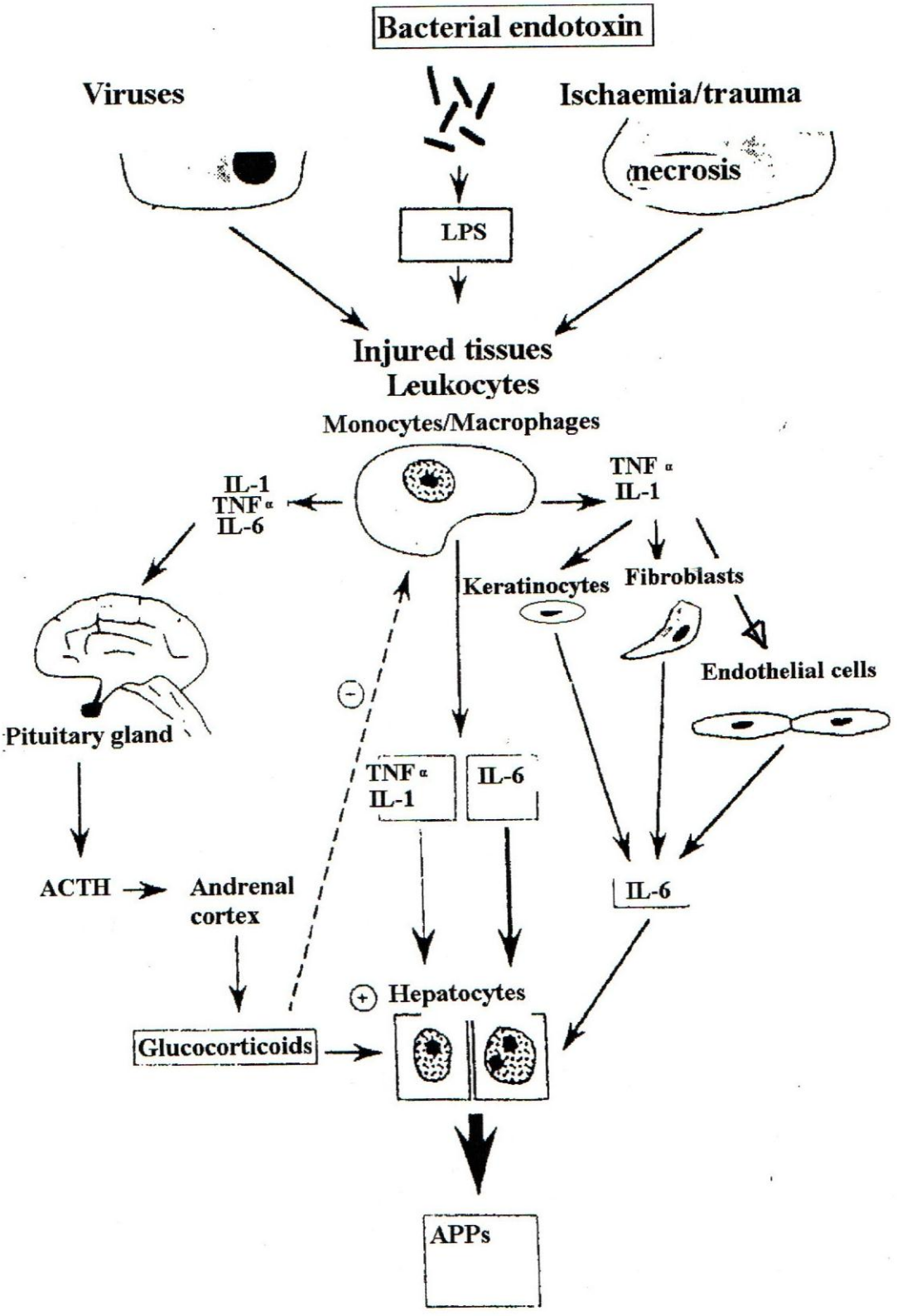


Figure 1: Acute Phase Response

2.2.2 Initiation of the APR

The initiation of the APR takes place at the site of injury. Mononuclear cells usually start the inflammatory cascade. They are able to release a broad spectrum of inflammatory mediators, such as cytokines, lipid mediators, vasoactive amines, products of the complement and coagulation cascades, proteases, reactive oxygen species and nitric oxide (Olson *et al,* 1995; Monshouwer *et al,* 1996). The inflammatory mediators trigger both the local and systemic inflammatory reactions. The local reactions include increase in capillary permeability, and infiltration of leucocytes to the area of inflammation. The increased capillary permeability allows the transport of different molecules between circulation and the area of tissue injury. These molecules consist of many serum proteins, such as proteinase inhibitors, transport proteins and other binding proteins. Also many ions, such as sodium and chloride, are transferred to the area of inflammation (Hirvonen, 2000).

Table 2: Initiation of the APR

Tissue trauma	Injury Infection Necrosis Neoplasm Immunological disorders
Elaboration of alarm molecules	Proteases Free radicals Lipid derivatives Vasoactive amines Products of complement and coagulation cascades
Activation of responsive cells	Macrophages Fibroblasts Endothelial cells Other cells
Synthesis and release of pro-inflammatory Cytokines	Interleukins Tumour necrosis factor-α Interferon-γ
Initiation of systemic APR and APP production	Clinical signs (e.g. fever) Endocrinological changes (e.g. glucagon & insulin) Metabolic changes (e.g. hepatic production of APP) Haematological changes (e.g. Zinc, iron, copper) Immunological changes (e.g.macrophage phagocytosis) Neurological changes (e.g. depression of CNS)

2.2.3 Continuation of the APR

The systemic inflammatory signs reflect multiple changes in the homeostatic control of the diseased individual (Hirvonen, 2000). These include: endocrinological changes, metabolic changes, haematological changes, neurological changes, immunomodulation and changes in cytokine profiles.

2.2.3.1 Endocrinological Changes

Serum concentrations of many hormones increase during the APR. These include adreno corticotrophic hormone, adrenal catecholamines, glucagon, insulin, growth hormone, adolsterone, vasopressin and prolactin (Kushner, 1982; Dinarello, 1984; Mandrup-Poulsen *et al,* 1995). Some other hormones decrease in concentrations during the acute phase. These are rennin, thyroxin, as well as gonadal steroids (Mandrup-Poulsen *et al,* 1995). These changes are however still partly controversial and their background is poorly understood. But one aim of these changes could be stimulation of the energy metabolism of the host (Hirvonen, 2000).

2.2.3.2 Metabolic Changes

The main metabolic changes during the APR are increased protein catabolism and gluconeogenesis. The muscle proteins are catabolised to amino acids that are required for synthesis of new proteins at a time when food intake is reduced. Amino acids are required for synthesis of hepatic acute phase proteins, immunoglobulins, and collagen for tissue repair, and the proliferation of lymphocyte and fibroblast cells. Amino acids are also used for gluconeogenesis and energy. Despite the anabolic processes, the catabolism of muscle proteins results in negative nitrogen balance and weight loss. Some central organs, such as kidney, liver and lung, are preferentially preserved from this catabolism. The reason for this is not clear, but these tissues are major components of the reticuloendothelial system, which frequently increases its activity during APR (Jennings & Elia, 1996). One of the most important metabolic changes is the strongly increased synthesis of a group of serum proteins, namely acute phase proteins, in the liver (Kushner, 1982; Eckersall & Conner, 1988; Baumann & Gauldie, 1994; Gruys *et al,* 1994; Raynes, 1994; Pannen & Robotham, 1995).

2.2.3.3 Haematological Changes

Alterations in serum cation concentrations occur during the APR (Kushner, 1982). Zinc and iron concentrations decline substantially, whereas serum copper may increase (Lohuis *et al,* 1988 and 1988b; Hayes, 1994). These ion changes reflect changes in cation binding of serum proteins, and more importantly, alterations in cellular uptake mechanisms (Hirvonen, 2000).

2.2.3.4 Neurological Changes

APR initiates many neurological changes in the host. Somnolence during the acute phase is due to the depression of the CNS. The inflamed area is usually painful; pain is mediated through vasoactive amines, such as bradykinin (Baumann & Gauldie, 1994).

2.2.3.5 Immunomodulation

APR has many immunosuppressive properties such as lymphocyte hyporeactivity, and decrease of neutrophil bactericidal and macrophage phagocytic activity (Kohler & Prokop, 1978; Kushner, 1982). The cytokines produced in the APR play a major role as immunomodulators (Koj, 1996), and also many liver-derived APP are known to have such properties (James, 1990; Motoi *et al,* 1992; Sato *et al,* 1995). Acute phase proteins, therefore, could also play a role in the pathogenesis of the disease in which they are produced. Haptoglobin has been shown to affect the immune system, including, mediating non-specific immune suppression (Oh *et al,* 1990). C-reactive protein and serum amyloid-P can stimulate the production of pro-inflammatory molecules from monocytes (Sarlo & Mortensen, 1985; Ballou & Lozanski, 1992). α_2-macroglobulin can modulate the immune response and inhibit mitogen induced lymphocyte proliferation (Gravagna *et al,* 1982), and also binds to cytokines thus modifying their properties (Matsuda *et al,* 1989; Keith, 1990). Indeed, haptoglobin has been shown to be produced in higher amounts during trypanosome infection in resistant as compared to susceptible mice (Shapiro & Black, 1992), while α_2-macroglobulin levels in *Trypanosoma cruzi* infections were found to correlate well with resistance to acute infection (Araujo-Jorge *et al,* 1992). C-reactive protein injected into mice

provided protection against *Streptococcus pneumoniae* (Mold *et al,* 1981), while serum amyloid protein enhanced macrophage listericidal activity (Singh *et al,* 1986).

The APR is followed by the specific immune response, including antigen processing and presentation, T-cell proliferation, and B-cell originated antibody production. It is evident that these two mechanisms interact strongly (Hirvonen, 2000).

2.2.3.6 Changes in Cytokine Profiles

The cytokines elicited *in S. mansoni* infection vary with the animal species, as Correa-Oliviera *et al,* (1998) demonstrated. They found that in humans, both cell-mediated (Th1-type) and humoral (Th2-type) responses are needed in the elimination of *S. mansoni* infection. This contrasts with the situation in the mouse model, where Th1-type responses are associated with protection and Th-2 type responses are associated with pathology, and demonstrates that human studies, though logistically more difficult, are absolutely needed.

The type and magnitude of cytokine production also depends on the stage of the infection (Chitsulo, 2000). It has been shown in murine intestinal schistosomiasis that a type I (Th-1 type) immune response with increased interferon-gamma (IFN-γ) predominates in early infection, but the type of immune response changes as egg production and tissue reaction begin. Decrease in IFN- γ as infection progresses is accompanied by an increase in IL-10 and granuloma formation (Coutinho *et al*, 2000). The earliest hepatic granulomas, therefore, form in a Th-1 environment, with down-regulation of Th-1 and up-regulation of Th-2 responses six weeks after infection (Todt *et al,* 2000). The granuloma is conceptualised as a Th-2 dominant reaction, but under some conditions, Th-1 granulomatous response may be predominant and damaging (Rutitzky *et al,* 2001). In chronic infections, considered as 20 weeks and over, these responses are blunted (Henderson *et al*, 1992). Borojevic (1992) regards the chronic phase of murine schistosomiasis as predominantly Th-1 mediated.

Studies in murine schistosomiasis demonstrate that the development of fibrosis requires the production of the profibrotic cytokines IL-2 and IL-4, and is suppressed by IL-12 and IFN-γ. Cytokine regulation of fibrosis and granuloma formation appear to differ. Whether these cytokines participate in the development of schistosome-induced hepatic

fibrosis in humans or non-human primates has never been directly tested (Farah *et al*, 2000a). However, IL-10 has been found to be an important cytokine in regulating the immune response and possibly controlling morbidity in human *Schistosoma mansoni* infection, and that the production of IFN-γ may be associated with resistance to infection (Correa-Oliveira *et al*, 1998).

Repeated exposure or challenge has also been found to be a risk factor for periportal fibrosis in baboons by a mechanism that primes lymphocytes to produce increased levels of profibrotic molecules that include TGF-β and IL-4 in baboons (Farah *et al*, 2000a).

In vitro assays showed that when inflammatory cells collected at various stages of infection are stimulated by egg or adult worm antigens, antigen-specific cytokine responses predominate in the acute infection stage. Blocking of IL-10 using monoclonal antibodies found presence of IFN-γ in chronic infection, but at reduced levels (Coutinho *et al,* 2000).

Boros & Lukacs (1992) found that soluble egg antigens (SEA) elicited IL-2 and IL-1 lymphokine production at the acute stage of murine *S. mansoni* infection. At the chronic stage, there was an overall decrease in the production of the two lymphokines. Cheever *et al* (1992), also observed that type Th-2 cytokines, such as IL-4 and IL-5, are augmented after egg laying begins, while type Th-1 cytokines, such as IL-2 and IFN-γ, are downregulated in *S. mansoni* infected mice.

The production and role of other cytokines in schistosomiasis is still a fertile area of study, especially with respect to treatment (Chitsulo, 2000).

2.2.4 Down Regulation of the APR

The down regulation of the APR involves many inflammatory mediators, such as glucorticoids, cytokines (e.g. IL-4 and IL-6) and receptor antagonists for certain pro-inflammatory cytokines (Besedovsky *et al,* 1986; Baumann & Gauldie, 1994; Koj, 1996). The APR subsides after removal of the factors causing tissue damage, usually after 1-2 days, and the host returns to normal function. The APR can also be prolonged if acute

inflammation develops to chronic state (Baumann & Gauldie, 1994). Several physiological and pathophysiological phenomena affect the manifestation of APR. For example, malnourishment can attenuate APR (Jennings & Elia, 1996).

2.2.5 Haptoglobin & Albumin in the APR

Haptoglobin (Hp) is a moderate acute phase protein in man i.e. it increases 2-10 fold in the acute phase. It has been documented as a good indicator of infection and inflammation (Solter *et al,* 1991; Gruys *et al,* 1993). Haptoglobin is important in the APR because of its ability to bind haemoglobin (Hb) and the transportation of the Hp-Hb complex to the liver. This process prevents the loss of iron. In addition, the reduction of iron availability might be of importance in resistance of infections whose causative organisms require iron for growth (Hirvonen, 2000). However, Hp could also promote the pathogenesis of the disease in which it is produced. It has been shown to affect the immune system, including, mediating non-specific immune suppression (Oh *et al*, 1990).

Serum albumin levels fall in the acute phase due to decreased synthesis by an inflammed/diseased liver. Other causes of hypoalbuminemia apart from liver inflammation/disease are protein deficiency, increased loss due to renal disease, intestinal parasitism or gastrointestinal disease or sequestration due to decreased oncotic pressure or increased hydrostatic pressure. Increases in serum albumin are not seen as a natural condition. They may be an artefact of sample haemolysis or may be due to dehydration or haemoconcentration (Cooper, 1990).

2.2.6 Serum Cations in the APR

During the acute phase response, there are alterations in serum cation concentrations (Kushner, 1982). Zinc and iron concentrations decline substantially, whereas serum copper concentrations may increase (Lohuis *et al,* 1988 and 1988b; Hayes, 1994). These ion changes reflect changes in cation binding of serum proteins, and more importantly, alterations in cellular uptake mechanisms (Hirvonen, 2000).

Serum iron has been found to decline substantially in the APR of a number of infections (Lohuis *et al,* 1988 and 1988b; Alsemgeest, 1994; Hayes, 1994; Mwangi *et al,* 1995).

Decline of serum iron during the APR is a reflection of the increase in the iron binding protein, haptoglobin, and impaired intestinal iron transport during the acute phase reaction. The rapid decrease in iron absorption accompanying the APR results from diminished brush border iron uptake (Anderson *et al,* 2000). Reduction of iron might be of importance in the resistance of infections whose causative organisms require iron for growth (Hirvonen, 2000).

The body distribution of zinc responds in a unique and unusual manner during the APR. Zinc concentrations in serum show an abrupt decline, falling to about half their normal values. This hypozincemia occurs because of a sequestration of zinc within hepatocytes, thymocytes and marrow cells. Zinc sequestration then persists throughout the duration of the illness. The sequestration of zinc is the secondary consequence of the induced expression, caused by pro-inflammatory cytokines, of metallothionein 1 and 2 genes within these responding cells. Newly synthesized metallothionein proteins generate binding sites for zinc, and lead to the hypozincemia, which characterizes acute phase reactions. Biological reasons for this unique sequestration of zinc remain unknown, although macrophage activation may be enhanced by low serum zinc values (Lao *et al,* 1987; Rhodes & Kluh, 1993; Vallee & Falchuk, 1993; Taylor, 1996). Furthermore, zinc is a component of many enzymes, including thymulin, and other protein structures such as the zinc fingers in transcription activation factors. Dramatic loss of zinc may therefore occur during catabolic processes, especially following severe infections. Acute zinc deficiency may then be precipitated as anabolic processes requiring zinc supervene (Vallee & Falchuk, 1993).

The major circulating form of copper is the blue glycoprotein, ceruloplasmin, which is synthesized in the liver. Each molecule of ceruloplasmin contains 6-8 atoms of copper. The functions of this protein are still unclear, but it is important in iron metabolism as a ferroxidase, and may have a role in regulating copper transport (Danks, 1995). It is an acute phase reactant and can increase greatly in response to infection, injury, chronic inflammatory conditions or steroid hormones. Serum copper and ceruloplasmin are both

increased in these circumstances as ceruloplasmin normally carries about 95% of the circulating copper (Taylor, 1996).

2.2.7 Physiological Functions of Acute Phase Reactants

Acute phase reactants have a wide range of activities that contribute to host defence: They can directly neutralize inflammatory agents, help to minimise the extent of local tissue damage, as well as participate in tissue repair and regeneration. There is a rapid increase in the serum concentration of many complement cascade components, the activation of which ultimately results in the local accumulation of neutrophils, macrophages and serum proteins. These participate in the killing of infectious agents, the clearance of foreign and host cellular debris, and the repair of damaged tissue. Coagulation components, such as fibrinogen, play an essential role in promoting wound healing (Pannen & Robotham, 1995).

