SERUM LIPID CHANGES IN RABBITS WITH SINGLE AND MIXED TRYPANOSOMA BRUCEI BRUCEI AND TRYPANOSOMA CONGOLENSE INFECTION.

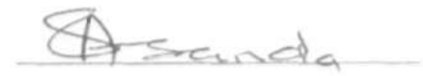
SAFIYA ASIBI SANDA

A thesis submitted to the postgraduate school, Ahmadu Bello University in partial fulfilment of the requirements for the award of Master of Science (M.Sc.) Biochemistry.

Department of Biochemistry
Faculty of Science
Ahmadu Bello University
Zaria, Nigeria.

DECLARATION

I hereby declare that this thesis is a record of my own research work and has not been presented in any previous application for a higher degree at any other University. All information and excerpts from the work of others have been acknowledged by means of references.



Safiya A. Sanda (Mrs)

DIGITIZED

Date: 24th July 1997

CERTIFICATION

This thesis titled "Serum Lipid changes in rabbits with single and mixed Trypanosoma brucei brucei and Trypanosoma congolense infections" meets the regulation governing the award of the degree of M.Sc. Biochemistry Ahmadu Bello University and is approved for its contribution to Scientific knowledge and literary presentation.

Dr. D.A. Ameh
Chairman Supervisory Committee Date 24-7-97

Dr. S. Ibrahim
Member Supervisory Committee Date

The Head
Dept. of Biochemistry. Date 24-7-97

The Dean
Postgraduate School Date 24/10/97

DEDICATION

This thesis is dedicated to the Almighty God, and to my children Salamatu, Aliyu, Malik and to my beloved husband Umaru Sanda.

ACKNOWLEDGEMENTS

I wish to acknowledge the Director Nigerian Institute for Trypanosomiasis Research for funds used for this study. I am very grateful to my supervisors Drs D.A. Ameh and S. Ibrahim for their useful criticisms and suggestions without which this work would have been impossible. Their thorough scrutiny of this write up is highly acknowledged. I also wish to acknowledge Drs H.E. Edeghere, E.O. Elhassan, F.A.G. Lawani and Mr. R. Uzoigwe for their wealth of experience which they were ready to share with me at all times. The technical assistance of Messrs M. Ikenga, M. Maikaje, D. Obaje and Mr. Ikemere is acknowledged. I am grateful to Mrs S. Ibrahim for typing the manuscript. My thanks also go to several others who in one way or the other contributed to the successful completion of this work.

ABSTRACT

The packed cell volume and serum lipids of male New Zealand rabbits with single and mixed infections of Trypanosoma brucei and Trypanosoma congolense were investigated. In the mixed infection group, T.brucei was the predominant parasite species and the clinical picture tended towards that of single T.brucei infection. Anaemia was a significant feature of the disease in all the infected animals with PCV values significantly lower than that of control animals. There was no significant ($P>0.05$) difference between the PCV of all the infected animals. All the infections were characterized by hypercholesterolemia, hypertriglyceridemia, hypoalbuminemia and elevated serum free fatty acid levels which were significantly higher ($P<0.05$) in all the infected groups compared to the control group. Elevated serum total cholesterol and triglyceride serve as predictors of coronary heart disease and may therefore be a cause of death in the infected animals.

TABLE OF CONTENTS

PAGE

Title i

Declaration ii

Certification iii

Dedication iv

Acknowledgements.. v

Abstract vi

Table of contents vii

List of figures ix

1.0 Introduction 1

2.0 Literature Review.. 5

2.1 Morphology of trypanosomes.. 5

2.2 Structure of trypanosomes.. 9

2.3 Transmission of trypanosome infection...... 13

2.4 Antigenic variation and the problem with vaccine development 14

2.5 Trypanosome metabolism.. 16

2.6 Lipid metabolism in African Trypanosomes... 18

2.7 Trypanosomiases.. 20

2.7a Human trypanosomiasis.. 20

2.7b Animal trypanosomiasis.. 21

2.8 Anaemia in trypanosomiasis.. 22

2.8a Immune haemolysis or erythrophagocytosis .. 22

2.9 Control of African trypanosomiasis.......... 26

2.9a Chemotherapy of human trypanosomiasis....... 26

PAGE

2.9b Chemotherapy of Animal trypanosomiasis...... 28

2.9c Vector control............................ 29

2.9d Trypanotolerance........................... 30

2.9e The role of trypanosomal phospholipases on host lipid metabolism 31

3.0 Materials and methods...................... 33

3.1 Materials.................................. 33

3.2 Animal inoculation......................... 34

3.3 PCV determination.......................... 35

3.4 Free fatty acid determination.............. 35

3.5 Albumin determination...................... 36

3.6 Triglyceride determination................. 37

3.7 Cholesterol determination.................. 38

4.0 Results and Discussion..................... 40

5.0 Conclusion and suggestions................. 53

References 55

LIST OF FIGURES

PAGE

Fig. 1 Mean weekly parasitaemia of rabbits infected with T.brucei and T.congolense 41

Fig. 2 Mean weekly PCV (%) of rabbits infected with T.brucei and T.congolense 44

Fig. 3 Mean weekly serum free fatty acid levels of rabbits infected with T.brucei and T.congolense 46

Fig. 4 Mean weekly serum triglycerides levels of rabbits infected with T.brucei and T.congolense 48

Fig. 5 Mean weekly serum Cholesterol levels.of rabbits infected with T.brucei and T. congolense......... 50

Fig. 6 Mean weekly serum Albumin .level of rabbits infected with T.brucei and T.congolense......... 52

CHAPTER ONE

INTRODUCTION

Trypanosomiasis is a disease of immense socio-economic importance affecting both man and animals in sub-Saharan Africa. The disease which is transmitted mainly by the bite of an infected tsetse fly (Glossina species) is caused in man by Trypanosoma brucei gambiense in the West African sub region and Trypanosoma brucei rhodesiense in the East African sub region. In animals the causative organisms are Trypanosoma brucei brucei, Trypanosoma vivax and Trypanosoma congolense. If untreated the disease is fatal in both man and animals.

Animal trypanosomiasis constraints agricultural development in more than a third of the African continent by causing livestock production losses through poor weight gain, stunted growth (ILRAD Report, 1990) poor milk production (Agyemang et al; 1990), reproductive failure (Anene and Omamegbe, 1984) and death. Total current annual costs of the disease including production losses and control costs are estimated to be more than 500 million U.S. dollars (ILRAD reports, 1993).

The clinical manifestations of trypanosomiasis are anaemia (Fiennes, 1953) hepatosplenomegaly, fever and lymphadenopathy (Fiennes, 1970; Stephen, 1970). Even though anaemia is the primary disease manifestation in trypanosomiasis, the mechanism of the anaemia is not fully understood (Jenkins et al, 1974; Facer, 1976; Anosa and Isoun, 1980). Certain mechanisms have been postulated to explain the development of anaemia during trypanosomiasis. These

include haemodilution (Holmes and Jennings, 1976), haemolysis (Naylor, 1971), poor haemopoetic response (Igbokwe, 1989) and haemorrhage (Dirie et al, 1988; Gardiner,et al; 1989; Anosa et al, 1992). Trypanosomes are believed to produce neuraminidases which cleave off sialic acid residues from erythrocyte membranes (Esievo, 1979); causing physical damage to the red blood cells during infection as well as binding of trypanosome antigens or host antibody trypanosome complexes to red blood cells (Kobayakawa et al, 1979). This leads to the identification of the affected red blood cells as foreign bodies and subsequent phagocytosis by macrophages and their removal from circulation.

Pharmacologically active substances released by dead and dying trypanosomes have also been shown to be hemolytic in nature (Tizard et al, 1978a). Among these are the kinins and kallikrein (Boreham, 1980) and free fatty acids (Tizard et al, 1978a). The trypanosomal enzyme phospholipase A has been shown to have hemolytic activity (Mellors and Samad, 1989).

Presently the mechanisms of the overall pathogenesis of trypanosomiasis remains unravelled, although activated macrophages have been implicated.

Trypanosomes have been shown to produce substances which could cause most of the observed pathological symptoms (Rouzer and Cerami, 1980; Pentreath, 1991).

These multifactorial events produce different changes in the blood chemistry of the host in different infections. Among these are the changes in lipid metabolism. The changes in serum lipids

observed during trypanosomiasis have been attributed to depressed lipoprotein lipase activity and consequent reduction in the ability of the host to utilize lipoprotein (Roberts, 1975). Phospholipase A released by dead or dying trypanosomes breaks down phospholipids releasing free fatty acids which are hemolytic in nature (Tizard et al, 1978a). Reduced thyroid output has also been implicated in serum lipid changes (Huet, et al, 1990).

It is known that trypanosomes are unable to synthesize lipids de novo, they therefore depend on host lipids (Mellors and Samad, 1989). The dependence of trypanosomes on host lipids suggests that the survival of the parasite requires the appropriate concentration of lipids in the host blood. In eukaryotes, pyruvate dehydrogenase occurs in the mitochondrion and the acetyl CoA produced is used for fatty acid synthesis in the cytoplasm. The absence of lipid synthesis in the blood stream forms of trypanosomes correlates with the fact that blood stream forms of trypanosomes lack functional mitochondria and therefore depend on anaerobic glycolysis for its energy supply (Mellors and Samad, 1989).

Changes in lipid metabolism in ruminants have been documented (Roberts, 1975). Reduced serum lipids have been associated with T. congolense infection in ruminants (Roberts, 1975) while T.brucei infections lead to increased serum lipids (Katunguk-Rwakishaya et al, 1991). Progressive increases in serum lipids in dogs with experimental T. brucei infection has been reported (Ndung'u et al, 1991). Changes in plasma cholesterol and serum lipids were correlated with levels of parasitaemia in sheep with T. congolense

infection. In all these studies however none has assessed the effect of infection with mixed trypanosome species although animals in the field are known to harbour more than one species of trypanosomes (Nyeko _et al_, 1990; Chitambo and Arakawa, 1991).

In order to simulate field conditions,where mixed infections is of common occurence, The effect of experimental single and mixed _T. brucei brucei_ and _T. congolense_ infections on the host serum lipids have been investigated in this study.

CHAPTER TWO

2.0 LITERATURE REVIEW

Trypanosomiasis is a debilitating and commonly fatal disease of man and animals. It occurs in tropical and subtropical regions and is caused by infection with the protozoan trypanosome parasite. In sub-Saharan Africa the disease is spread mainly by the bite of trypanosomes infected tsetse flies. Trypanosomiasis is considered a major infectious disease holding back the development of livestock production in much of Africa.(ILRAD reports 1993).

2.1 MORPHOLOGY OF TRYPANOSOMES

Trypanosome is a microscopic, elongated unicellular organism that move with the help of a single flagellum directed forward at the base of which is found a characteristic structure the kinetoplast. They are obligatory parasites usually having two hosts. They multiply in the body fluid especially blood of the vertebrate host and live in the digestive tract of the invertebrate host which is generally a biting insect. The classification according to Hoare (1972) is given below.

Phylum: protozoa

Subphylum: Sarcomastigophora

Super class: Mastigophora

Class: Zoomastigophora

Order: Kinetoplastida

Suborder: Trypanosomida

Family: Typanosomatidae

Genus: Trypanosoma

The most common form of trypanosomatidae is the bloodstream form of the mammalian parasites. The sizes and shapes vary according to the genus. The length ranges from 2 to 3μm for the smallest and up to 120μm for some reptilian and mammalian trypanosomes. The parasite is typically an elongated and flattened cell, with an undulating membrane wrapped loosely around the body and a free flagellum at the anterior end (Vickerman,1962).

The shape changes not only with the genus but also during the different stages of the *life cycle* in the insect vector and in the mammalian host. The following configurations (fig.1a) are found in the insect vector characterized by the position of the flagellum.

(a) Amastigote

These are small round cells of which only the nucleus and kinetoplast are visible through an optical microscope.

(b) Promastigote

The flagellum emerges from the anterior end of the cell. The kinetoplast situated in front of the nucleus lies between it and the base of the flagellum. This exists especially in culture forms.