The acute phase response (APR) alters the synthesis and release of many proteins synthesised by the liver; some of which decrease and others increase. Those proteins that decrease are termed negative acute phase proteins; to this group belong albumin and many other binding proteins e.g. transferrin and retinol binding protein (Hayes, 1994; Gruys *et al,* 1994). Those proteins that increase over 25% are termed positive acute phase proteins; they include the metal-binding proteins haptoglobin and ceruloplasmin (Kushner, 1982).

Haptoglobin dynamics in the acute phase have been described above. The increase in haptoglobin results in reduction of serum iron. This reduction in iron availability may be of importance in host resistance to bacterial and parasitic infections which require iron for growth (Hayes, 1994).

Albumin serves as the major reservoir of protein, is a main contributor of colloidal osmotic pressure, is involved in acid-base balance, and acts as a transport carrier for small molecules such as vitamins, minerals, hormones, fatty acids and many drugs (Kushner, 1982). During the acute phase of disease, albumin synthesis by the liver is decreased in deference to increased synthesis of positive acute phase proteins, mainly the globulins.

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2.2.8 *Applications of the APR*

In mammals, the concentrations of acute phase reactants can increase by between 2 and 10 fold in response to many different types of tissue injury, including immune and non-immune mediated inflammation, parasitic infections and neoplasms (Stadnyk & Gauldie, 1991; Whicher & Westacott, 1992; Baumann & Gauldie, 1994).

In its simplest application, the acute phase analysis will assist in the diagnosis of inflammatory or infectious disease. Estimation of C-reactive protein (an acute phase protein) in cerebrospinal fluid and serum, for example, is used as an early marker for rapid diagnosis of pyogenic meningitis (Tankhiwale *et al*, 2001). Indeed, the measurement of acute phase reactants is becoming of increasing importance in the diagnosis and assessment of disease activity (Kent, 1992; Gruys *et al,* 1994), and as an indicator of tissue damage (Kushner & Mackiewicz, 1987; Mozez *et al,* 1989). Measurement of specific acute phase reactants would therefore, in addition to providing useful diagnostic information, give a good indication of the prognosis of the disease.

The stage of the disease can also be evaluated by monitoring several acute phase reactants, so chronic as well as acute conditions can be evaluated and characterised by acute phase profiling (Eckersall *et al,* 1988; Scott *et al,* 1992). Alsemgeest (1994) found acute phase proteins useful for distinguishing between acute, sub-acute and chronic inflammatory disease.

The reactants may also serve in the follow-up of medical treatment, where sequential determinations of the reactants would provide accurate information on the course of the disease (Kaneti *et al,* 1984; Allen & Kold, 1988;).

It is also possible that sub-clinical inflammation will be identified by acute phase assays of high sensitivity. This is currently an area of intense study in human medicine as it is becoming clear that major conditions, such as cardiovascular disease and type II *diabetes mellitus*, may have an inflammatory origin and can be predicted by the determination of concentrations of acute phase reactants in serum (Murata *et al,* 2004).

The components and magnitude of the acute phase response depend on both the stimulating factors and the host species. Bacterial endotoxin, for example, is one of the most potent stimulators of hepatic synthesis of acute phase proteins (Whicher & Dieppe, 1985). Measurement of acute phase reactants can therefore be used to distinguish bacterial from

other non-bacterial infections. Stearman & Southgate (1994) demonstrated that C-reactive protein levels in cerebrospinal fluid were raised in cases of bacterial meningitis, but not in viral meningitis.

Acute phase reactants have found great use in health screening of companion animals, where the wide nature of conditions that can stimulate their production is a distinct advantage. Any positive result then requires secondary investigation to identify its cause (Eckersall *et al,* 1988)

For production animals, monitoring acute phase reactants is logical not only for the identification of individual animals with disease, but also as a means to categorise any sub-clinical disease present The use of acute phase reactants for screening ante or post mortem inspection to identify those animals which should be given a more thorough inspection or to ensure the health of animals prior to entry to the human food chain has been postulated (Murata *et al*, 2004). The recent recognition that APPs are produced in the mammary gland in response to the bacteria that cause bovine mastitis has exciting potential for their use in the detection of this economically important disease (Alsemgeest, 1994).

Acute phase reactants are now being included in experimental studies as end-point biomarkers for the activity of the pro-inflammatory cytokine network. Indeed with the difficulty of measuring these cytokines, such as interleukins 1 and 6, and tumor necrosis factor-α, in animals due to lack of species-specific reagents, limited stability and interfering factors, monitoring the APR is more expeditious. Therefore, any experiments using experimental models of inflammatory or infectious disease in assessments of anti-microbial or anti-inflammatory agents, or vaccine development could benefit from the quantitative data that can be derived from measuring the APR. In addition, confirmation of the health status of animals going into experimental procedures should routinely include acute phase reactant assays (Murata *et al,* 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Design

The study was done using two animal models: mice and baboons.

The mice were used for preliminary studies, which were carried out over 11 weeks. This entailed an infection and treatment experiment. Fifty-five mice were infected with *S. mansoni* cercariae. A similar number of mice were left uninfected for use as controls. Five mice were sacrificed at each sampling point. All infected mice that had not been sacrificed by Day 42 post-infection were treated. A booster dose of treatment was given 48 hours later. Sampling was carried out every week following infection.

The primate studies were done using two sets of five baboons each. The first baboon experiment, the Infection Experiment was a straightforward infection of the baboons with *S. mansoni* cercariae. The experiment was carried out over a period of six weeks, after which the animals were sacrificed. The baboons were infected with *S. mansoni* cercariae and sampled before infection and every two weeks following infection. This experiment was used as a pilot for the subsequent more complex study, which was an infection, treatment and challenge experiment. The Infection/Treatment/Challenge Experiment also involved five baboons. The baboons were infected similarly. They were however treated for the infection on Day 88 post-infection. They got a booster treatment 14 days after the first treatment to ensure complete cure. They were then challenged with a second cercarial infection 228 days after the first infection and following curative treatment. The baboons in this experiment were sampled before infection and serially following infection on days 27, 54, 81, 88,102, 123, 136, 164, 221, 228, 242, 256, 270, 284 and 298.

3.2 Laboratory Maintenance and Infection of Snails

Biomphalaria pfeifferi snails were collected from Kakuyuni in Kagundo, Machakos District. They were screened to ensure that they did not have any schistosomes. This was

done by putting them under strong light (100 watt lamp) for two hours to induce cercariae production. The process was repeated once every week for five weeks since the pre-patent period of *S. mansoni* in snails is 4-5 weeks. The snails were housed in a temperature-controlled room at the Institute of Primate Research (IPR), Nairobi. The temperature was maintained at 25-28 °C for 12 hours of light /12 hours of darkness. Plastic trays, for housing the snails, were washed in 3% hydrochloric acid and subsequently rinsed thoroughly with unchlorinated tap water. Soil from the riverbed where the snails were collected was taken and sterilized by heating at 150°C for 11 hours in an oven. It was allowed to cool and then layered at the bottom of the snail trays. The water was added up to three quarter tank full and the snails washed with water and distributed to the prepared aquatic tank. The snails were fed on lettuce throughout the experimental period.

Schistosome-free snails were then infected individually with 3-6 miracidia, artificially hatched from harvested eggs of *Schistosoma mansoni* from infected baboon faeces. The infected snails were maintained in the snail room for four weeks in the conditions described above. After the fourth week, the snails were put in the dark until they were required for cercarial shedding.

To obtain cercariae for infection of hosts, the snails were transferred into 100ml beakers containing 20ml of water. The snails were then exposed to artificial heat (28°C) and light (100 watt lamp) for 1 hour to induce them to shed the cercariae. The number of cercariae in an aliquot of 50 µl of the suspension was then determined using a dissecting microscope. These cercariae were then used to infect the mice and baboons.

3.3 Selection, Laboratory Maintenance, Infection and Treatment of Mice

Seventy six-week-old male BALB/c mice bred at the Institute of Primate Research (Nairobi, Kenya) were used for this study. The animals were housed in groups of ten per cage and fed on commercial pellets and provided with water *ad libitum*. They were kept under a natural dark: light cycle of about 12/12 hours, at an ambient temperature of 20±1°C and relative humidity of 50-60%.

The mice were each anaesthetised by intra-peritoneal injection of 0.04ml of Ketamine/xylaxine combined in the ratio 20:1. Each mouse was shaved on the abdomen and

a metal ring secured in place with masking tape. An aliquot containing an average of 110 cercariae was placed in this metal ring and the parasites allowed 30 minutes to penetrate, and thus infect the mice (Smithers & Terry, 1965). Uninfected mice served as controls.

Five mice were sacrificed at every sampling point. The mice were sampled before infection, and weekly following infection. Thirty mice were treated on Days 42 and 44 post-infection with two equal oral doses of praziquantel, at a dosage rate of 450mg/kg body weight. Being smaller sized animals, this dosage was much higher than that used in the primates. The mice were also sampled weekly thereafter.

3.4 Selection, Laboratory Maintenance, Infection & Treatment of Baboons

Ten Kenyan olive baboons, *Papio anubis,* weighing about 6.5kg and caught in a non-schistosomiasis-endemic region, were used for the study. The animals were quarantined for 90 days, prior to initiation of experiments, during which time they were screened for common bacterial, viral and parasitic infections. They were also tuberculin tested according to the standard procedures at the Institute of Primate Research, Nairobi, Kenya. They were tested for prior patent schistosomiasis infection by the Kato technique (Katz *et al,* 1972).

As a further safeguard against the possibility of prior exposure to schistosomiasis, serum was obtained from each animal for the enzyme linked immunosorbent assay of specific IgG antibody against SWAP (Nyindo *et al,* 1999). Only animals with antibody concentrations below 22μg/ml $\pm$ 3 standard deviations of the value, obtained from a laboratory colony-born baboon and negative on the Kato test were used in the study. The animals were individually caged and fed on baboon pellets twice a day. They were also fed on fruits and vegetables twice a week, while water was provided *ad libitum*.

Blood samples were collected from the baboons prior to infection. These served as a baseline for the various analyses. Infection schedules comprised a large cercarial dose, of about 1000 cercariae, given once as single-dose infection. All infections were done percutaneously by the pouch method (Sturrock *et al,* 1976). A pouch was made in the inguinal region of anaesthetized baboons by folding the surrounding skin with pegs. The cercarial suspension was then poured into the pouch and left for 30 minutes to allow for skin

penetration of the cercariae. The baboons were anaesthetised with xylaxine 2% and ketamine (100mg/ml) at a dose of 0.1ml/kg body weight (Farah, 2000).

The five baboons comprising the infection experiment were all sampled before infection, and once every two weeks following infection. The other five uninfected control baboons were sampled synchronously with the infected baboons.

The five baboons comprising the infection/treatment/challenge experiment, as well as their uninfected controls, were all sampled on Day 0 (pre-infection) and on Days 27, 54, 81 and 88 post-infection. The infected baboons were then all treated with a dose of praziquantel on Day 88 post-infection. This treatment was repeated 14 days later and was accompanied by sampling. All the baboons were subsequently sampled on Days 123, 136, 164, 221 and 228 post-infection. On Day 228, the treated baboons were infected with a challenge dose of 1,000 cercariae. The infection was done as previously described. The baboons were then sampled on Days 242, 256, 270, 284 and 298 post-infection.

At every sampling point in both experiments, the non-infected control baboons were also bled by the same method. Control baboons were subjected to the same conditions as the infected baboons throughout the course of the experiments.

3.5 Collection of Blood Samples

Blood from the mice was collected by heart puncture at weekly intervals for eleven weeks. Five mice were sacrificed at each sampling point. The blood collected at each sampling point was pooled, allowed to clot and centrifuged. The serum collected was stored at -70°C ready for analysis. Since the blood from mice was pooled, only single values were obtained for each sampling point (no means were calculated).

Sampling for blood in the baboon models was done every two weeks in the Infection Experiment and variably in the Infection/Treatment/ Challenge Experiment. This was for a total period of eight weeks in the infection experiment, and for a total period of forty-seven weeks post-infection and thirty-five weeks post-treatment in the Infection/Treatment/Challenge Experiment. The baboons were anaesthetised as described above. Ten ml of blood from the inguinal vein was then collected, allowed to clot and centrifuged. The serum separated out was then stored at -70°C ready for analysis (Mwangi *et*

al, 1995; Yole *et al,* 1996; Farah, 2000). Serum from each baboon was assayed on its own and the mean and standard error for the group was worked out.

3.6 Haptoglobin Assay

Haptoglobin (Hp) was measured using the method described by Makimura & Suzuki (1982) with modifications by Conner *et al,* (1988). The assay uses purified bovine Hp as standard. This test is based on the ability of Hp to bind to haemoglobin (Hb) and retain peroxidase activity at acidic pH, whereas free Hb loses its peroxidase activity.

Haemoglobin (met haemoglobin) was prepared according to Makimura & Suzuki (1982), and stored as stock solution at a concentration of 3g/100ml in normal saline. Tetra methyl benzidine (TMB) in phosphate buffer, pH 3.8, was prepared according to Conner *et al,* (1988). TMB served as the chromogen. A stock sample of bovine haptoglobin of known concentration was used as the standard. This was serially diluted in normal saline to produce haptoglobin concentrations ranging between 1.0 and 7.0g/l.

An aliquot of 20µl of the test samples and standards was pipetted into test tubes. Into each tube was added 100µl of the 30mg/ml (1:100 dilution of stock solution) Hb solution, which was mixed and allowed to react for 10 minutes at room temperature. Normal saline was then added to each test tube, 5ml per tube, and mixed. Using a micro-pipette, 20 µl of the mixture from each test-tube was then measured into the wells of a 96-well pyrex® microtitre plate, each sample being prepared in duplicate. This was followed by adding 200µl of TMB solution into each well, mixing and incubating at 37°C for 1 hour. At the end of the incubation period, 50 µl of 1% hydrogen peroxide, as the substrate, was added to each well. This was left to react until a strong blue colour developed in the high standard. The reaction was terminated by adding 50µl of 2M sulphuric acid per well. The plates were then read on an Olympus® ELISA plate reader at 450nm. The Hp levels within the samples were extrapolated from the standard curve, which was obtained from the standard optical readings, using a PC computer connected to the reader.

In each assay, control samples containing a high and low concentration of Hp were included.

3.7 Albumin Assay

Serum albumin was measured using the BCG® photometric colorimetric method. The test uses bovine albumin as a standard. It is based on the formation of a coloured complex by albumin in citrate buffer and bromocresol green. The absorbance of this complex is proportional to the albumin concentration in the sample.

10µl of the sample and of the standard were pipetted into separate cuvettes. Into each of these was added 1000µl of the reagent, consisting of 30mmol/l of citrate buffer (pH 4.2), and 260µmol/l of bromocresol green. The contents of the cuvettes were mixed and incubated for 5 minutes at 25°C. The absorbance of the sample and standard were then measured at 578nm against a blank of the reagent within 30 minutes.

Albumin concentration (C) was calculated using the formula below:

$$C = \frac{40 \times \Delta A \text{ Sample}}{\Delta A \text{ Standard}} g/l$$

Where A is the absorbance at 578nm.

3.8 Assays for Serum Cations

All apparatus used for handling serum for the determination of iron, zinc and copper, were acid-washed and tested spectrophotometrically for contamination by the metals. Iron, zinc and copper concentration determinations were essentially carried out as described by Passey *et al,* (1985). All the samples were prepared in duplicate. Briefly, 0.5ml serum was drawn into a 3.5ml test-tube, mixed with 1ml spectrosol grade concentrated nitric acid, and heated at 80°C for 1 hour. The resulting solutions were diluted with 1ml de-ionised water and analysed for iron, zinc or copper by aspiration into an atomic absorption spectrophotometer (Thermo Jarell Ash model). Iron concentrations were measured at a wavelength of 248.3nm and a current of 8mAmps, within a linear range of up to 5 parts per million (ppm). Zinc concentrations were measured at wavelength 213.9nm, current 3mAmps, within a linear range of up to 0.4ppm. Copper concentrations were measured at wavelength 324.7nm, current 5mAmps, within a linear range of up to 4ppm. Serum from uninfected animals was used as a control.

3.9 Histopathological Examination of Tissues

From Day 42 (Week six) post-infection and following treatment, livers from sacrificed mice were collected and fixed in 10% buffered formalin. This was also done for livers from five uninfected controls. The fixed tissue samples were dehydrated using ethyl alcohol, and then embedded in paraffin wax. Six-micrometer thick sections were cut using a rotary microtome, and stained with haematoxylin and eosin (H/E). The sections were then studied for histopathological changes under a light microscope.

The baboons were euthanised at the end of the experiments by intravenous administration of heparinized sodium pentobarbital. For each animal in the Infection Experiment, the viscera were exposed after median incision. Observing the extent of tissue inflammation, density of granulomas, mucosal and serosal integrity of the colon and size of mesenteric lymphnodes made a subjective assessment of gross pathology.

3.10 Statistical Data Analysis

Statistical analysis of the data was performed using SPSS and Excel software programmes. Excel was used to graphically depict trends in the analytes measured in infected animals and in controls. One-tailed ANOVA tests were used to compare differences in analyte concentrations between infected animals and uninfected controls. Duncan's Multiple Range Test (DMRT) was used to compare differences in concentrations of the proteins and cations between controls, and following infection, treatment and/or challenge with a second caercarial dose.

CHAPTER FOUR

RESULTS

4.1 Studies involving mice

4.1.1 Changes in Protein Concentrations

Following infection of the mice, there was a gradual increase in mean serum haptoglobin concentrations, as shown in Figure 2. The increase was observed from Day 7 post-infection, where levels rose from pre-infection concentrations of 2.10g/l to 2.6g/l. The increase was sustained until Day 42, when mean concentrations of haptoglobin reached 7.30g/l. Following curative treatment with praziquantel on Day 42, the haptoglobin levels continued to increase until Day 49 post-infection, to reach 67.5 g/l. Thereafter, there was a gradual decline in haptoglobin levels. However, the levels were still higher than control concentrations and did not reach pre-infection levels by the time of termination of the experiment at Day 77 post-infection. The pre-infection concentrations were statistically different from all post-infection concentrations except those on Days 7 and 28, as shown in Table 3. Statistical significance was determined by the Duncan's Multiple Range Test (DMRT), at a P value of < 0.05.

Changes in mean albumin concentrations of mice infected with *S. mansoni* and treated curatively at Day 42 post-infection, and uninfected controls, are depicted in Figure 3. Following infection, there was a gradual decline in albumin concentrations from a mean pre-infection concentration of 39g/l. This persisted until Day 42 post-infection when the mean albumin concentration was down to 20g/l. Following treatment at Day 42 post-infection, the albumin levels drastically increased to reach pre-infection levels of 40g/l by Day 77 when the experiment was terminated. As shown in Table 3, post-infection albumin levels were significantly lower than pre-infection levels up to the third week following treatment (i.e. 63 days post-infection).

4.1.2 Changes in Cation Concentrations

Changes in mean iron concentrations of mice infected with *S. mansoni* and treated curatively at Day 42 post-infection, and uninfected controls, are depicted in Figure 4. The mean iron concentrations in the infected animals decreased gradually from pre-infection

levels of 217μg/dl to reach levels of 114μg/dl by Day 42 post-infection, when the mice were curatively treated with praziquantel. The iron levels remained around treatment concentrations for two weeks (i.e. until 56 days post-infection) after which they gradually increased to 160μg/dl by Day 77 post-infection. This concentration was, however, still lower than pre-infection concentrations. Post-infection concentrations became significantly different from the mean pre-infection concentration from Day 14 post-infection, as shown in Table 3.

Changes in mean zinc concentrations of mice infected with *S. mansoni* and treated curatively at Day 42 post-infection, and uninfected controls, are depicted in Figure 5. Zinc concentrations in the infected mice gradually declined from pre-infection concentrations of 232μg/dl in the initial two weeks (i.e. up to Day 14 post-infection). This was followed by a drastic decline where concentrations dropped to reach 78 μg/dl by the time of treatment. Following curative treatment with praziquantel, zinc concentrations remained at treatment levels of 78μg/dl for about two weeks (i.e. until Day 56 post-infection) after which the levels drastically increased to 171μg/dl at 77 days post-infection, when the experiment was terminated. Though post-treatment levels demonstrated drastic increases from Day 56 onwards, they were still significantly lower than the control levels. Post infection concentrations became significantly different from the mean pre-infection concentration ($P<0.05$) from Day 7 post-infection, as shown in Table 3, and remained so throughout the experiment.

Changes in mean copper concentrations of mice infected with *S. mansoni* and treated curatively at Day 42 post-infection, and uninfected controls, are depicted in Figure 6. Copper concentrations in the infected animals increased gradually following infection until Day 21, from pre-infection concentrations of 83.2μg/l to 96.0μg/l. The levels, however, did not significantly differ from those of the uninfected controls during this period. Between 21 days and 42 days post-infection, the copper levels showed a drastic increase to reach a concentration of 134.0μg/l. Following curative treatment with praziquantel on Day 42 post-infection, copper levels showed a slow but steady decline until the completion of the experiment on Day 77 post-infection, when mean concentrations were down to 97.0μg/l. Although copper levels in the infected and treated animals approached those of the uninfected controls at this point, they did not attain pre-infection concentrations. As shown

in Table 3, copper concentrations in infected animals became significantly different from pre-infection levels on Day 21 post-infection, and remained statistically different (at $P<0.05$) throughout the experiment. Levels in the infected animals were significantly different from those of the uninfected controls from Day 28 through to Day 70. Levels before treatment were not statistically different from those following treatment.

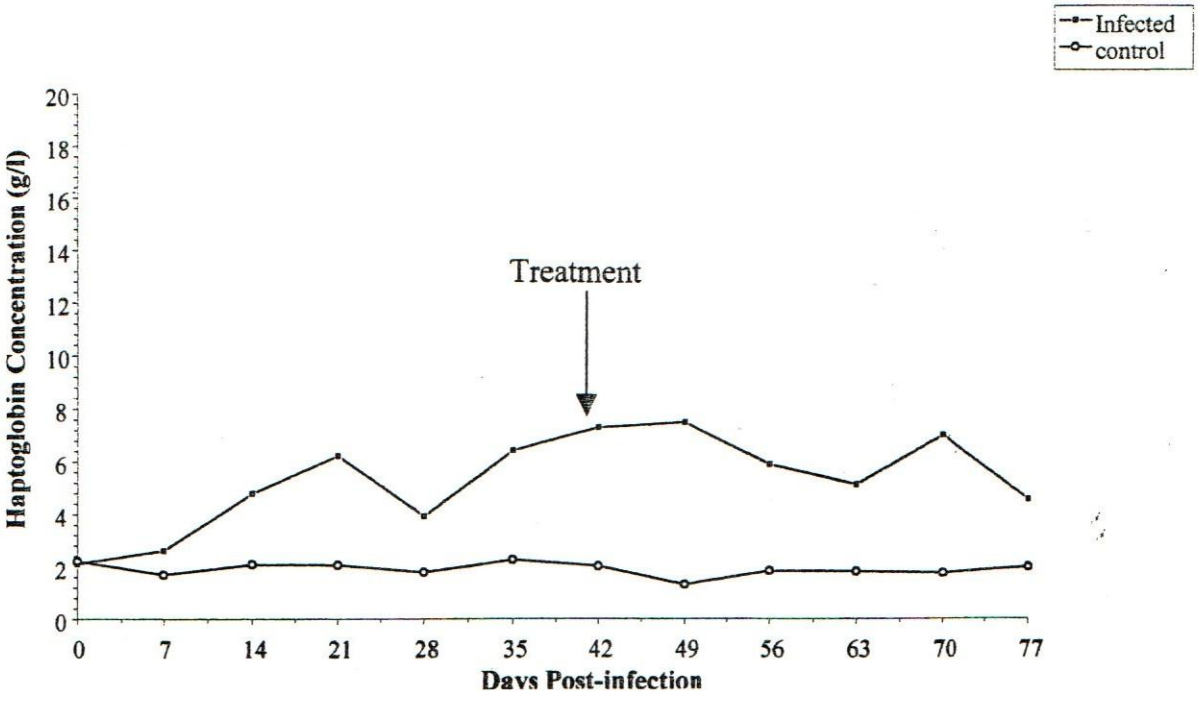


Figure 2: Mean serum haptoglobin concentrations in control and *S. mansoni* infected mice.

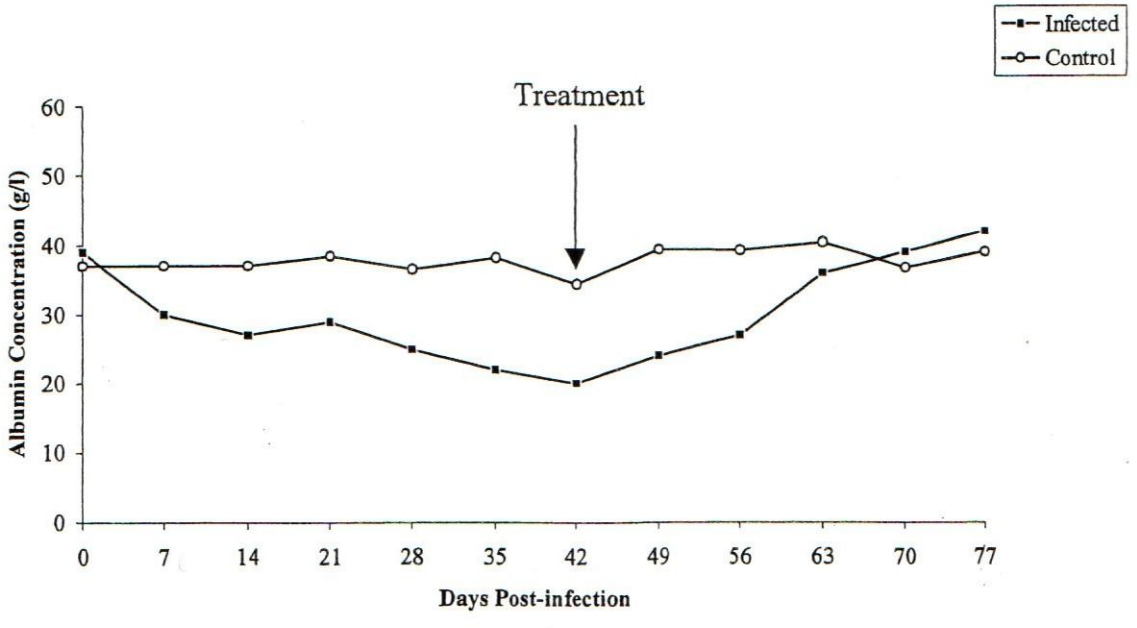


Figure 3: Mean Serum albumin concentrations in control and *S. mansoni* infected mice

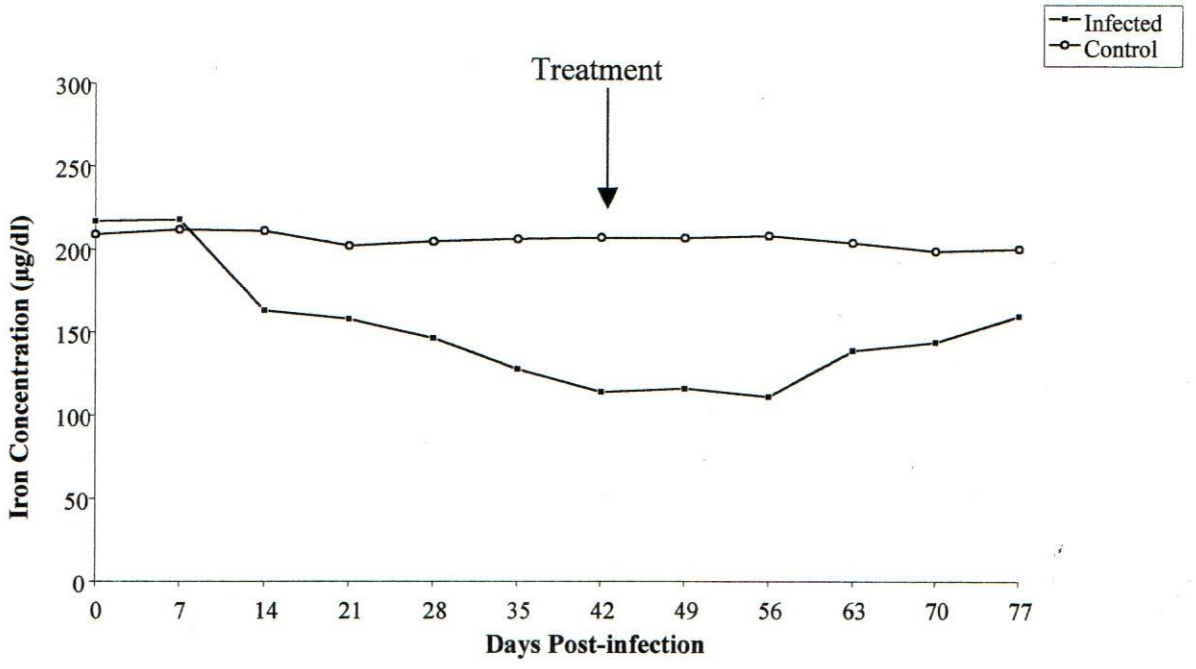


Figure 4: Mean serum iron concentrations in control and *S. mansoni* infected mice.

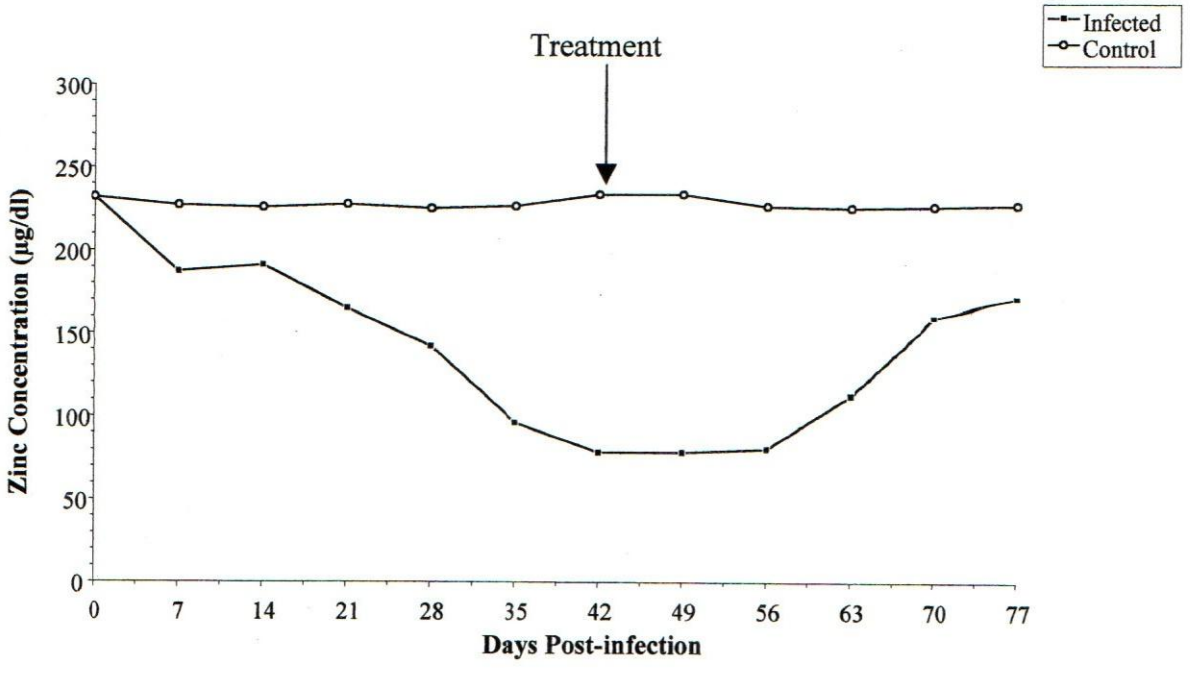


Figure 5: Mean serum zinc concentrations in control and *S. mansoni* infected mice.

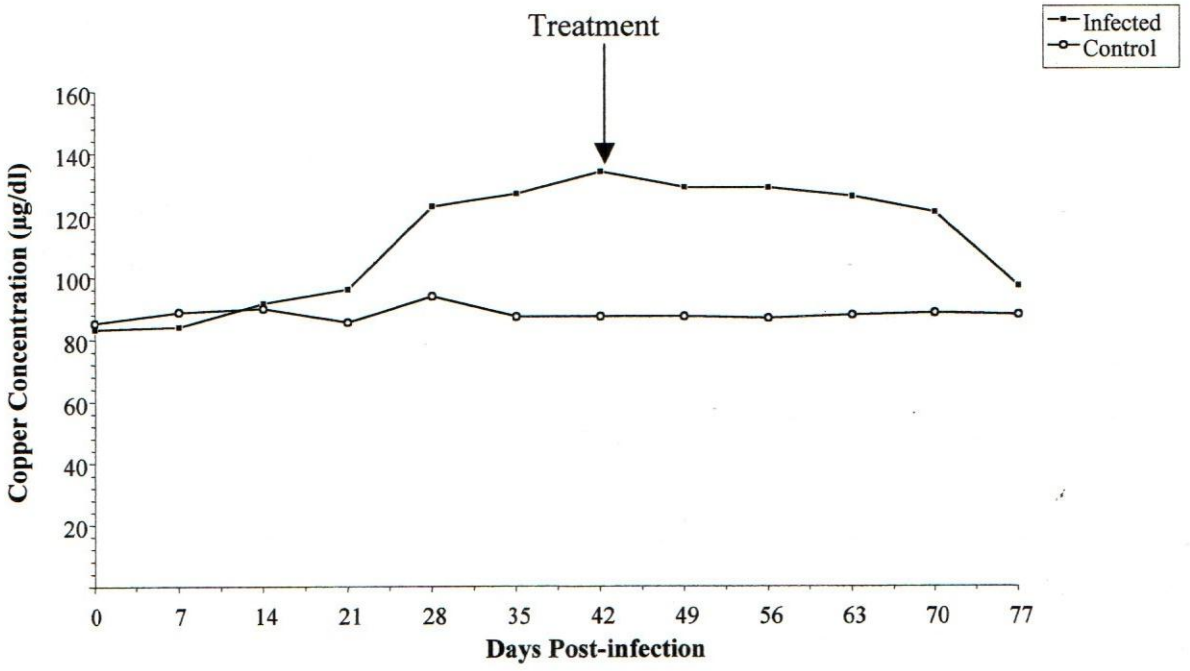


Figure 6: Mean serum copper concentrations in control and *S. mansoni* infected mice.

Table 3: Mean serum concentrations of analytes in *S. mansoni* infected and treated mice

Days	Haptoglobin	Albumin	Iron	zinc	copper
0	2.10^{a}	39^{d}	217.0^{f}	232.0^{h}	83.2^{a}
7	2.60^{a}	30^{bc}	218.0^{f}	187.0^{g}	84.0^{a}
14	4.80^{bc}	27^{ab}	163.0^{e}	191.0^{g}	91.6^{ab}
21	6.20^{cde}	29^{bc}	158.0^{de}	165.0^{ef}	96.0^{b}
28	3.90^{ab}	25^{ab}	146.4^{cde}	142.0^{d}	123.0^{cd}
35	6.40^{cde}	22^{ab}	127.6^{ab}	96.0^{b}	127.0^{cd}
42*	7.30^{e}	20^{a}	114.0^{a}	78.0^{a}	134.0^{d}
49	7.50^{e}	24^{ab}	116.0^{a}	78.0^{a}	129.0^{cd}
56	5.90^{bcde}	27^{ab}	111.0^{a}	80.0^{a}	128.8^{cd}
63	5.10^{bcd}	36^{cd}	139.0^{bc}	112.0^{c}	126.0^{cd}
70	7.00^{de}	39^{d}	144.0^{cd}	159.0^{e}	121.0^{c}
77	4.56^{bc}	42^{d}	160.0^{de}	171.0^{f}	97.0^{b}

Mean values in the same column followed by the same superscript are not significantly different ($P<0.05$)

* Treatment with praziquantel

4.1.3 Pathological Findings

4.1.3.1 Gross Pathology

The infected mice started showing loss of body condition, evidenced by weight loss and malaise, by Day 42 post-infection with *S. mansoni* cercariae. Their body condition continued to deteriorate with progress of the disease, until after curative treatment with praziquantel.