(c) Epimastigote

This form is elongated with the kinetoplast near to and located in front of the nucleus which is situated at the posterior end. The flagellum emerges from one side of the body and runs along a short undulating membrane towards the anterior end.

(d) Trypomastigote

This is the typical trypanosome form in the fly which is infective to mammals. The body is elongated, with a kinetoplast situated behind the nucleus. The flagellum, emerges from one side of the body and runs along an undulating membrane. In the animal host, slender forms of the parasite multiply by binary fission until large parasite numbers build up in the blood. The trypanosomes then transform into intermediate forms and then into stumpy forms which are able to infect tsetse flies.

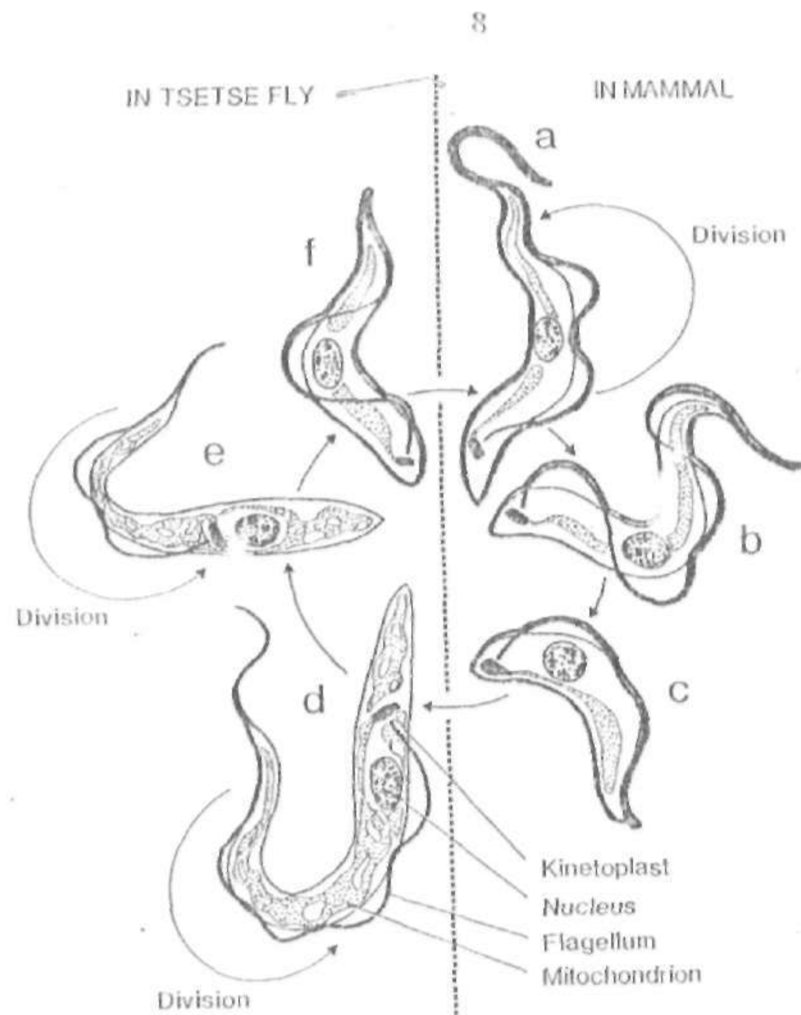


Fig. 1a The life cycle of Trypanosoma brucei. In the animal host, slender forms of the parasites (a) multiply by binary fission until large parasite numbers build up in the blood. The trypanosomes then transform first into intermediate forms (b) and then into stumpy forms (c) which infect tsetse flies. Procyclic forms (d) arise and undergo division in the fly . The parasite then enter the proventriculus and later the salivary glands where they assume epimastigote forms (e) and undergo further division. Finally metacyclic forms (f) arise in the salivary glands. These are infective to mammals (Adapted from Vickerman 1962).

2.2 STRUCTURE OF TRYPANOSOMES

Trypanosomes consist of a single cell which is an autonomous body carrying out all vital functions on its own. The cell body is made up of a mass of cytoplasm containing various organelle inclusions including a nucleus. This mass of cytoplasm is bounded by a cell membrane. The structure of the different cell constituents can now be determined by electron microscopy. The body of the trypanosome is bounded by a wall, called the periblast, comprising of a plasma membrane 8 - 10nm thick and a layer of pellicular microtubules. The plasma membrane made up of three layers, the external and internal being denser than the middle layer. The surface coat of the membrane may be covered by amorphous layer 12 - 15nm thick Secreted by the cell. This layer is made up of glycoprotein with a molecular weight of about 65,000 daltons which act as surface antigens in the bloodstream forms of mammalian trypanosomes. This layer can be discarded and reformed by the trypanosome which enables it to evade antibodies formed by the host (Englund et al; 1982). However it does not occur in the developmental stages in the intermediate host, but reappears when the epimastigote are transforming into infective metacyclic forms. There is an infolding of the cell membrane where the flagellum emerges forming an open cavity, the flagellar pocket. The flagellum originates at the base of this cavity. The flagellum includes a short, proximal intracytoplasmic portion known as the basal body, a transition zone and the actual

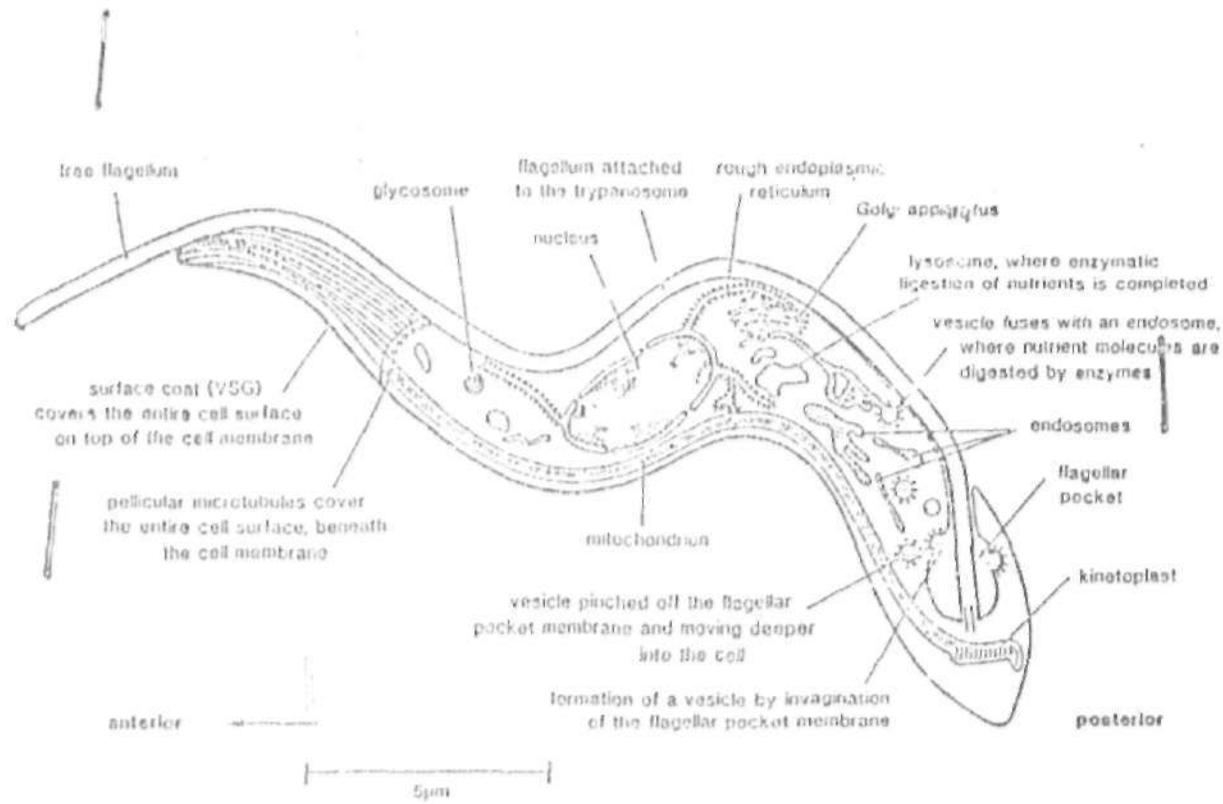


Fig. 1b Schematic diagram of a trypanosome of the Trypano: brucei group in its intermediate bloodstream form, illustrating major organelles (from ILRAD report 1990).

flagellum. Along some part of the flagellum is another system of 5nm thick filaments arranged longitudinally and transversely at several levels. This striated band of the flagellum or para-axial rod is only found with the axoneme where the flagellum adheres to the surface of the body to form the undulating membrane. It is not found where the flagellum is free or enclosed in the flagellar pocket (Itard,1989).

The position of the nucleus varies but in the bloodstream form it is generally found in the centre or anterior part of the cell. Under optical microscope the nucleus appears as a small spherical or oval vesicle. It consists of a rounded central mass the karyosome; enclosed in a nuclear membrane lined inside with chromatin granules.

The mitochondrion which contains the enzymes necessary for respiration is absent in the blood stream forms of the trypanosomes but present in the forms found in the insect vector (Itard,1989). The basal body is intimately associated with the kinetoplast. The kinetoplast is a unique cytoplasmic structure containing DNA. The kinetoplast forms part of a single mitochondrion extending from one end of the trypanosome to the other. In some trypanosomes it is often difficult to distinguish a kinetoplast capsule region in the mitochondrion.

Apart from the generalized morphology of trypanosomes given above, there are individual morphological differences between different species of trypanosomes. The bloodstream forms of T.congolense are relatively small with a range in total length of 8-24um. An important characteristic is the absence of a free flagellum. Although some individuals possess a very short flagellum T.congolense is not very motile, rather it wriggles on one spot when examined under wet film. The medium sized kinetoplast in T.congolense is larger than in T.brucei. In most cases it occupies a marginal and subterminal position. The nucleus is usually situated in the centre of the body.

The mammalian form of the T.brucei organisms exist in three forms; long, intermediate and short forms. They are therefore described as pleomorphic (Vickerman,1965). The kinetoplast is small and subterminal and the undulating membrane is well developed. The long forms have a long free flagellum while the short forms do not.

The kinetoplast is near the rounded or sometimes blunt posterior end. In the intermediate forms, the free flagellum is shorter than in the long forms. The posterior end is more blunt and the kinetoplast more nearly terminal. The long form have an average length of 23-30μm including a 6μm flagellum (Itard,1989). The proportion of the three forms changes during the course of infection. At high parasitaemia, long forms predominates while at suppressed parasitaemia the short form predominates and the intermediate forms are merely transitional.

Members of the T.brucei species are morphologically indistinguishable but differ in host specificity and pathogenicity.

2.3 TRANSMISSION OF TRYPANOSOME INFECTION

Cyclically transmitted trypanosomes infection of the mammal is initiated by the metacyclic stages injected with saliva into the blood of the animal host by the tsetse fly during feeding. In the tsetse borne trypanosomes, the metacyclic trypanosomes develop further in the chancre that forms in the host's skin at the site of the bite. T.brucei metacyclic forms transform into long thin slender forms that invade the local lymph vessels and then the blood stream where they multiply by binary fission until large parasite numbers build up in the blood. The trypanosomes then transform into intermediate form and then into stumpy forms which are able to infect tsetse flies. Stumpy forms of the parasite are ingested by tsetse fly as it feeds on an infected animal. In the midgut of the tsetse fly procyclic forms arise and undergo division after which the parasite enter the proventriculus and later the salivary glands of the fly where they assume epimastigote forms and undergo further division. Finally the metacyclic forms arise in the salivary gland (ILRAD Reports, 1990). Metacyclic forms are able to infect mammals and the life cycle is repeated.

The mitochondrion which is inactive in the slender forms begins to become active in the stumpy forms and is fully

active in forms that occur in the tsetse fly. Parasite forms that live in the mammalian bloodstream have a glycoprotein surface coat. This surface coat disappears in the procyclic forms that arise in the midgut of the tsetse fly and is later reformed in the metacyclic forms in the tsetse salivary gland (Vickerman, 1962; ILRAD report, 1990).