The animals showed no visible granulomas on gross examination up to Day 42 post-infection. By Day 49 post-infection, their livers showed presence of moderate-to-severe granulomas. Day 56 post-infection animals had very poor body condition and numerous liver granulomas. The treated animals however had better body condition and their livers had fewer granulomas. However, both treated and untreated animals had enlarged livers and spleens. By Day 63 post-infection, the treated animals had regained good body condition. They showed only few granulomas, but still showed hepatomegaly and splenomegally. Only one of the animals was found dead. It had enlarged spleen, liver, caecum and stomach.

The animals at Day 70 post-infection generally had good body condition. They showed very few liver granulomas. However, they had pronounced enlargement of the liver, spleen, and appendix, with marked ascites. One of the mice was found dead with a very enlarged abdomen.

On Day 77 post-infection, all animals had ascites, enlarged livers and spleens. The appendix was enlarged in two of the animals. One mouse showed no visible granulomas, while the other four showed only very few. The liver in one of the mice appeared 'cooked', and in another, the blood appeared darker than normal.

4.1.3.2 Histopathology

Histopathological examination of hematoxylin and eosin (H & E) liver sections of the mice models yielded the following results:

As shown in Plate 1, the hepatic tissue in the uninfected controls was compact, because of little cellular infiltration, with a high content of erythrocytes.

Liver sections from animals in Day 42 post-infection showed many trapped eggs with little tissue reaction around them, as shown in Plate 2. There were also some adult worms trapped in the tissues (Plate 3). Minimal infiltrations by leukocytes, mainly eosinophils, were seen in the hepatic and around the blood vessels (Plate 4).

Some liver sections of Day 49 post-infection animals showed trapped eggs with little cellular infiltration, similar to those seen in Day 42 sections. This is evidenced in Plate 5. Other sections of the animals sacrificed on the same day, however, revealed numerous pronounced granulomas (eggs with massive tissue reaction around them), as seen in Plate 6.

Plate 7 and 8 show light tissue fibrosis and resolving granulomas seen in liver sections from all Day 56 post-infection animals. There was evident cellular infiltration, with the predominant cell-type changing from the eosinophils seen in previous sections to lymphocytes. Numerous pockets of lymphocytes were seen amidst normal hepatic tissue. In addition, unlike in the previous week, periportal fibrosis was observed.

Plate 9 shows a Day 63 post-infection liver section. There was a slight reduction of fibrosis, compared to that seen in Day 56. Small regions of normal hepatic tissue could be seen in-between the fibrotic areas. There was, however, still the presence of periportal fibrosis and lymphocyte infiltration. There was also massive bile duct hyperplasia, as shown in Plate 11. This had not been observed in sections from previous weeks.

Compared to Day 63, tissue fibrosis was significantly decreased in Day 70 post-infection, although periportal fibrosis was still present. This is evidenced in Plate10. Large areas of normal liver tissue with only little cellular infiltration could be seen. However, bile duct hyperplasia was still present, though less pronounced. As evidenced in Plate 12,, a few adult worms were still trapped in blood vessels.

Plate 13, 14 and 15 show that by Day 77 post-infection, fibrosis was greatly decreased, with most of the hepatic tissue having normalised. There was very little cellular infiltration. Only a few resolving granulomas were seen. No bile duct hyperplasia was evident, but periportal fibrosis could still be seen.

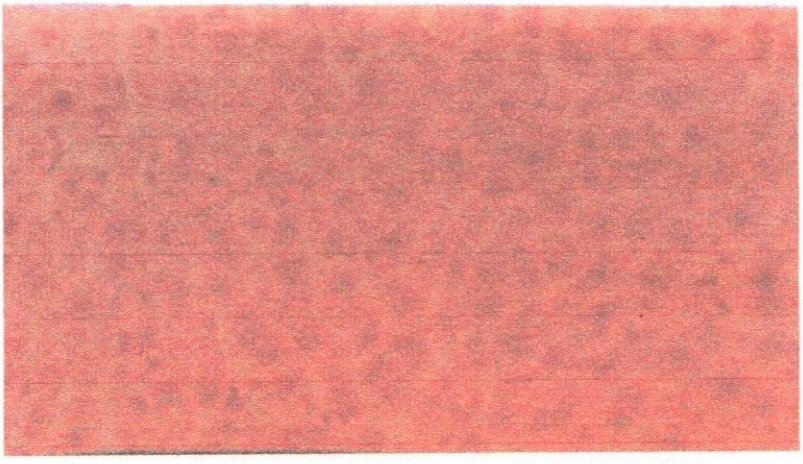


Plate 1: Liver section showing normal hepatic tissue in uninfected mouse.

(H & E stain ×100)

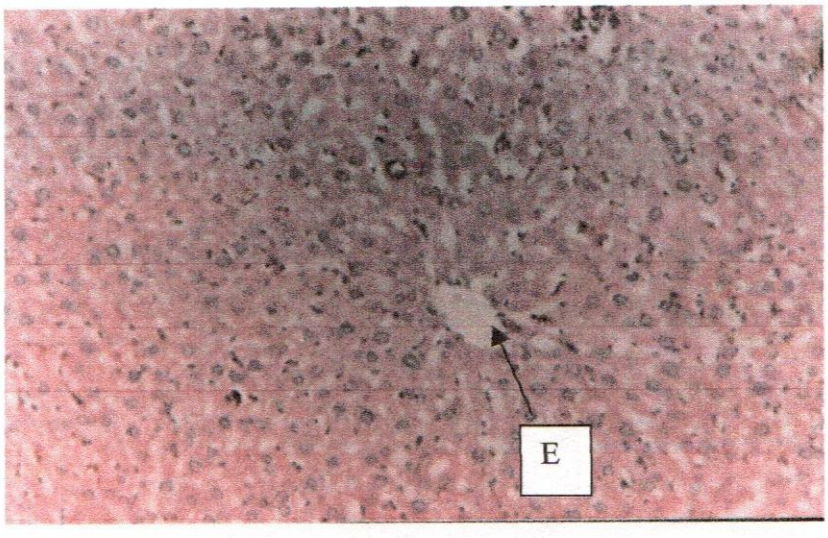


Plate 2: Liver section of from *S. mansoni* infected mouse at Day 42 post-infection, showing presence of trapped egg (E) with minimum tissue reaction.

(H & E stain ×100).

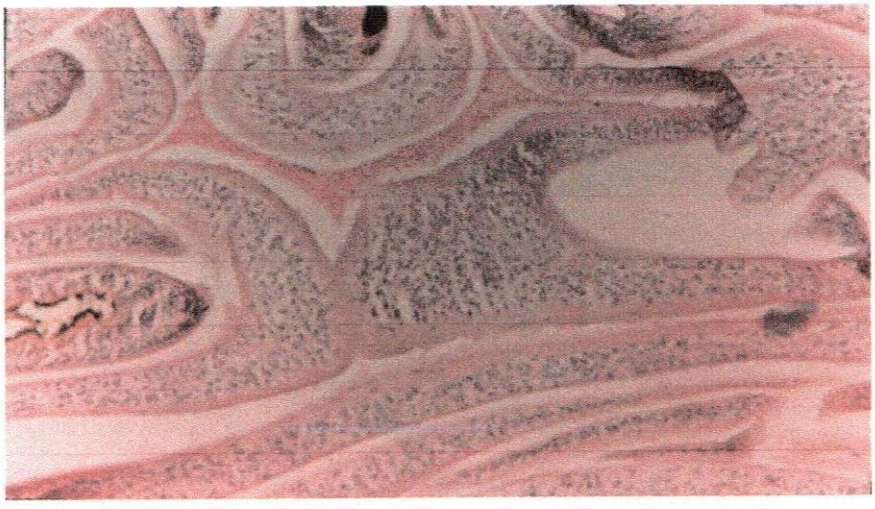


Plate 3: Liver section from *S. mansoni* infected mouse at Day 42 post-infection, showing an adult worm trapped in the hepatic tissues.

(H & E stain ×100).



Plate 4: Liver section from *S. mansoni* infected mouse at Day 42 post-infection, showing portal triad with slight cellular infiltration .

(H & E stain ×100).

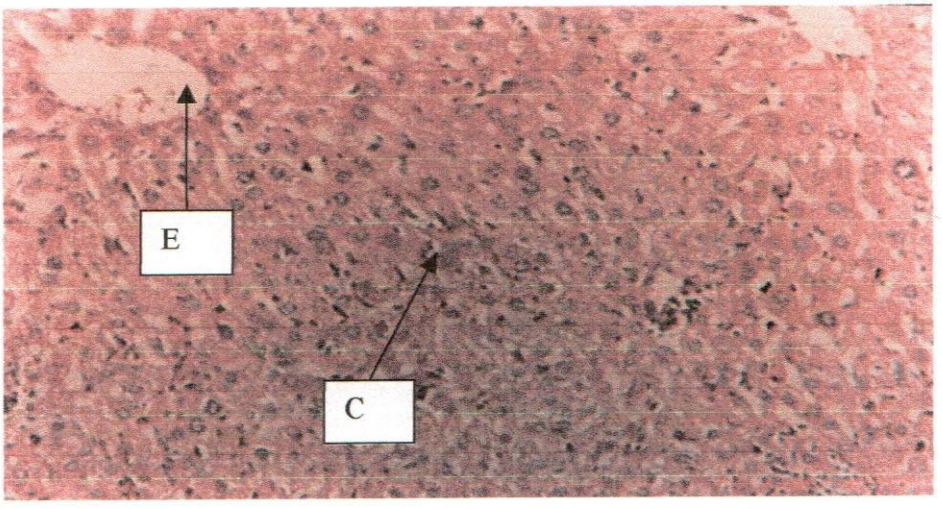


Plate 5: Liver section from *S. mansoni* infected mouse at Day 49 post-infection and Day 7 after curative treatment with praziquantel, showing trapped eggs (E) and cellular infiltration (C).

(H & E stain ×100).

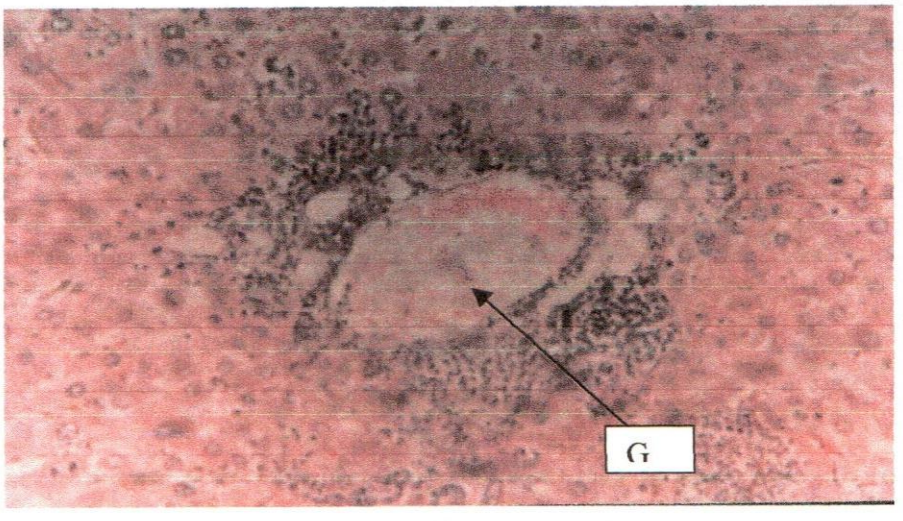


Plate 6: Liver section from *S. mansoni* infected mouse at Day 49 post-infection and Day 7 after curative treatment with praziquantel, showing presence of granuloma (G).

(H & E stain ×100).

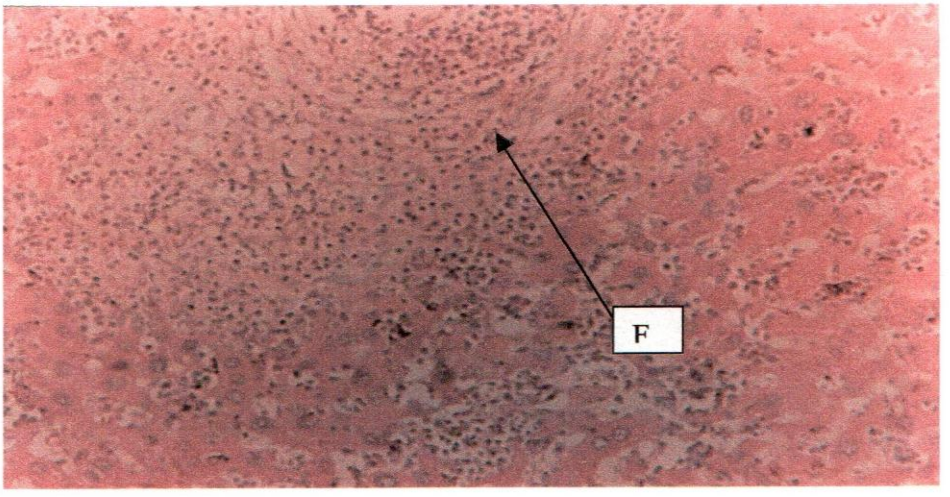


Plate 7: Liver section from *S. mansoni* infected mouse at Day 56 post-infection and Day 14 after curative treatment with praziquantel, showing massive tissue fibrosis (F).

(H & E stain ×100).

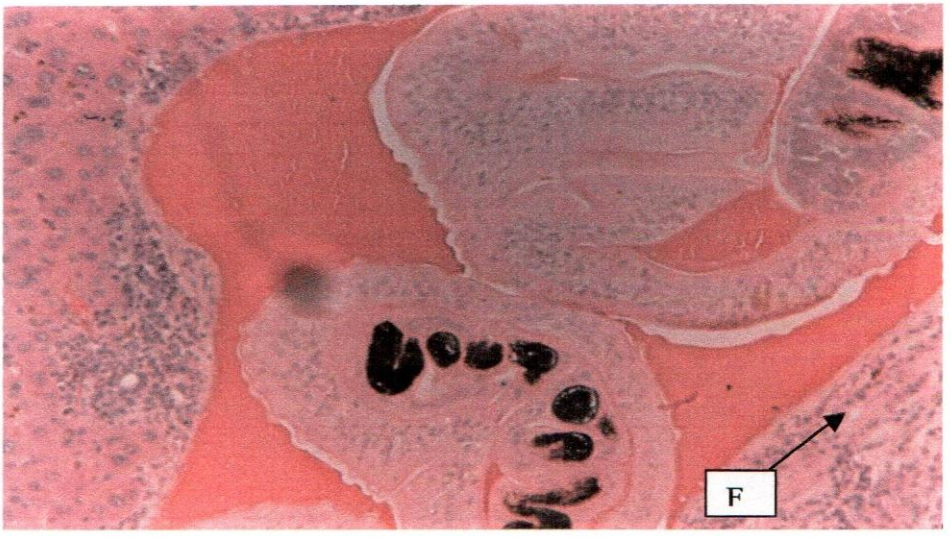


Plate 8: Liver section from *S. mansoni* infected mouse at Day 56 post-infection and Day 14 after curative treatment with praziquantel, showing

light periportal fibrosis (F).

(H & E stain ×100).

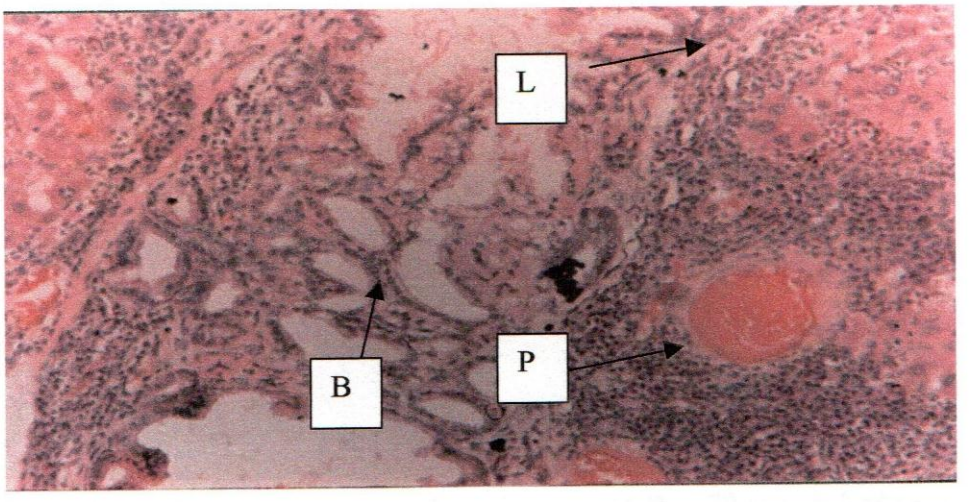


Plate 9: Liver section from *S. mansoni* infected mouse at Day 63 post-infection and Day 21 after curative treatment with praziquantel, showing bile duct hyperplasia (B), periportal fibrosis (P) and lymphocyte infiltration (L).

(H & E stain ×100).