2.4 ANTIGENIC VARIATION AND THE PROBLEM WITH VACCINE DEVELOPMENT.

The most cost-effective way of controlling infectious diseases is usually through immunization. Successful vaccination against a pathogen exploits the ability of animals to control disease causing organisms by mounting an immune response (Borst, 1986). When an animal is infected with a parasite, the host immune system recognizes molecules exposed on the surface of the parasite and targets an immune response to those molecules (antigens). This leads to the destruction and removal of the pathogen by effector components of the immune system. Most vaccines contain one or more of these accessible molecules purified from the parasite. These stimulate an immune response. The immune system recognises these antigens and if the animal is exposed to this parasite, the animal is able to control the infection. In this way vaccinated animals are able to eliminate parasites quickly and efficiently without the development of disease.

In trypanosomes a defence mechanism known as antigenic variation exists which enables them to avoid elimination by

the immune system. Antigenic variation involves a switching of the surface antigens (variant specific antigen) of the trypanosomes during infection. A mammalian host can usually make good immune responses to the first wave of invading trypanosomes by producing antibodies against antigenic glycoprotein molecules displayed on the surface of parasites. However, before all the parasites can be eliminated the trypanosomes develop different surface molecules not recognized by the animal's initial immune response. By the time the host produces new antibodies against the 2nd kind of glycoprotein, other parasites appear displaying yet another glycoprotein and so the process continues. This leads to a series of a high parasitaemia followed by relapses (Vickerman et al, 1976). Each peak population observed in the blood is serologically unique. The different successive coats, variable surface glycoprotein (VSG) are expressed by variable antigenic types VAT (Turner et al, 1991). The VSG constitute a family of related glycoproteins tightly packed on the cell surface (Coppens et al, 1992). As a result of the high evolution rate of the antigenic repertoire VSG (Mitchels et al, 1990) as well as the rapid and unpredictable changes of its expression (Borst, 1986), programmes aimed at vaccine development against trypanosomes have not yielded satisfactory result.

way that the enzymes for the conversion of glucose into phosphoglycerate are inside the glycosome while those catabolizing the last part of the pathway are located in the cytosol. As a consequence, net ATP synthesis occurs in the cytosol but the consumption and production of ATP are balanced within the organelle (Opperdoes and Borst, 1977).

African trypanosomes are also known to catabolize aromatic amino acids, producing compounds which may contribute to the pathological conditions seen during trypanosomiasis (Stibbs and Seed, 1976). Trypanosoma gambiense catabolizes tryptophan to indole-3-ethanol (Tryptophol), indole-3-lactic acid and 5-hydroxytryptophan to 5-hydroxytryptophol. These indoles produce a sleep-like or comatose state which is a characteristic feature of trypanosomiasis (Stibbs and Seed, 1976). Tryptophol lowers body temperature (Seed and Sechelski, 1977) and suppresses the human immune response. It also alters the permeability of synaptic membranes leading to changes in the transmission of nerve impulses.

In trypanosomes unlike the mammalian host, the organism is protected from free radicals by trypanothione peroxidase, analogous to glutathione peroxidase (Shames et.al 1986). Trypanothione reductase serves to maintain the thiol disulphide balance. It has been shown that melarsen oxide is an effective inhibitor of trypanothione reductase (Fairlamb et.al, 1989).

2.6 LIPID METABOLISM IN AFRICAN TRYPANOSOME

Lipids are a heterogenous class of molecules whose roles in membrane structure have been well documented and in recent years the functions of lipids in cell activation and signal transmission have been the focus of many studies (Mellors and Samad, 1989). Lipid residues have been found to be important in the anchoring of many membrane proteins such as the variant specific glycoprotein (VSG) of trypanosomes. The lipid rich high density lipoprotein (HDL) of human serum has been found to be trypanolytic for animal strains of *T.brucei* (Rifkin, 1978). Trypanosomes contain the usual range of lipids found in eukaryotes. Although the fatty acid composition of bloodstream trypanosome is similar to that of lipids found in the plasma of their mammalian hosts. Some essential differences suggest that trypanosomes can regulate their fatty acid composition (Mellors and Samad, 1989). The fatty acids of trypanosomes are mainly esterified as phosphoglycerides or as cholesterol esters though they also exist as free fatty acids (Mellors and Samad, 1989).

African trypanosomes have very limited ability to synthesize fatty acids *de novo*. *Trypanosoma.b.rhodesiense* can not incorporate non-lipid precursors into cellular fatty acid and is dependent on an exogenous supply of fatty acids. Glucose and acetate incorporation into phospholipids were negligible and glycerol incorporation into phospholipids was minor compared with the uptake of large amounts of the fatty

acids, palmitic acid and linoleic acid (Flynn and Bowman, 1973). Trypanosoma.b.brucei and other trypanosomes readily incorporate exogenous fatty acids but with some selectivity (Williamson and Brown, 1964). Phospholipids are essential components of the plasma membrane of all life forms and in trypanosomes they constitute 80% of the trypanosomal lipid (Venkatsen and Ormerod, 1976).

Early studies suggested that trypanosomes produce haemolytic factor or toxins. The haemolytic activities of T.congolense and T.brucei suspensions incubated in vitro were shown to be due to the release of free fatty acids (Tizard et.al, 1978a). The role of the free fatty acids in the anaemia of African trypanosomiasis has been well studied (Tizard et.al, 1977). One source of free fatty acid is a potent phospholipase which is found at very high levels in trypanosomes. This enzyme can hydrolyze exogenous phospholipids to yield glycerolphosphocholine and free fatty acids and in the presence of amphiphile it can cleave phosphatidylcholine to produce 2-acylglycerophosphocholine and a fatty acid. (Tizard and Holmes, 1976).

The 2-acylglycerophosphocholine undergoes rearrangement to form 1-acylglycerophosphocholine which can serve as a substrate for a second hydrolysis by the phospholipase A. Recently it has been shown that phospholipase A from T.congolense has haemolytic activity (Andrew et.al, 1992). It has been suggested that a major role of the trypanosomal

phospholipase action is to detoxify membrane lytic lysophospholipids. Phospholipases have been suggested as target for chemotherapy (Andrew et al, 1992). It has been found that trypanosomes also produce neuraminidase which cleaves off sialic acid residues from the surface of erythrocytes (Esievo et.al, 1982) rendering erythrocytes more prone to phagocytosis.

2.7 TRYPANOSOMIASES

Trypanosomiases is a group of diseases transmitted by certain species of tsetse fly (Glossina spp). Potentially pathogenic infections caused by African trypanosomes start when the vector deposits the metacyclic forms in the subcutaneous tissues of mammals; from where the trypanosomes are disseminated throughout to colonize and multiply in selected tissues. Infections by pathogenic trypanosomes cause diseases which vary in severity depending on the virulence of the organism and susceptibility of the host. This gives rise to a range of responses including acute, chronic and asymptomatic carrier syndromes (Stephen, 1970).

2.7a Human trypanosomiasis

The human disease is caused by two species, *T.gambiense* and *T.rhodesiense*. The rhodesiense disease present an acute syndrome while the gambiense disease is of a chronic form. The first sign of a trypanosome infection may be the presence

of a chancre at the site of the bite. The chancre is a round inflamed area about 3cm in diameter with a red spot in the middle. This is usually followed by fever which signals the invasion of the bloodstream by the trypanosomes. Other symptoms of the disease in humans include weakness, enlargement of lymph nodes, irritability, splenomegaly, local oedema, endocrine dysfunction and anaemia (Apted, 1970). Nervous system involvement is manifested by the daytime somnolence which gives the disease its name, sleeping sickness.

American trypanosomiasis otherwise called chagas disease is caused by Trypanosoma cruzi and transmitted by domiciliary vectors such as Rhodnius prolixus and Triatoma infestans. This disease which occurs in South America is characterized by cardiopathy.

2.7b Animal Trypanosomiasis

Three species of trypanosomes, T.congolense, T.brucei and T.vivax are the important causative agents of animal trypanosomiasis in Africa. The severity of diseases caused by these organisms depend on the virulence of that species and the animal host infected. At the same time, host factors such as age, nutritional status and breed are important (Murray, et al, 1982). Animal trypanosomiasis are characterized by splenomegaly, cachexia, weakness, enlargement of superficial lymph nodes, endocrine dysfunction, fever and anaemia (Fiennes, 1970; Stephen, 1970).

2.8 ANAEMIA IN TRYPANOSOMIASIS

Anaemia is the most important pathological change in African trypanosomiasis (Murray, 1974). The onset of anaemia is closely related to the onset of fever, appearance, intensity and duration of parasitaemia. The severity of the anaemia which follows infection is affected by several factors. These include differences in virulence that exist among different species of trypanosomes and among the large number of strains belonging to each species. At the same time, host factors such as age, nutritional status and breed are important (Murray et.al, 1982). Anaemia in African Trypanosomiasis is of multifactorial origin with the following mechanisms implicated in the aetiology. These include haemolysis (De Gruchy, 1970) Dyshaemopoiesis (Dargie et.al, 1979a;and Igbokwe, 1989) haemorrhage (Dirie et.al 1988 and Anosa et al, 1992) and haemodilution (Holmes and Jennings, 1976).

Haemolytic anaemia in trypanosomiasis has two components, immune haemolysis and the activity of haemolytic factors.

2.8a Immune haemolysis or erythrophagocytosis

Some of the disease processes accompanying trypanosomiasis cause the alteration of erythrocytes leading to their identification as non-self and subsequent phagocytosis. The membrane of red blood cells may be damaged during infection by direct trauma to red blood cells by the

lashing action of trypanosomes when parasitaemia is severe (Jenkins, 1980). Febrile responses which accompany parasitaemia lead to decreased erythrocytes due to increased osmotic fragility, decreased plasticity and increased membrane permeability. Trypanosomes produce neuraminidases which cleave off sialic acid residues from erythrocyte surfaces (Esievo, 1979) rendering them more prone to phagocytosis directly (Durocher et.al, 1975) by immunoglobulins and complement opsonization (Jancik et.al, 1978) or by activation of the classical or alternate pathway of complement (Brown et.al, 1983). Furthermore, cleavage of sialic acid from red cells could theoretically lead to antibody production against these exposed epitopes and increased erythrophagocytosis. Splenomegaly is one of the clinical findings in African trypanosomiasis. Splenomegaly results mainly from red blood cell sequestration and massive macrophage population phagocytosing red blood cells (Anosa and Kaneko, 1983). This situation predisposes to the phenomenon of hypersplenism in which the transit time of the erythrocyte is increased during their repeated passages through the spleen; so that the cells are exposed to the injurious factors of the splenic environment including low pH, high lactic acid concentration, low glucose level and low cholesterol levels for longer periods than normal. The cells become prematuredly damaged by these factors and are destroyed (Weinberg and Weiss, 1969). The observation that injection of the immunodepressive agent

cyclophosphamide did not alleviate the anaemia due to *T.brucei* infection suggests that factors other than immune haemolysis are also important in the development of anaemia.

On autolysis, *T.congolense* and *T.brucei* have been shown to generate an active phospholipase A. While phospholipases themselves have direct effect on red blood cell membranes (Andrew *et.al*, 1992) their main activity would be through their action on endogenous phosphatidylcholine with their release of large quantities of free fatty acid (FFA) including palmitic, stearic and linoleic acid, with lesser amounts of oleic and arachidonic acids. As a result of their detergent qualities FFA, particularly linoleic acid, are haemolytic (Tizard *et.al*, 1978a).

2.8b Haemorrhage

Haemorrhages have been reported in some cases of human trypanosomiasis caused by *T.rhodesiense* (Banks, 1980) and in acute *T.vivax* infections of cattle, sheep and goats (Anosa and Isoun, 1983). The contribution of haemorrhagic *T.vivax* infection to anaemia in Kenya has been reported (Kimeto *et.al*, 1990).