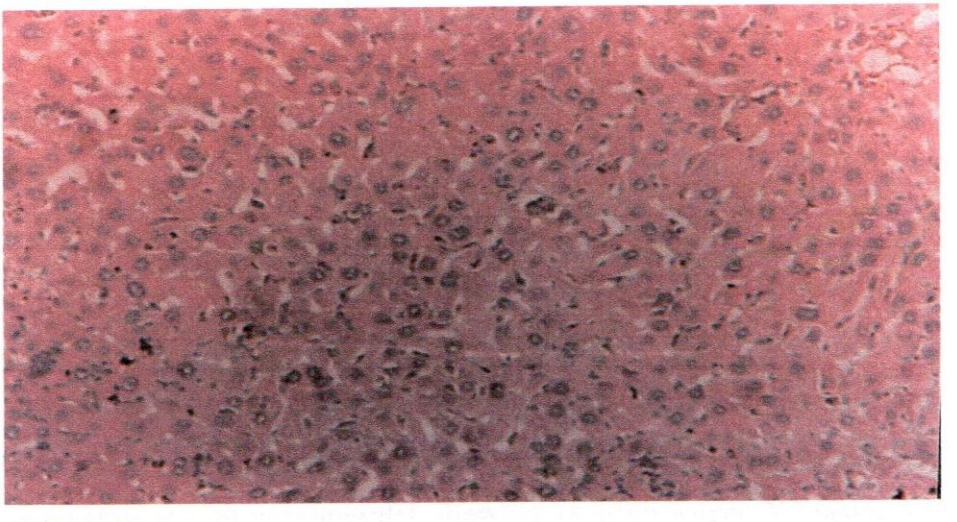


Plate 10: Liver section from *S. mansoni* infected mouse at Day 70 post-infection and Day 28 after curative treatment with praziquantel, showing a near normal hepatic tissue with minimal cellular infiltration.

(H & E stain ×100).

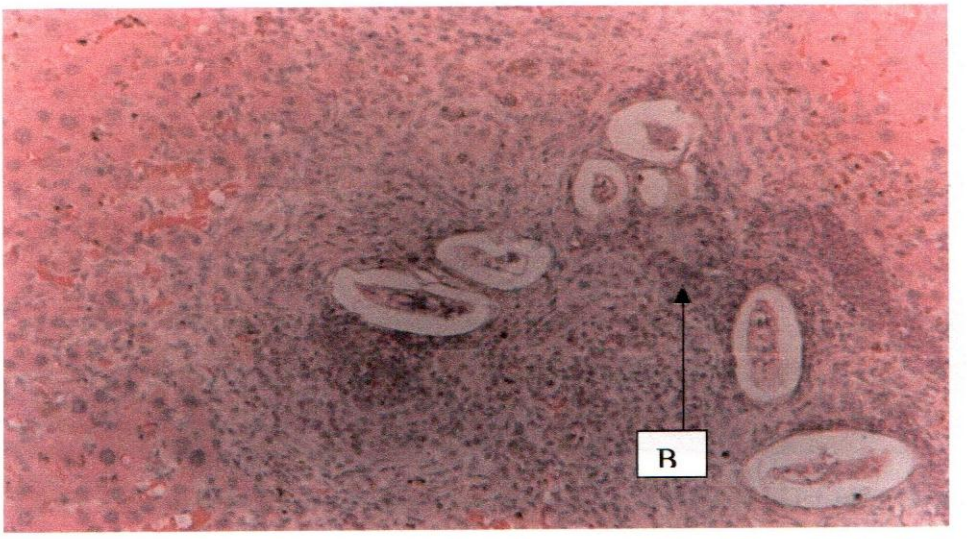


Plate 11: Liver section from *S. mansoni* infected mouse at Day 70 post-infection and Day 28 after curative treatment with praziquantel, showing bile duct hyperplasia (B).

(H & E stain ×100).

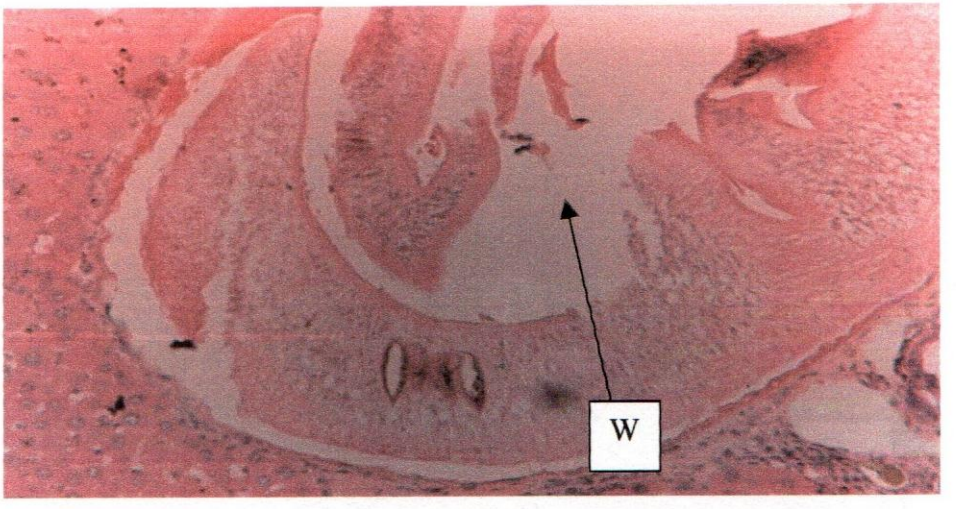


Plate 12: Liver section from *S. mansoni* infected mouse at Day 70 post-infection and Day 28 after curative treatment with praziquantel, showing an adult worm (W) inside a blood vessel.

(H & E stain ×100).

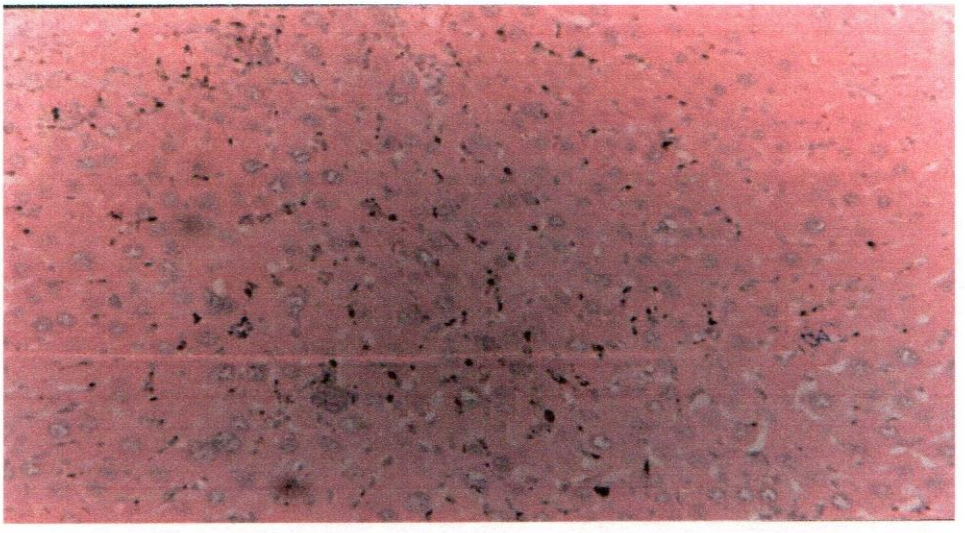


Plate 13: Liver section from *S. mansoni* infected mouse at Day 77 post-infection and Day 35 after curative treatment with praziquantel, showing normalization of hepatic tissue.

(H & E stain ×100).

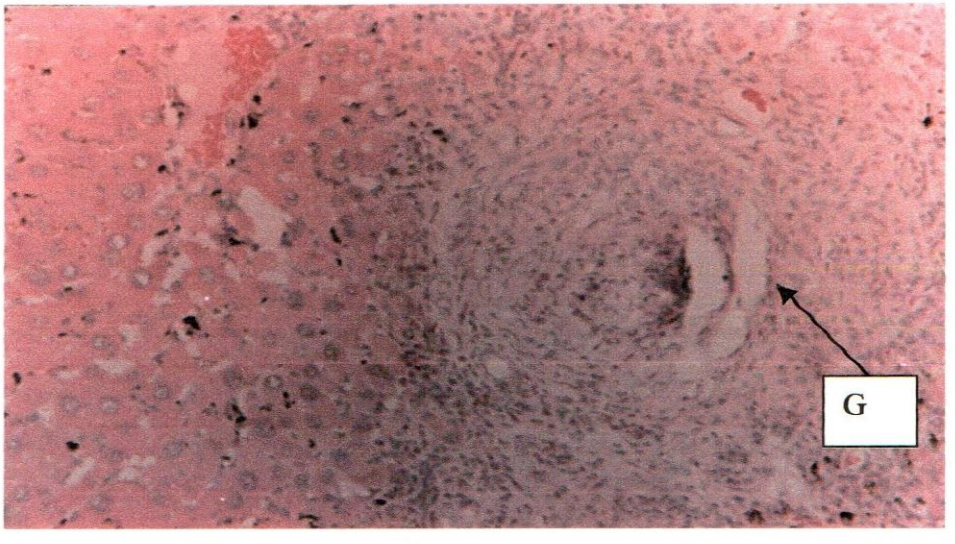


Plate 14: Liver section from *S. mansoni* infected mouse at Day 77 post-infection and Day 35 after curative treatment with praziquantel, showing a resolving granuloma (G).

(H & E stain ×100).

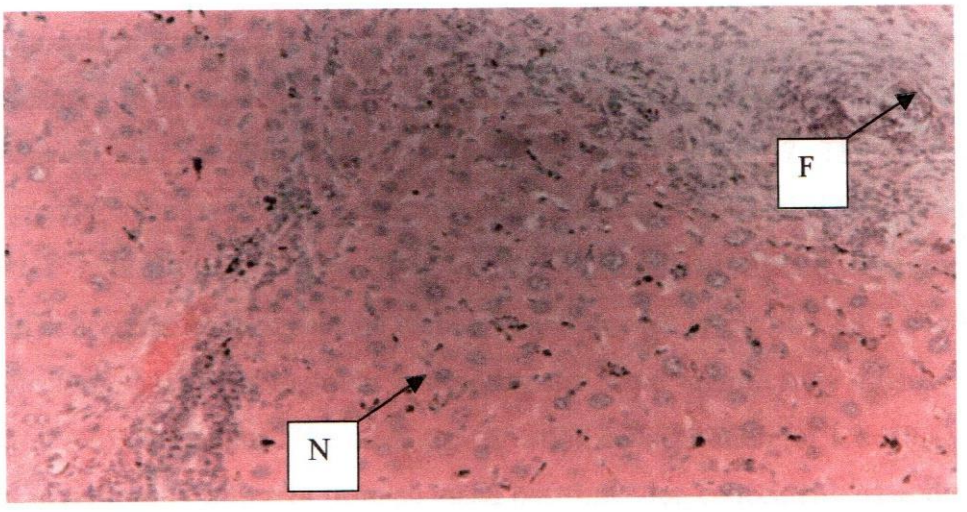


Plate 15: Liver section from *S. mansoni* infected mouse at Day 77 post-infection and Day 35 after curative treatment with praziquantel, showing resolving fibrosis (F) and near normalized hepatic tissue (N).

(H & E stain ×100).

4.2 Studies involving baboons

4.2.1 Infection Experiment

4.2.1.1 Changes in Protein Concentrations

Changes in mean serum haptoglobin concentrations (±SEM) of baboons infected with *S. mansoni*, and uninfected controls, are depicted in Figure 7. The concentrations showed a gradual increase in the infected baboons. The levels had tripled from pre-infection levels of 1.1 ± 0.43g/l, to 3.6 ± 0.45g/l by 14 days post-infection. The increase in haptoglobin concentration then became more gradual to reach levels of 4.2 ± 0.64g/l by Day 42 post-infection. Mean haptoglobin concentrations were significantly higher than the controls at all sampling points. The mean pre-infection concentration (of 1.1 ± 0.43 g/l) was found to be significantly different from all mean post-infection concentrations as determined by the Duncan's multiple range test (where $P < 0.05$).

Changes in mean serum albumin concentrations (±SEM) of baboons infected with *S. mansoni*, and uninfected controls, are depicted in Figure 8. The serum albumin concentration of the infected baboons did not vary significantly from pre-infection levels of 42 ± 3.1g/l, except at Day 28 when it decreased to 39.4 ± 2.9g/l. Thus the levels were not statistically different from the controls. As shown in Table 4, post-infection levels were also not significantly different from the pre-infection concentration at a P value of < 0.05.

4.2.1.2 Changes in Cation Concentrations

Changes in mean serum iron concentrations (±SEM) of baboons infected with *S. mansoni*, and uninfected controls, are depicted in Figure 9. Mean iron concentrations in infected animals decreased gradually from pre-infection values of 215.0 ± 2.4µg/dl by Day 14 post-infection. The decrease continued linearly to Day 42 post-infection, when mean concentrations were down to 140 ± 4.3g/dl. Post-infection levels were significantly different from those of controls.

Changes in mean serum zinc concentrations (±SEM) of baboons infected with *S. mansoni*, and uninfected controls, are depicted in Figure 10. The mean zinc concentrations of infected animals decreased below control levels to 123.2 ± 4.9µg/dl by 14 days post-

infection. The levels slightly increased to 133.5 ± 3.1μg/dl by Day 28, but later decreased to 85.0 ± 2.3μg/dl by Day 42 when the experiment was terminated. Zinc levels in the infected baboons were significantly ($P< 0.05$) different from controls through out the infection.

Changes in mean serum copper concentrations (±SEM) of baboons infected with *S. mansoni*, and uninfected controls, are depicted in Figure 11. The copper concentrations in infected baboons decreased slightly from pre-infection levels of 99.0 ± 2.3μg/dl to 98.2 ± 2.0μg/dl at Day 14 post-infection. The concentrations then rose to 114 ± 2.6μg/dl by Day 28 post-infection and slightly more at Day 42 post-infection. These levels were not significantly different from levels in the controls. As shown in Table 4, post-infection concentrations only became significantly different from pre-infection concentrations at Day 28 post-infection.

4.2.1.3 Gross Pathology

All the baboons in the infection experiment had severe pathology with granulomas extending throughout the liver. At Day 42 post-infection, granulomas appeared as numerous raised pinhead-sized foci distributed over the surface of the liver lobes. In addition, the liver was friable, oedematous, with rounded edges, which indicated a state of acute inflammation. At termination of the experiment (6 weeks post-infection), the colon had only rare, scattered, nodular lesions on the serosal surface, but otherwise appeared normal. The mesenteric lymph nodes were slightly enlarged.

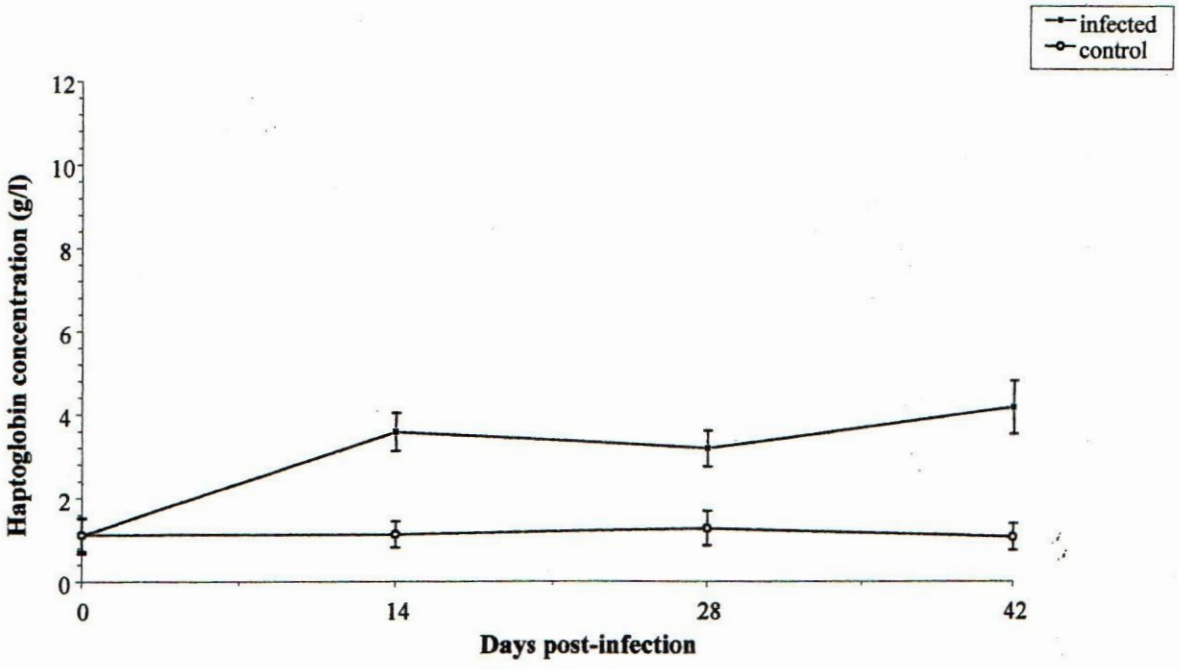


Figure 7: Mean serum haptoglobin concentrations (± S.E.M) in control and *S. mansoni* infected baboons.

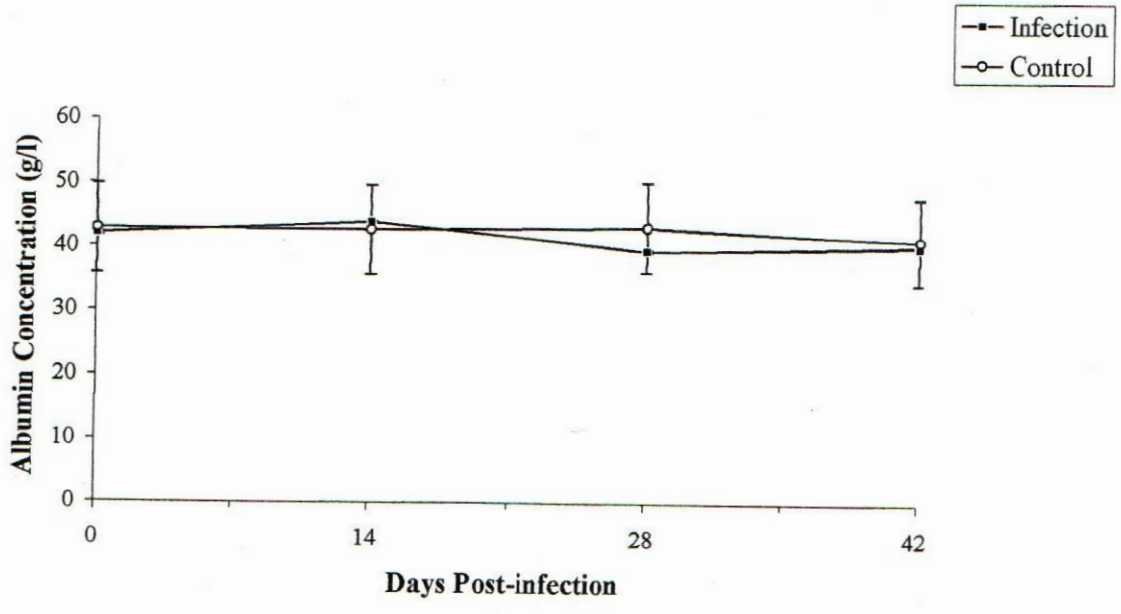


Figure 8: Mean serum albumin concentrations (± S.E.M) in control and *S. mansoni* infected baboons.

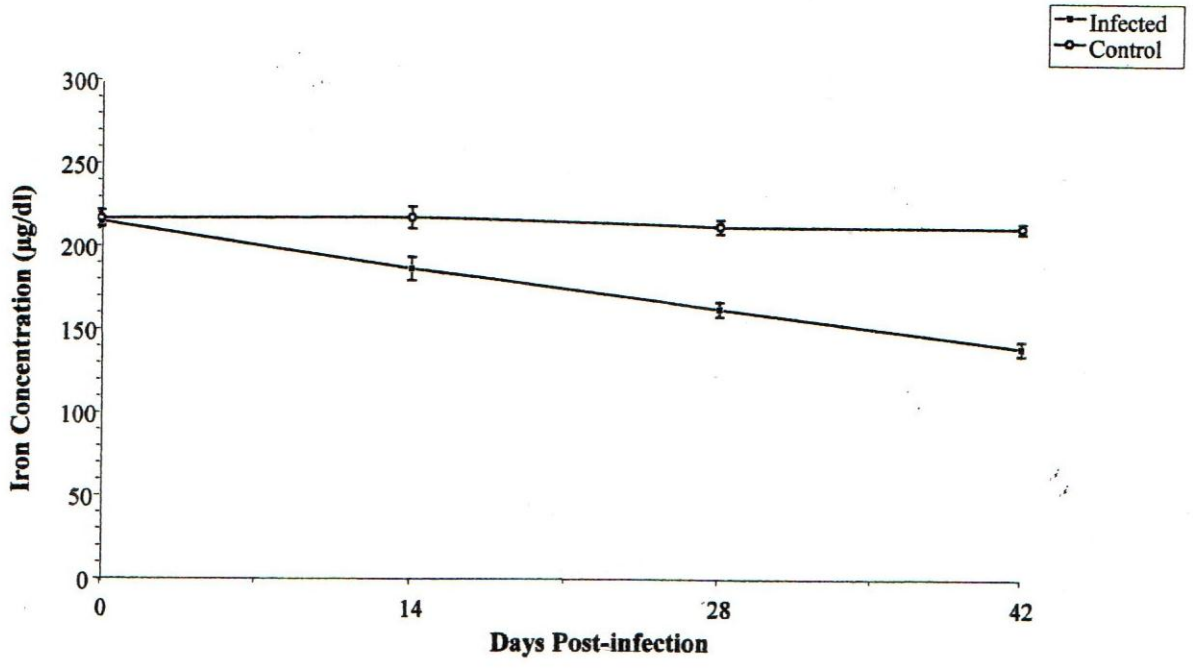


Figure 9: Mean serum iron concentrations (± S.E.M) in control and *S. mansoni* infected baboons.

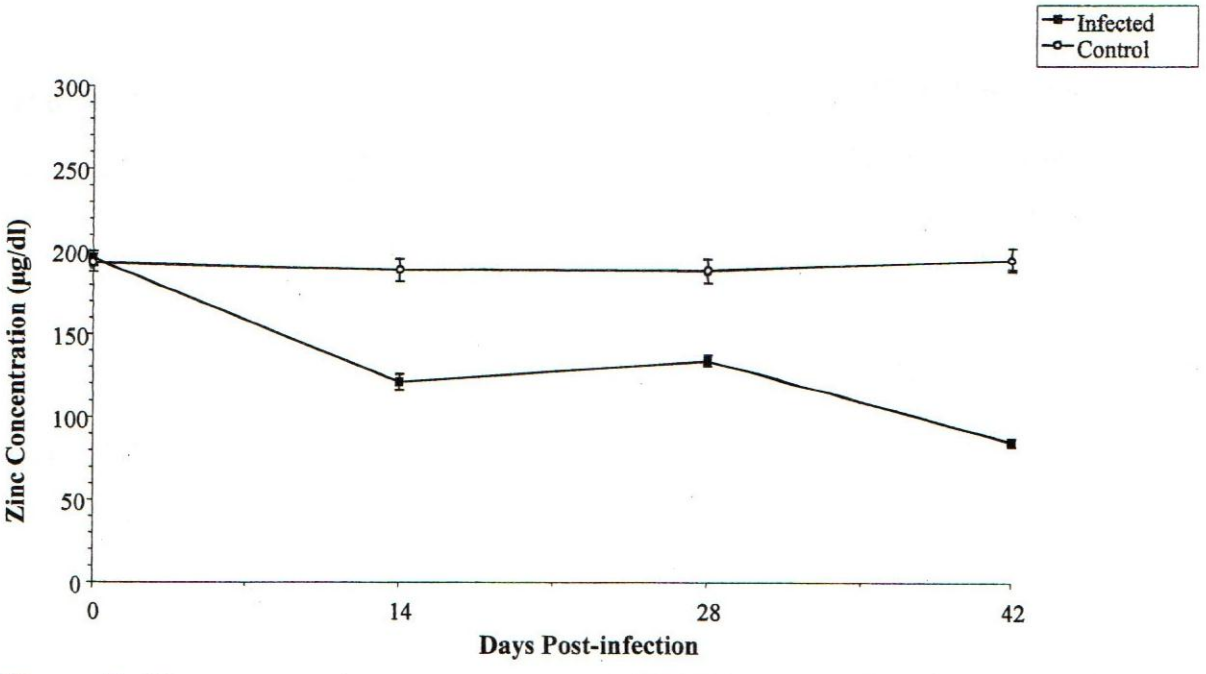


Figure 10: Mean serum zinc concentrations (± S.E.M) in control and *S. mansoni* Infected baboons.

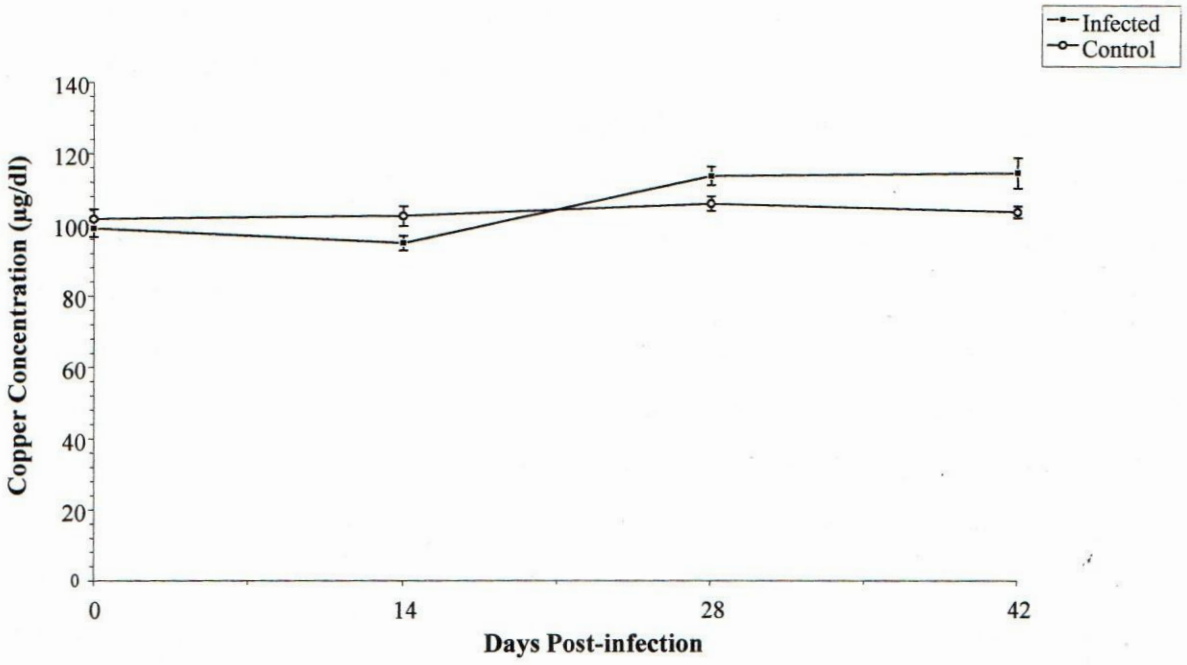


Figure 11: Mean serum copper concentrations (± S.E.M) in control and *S. mansoni* infected baboons.

Table 4: Mean serum concentrations of analytes in *S. mansoni* infected baboons

Days/Treatment	Haptoglobin	Albumin	Iron	Zinc	Copper
0	1.1 ± 0.43^{a}	42.0 ± 3.1^{a}	215.0 ± 2.4^{d}	196.0 ± 4.5^{c}	99.0 ± 2.3^{a}
14	3.6 ± 0.45^{b}	43.8 ± 1.9^{a}	183.3 ± 7.0^{c}	123.2 ± 4.9^{b}	98.2 ± 2.0^{a}
28	3.2 ± 0.43^{b}	39.4 ± 2.9^{a}	163.0 ± 4.5^{b}	133.5 ± 3.1^{b}	114.0 ± 2.6^{b}
42	4.2 ± 0.64^{b}	40.2 ± 0.9^{a}	140.0 ± 4.3^{a}	85.0 ± 2.3^{a}	115.0 ± 4.3^{b}

Mean ± S.E.M values in the same column followed by the same superscript are not significantly different ($P<0.05$).

4.2.2 Infection/Treatment/Challenge Experiment

4.2.2.1 Changes in Protein Concentrations

Changes in the mean serum haptoglobin concentrations (±SEM) in *S. mansoni* infected baboons, treated with praziquantel at Day 88 and challenged at Day 228, and the uninfected controls, are depicted Figure 12. The mean haptoglobin concentrations showed a four-fold increase from pre-infection concentrations of 1.68±0.23g/l to 8.2±0.81g/l by Day 88 post-infection when treatment was instituted. Following treatment, the levels slightly increased to 8.4g/l±0.86g/l on Day 102. After this, they gradually decreased to near pre-infection levels of 2.3±0.71g/l on Day 228, when the animals were challenged with *S. mansoni* cercariae. On challenge with the parasites the haptoglobin levels showed a slow but gradual increase to reach a concentration of 6.1±0.95g/l on Day 298, when the experiment was terminated. As shown in Table 5, the haptoglobin concentrations following infection, and thereafter following challenge, were statistically different from pre-infection concentrations, until at Day 221 post-infection (5 weeks post-treatment). Mean haptoglobin concentrations of the infected baboons were also significantly different (P <0.05) from those of uninfected controls.

Changes in the mean serum albumin concentrations (±SEM) in *S. mansoni* infected baboons, treated with praziquantel at Day 88 and challenged at Day 228, and uninfected controls, are depicted Figure 13. The mean albumin concentrations in infected baboons showed a gradual decrease from a pre-infection value of 47 ± 2.8g/l to 34 ± 3.9g/l at Day 88, when the baboons were treated curatively with praziquantel. Following treatment, the albumin levels showed minimal changes from pre-treatment concentrations up to the time of challenge with *S. mansoni* cercariae. Following the challenge, the mean albumin concentrations of the challenged baboons then gradually started decreasing from 242 days post-infection (2 weeks post-challenge) to reach mean serum concentrations of 22.8 ± 1.5g/l on Day 298 post-infection when the experiment was terminated. The mean serum albumin concentration in the infected baboons was significantly different from control levels throughout the experiment. As shown in Table 5, post-infection concentrations only became statistically different (at P<0.05) from pre-infection concentrations on Day 81 post-infection. All post-challenge concentrations were, however, significantly different from pre-infection concentrations.

4.2.2.2 Changes in Cation Concentrations

Changes in the mean serum iron concentrations (±SEM) in *S. mansoni* infected baboons, treated with praziquantel at Day 88 and challenged at Day 228, and uninfected controls, are depicted Figure 14. The mean iron concentration of the infected animals decreased gradually following infection to reach 162.0 ±3.4 μg/dl at Day 88 post-infection, when the baboons were treated. Following treatment, the mean levels continued to decrease until Day 123, when the mean iron concentration was down to 109.6 ± 2.2μg/dl. After this, the levels started to increase, reaching a mean concentration of 178.0 ± 3.4μg/dl at Day 228 post-infection, when the baboons were challenged with *S. mansoni* cercariae. Following challenge, the levels immediately begun a gradual decrease to reach mean concentrations of 137.0 ± 2.4μg/dl by Day 298, when the experiment was terminated. The mean pre-infection concentration, of 192.0 ± 4.8μg/dl, was significantly different from post-infection, post-treatment and post-challenge concentrations, as shown in Table 5. Mean serum concentrations in the infected animals also showed significant differences from those of the uninfected controls.

Changes in the mean serum zinc concentrations (±SEM) in *S. mansoni* infected baboons, treated with praziquantel at Day 88 and challenged at Day 228, and uninfected controls, are depicted Figure 15. Zinc concentrations of infected animals drastically fell from a mean pre-infection concentration of 230.0 ± 3.5μg/dl to 149.0 ± 3.2 μg/dl at Day 27 post-infection. These levels continued to decline to reach 74.0 ± 2.7μg/dl at Day 88 post-infection, when the baboons were treated. Following treatment, the zinc levels gradually increased, reaching levels of 182.0 ± 3.6μg/dl by Day 228, when the animals were challenged by a second infection with cercariae. Following challenge, the zinc levels showed gradual, fluctuated decrease to reach a concentration of 177.0 ± 8.4μg/dl at Day 298 post-infection, when the experiment was terminated. As depicted in Figure 15, mean serum zinc concentrations of infected animals differed significantly from those of uninfected controls. Table 5 shows that all post-infection mean zinc concentrations differed statistically from pre-infection concentrations at a P value of <0.05. Mean zinc post-challenge concentrations were, however, not significantly different from pre- challenge concentrations.

Changes in the mean serum copper concentrations (±SEM) in *S. mansoni* infected baboons, treated with praziquantel at Day 88 and challenged at Day 228, and uninfected controls, are depicted Figure 16. Following infection, mean copper concentrations showed a drastic increase from Day 27 post-infection to reach levels of 128.0 ± 5.0μg/dl at Day 88 post-infection, when the baboons were treated. Following treatment, the copper levels continued to increase to reach a peak of 143 ± 5.8μg/dl at Day 102 post-infection. After this, they drastically declined to reach levels of 83 ± 1.7μg/dl by Day 228, when the baboons were challenged by a second cercarial dose. Following challenge, the mean copper levels started to gradually increase, reaching levels of 96.0 ± 2.8μg/dl by Day 298 post-infection when the experiment was terminated. From Day 27 post-infection, mean copper concentrations became statistically different from pre-infection concentrations at $P< 0.05$, as shown in Table 5. Both post-infection and post-challenge concentrations differed significantly from uninfected controls as shown in Table 5.

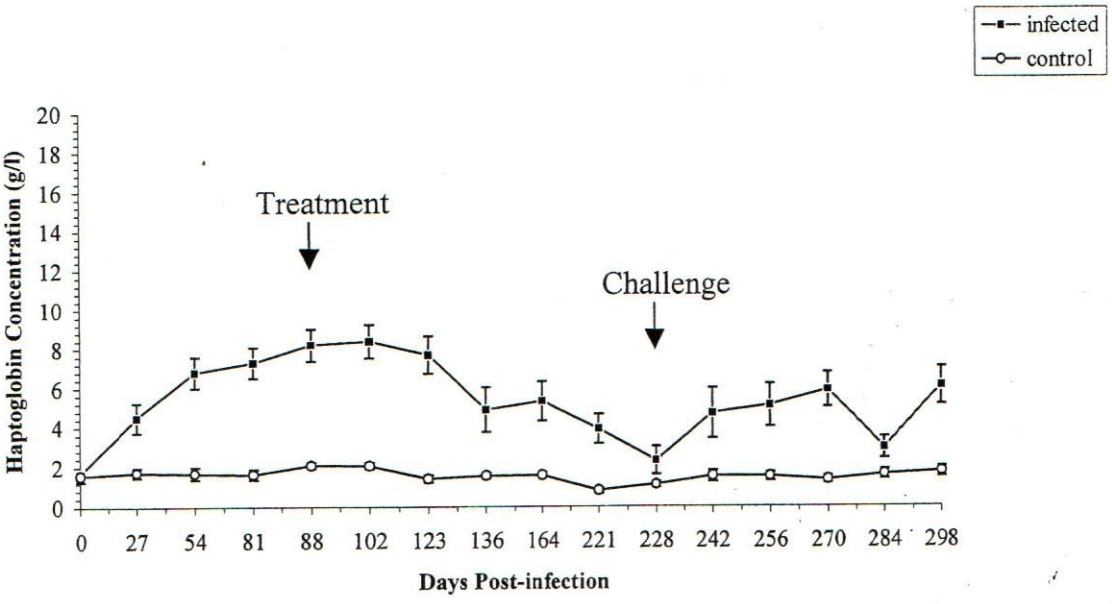


Figure 12: Mean serum haptoglobin concentrations (± S.E.M) in control and *S. mansoni* infected, treated and challenged baboons.