2.8c Dyshaemopoiesis

Conflicting reports on the erythropoietic system in trypanosome infection are documented. Expansion of red bone marrow in the long bones of infected animals, such as *T.brucei*

infection of horses, T.congolense infection of cattle, sheep and goats (Maxie and Valli, 1979) and T.vivax infection of sheep and goats (Anosa and Isoun, 1980) indicate positive erythropoietic response. Other reports show that the bone marrow hyperplasia was evident in acute cases or early part of the infection but in chronic cases becoming hypoplastic indicating unresponsiveness (Fiennes, 1953; 1954).

The above mechanisms act in concert to produce anaemia which is a common feature of trypanosomiasis, the severity of which depends on the parasite species and its infectivity to the host.

2.8d Haemodilution

While increases in plasma volumes have consistently been reported, there is a divergence of opinion on the status of the total blood volume. Some workers reported that it increased, for example in T.brucei infections of mice (Amole et.al, 1982) and cattle (Dargie, 1978) and T.vivax infection of sheep and goats (Anosa and Isoun, 1974) while others reported that it remained normal as in T.congolense and in T.vivax infection of sheep (Dargie et.al, 1979). It is however worthy to note that trypanosome anaemia has a haemodilutionary component because whether the blood volume remains normal so that the increase in plasma volume is only a pathophysiological compensatory mechanism to maintain total blood volume, the total blood volume is increased amounting to

over-compensation, the erythrocyte values are depressed below a level they should attain had the plasma volume remained at the normal level existing prior to the destruction of RBC.

2.9 CONTROL OF AFRICAN TRYPANOSOMIASIS

The methods of control of African trypanosomiasis are based on the epizootiology of these diseases. They concern the three factors, parasite, mammalian hosts and vectors that affect disease incidence.

2.9a Chemotherapy of human trypanosomiasis

Effectiveness of chemotherapy depends on early diagnosis. The definite diagnosis of trypanosomiasis depends on demonstration of the parasite in body fluids using various parasitological techniques. However, very low parasitaemia might not be detected by these methods hence the use of serological techniques for detecting parasite antigens or host antibodies raised against the antigens. As a result of the persistence of antibodies in circulation for long periods after treatment, antibody detection cannot differentiate current from past infections (Nantulya et al,1992). However, recently Nantulya et.al, (1992) have developed the antigen detection enzyme linked immunoassay based on species specific monoclonal antibodies against non-viable, somatic antigens of trypanosomes. Another promising area is the use of species specific DNA probes for diagnosis (Nyeko et.al, 1990). In

view of the existence of mixed trypanosome infection in the field, it is important that in epidemiological surveys, combined parasitological and serological diagnostic test are carried out to enhance the diagnosis of mixed infection. This becomes very important in areas where drug resistance has been established for one of the parasites in the mixed infection; as treatment with such a drug will lead to the elimination of the sensitive trypanosome and subsequent relapse resulting from the resistant trypanosomes. This would have serious shortcomings on the use of chemotherapy for the control of trypanosomiasis.

Current treatment of African human trypanosomiasis leaves much to be desired. Most drugs still in use were developed in the early part of this century. Suramin and pentamidine for early stages of the disease and arsenicals and antimonials for the late stage of the disease when there is neurological involvement. Some of these drugs would not pass some of today's stringent standards for drug safety. One noticeable exception is the introduction of alpha difluoromethyl ornithine (DFMO) for treatment of late stage Gambian sleeping sickness, especially in cases refractory to melarsoprol (Schechter and Sjoerdsma, 1986). Increasing interest in rational drug design has led to the identification of trypanothione reductase as one of the possible target for new drugs (Fairlamb, 1990) because of the physicochemical differences between the parasite enzyme and the analogous

human enzyme glutathione reductase. This has led to the synthesis of a series of nitrofurans substituted with basic functional side-chain which can inhibit the parasite enzyme. There is pronounced synergism between DFMO and arsenical drugs. The arsenical, melarsoprol (Mel B) the main drug in use for treatment of late stage human trypanosomiasis is associated with a number of serious side effects, such as arsenical induced encephalopathy which is fatal. A combination of DFMO with reduced doses of Melarsoprol could be used to reduce the number of relapse cases (Jennings, 1991).

2.9b Chemotherapy of Animal Trypanosomiasis.

As at now treatment of infected animals is the most important control method of trypanosomiasis as it aims at reducing the transmission potential of the disease. Drug control of animal trypanosomiasis relies essentially on four drugs, namely Novidium (Homidium Chloride); Ethidium (Homdium bromide); Berenil (Diminazene aceturate) and Samorin (Isometamidium Chloride) (Ilemobade, 1987). Apart from treatment of infected animals samorin has been successfully used for chemoprophylaxis (Jibbo et.al, 1987). The success of trypanocidal drugs has been undermined by the development of drug resistance by some strains of trypanosomes to these common drugs (Pinder and Authie, 1984; Kaggwa et.al, 1988). Relapse following chemotherapy of animal trypanosomiasis has led to search for new drugs.

Current understanding of the metabolism of trypanosomes had led to sourcing of compounds which could block lipid uptake by trypanosomes (Fairlamb, 1990). This development reveals the importance of an understanding of lipid metabolism in both host and parasite as these would lead to the development of drugs that are structural analogues of lipids that are selectively toxic to the parasite.

2.9c **Vector Control**

Vector control is one of the methods used for the control of trypanosomiasis. This has been done using various methods which include the use of insecticides such as DDT, dieldrin and endosulphan to kill the adult tsetse fly (Lee et.al, 1980) either as aerosol sprayed directly onto the fly or as residual deposits on vegetation or traps (WHO, 1980). Other methods include the use of traps like the biconical traps, langridge and Moloo traps (Owago, 1989). At times the traps are operated with odour baits. Recently a combination of the various vector control methods in integrated pest management such as baiting traps with either female sex pheromone or food and may be with insecticides or sterilant have been successfully used in the control of tsetse (Barclay and Driessche, 1989).

As a practical approach for exterminating tsetse, bush clearing has been undertaken with the objective of destroying vegetation that constitutes the resting haunt of adult fly.

Vegetation clearing pushes back the fly front and creates barrier zones that restrict and in some cases prevent migration of adult fly (WHO, 1980). Game destruction was aimed at depriving the fly of its main source of nutrition thus resulting in starvation and eventual elimination of the fly. Some game animals serve as reservoir host for the parasite that causes the disease in man and his domestic animals (Kageruka et.al, 1993). These traditional methods have serious disadvantages as a result of the environmental problems they pose which therefore require that their continual use be reviewed. As a result of these shortcomings several alternatives have been suggested including the use of parasites and pathogens affecting the immature and adult stages of the fly (Greathead, 1980). One of the most promising and practical of the approaches so far investigated is the sterile insect technique (SIT). Essentially, the technique consists of releasing large number of laboratory bred gamma irradiated male flies into the natural population (Politzer et.al, 1980) so that when these sterile males mate with sexually normal wild strains the latter fails to produce offspring. SIT has been found to be effective in areas with low tsetse density.

2.9d Trypanotolerance

In Africa it has long been recognized that the Baoule taurine (Bos tauru's) breed of cattle are able to survive in

tsetse infected areas where other breeds readily succumb (Roberts and Gray, 1973b). Furthermore in many instances under these circumstances they also appeared to be productive. This trait has been termed trypanotolerance and is generally attributed to the indigenous taurine breeds of cattle in West and Central Africa, namely the N'dama and West African short horn. The resistance of these breeds to anaemia especially the N'dama appeared to be correlated with their ability to limit the intensity, prevalence and duration of parasitaemia (Dargie et.al, 1979). It is generally assumed that the superior capacity of trypanotolerant animals to control parasitaemia is associated with a better immune response (Weitz, 1970) acquired as a result of exposure to trypanosomes (Desowitz, 1959) and this is believed to be genetically determined (Maillard et.al, 1992).

The breeding of trypanotolerant animals is one of the ways of controlling trypanosomiasis.

2.9e The role of trypanosomal phospholipases on host lipid metabolism

Trypanosomal phospholipases act on host erythrocyte phospholipids to generate free fatty acids which as surface active agents can cause hemolysis leading to anaemia (Tizard et. al, 1978a) in the infected host. The lysophospholipase of T.congolense is potentially capable of hydrolyzing lysophosphotidylcholine producing more free fatty acids.

Lysophospholipases are responsible for the dramatic drop in serum lysophosphotidylcholine observed in T.congolense infected cattle (Roberts et. al, 1977).

Apart from the hemolysis which results from increased serum free fatty acids, it also leads to increased cholesterol synthesis via acetyl CoA. Lysophosphotidylcholine is required for the utilization of cholesterol and other lipids; its absence therefore blocks lipid utilization.
The consequences of these are that it is expected that all trypanosomal infections should lead to elevated serum free fatty acids and consequent anaemia with accompanying hypercholesterolemia. However this has not been the case as conflicting reports have been given. T.brucei infections of sheep leads to hypercholesterolemia, while T.congolense in sheep leads to hypocholesterolemia (Roberts, 1975).

In rabbits most reports have been on serum lipids changes in T.brucei infections (Godwin and Guy, 1973; Arowolo et. al, 1988).

In order to assess the relative contributions of parasitaemia to free fatty acid generation and anaemia in rabbits and also the chnages of host lipid metabolism in rabbits under field conditions, serum lipid changes in rabbits with mixed and single infections of T.brucei and T.congolense were studied.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Twenty New Zealand rabbits were used in the experiment. *Trypanosoma congolense* stabilate 158 isolated from a goat, and *Trypanosoma brucei brucei* stabilate 159 isolated from a goat were obtained from the Department of Veterinary Parasitology Ahmadu Bello University, Zaria.

3.1a Chemicals

Bromocresol green (BCG), acetylacetone, cholesterol standard, Triolein standard and albumin standard were purchased from Sigma Chemical Company, St. Louis, U.S.A.
Chloroform, sulphuric acid and isopropanol were purchased from May and Baker, Dagenbam, England.
Aluminium oxide was purchased from Hopkins and Williams, Chadwell Heath, Essex, England.
1,5-Diphenyl carbazide, ammonium acetate, Triethanolamine, Absolute ethanol, sodium metaperiodate, palmitic acid, Heptane, methanol, succinic acid, sodium azide and Brij 35 were purchased from British Drug House chemicals Ltd, Poole England.

3.1c Equipment

i Metler top loading balance

ii Gallemkamp Hot plate-magnetic stirrer

iii pH meter Prazisions-pH meter E510 Metrohm Herisau.

iv International clinical centrifuge. International equipment Co. Needham, Mass. U.S.A.

v Spectrophotometer model SD104 WPH Linton Cambridge U.K.

vi Thermonics mixer model 105 Thermonics Co. Tokyo Japan.

3.2 Animal inoculation and Sample collection

The rabbits weighing between 1.45 to 2.10kg were housed in rabbit cages and supplied with feed and water ad libitum. The rabbits were acclimatized for four weeks after which they were screened for haemoparasites. Thereafter blood samples were collected from the marginal ear vein, for PCV determination and serum lipid assay for six weeks prior to infection. The trypanosome stabilates were grown in mice. Parasite enumeration was done using the Naubeur haemocytometer after which they were diluted to the appropriate concentration using phosphate bufferred saline glucose.

The rabbits were then divided into four groups of five animals each, Group A was infected with T.brucei, group B with T congolense, group C was the mixed infection (T.brucei and T.congolense) and group D served as the uninfected control. The rabbits were inoculated with $1x10^4$ trypanosomes each in the case of the single infections while the mixed infection group were inoculated with $1.0x10^4$ trypanosoma brucei brucei and $1.0x10^4$ trypanosoma congolense. Animals in group D served as uninfected control. After parasite inoculation parasitaemia was estimated daily using wet film method and

blood was collected weekly for PCV determination. The blood was spun at 700g and serum collected was stored at - 20°C until when required.

3.3 PCV Determination

Blood was collected in heparinized capillary tubes. The tubes were sealed at one end with crystaseal and centrifuged at 2000g for five minutes in microhaematocrit centrifuge. The PCV was then read using the PCV reader.