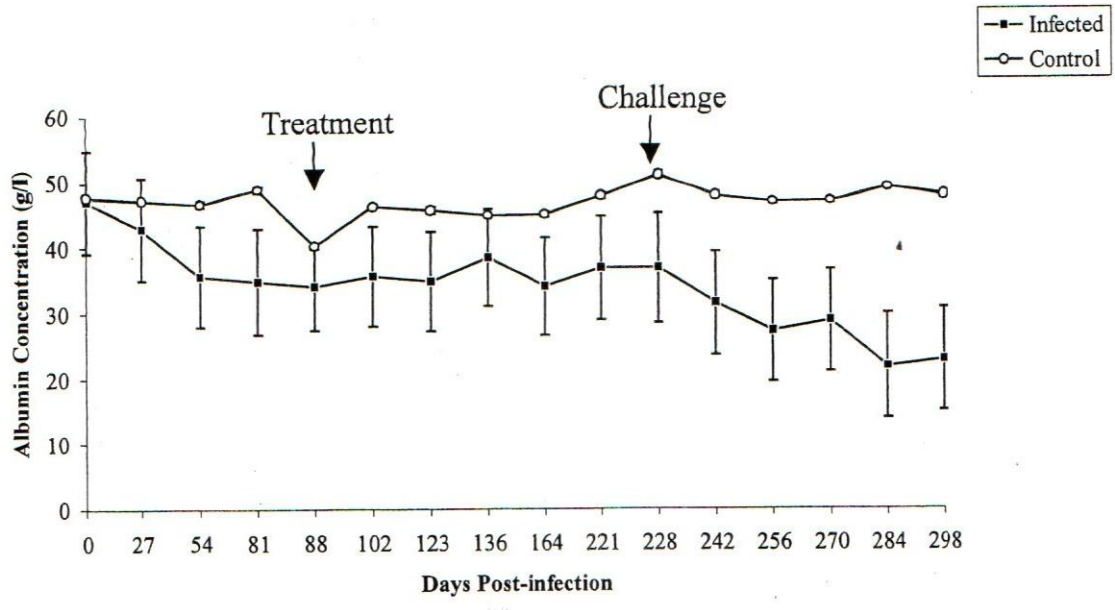


Figure 13: Mean serum albumin concentrations (± S.E.M) in control and *S. mansoni* infected, treated and challenged baboons.

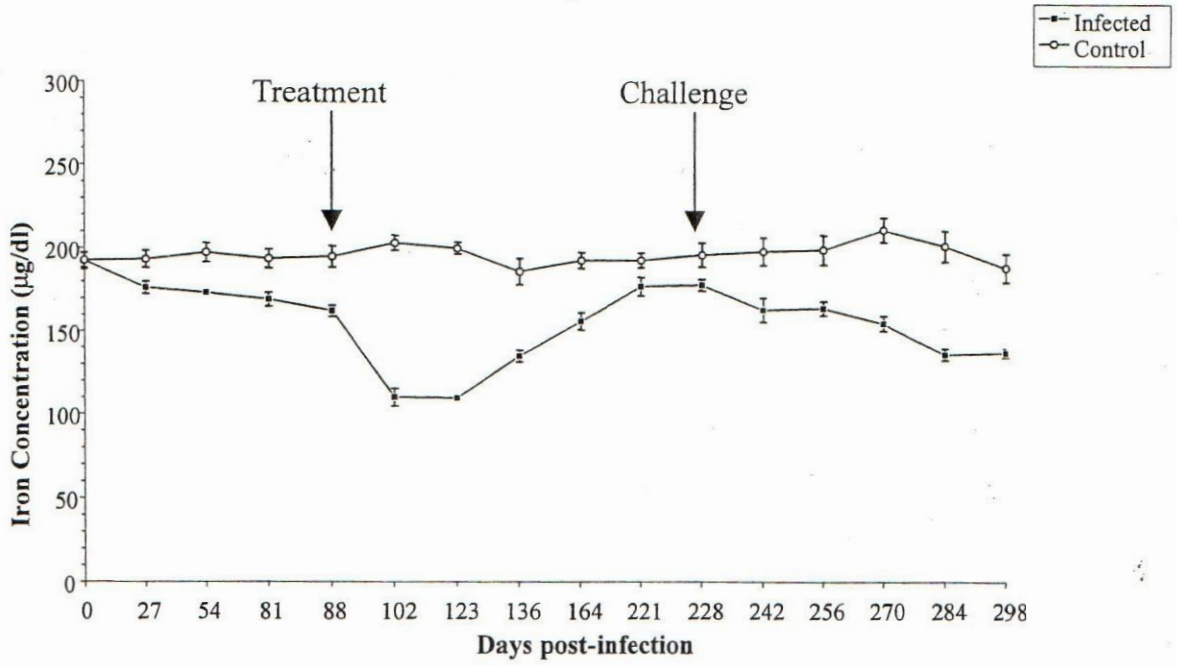


Figure 14: Mean serum iron concentrations (± S.E.M) in control and *S. mansoni* infected baboons.

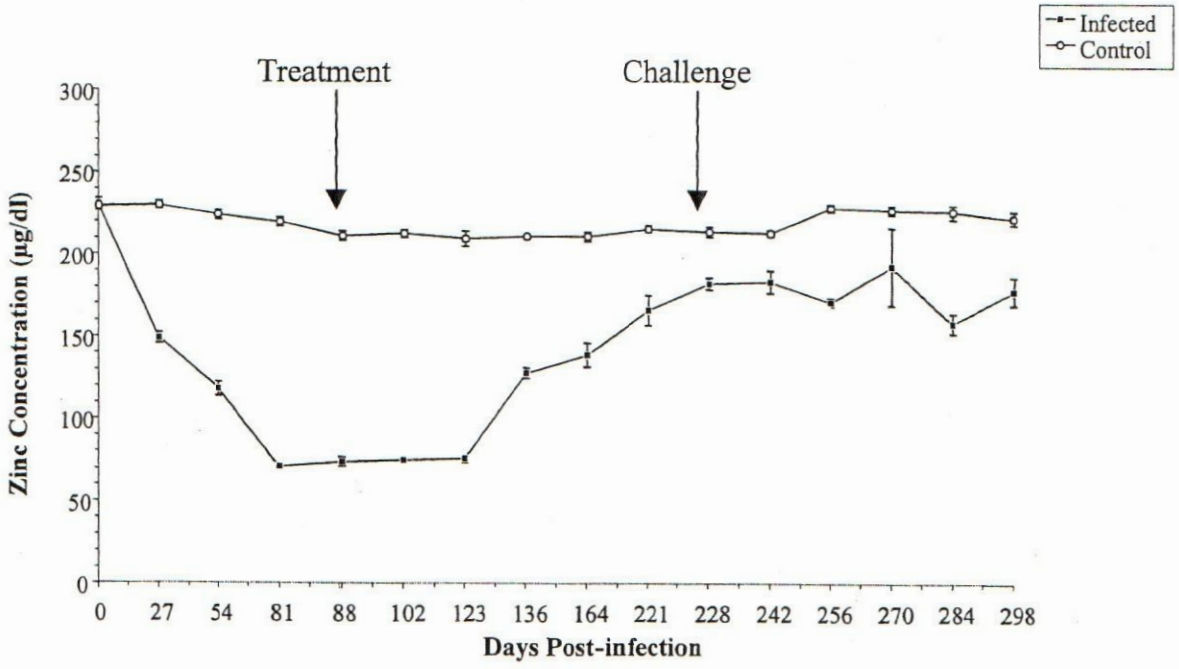


Figure 15: Mean serum zinc concentrations (± S.E.M) in control and *S. mansoni* infected, treated and challenged baboons.

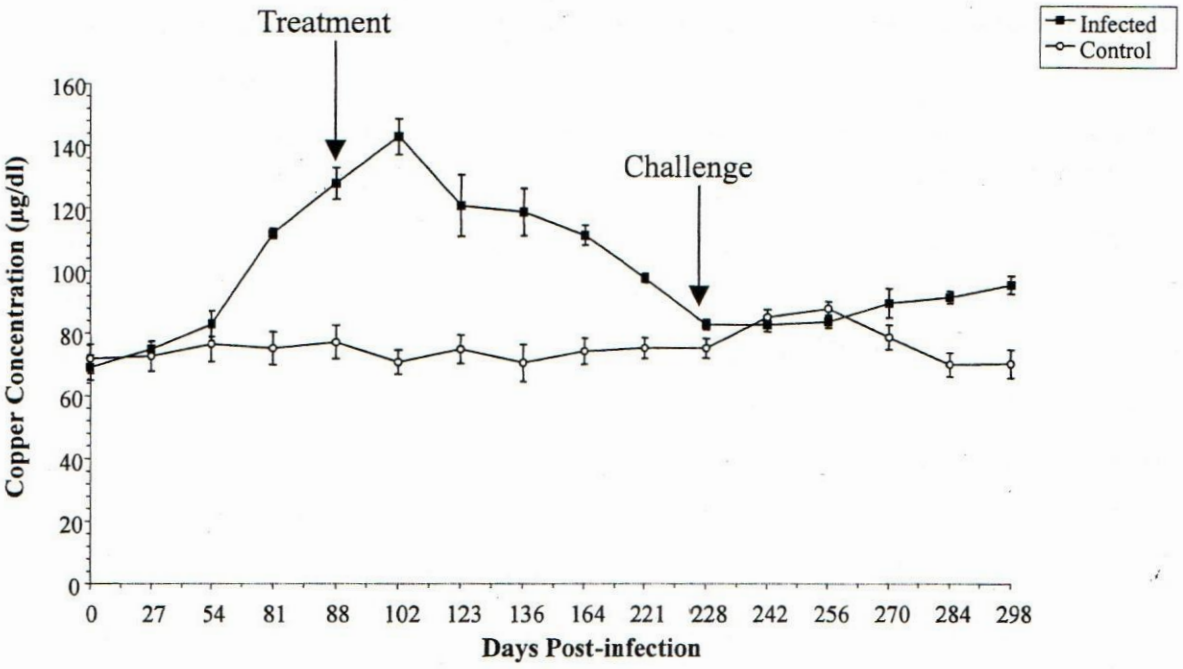


Figure 16: Mean serum copper concentrations (± S.E.M) in control and *S. mansoni* infected, treated and challenged baboons

Table 5: Mean serum concentrations of analytes in *S. mansoni* infected, treated and challenged baboons

Days	Haptoglobin	Albumin	Iron	Zinc	Copper
0	1.68 ± 0.23^{a}	47.0 ± 2.8^{e}	192.0 ± 4.8^{l}	230.0 ± 3.5^{l}	69.0 ± 3.9^{a}
27	4.48 ± 0.75^{bcde}	42.8 ± 1.9^{de}	176.0 ± 3.7^{fgh}	149.0 ± 3.2^{cde}	75.0 ± 2.5^{ab}
54	6.80 ± 0.79^{defg}	35.6 ± 5.1^{cde}	173.0 ± 1.8^{efgh}	118.0 ± 4.2^{b}	83.0 ± 4.1^{bc}
81	7.30 ± 0.79^{efg}	34.8 ± 5.4^{cd}	169.0 ± 4.2^{defgh}	71.0 ± 1.7^{a}	112.0 ± 1.6^{e}
88*	8.20 ± 0.81^{g}	34.0 ± 3.9^{bcd}	162.0 ± 3.4^{cde}	74.0 ± 2.7^{a}	128.0 ± 5.0^{f}
102	8.40 ± 0.86^{g}	35.6 ± 3.2^{cde}	110.0 ± 5.4^{a}	75.0 ± 1.4^{a}	143.0 ± 5.8^{g}
123	7.70 ± 0.95^{fg}	34.8 ± 5.2^{cd}	109.6 ± 2.2^{a}	76.0 ± 2.3^{a}	121.0 ± 9.8^{ef}
136	4.90 ± 1.14^{bcdef}	38.4 ± 1.8^{cde}	135.0 ± 3.6^{b}	128.0 ± 3.3^{bc}	119.0 ± 7.5^{ef}
164	5.32 ± 0.99^{cdef}	34.0 ± 3.6^{bcd}	156.0 ± 5.1^{cd}	139.0 ± 7.3^{bcd}	111.0 ± 3.1^{e}
221	3.90 ± 0.74^{abcd}	36.8 ± 4.0^{cde}	177.0 ± 5.5^{gh}	166.0 ± 9.1^{efg}	98.0 ± 1.4^{d}
228#	2.30 ± 0.71^{ab}	36.8 ± 4.5^{cde}	178.0 ± 3.4^{h}	182.0 ± 3.6^{gh}	83.0 ± 1.7^{bc}
242	4.70 ± 1.28^{bcde}	31.4 ± 2.2^{abcd}	163.0 ± 7.2^{cdef}	183.0 ± 6.8^{gh}	83.0 ± 2.2^{bc}
256	5.10 ± 1.09^{bcdef}	27.2 ± 3.6^{abc}	164.0 ± 4.3^{cdefg}	171.0 ± 2.3^{efgh}	84.0 ± 2.1^{bc}
270	5.90 ± 0.89^{defg}	28.8 ± 3.8^{abc}	155.0 ± 4.4^{c}	192.4 ± 23.3^{h}	90.0 ± 4.7^{cd}
284	2.94 ± 0.54^{abc}	21.8 ± 1.2^{a}	136.0 ± 3.5^{b}	158.0 ± 5.9^{def}	92.0 ± 2.0^{cd}
298	6.10 ± 0.95^{defg}	22.8 ± 1.5^{ab}	137.0 ± 2.4^{b}	177.0 ± 8.4^{fgh}	96.0 ± 2.8^{cd}

Mean ± S.E.M values in the same column followed by the same superscript are not significantly different (***P<0.05***).

* Treatment with praziquantel

\# Challenge with a second infection of *S. mansoni* cercariae

4.3 Correlations of Mice and Baboon Acute Responses to *S. mansoni* Infection

The correlations of various analyte responses in the mice experiment up to Day 42 post-infection, with those in the baboon infection experiment, which was terminated on Day 42 post-infection, were determined statistically by SPSS using Pearson correlation method.

There were strong positive correlations in the mean serum concentrations of haptoglobin (R= 0.720), albumin (R= 0.411), iron (R= 0.902), zinc (R=0.998) and copper (R= 0.945) between mice and baboons in the first 42 days following infection with *S. mansoni* cercariae. These correlations were significant at a P value of <0.05.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The symptomatology of *Schistosoma mansoni* infection is fairly non-specific. Furthermore, these symptoms are not evident until later in the pathogenesis of the disease when egg deposition begins. As a result, many infections go undiagnosed, especially during the early stages when complete cure can still be attained. In addition, current diagnosis is based on the identification of scant eggs in infected faeces, or immunological tests which also show positive when there have been prior exposures. There is therefore much need for additional methods that will improve early detection of the disease, even before the appearance of definitive signs and symptoms. Profiling of the biochemical changes that occur in the acute phase of Schistosomiasis may provide such a solution. Though sadly, this still largely remains an unexplored frontier.

Acute phase reactants are produced in response to a variety of disease conditions stimulated by the pro-inflammatory cytokines and in response to infection, inflammation or trauma. The wide nature of the acute phase response can be seen as a disadvantage in that the acute phase assays are not specific for one disease, but this is shared with other long established diagnostic tests in the clinicians' repertoire. For example, measuring temperature and heart rate are not tests for specific disease but have nevertheless proved useful over centuries of use. Indeed, the acute phase reactants have been called 'chemical thermometers', but they are actually more stable than the measurement of temperature as mammals have strong homeostatic regulatory mechanisms in operation and the acute phase response can be more closely related to disease progression or recovery. They also have an advantage over liver enzymes that are used to assess liver pathology, such as aspartate amino tranferase and alanine aminotransferase, in that they are less sensitive to physiological variations including age, sex and weight. Over the last few decades, acute phase reactants have become the biomarkers of choice in human medicine for monitoring inflammation and infection. (Murata *et al,* 2004).

The study demonstrated that the pathogenetic process of *S. mansoni* could be faithfully produced in animal models. Histopathological studies of hepatic tissue of infected mice revealed that granuloma formation peaks around weeks 6-7 post-infection. A similar observation was made in baboons by Farah *et al,* (1997). The pathogenetic process of *Schistosoma mansoni* studied in the mice models also seemed to closely resemble that seen in humans. The development and modulation of granulomas, and eventual fibrosis in the liver that was experimentally induced in the mice is also seen in humans infected with *S. mansoni.* Studies in murine schistosomiasis demonstrate that the development of fibrosis recquires the production of the profibrotic cytokines IL-2 and IL-4, and is suppressed by IL-12 and IFN-γ. Cytokine regulation of fibrosis and granuloma formation appear to differ. Whether these cytokines participate in the development of schistosome-induced hepatic fibrosis in humans or non-human primates has never been directly tested (Farah *et al,* 2000a). However, IL-10 has been found to be an important cytokine in regulating the immune response and possibly controlling morbidity in human *S. mansoni* infection, and that the production of IFN-γ may be associated with resistance to infection (Correa-Oliveira *et al*, 1998).

Portal space enlargement and the preliminary stages of "pipestem" fibrosis were also seen in the infected mice models. This deducedly led to portal hypertension and the subsequent hepatomegally and splenomegally observed. Hepatic fibrosis may also be credited for increased pressure on the bile ducts, leading to the bile duct hyperplasia observed in the livers of some mice. The development of "pipestem" fibrosis agrees with studies done on *S. mansoni* infected mice by Coutinho (2004).