3.4 Free fatty Acid Determination

Free fatty acid assay was carried out using the method of Falholt *et al*, 1973. In this technique serum was extracted with chloroform, heptane and methanol mixture (5:5:1, v/v) in the presence of a phosphate buffer to eliminate interference from phospholipids and the reagent shaken with a high density copper reagent, pH 8.1. The copper soap remained in the upper organic layer from which an aliquot was removed and the copper determined colorimetrically with 1,5 - diphenylcarbazide.

Procedure

The frozen serum was thawed. One millilitre phosphate buffer and 6ml extraction solvent were added to each of 50μl serum, 50μl water and 50μl working standard in stoppered centrifuge tubes respectively. The tubes were shaken vigorously for 90 seconds using a thermonics mixer and allowed

to stand for 15 minutes. This was followed by centrifugation for 10min. at 1250g after which the buffer was removed by suction and 5ml extract was transferred to a similar dry centrifuge tube. Two mililitre of copper reagent (made up of 10ml of 500mM copper nitrate trihydrate,10ml of 1M triethanolamine and 6ml of 1M sodium hydroxide were diluted to 100ml with water and 33g sodium chloride was added and the pH adjusted to 8.1 with sodium hydroxide) were added and the mixture shaken vigorously for 5min, followed by centrifugation at 1250g for 5min. Three millilitre of the upper layer was transferred to a tube containing 0.5ml phenylcarbazide solution and carefully mixed. The absorbance was read after 15min. at 550nm. The free fatty acid in the serum was estimated using a calibration curve.

3.4 Albumin Determination

Albumin was assayed using the method of Doumas *et al*, (1971) as modified by Price and Spencer(1977).

Principle of the method

In this technique, the dye Bromocresol green (BCG) binds albumin to give a coloured complex that is read colorimetrically at 632nm.

Procedure

Twenty microlitre serum was added to 4ml BCG solution, (containing BCG, succinate buffer pH 4.10 sodium azide and Brij 35) and the mixture was allowed to stand for 10min at 25°C and the absorbance was then read against a blank of working dye solution. The albumin was estimated using a calibration curve.

3.6 Triglyceride Determination

This was determined colorimetrically using the Hantzsch reaction (Fletcher, 1968; Soloni, 1971; Foster and Dunn, 1973).

Principle of the method

In this technique phospholipids are absorbed unto Alumina. The triglycerides were extracted with isopropanol and saponified with KOH. The glycerol released is oxidized to formaldehyde. The formaldehyde released is determined colorimetrically after treatment with acetylacetone and ammonia in the Hantzsch reaction.

```
            CH2
           / \
         /     \
CH3OC - C       C - COCH3                +    3H2O
        ||      ||
  CH3 - C       C - CH3  3,5 -diacetyl - 1,4, - dihydrolictidine
         \     /
           NH
```

Procedure

One hundred microliters of serum, standard and water (for reagent blank) were placed respectively in screw-capped tubes and 4ml isopropanol was added to each and mixed well. Four hundred milligrams of washed alumina was added to each tube and placed in a mechanical rotator for 15min. They were centrifuged at 700g for 7 min. Then 2ml of the supernatant were transferred into dry screw capped tubes. To these was added 0.6ml KOH, stoppered and incubated at 65°C for 15min. in a water bath after which the tubes were cooled to room temperature. Upon cooling 1ml metaperiodate solution was added and mixed followed by 0.5ml acetylacetone. The mixture was incubated at 50°C for 30 min. This was followed by cooling and the absorbance read at 405nm.

3.7 Cholesterol determination

Serum cholesterol was determined colorimetrically according to the method of Zlatkis et al, (1953). In this technique cholesterol reacts with concentrated sulphuric acid in the presence of ferric chloride to give a coloured compound that is read at 560nm. The sulphuric acid serves as a dehydrating agent.

Procedure

One hundred microliter serum, cholesterol standard and water (for reagent blank) were placed respectively in stoppered centrifuge tubes. To these was added 4.9ml solution of 10% $FeCl^3$; Acetic acid (1:2 v/v). They were mixed and allowed to stand for 15min. To 2.5ml of the mixture was added 1.5ml concentrated H_2SO_4. The tubes were cooled in a water bath and the absorbance read at 560nm.

Total serum cholesterol as mg total serum cholesterol per 100ml of serum was calculated as $\frac{Au \times Cs}{As}$

where

Au = Absorbance of sample

Cs = Concentration of standard

As = Absorbance of standard

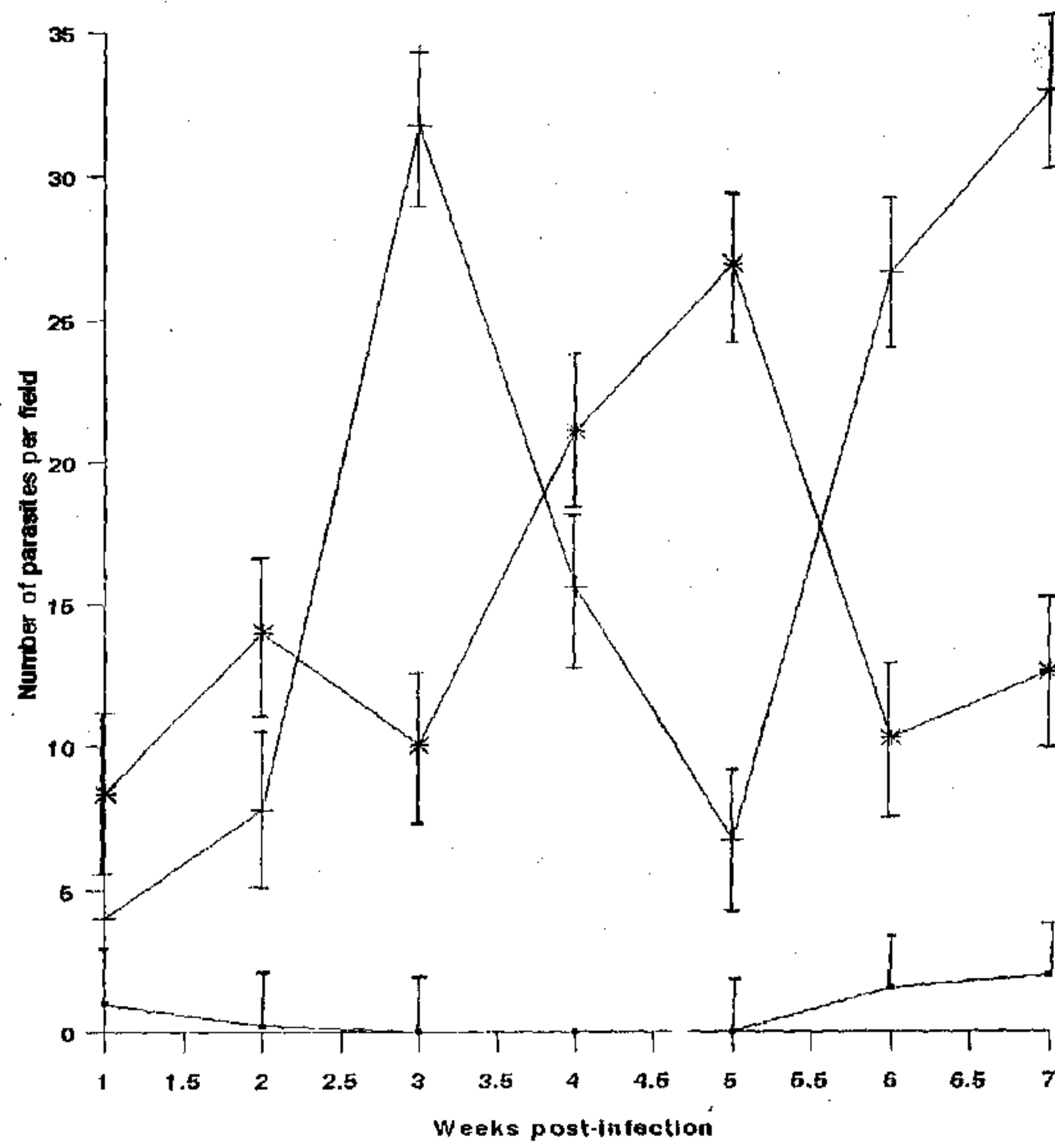
CHAPTER FOUR

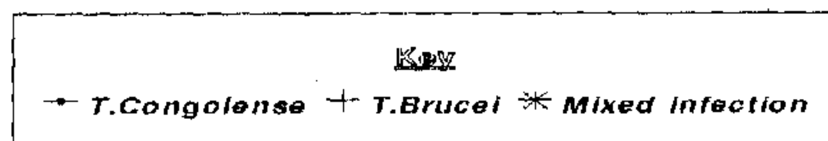
RESULTS AND DISCUSSION

4.1 Parasitaemia

In the *T.brucei brucei* infected group, the animals became positive for the infection at 5 days post infection (Pi) while the *T.congolense* infected and mixed infection groups became positive at 6 days post infection. Parasitaemia was high and intermittent in the *T.brucei* and mixed infections groups. In the *T.congolense* group parasites were detected in blood from 6 days post infection up to 12 days post infection and disappeared completely with resolution of clinical signs. After 3 weeks of aparasitaemia, re-inoculation with a homologous strain of *T.congolense* produced infection in 2 out of 5 animals in this group as shown in fig 1, in the *T.congolense* group parasitaemia was generally low and intermittent. In the mixed infection group, Giemsa stained thin and thick films revealed a predominance of the *T.brucei* species. In most cases massive parasitaemia was followed by death of the animals within 1 to 3 days.

Previous studies on animal trypanosomiasis (Weitz, 1970) have indicated that the course and nature of this disease depends on the interaction between the parasite and the host giving rise to clinical diseases ranging from asymptomatic carrier to rapidly progressive fatal diseases. The disappearance of parasites from the blood of *T.congolense* infected animals and the resistance of 3 out of the 5 animals





Values are mean ± SEM

Fig 1. Mean Weekly parasitaemia in rabbits infected with T.brucei and T.congolense

to re-infection with a homologous strain of T.congolense suggests that the immune mechanisms of the rabbit are more efficient against T.congolense than T.brucei and this varies for individual animals. This finding is consistent with that of Luckins et al, (1986) who reported the resistance of rabbits to homologous T.congolense challenge after treatment with a trypanocidal drug. Dwinger et al, (1986) have reported that goats that had been infected simultaneously with two serodemes were immune to the homologous challenge.

4.2 Disease Pattern

T.brucei and mixed infection groups showed subacute disease with death occurring within 4-6 weeks post infection; by which time 60% of the animals in these groups had died. In the mixed infection group one animal exhibited chronic disease as the infection lasted up to 10 weeks. The rabbits with T.brucei and mixed infection died before those with T.congolense infection possibly because of the low infectivity of T.congolense stabilate to rabbits.

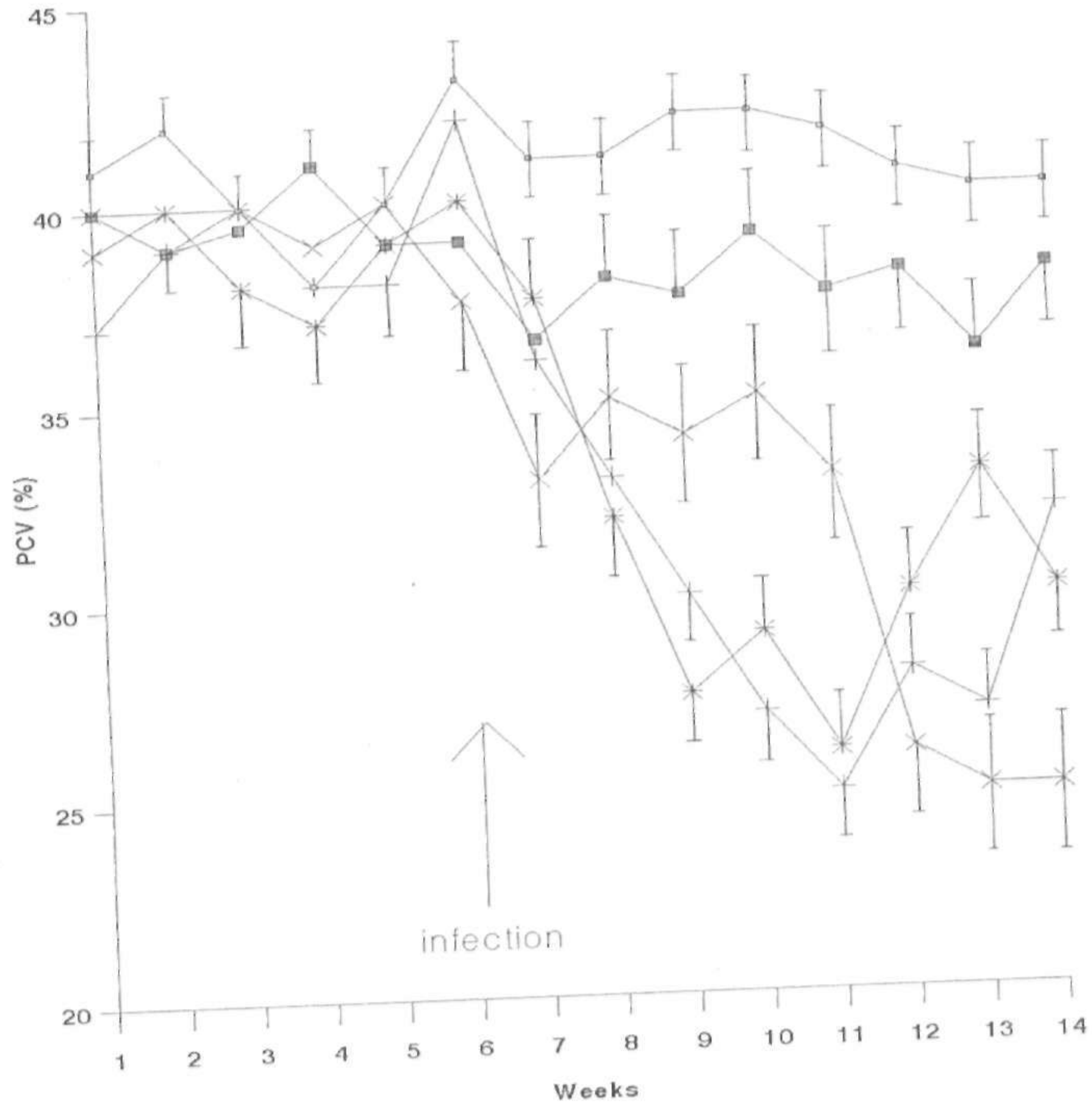
4.2 Clinical Signs

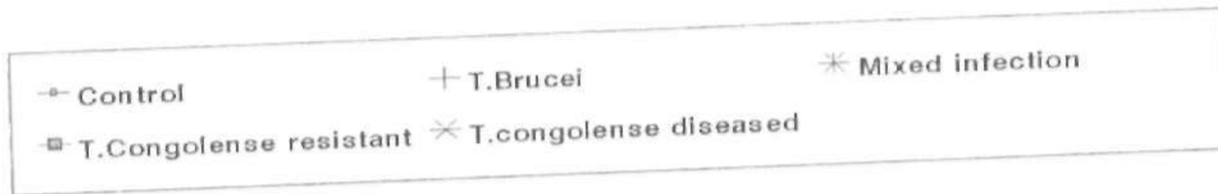
Clinical signs became apparent in the T. brucei and mixed infection groups at 3 weeks post infection. These were characterized by droopy ears, ocular and nasal discharges and weakness. The scrotum was Oedematous and warm to touch. The ocular discharge was more severe in the mixed infection group,

one animal in this group had massive and offensive ocular discharge and exhibited uncoordinated movement as if it were blind. Ocular and nasal discharges observed in the T.congolense group were milder than the other two infected groups. The clinical signs observed in all the infected groups were directly related to the level of parasitaemia. The group with least parasitaemia had milder clinical signs.

4.3 Anaemia

Anaemia was of common occurrence in the three infected groups. Right from the 2nd week of infection there was a significant ($p<0.05$)drop in PCV in all the infected animals compared to the control. A comparison of all the infected groups showed no significant difference between the T.brucei, mixed infection and T.congolense diseased animals ($P>0.05$). While animals resistant to T.congolense infection had low PCV values which were not significantly different from those of control animals (Fig.2). This finding agrees with that of Jenkins and Facer,(1985) who reported different patterns of parasitaemia (given the same inoculum) for different stocks of T.congolense in rabbits and this was reflected in the degree of anaemia.





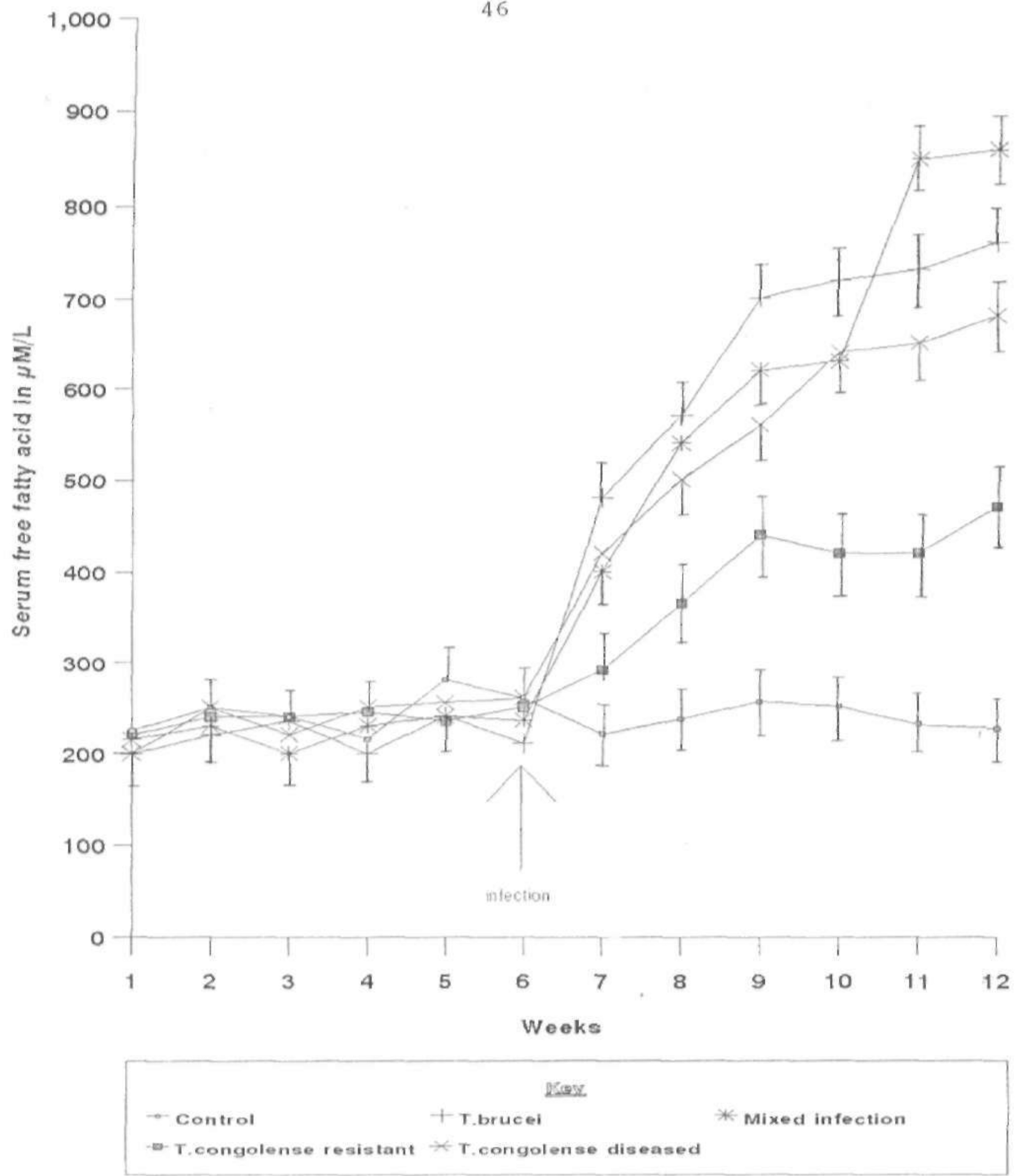
Values are mean ± SEM

Fig 2. Mean Weekly PCV (%)in rabbits infected with _T_. _brucei_ and _T_. _congolense_

4.4 Free Fatty Acid (FFA)

Using the analysis of variance, there was a significant difference between the control group and the infected groups ($P<0.05$). Further statistical analysis showed a significant difference ($P<0.05$) between those infected and those resistant to *T.congolense* infection.

Free fatty acids are generated by the activity of phospholipases on endogenous phosphatidylcholine and from autolysing trypanosomes. A pooled mean of the FFA of all the animals inoculated with *T.congolense* showed that it had a lower FFA level compared to the other infected groups.
The highest FFA level was observed in the *T.brucei* group. The fact that autolysing trypanosomes account for the major portion of haemolytic activity (FFA) possessed by these organisms (Tizard *et al*, 1978a), suggests that the more the number of autolysing trypanosomes the more the FFA generated. And since *T.congolense* group had the least parasitaemia it means the number of autolysing trypanosomes were fewer and so gave rise to low FFA levels. This however was not



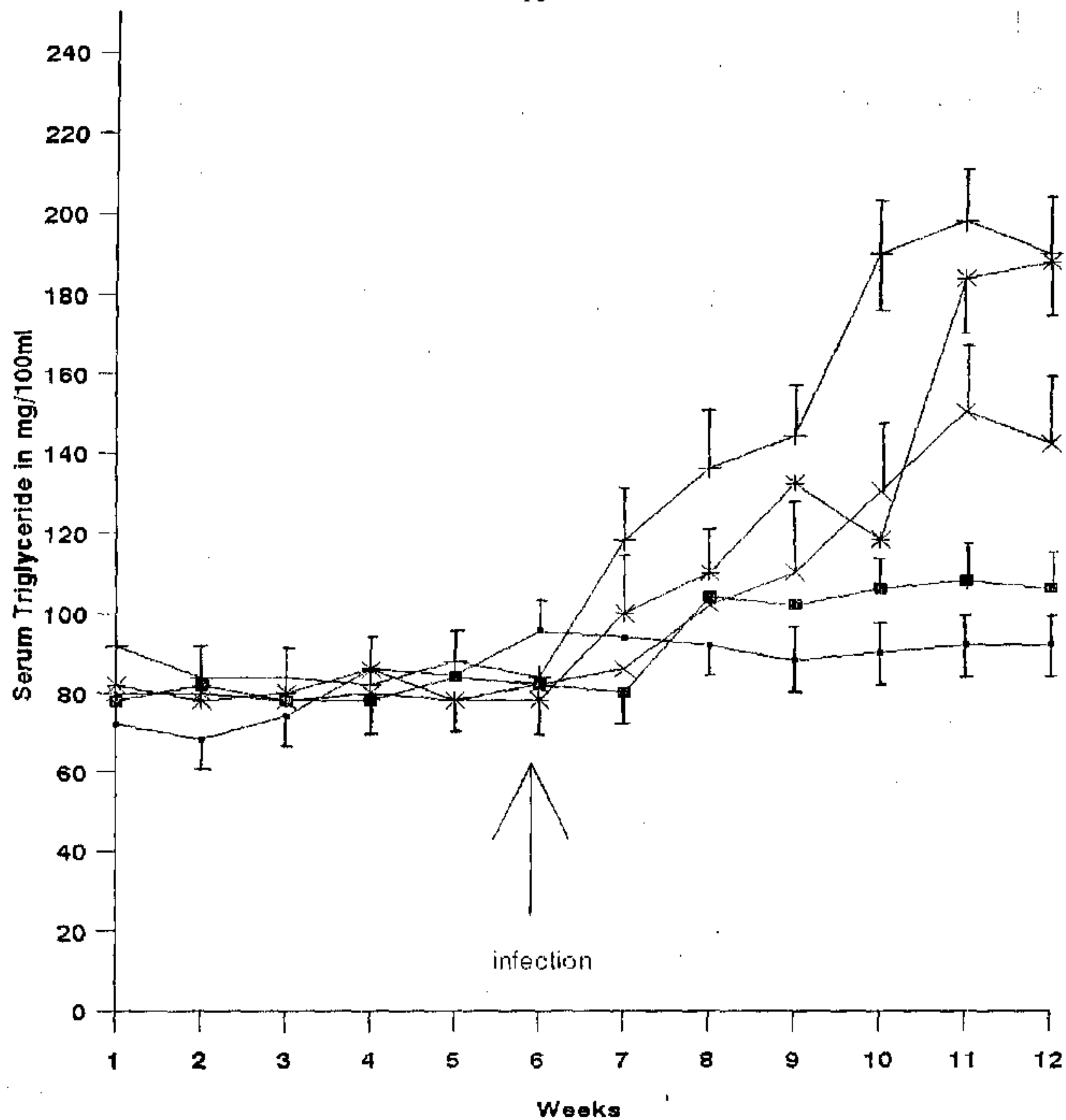
Values are mean ± SEM

Fig 3. Mean Weekly Serum Free Fatty Acid in rabbits with *T.brucei* and *T.congolense* infections

statistically significant. The observation that serum FFA elevation was observed in all the infected animals shows the importance of FFA elevation in the pathology of trypanosomiasis especially with regards to anaemia.