Following treatment with a curative dose of praziquantel, the histopathological effects of the schistosomal inflammatory processes were seen to regress. This supports the assertion by Alsemgeest *et al*, (1994), that acute inflammatory processes are usually reversible. By 2 weeks after treatment, the granulomas in the liver had begun to resolve. One week later, hepatic tissue fibrosis showed slight resolution. Bile duct hyperplasia greatly reduced by the twenty-eigth day post-treatment. By the fifth week following treatment, which was the final sampling point, fibrosis was greatly decreased, with most of the hepatic tissue having normalised. Only a few resolving granulomas were left. However, there was still some peri-portal fibrosis. This could explain the continued hepatomegally,

splenomegally and ascites observed in the treated animals. Furthermore, these complications mitigated the inability to attain pre-infection levels seen in the various analytes. Zwingenberger *et al,* (1990), assessed serological indicators for disease activity and portal vein obstruction and/or deviation in 23 patients with hepatosplenic schistosomiasis before and up to 18 months after praziquantel treatment. The results provided evidence that the fibrogenetic process ceases after specific chemotherapy, and that portal vein pathology regresses in a substantial proportion of hepatosplenic schistosomiasis cases. This study collaborates Zwingenberger's findings of caesation of the fibrogenetic processes following specific treatment. Furthermore, slight regression of portal pathology was observed by the time the experiment was terminated. It may be inferred that with time, keeping in mind that this study covered a period up to only 5 weeks following treatment, more obvious regression of the periportal fibrosis may have been evident.

The results of the study have shown that there are indeed significant changes that occur in some serum proteins and cations during the acute phase of *S. mansoni* infection. These changes were shown to increase with increased tissue inflammation and to decrease with resolving inflammation and fibrosis following curative treatment. The changes were however notably less pronounced in cases following challenge by a second cercarial dose, signalling that there are differences between acute phase and chronic phase profiles of these elements.

The synthesis, secretion and clearance of different acute phase reactants varies greatly. The first acute phase reactants are produced within a few hours of tissue injury and their peak values can be reached within one day after the onset of the injury (Coutinho, 2004). Constitutively secreted plasma proteins respond with delay followed by sustained responses due to longer half-lives (Hayes, 1994). In some inflammatory diseases, certain acute phase reactants may be more actively consumed, thus resulting in relatively low serum levels considering the stage of inflammation (Dinarello, 1984).

The response patterns of the acute phase reactants vary with the acute phase stimulator and the host species. The responses may also be affected by age, sex, pregnancy and genetic polymorphisms (Alsemgeest *et al,* 1994; Hayes, 1994). In order to minimise these variables, the mice used in the study were in-bred, of about the same age, and were all males. The study showed haptoglobin and copper to be positive acute phase responders,

while albumin, zinc and iron were negative acute phase responders in *S. mansoni* infections of both the baboon and mice models. These changes correlated well with tissue pathology following infection and with recovery following treatment. There were strong positive correlations between mice and baboon analytes following infection. These correlations were significant at a P value of <0.05.

Of the studied molecules, haptoglobin appeared to be the most sensitive marker for inflammation in both baboon and mice models. In baboons, Hp showed increases of up to 281% on Day 42 post-infection of the infection experiment and 400% on Day 102 of the infection/treatment/challenge experiment. In the mice, increases of 257% were achieved on Day 49 post--infection, with reference to the initial mean pre-infection concentrations. These increases were however not as sustained because the haptoglobin-haemoglobin complex is rapidly removed from the plasma, for example, its half-life in humans is only 10-30 minutes (Koj, 1974). These increases were seen to diminish significantly following effective treatment with praziquantel and to increase gradually following challenge with a second cercarial dose. Post-challenge increases were, however not as pronounced as those following the first infection, probably because the liver, which is the main source of haptoglobin had not completely recovered from fibrosis, thus reducing its potential to produce the acute phase proteins. However, normal pre-infection concentrations were not realised following curative treatment, even after the liver damage and inflammation had resolved as histologically evidenced in week 11 of the mice experiments. Haptoglobin therefore proved to be a good indicator of residual tissue pathology. Though much work has been done in investigating the response of haptoglobin to various infections, little appears to have been done to study the protein in schistosomiasis.

Albumin showed the least sensitive responses to the infection of all the reactants studied in the experiments. The baboon Infection Experiment only showed decreases of up to 14% on Day 28 post-infection from the concentrations seen in the uninfected controls. Decreases in the baboon Infection/Treatment/Challenge Experiment and in the mice experiment were however more pronounced at 53.6% on Day 282 and 48.7% on Day 42 post-infection respectively. The liver has large reserves of albumin synthetic capacity. In addition, albumin generally has a long plasma half-life (20 days in humans). These attributes could account for the slow responses of serum albumin concentrations observed. In addition,

the differences in albumin responses between mouse and baboon models could be due to variation in plasma half-life in the species. The effects of low serum albumin are related to maintenance of fluid in the circulating compartment (albumin provides 80% of plasma oncotic pressure). With reduced levels of serum albumin, fluid may escape into the tissues to cause oedema or into body cavities to cause ascites/pleural effusions. This, in addition to periportal fibrosis, could have contributed to the ascites seen in the gross pathology seen of the infected mice.

These changes in haptoglobin and albumin concentrations during schistosomiasis may have a useful role both in the murine and primate models as sensitive markers for inflammation and disease activity. However, it will be important to identify the mechanisms responsible for the development of the APR. This study has shown that a similar APR occurred in both mice and baboon models infected with *S. mansoni.* It should be noted that these increases could be demonstrated in circulation fairly early in the infection, long before the development of definitive signs and symptoms. Moreover, the acute phase reactants responded sensitively to treatment and also indicated the presence of residual pathology.

Acute phase proteins might have pathophysiological functions during disease conditions such as schistosomiasis. Haptoglobin can influence the pathogenesis of disease in several ways. In the circulation, it binds iron, an effect that in the long run could improve iron conservation (Ngure *et al*, 1997), which would mitigate the anaemia seen in schistosomiasis. Also, the peroxidase activity of the haemoglobin-haptoglobin complexes, might locally inactivate inflammatory cell products which cause tissue damage in chronic disease (Koj, 1974). Haptoglobin, at acute phase response concentrations, has been shown to be able to significantly depress T-cell stimulation (Oh *et al*, 1990).

Haptoglobin has been described as a sensitive, specific and efficient disease marker in several animal species including cattle, sheep and dogs. It is also less likely to give false positive and negative results in comparison to other indicators such as haematology (Skinner & Roberts, 1994). These findings have been echoed in the present study in both mice and baboon models, indicating that it is valuable in determining pathology and response to treatment in *S. mansoni* infections.

The response sensitivity of iron was found to be relatively moderate with the greatest decrease of 48.9% being seen on Day 56 post-infection of the mice experiment.

The most pronounced decrease in the baboon Infection/Treatment/Challenge Experiment was of 43.2% on Day 102, and in the Infection Experiment was 34.9% on Day 42 post-infection. These results agree with a study by Laudage & Schirp (1996), who found that schistosomiasis was a rare cause of iron-deficiency anaemia. This could partially be credited to the increase in haptoglobin observed in the study, as the haemoglobin-haptoglobin complex binds iron and preserves it, thus preventing iron deficiency anaemia. Serum iron has also been found to decline substantially in the APR of a number of infections (Lohuis *et al,* 1988 and 1988b; Alsemgeest, 1994; Hayes, 1994; Mwangi *et al,* 1995).

Zinc also was seen to drop fairly rapidly following infection with *S. mansoni.* There was also little variation in peak levels between experiments. There were decreases of up to 66.4% on Day 42 & 49 post-infection in the mice and 56.6% on Day 42 post-infection of the Infection experiment & 69.1% on Day 81 in the Infection/Treatment/Challenge Experiment of the baboons. This hypozincemia occurs because of a sequestration of zinc within hepatocytes, thymocytes and marrow cells. The sequestration of zinc is the secondary consequence of the induced expression, caused by pro-inflammatory cytokines, of metallothionein 1 and 2 genes within these responding cells (Lao *et al,* 1987; Rhodes & Kluh, 1993; Vallee & Falchuk, 1993; Taylor, 1996).

Copper appeared to have the greatest response in baboons, where increases of up to 107% were seen in the baboon infection experiment on Day 102 post-infection. The baboon Infection/Treatment/Challenge Experiment saw peak levels reaching up to increases of 61% on Day 42 post- infection. Responses in the mice experiment were however poor with increases of only up to 16% on Day 42 post- infection. The differences in responses between mice and baboon models could be due to variations in plasma half-lives of copper between the two species. Increase in serum copper is concomitant with increase in ceruloplasmin, as ceruloplasmin normally carries about 95% of the circulating copper (Taylor, 1996).

A study by Mikhail & Mansour (1982), found plasma copper in patients with active *S. mansoni* infection was significantly elevated and zinc was significantly depressed, and the degree of this correlated with the associated hepato-splenic complications of the disease. At clinical recovery, levels of copper and zinc were significantly improved in all the patients from pre-treatment values, but were within the normal range only in patients

without complications. This indicated that complications associated with schistosomiasis could delay normalisation of copper and zinc levels beyond the clinical cure stage. These observations made by Mikhail & Mansour (1982), were echoed in this study when complications, including hepatomegally, splenomegally and ascites, were seen to hinder attainment of pre-infection concentrations of the acute phase reactants.

Although changes in concentrations of serum cations are recognized as one of the first, and most sensitive, indicators of a metabolic change in animals as a result of parasitic infection (Beisel, 1977), the pathophysiological mechanisms involved are not well understood. Moreover, the protection afforded to animals by the acute phase changes in trace mineral metabolism during infection has only been partially characterised. A defence system termed "nutritional immunity" was proposed in which the host prevents the multiplication of bacteria in body fluids by preventing their acquisition of nutritionally important trace minerals (Weinberg, 1978). Such a mechanism has been well characterised for iron which is tightly chelated in extracellular fluids by transferrin and lactoferrin (Weinberg, 1978). However, "nutritional immunity" involving zinc has not yet been demonstrated, although adequate zinc levels are known to be necessary for almost all manifestations of an animal's immune response, including macrophage phagocytosis and intracellular killing, and the early activation stages of antibody formation (Cousin, 1985). It would appear that the system proposed by Weinberg (1978) for preventing parasites from acquiring nutritionally important trace minerals would play a protective role during infections with *S. mansoni.* However, there was no evidence of protection in the infected animals in the study in spite of significant reduction in serum iron and zinc concentrations. This might have been due to the apparent slow pace at which the nutrients were being removed from the general circulation. Moreover, the levels to which plasma iron and zinc concentrations dropped might still have been adequate for parasite growth and expansion (Mwangi *et al,* 1995).

In all three experiments there were changes in the serum concentrations of all acute phase reactants immediately following infection. These changes may reflect the changing pattern of cytokine release at different stages of the disease, the variation in cytokines modifying the regulation of the acute phase protein genes. It is known that there are species variations in cytokine profiles in schistosomiasis (Correa-Oliveira, 1998).

However, cytokine profiles in primate/human schistosomiasis seem to be poorly understood, possibly due to the inherent difficulty of measuring cytokines. Cytokine profiles in murine schistosomiasis, however, appear to be quite well characterised. Coutinho *et al*, (2000), found that increased IFN-γ predominates in early infection in mice, but as the infection progresses and egg deposition and tissue reaction begin, IFN-γ production decreases and that of IL-10 increases. Stadecker (1992) proposed that IL-10 causes downregulation of the granuloma, and his colleagues showed down modulation of anti-egg immune responses and granuloma size in acute infection by IL-10 (Villanueva *et al,* 1994).

The post-infection concentrations of all the analytes were significantly different from those of uninfected controls, except for the analytes in the baboon experiments involving albumin. Haptoglobin, iron, zinc and copper proved to be good indicators of inflammation, as their concentrations in serum changed significantly following infection. In addition, the changes could be detected quite early in the infection, long before the onset of any tell-tale signs or symptoms. Significant changes in haptoglobin and iron concentrations could be detected from as early as 14 days post-infection for both baboon and mice models. Those of zinc could be detected by 14 days post-infection in the baboons and by 7 days post-infection in the mice. Those of copper could be detected 28 days post-infection in the baboons and 21 days post-infection in the mice. Of all the acute phase reactants analysed, copper appeared to have the slowest response to *S. mansoni* infection.

After treatment, in the baboon Infection/Treatment/Challenge Experiment and in the mice experiment, all the analytes monitored showed positive trends back towards their normal pre-infection concentrations. The post-treatment concentrations became significant ($P< 0.05$), with reference to the post-infection/pre-treatment concentrations, for all the reactants except for albumin concentrations in the baboon models. Significant recovery from post-infection/pre-treatment concentrations in the baboons was seen from Day 48 post-treatment for haptoglobin and zinc, Day 14 post-treatment for iron, and Day 76 post-treatment for copper. In the mice, significant recovery from post-infection/pre-treatment concentrations was noted from Day 21 post-treatment for haptoglobin, albumin, iron & zinc, and on Day 28 post-treatment for copper.

Although there were significant changes in the analytes, following treatment, back toward normal serum concentrations, pre-infection concentrations were never attained.

This may be attributed to the complications that still persisted, even after resolution of fibrosis and granulomas. Complications of hepatomegally, splenomegally and ascites were demonstrated in the infected and treated and mice. Moreover, the resolution of fibrosis was not absolute by the time of conclusion of the experiment, and hence it could be assumed that the liver was still performing sub-optimally.

In the baboon Infection/Treatment/Challenge Experiment, the baboons were challenged with a second cercarial dose 228 days after the first infection. The post-challenge serum concentrations of all the analytes attained significant differences ($P< 0.05$) from both post-infection/pre-challenge concentrations and pre-infection concentrations. This was excluding copper, whose post-challenge concentrations did not significantly differ from post-infection/pre-challenge concentrations. The post-challenge changes were notably less marked than those following the initial infection. This may be explained, in part, by the fact that the liver had not fully regained its original capacity for synthesis, following fibrosis caused by the first infection, by the time of the challenge infection.

The initial evidence of active *S. mansoni* disease is the Katayama syndrome. The incubation period of the Katayama syndrome ranges generally from 3-7 weeks in humans, and is associated with the onset of egg deposition. Suprisingly short incubation periods may however be encountered in non-immunes (Butterworth *et al,* 1989). The results of this study show that the acute phase reactants monitored begun to show changes in serum concentrations long before the onset of egg deposition, from as early as one week following infection. The changes however, became significant ($P< 0.05$) only after what may be assumed as the onset of egg deposition, at 3 weeks post-infection and later.

All the acute phase reactants monitored in the mice demonstrated changes that could be directly linked to the extent of tissue damage i.e. these changes appeared to be in direct response to severity of tissue damage. Haptoglobin, albumin, iron, zinc and copper, all showed maximum changes in serum concentration between week 6 and 8 post-infection. The histopathological studies of hepatic tissues of the mice revealed that it was in this period that there was peak granuloma presence, massive tissue fibrosis and periportal fibrosis. Thereafter, the effects of curative treatment with praziquantel begun to be evident as the granulomas and tissue fibrosis resolved. These improvements in the damaged hepatic tissues were also favourably reflected in the serum concentrations of the acute phase reactants. The

reactants showed recovery of normal serum concentrations with the healing of tissue damage. By week 5 post-treatment (week 11 post-infection), most of the hepatic tissue had healed and appeared normal. The acute phase reactants however did not achieve pre-infection concentrations. This may be explained by the continued hepatomegally, splenomegally and ascites seen in the gross pathology of the mice. In spite of the healing evident in the hepatic tissues, other inflammatory conditions still persisted in the animals. Hence, except for albumin, all the analytes displayed serum concentrations that, though improved, were still significantly different from pre- infection concentrations.

The results of the study proved right the hypothesis that during the acute phase of *Schistosoma mansoni* infection of baboon and mouse models, there is a characteristic increase in serum copper and haptoglobin concentrations, and a depression in serum albumin, iron and zinc concentrations. These changes were seen to reduce gradually following curative treatment. However, the concentrations did not attain normal haematological values probably because the studies were concluded before tissue trauma and inflammation had completely ceased. This was especially obvious in the mice experiment where complications such as ascites, hepatomegally and splenomegally were seen to persist to the end of the experimental period, even after resolution of liver fibrosis.

This study found haptoglobin, albumin, iron, zinc and copper to respond sensitively to tissue damage and inflammation. Albumin, however, seemed to be a slower respondent than the other reactants. This can be attributed to the fact that although albumin production decreases during the acute phase, the body has large reserves of albumin that cushion the decrease from seeming so obvious.

5.2 Conclusion

This study has shown that a similar acute phase response occurs in mice and baboon models experimentally infected with *S. mansoni.* This was demonstrated by an increase in haptoglobin and copper, and a decrease in albumin, iron and zinc following infection. Haptoglobin was the most sensitive responder to the infection of all the acute phase reactants studied. Moreover, changes in the reactants correlated well with liver pathology and curative treatment.

The results of this study have determined that the diagnosis, prognosis and monitoring of treatment in *S. mansoni* infection may indeed be enhanced by measuring haptoglobin, albumin, iron, zinc and copper. The study also proves that chronic, as well as acute conditions can be evaluated and characterised by profiling the biochemical changes that occur in the acute phase of *S. mansoni* infection.

The application of APP measurements in diagnostics and determination of disease prognosis would be of great value because the APPs are not species-specific, they are easy to measure and are not adversely affected by physiological variations. They are also good indicators of early disease and residual pathology. Furthermore, this study has proved that unlike the methods used currently for diagnosis, changes in acute phase reactants can be determined quite early in the development of the disease. Their inclusion in diagnosis would therefore greatly improve the prognosis of the disease as it would enhance early detection and intervention before extensive fibrosis has occurred.

5.3 Recommendations

There remain numerous avenues for further original research, not least to clearly define the acute phase response of *S. mansoni* infection, including the profiling of other reactants involved. This will lead to improved interpretation, and also to better understanding of the basic mechanisms of control and function of the acute phase reactants. Studies on human subjects should also be done in order to determine application of the findings of this study in human medicine.

Finally, the application of acute phase assays would be lifted to a higher plane if rapid, robust, multiplexed and inexpensive methodology could be developed so the levels of the proteins and cations in blood or other fluids could be measured in real-time at the point of investigation.

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