4.5 Triglycerides:

These trypanosomal infections were characterized by hypertriglyceridemia. The serum triglycerides levels were significantly ($P<0.05$) higher in the diseased animals than the control animals and the *T.congolense* (resistant) animals. Hypertriglyceridemia in trypanosomiasis has been attributed to defective triglyceride removal (Rouzer and Cerami, 1980). Godwin and Guy (1973) have reported hypertriglyceridemia in rabbits with trypanosomiasis and this they attributed to ultrastructural changes in the liver which alter lipid metabolism.

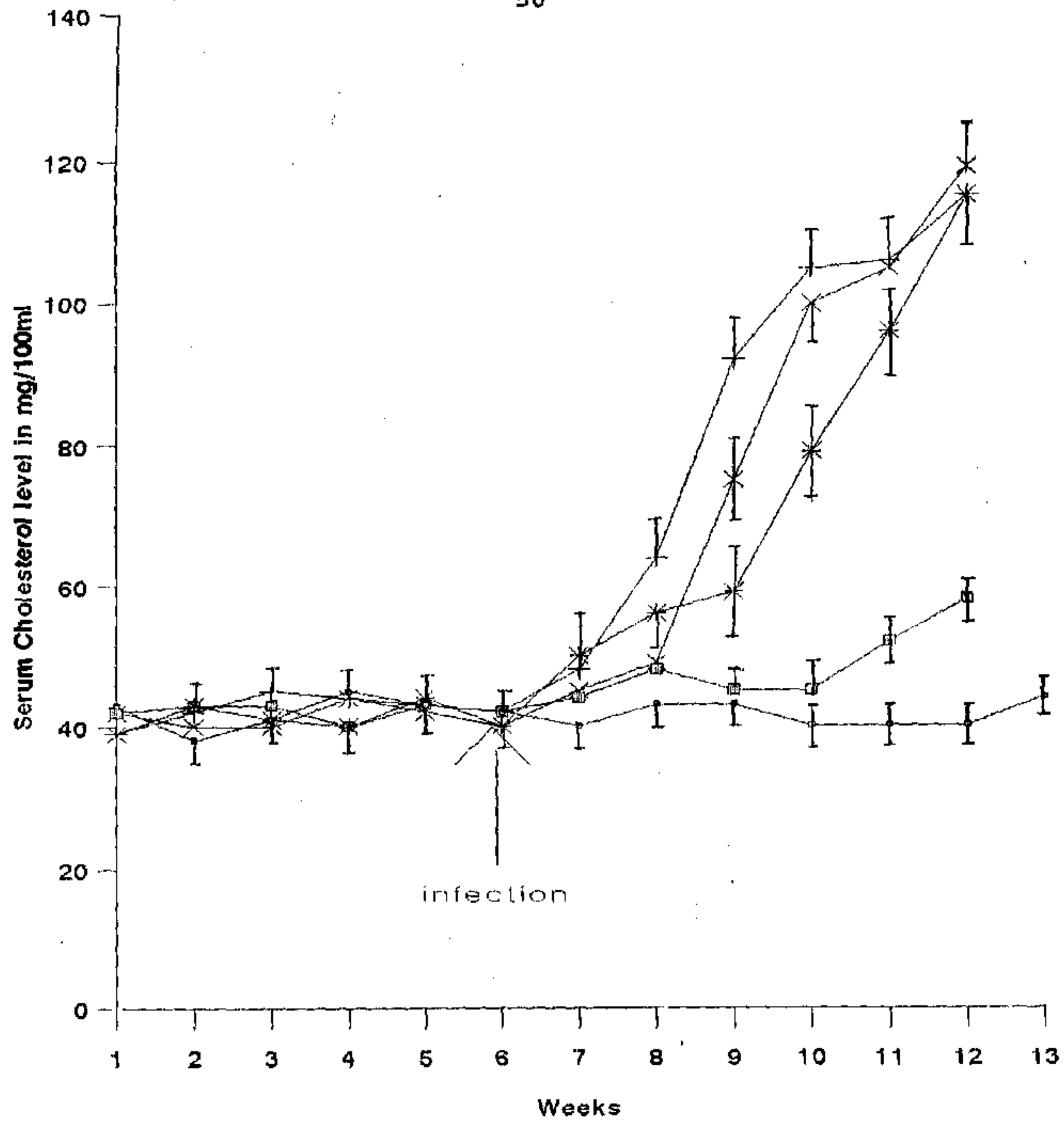


Values are mean ± SEM

Fig 4. Mean Weekly Serum Triglyceride Level in rabbits infected with T.brucei and T.congolense

4.6 Serum Cholesterol

All the infected animals had significantly ($P<0.05$) higher serum cholesterol levels compared to the control animals except those resistant to T.congolense whose values were close to that of the control group. Arowolo et al. (1988) have reported hypercholesterolemia in rabbits infected with T.brucei, which they attributed to an impairment of liver lipid metabolism. Accumulation of cholesterol in the atrium of dogs with T.brucei, infection is an important factor in the pathology of canine trypanosomiasis (Ndung'u et al. 1991) this has been confirmed by echocardiographic changes that accompany this disease in dogs. This could be the cause of death of infected animals.

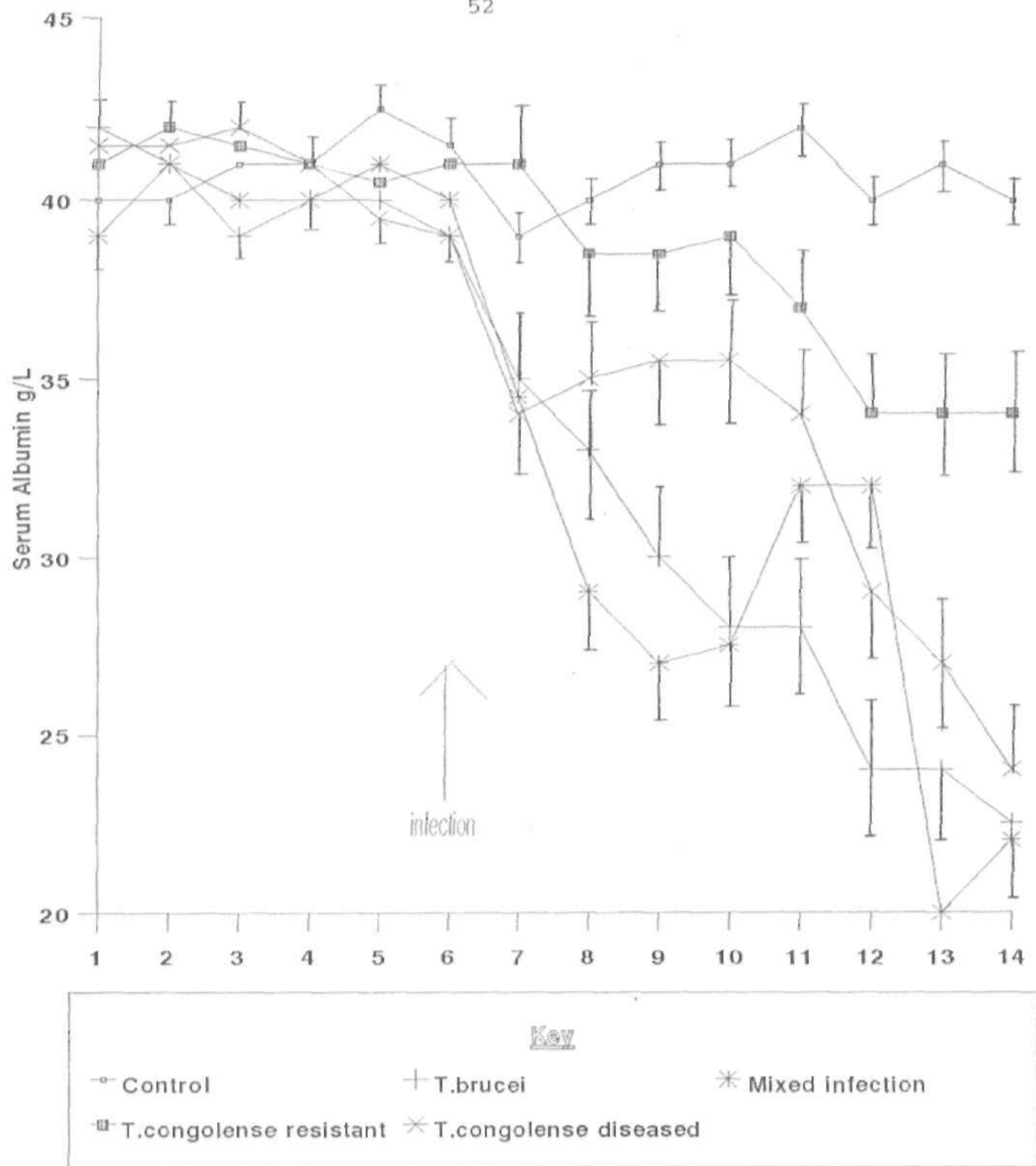


Values are mean ± SEM

Fig 5. Mean Weekly Serum Cholesterol Level in rabbits infected with T.brucei and T.congolenseM

4.7 Serum Albumin

The infections were characterized by hypo-albuminaemia. Using analysis of variance there was a significant difference ($P<0.05$) between the different experimental groups. Using the t-test there was no significant difference between the control and the *T.congolense* resistant groups. These two groups had significantly higher albumin levels compared to the other groups. Hypoalbuminaemia in trypanosomiasis has been attributed to decreased synthesis as a result of hepatic dysfunction (Arowolo *et al*, 1988).



Values are mean ± SEM

Fig 6. Mean Weekly Serum Albumin in rabbits infected with T.brucei and T.congolense

CHAPTER 5

CONCLUSION AND SUGGESTIONS

The rabbits were found to be more susceptible to infection with T.brucei than with T.congolense, this was manifested by the predominance of T.brucei in the mixed infection group. The differences between the T.congolense and T.brucei infected rabbits in the clinical outcome of the infection were associated with the ability of the rabbit to mount a better immune response to T.congolense infection and this varies with individual animal. The predominance of one parasite species in the mixed infection group implies that under field conditions, this could be mistaken for single infection and when treatment is effected by a drug to which only the predominant species is sensitive to, there could be a relapse of the other parasite, thus militating against chemotherapy of the disease.

It is therefore suggested that in epidemiological studies,as much as possible, parasitological and serological techniques be used in order to detect the presence of mixed infection in animals. Where one parasite species predominates the other, this will ensure that drugs to which all the trypanosome populations are sensitive are used.

It was remarkable that the rabbits that succumbed to infection with either or both trypanosome species had hyperlipidemia and this depended on the level of parasitaemia except for serum cholesterol in rabbits with T.congolense which was greatly increased when compared to the level of parasitaemia observed for this group.

This shows that in rodent trypanosomiasis, there is increased serum cholesterol level in both _T.brucei_ and _T.congolense_ infections unlike in ruminants where infection with _T.congolense_ is associated with decreases. It is therefore suggested that further studies be carried out to determine the different mechanisms by which _T.congolense_ alter cholesterol metabolism in different hosts.

Anaemia has been recognised as a major finding in animal trypanosomiasis. Anaemia was reflected by significant decreases in PCV in all the infected animals either with single or mixed parasite infection. However after 4 weeks post infection there were slight increases in the PCV of the _T.brucei_ and mixed infection groups thus reflecting good erythropoietic response to _T.brucei_ infection, since this was not recorded for single _T.congolense_ infection.

REFERENCES

Agyemang, K., Dwinger, R.H., Jeanin. P., Lespere P., Grieve; A.S., Bach. M.L. and Little, D.A. (1990). Biological and economic impact of trypanosome infection on milk production in N'dama cattle managed under village conditions in the Gambia. Animal Production 50(3), 383-389.

Amole B.O., Clarkson A.B., Shear, H.L. (1982) Pathogenesis of anaemia in Trypanosoma brucei infected mice. Infect. Immun. 36. 1060 - 1068.

Andrew, J.N., Esievo, K.A.N., Ibrahim, S., Ukoha, A.J. and Ikediobi C.O., (1992). Phospholipase A from T.congolense characterization and Haematological properties. Cell Biochem. and Function 11 125 - 130.

Anene, B.M., and Omamegbe, J.O. (1984). Abortion associated with T.b. brucei infection in an Alsatian bitch. A case report. Trop. Vet. 2(4) 211 - 213.

Anosa,V.O,and Isoun, T.T.(1974). Experimental T.vivax infection in sheep and goats:The relationship between the parasitaemia,the growth rate and the anaemia. J.Nig. Vet. Med. 3, 102-108.

Anosa V.O. Isoun T.T. (1980). Haematological studies on T.vivax infection of goats and in intact and splenectomized sheep. J. Camp. Path. 90. 151 - 160.

Anosa V.O. and Isoun T.T. (1983). Pathology of experimental T.vivax infection of sheep and goats. Zentl. Vet. Med. 30 685 - 700.

Anosa, V.O and Kaneko, J.J(1983). Ultrastructural pathology of haemopoietic organs in Trypanosoma vivax infection of goats. Vet. Pathol. 26 (1), 78-83

Anosa, V.O., Logan-Henfrey, L.L., Shaw, M.K., (1992). A light and electron microscopic study of changes in acute hemorrhagic T.vivax infection in calves. Vet. Path. 29 (1), 33 - 45.

Apted, F.I.C. (1970). Clinical manifestation and diagnosis of sleeping sickness. In: The African Trypanosomiasis. Mulligan H.W.ed George Allen & Unwin, London. pp. 661 - 683.

Arowolo, R.O.A., Elhassan, E.O., Amure, B.O. (1988). Assessing hepatic dysfunction in rabbits experimentally infected with Trypanosoma brucei. Rev. Elev. Med. Vet. Pays. trop; 41(3), 277 - 281.

Banks, K.L. (1980). Injury induced by T.congolense adhesion to cell membranes. J. parasit. 66, 34 - 37.

Barclay,H.J.and Driessche p.van den (1989).Pest control models of combinations of sterile releases and trapping. Insect Science and Its Applications 10 (2),107-116.

Boreham, P.F.L. (1980). Effects of pharmacologically active substances on the pathogenesis of trypanosomiasis. Trans. Roy. Soc. Trop. Med. & Hyg. 74 (2), 271 - 273.

Borst, P. (1986). Discontinuous transcription and antigenic variation in trypanosomes. Ann. Rev. of Biochem. 55, 701-731.

Brown, E.C., Joner, K.A., Frank, M.M., (1983). Interaction of desialated guinea pig erythrocytes with the classical and

alternative pathways of guinea pig complement in vivo and in vitro. J. Clin. Invest. 71, 1710 - 1719.

Chitambo, H. and Arakawa, A. (1991). Therapeutic effect of Berenil and Samorin in mice infected with four trypanosome populations isolated from Zambian cattle. Vet. Parasitol. 39, 43 - 52.

Coppens, I., Bastin, P.H., Opperdoes, F.R., Baudhin, P. and Courtoy P.J. (1992). Trypanosoma brucei brucei. Antigenic stability of its LDL receptor and immunological cross reactivity with the LDL receptor of the mammalian host. Exptl. Parasitol.74, 77-86.

Dargie, J.D. (1978). Effects of T.congolense and T.brucei on the circulatory volumes of cattle. In:Pathogenesis of trypanosomes Losos, G.J. and Chouirnard A. ed. Ottawa, Canada. IDRL, pp 141 - 144.

Dargie, J.D., Murray, P.K., Murray, M., McIntyre, W.I.M. (1979a). Bovine trypanosomiasis, the red cell kinetics of Ndama and Zebu cattle infected with T.congolense Parasitol. 78, 271 - 286.

De Gruchy, G.C,(1970).clinical haematology in medical practice, 3rd ed Oxford Blackwell Scientific Publications. pp 44-47

Desowitz, R.S. (1959). Studies on immunity and host parasite relationships 1. The immunological response of resistant and susceptible breeds of cattle to trypanosomal challenge. Ann. Trop. Med. & Parasitol. 53, 293 - 313.

Dirie, M.F., Wallbanks, K.R., Molyneux, R.H., Bornstein, S. and Omer H.A. (1988). Haemorrhagic lesions associated with T.vivax infections of cattle in Somalia. Acta. Tropica 45 (3), 291 - 292.

Doumas, B.T., Watson, W.A. and Biggs H.G. (1971). albumin standards and the measurement of serum Albumin with Bromocresol Green. Clin. Chim. Acta. 31, 87.

Durocher, J.R., Payre R.C., Conrad M.E., (1975). Role of Sialic acid in erythrocyte survival. Blood 45, 11 - 20.

Dwinger, R.H., Luckins, A.G., Murray M, Rue, P. and Moloo S.K. (1986). Interference between different serodemes of super infection in goats following transmission by tsetse. Parasite. Immunol. 8 (4), 293 - 305.

Englund, P.T., Hajduk, S.L. and Martini, J.C. (1982). The molecular biology of trypanosomes. Ann. Rev. Biochem 51 695 - 726.

Esievo, K.A.N. (1979). In vitro production of neuraminidase Sialidase) by T.vivax. International Scientific Council for Trypanosomiasis Research and control. 16th meeting Yaounde Cameron, 1979 OAU/STRC Publication No III, pp 205-210 (1981).

Esievo, K.A.N., Saror, D.I., Ilemobade A.A. and Hallaway M.H. (1982). Variation in erythrocyte surface and free serum sialic acid concentrations during experimental T.vivax infection in cattle. Res. Vet. Sci. 32, 1 - 5.

Facer, C.A. (1976). Blood hyperviscosity during Trypanosoma (Trypanozoon) brucei infection of rabbits. J. Camp. Pathol. 86, 393 - 408.

Fairlamb A.H. and Opperdoes. F.R. (1986). Carbohydrate metabolism in trypanosomes. In: Carbohydrate metabolism in cultured cells, Morgan M.J. ed Plenum publication Co. New York. pp 183 - 224.

Fairlamb, A.H. (1989). Novel biochemical pathways in parasitic protozoa. Parasitol 99 supplement 593 - 5112.

Fairlamb, A.H., Henderson, G.B. and Cerami, A. (1989). Trypanothione is the primary target for Arsenical drugs against African trypanosomiasis. Proc. Natl. Acad. Sci. USA 86, 2607 - 2611.

Fairlamb, A.H. (1990). Future prospects for the chemotherapy of human trypanosomiasis 1. Novel approaches to the chemotherapy of trypanosomiasis. Trans. Roy. Soc.Trop. Med.and Hyg.34 5),613-617

Falholt K, Lund, B. and Falholt, W. (1973). An easy colorimetric micromethod for routine determination of free fatty acids in plasma. Clin. Chim. Acta 46, 105 - 111.

Fiennes R.N.T.W. (1953). The result of autopsies on trypanosome infected cattle. Br. Vet. J. 107, 511 - 520.

Fiennes R.N.T.W. (1954). Haematological studies in trypanosomiasis of cattle. Vet. Res. 66, 423 - 434.

Fiennes R.N.T.W. (1970). Pathogenesis and pathology of animal trypanosomiasis In: The African trypanosomiasis. Mulligan

W.M.ed George allen and Unwin, London. pp 729-773.

Fletcher, M.J. (1968). A colorimetric method for establishing serum triglycerides. Clin. Chim. Acta 22, 393-395

Flynn, I.W. and Bowman, I.R.R. (1973). Purification and characterization of pyruvate kinase from T.brucei. Arch. Biochem. Biophys. 200(2) 401 - 409.

Foster, L.B. and Dunn, R.T. (1973). Stable reagents for determination of serum triglycerides by a colorimetric Hantzsh condensation method. Clin. Chem. 19, 338-340.

Gardiner, R.R., Assoku. R.K.G., Whitelaw D.D. and Murray M. (1989). Haemorrhagic lesions resulting from T.vivax infection in Ayrshire cattle. Vet. Parasitol. 31, (3-4), 187 - 197.

Godwin L.G. and Guy M.W. (1973). Tissue fluids in rabbits infected with T.brucei Parasitol. 66, 499 - 502.

Greathead, D.J. (1980).Biological control of tsetse flies as an assessment of insect parasitoids as control agents. Biocontrol News and information 1, 111 - 123.

Hart, D.T., Misset, O; Edwards, S.W. and Opperdoes, F.R. (1984). A comparison of the glycosomes (microbodies) isolated from Trypanosoma brucei bloodstream form and cultured procyclic trypanosomastigotes. Mol and Biochem. parasitol. 12, 25 - 35.

Hoare, C.A. (1972). The trypanosomes of mammals, a zoological monograph. Oxford and Edinburgh Blackweel Scientific publications. pp. 750

Holmes, p.H. and Jennings, F.W. (1976). The effect of treatment on the anaemia of African trypanosomiasis. In: pathophysiology of

parasitic infection Soulsby.E.L.L. (ed) Academic press London, New York. pp.199

Huet. G. Lemesre, K.L., Grard, G. Noireou, F. Bountignan, F; Dieu M .C., Janmin J. and Degand, D. (1990). Serum lipid and lipoprotein abnormalities in human African trypanosomiasis. Trans. Roy. Soc. Trop. Med. & Hyg. 84 (6),792-794.

Igbokwe, I.D. (1989). Dyserythropoiesis in animal trypanosomiasis, Revue d'Elev. de Med. Vet. des. pays, Trop. 42 (3),423-429.

Ilemobade, A.A. (1987). Chemotherapy against African trypanosomiasis: Its strength and limitations In: Proceedings of a meeting held on livestock production in tsetse affected areas of Africa in Nairobi, Kenya Nov. 1987 pp 23-27.

ILRAD reports (1990). Why do livestock infected with trypanosomes develop anaemia.July 1990 pp 1-2.

ILRAD reports (1993). Estimating the cost of Animal trypanosomiasis in Africa. April 1993 pp 2-4.

Itard, I. (1989). Afican Animal Trypanosomiasis. In Manual of Tropial Veterinary Parasitology. Published by C.A.B. International. 1989 pg 181 - 297.

Jancik, J.M., Schauer R., andres K.H., Von Dusing. M. (1978). Sequestration of neuraminidase-treated erythrocytes. Studies on its topographic, morphologic and immunologic aspects. Cell and Tissue Res. 186, 209-226.

Jenkins, G,C, Forsberg, C.M. Brown, J.L., Boulton F.E. (1974). The influence of the spleen in experimental T.brucei infection in rabbits. Trans. R. Soc. Trop. Med. Hyg. 68, 154-155.

Jenkins, G.C. (1980). Effects of trypanosome on the haemopoietic system. Trans. Roy. Soc. trop. Med. & Hyg. 74 (2), 268 - 269.

Jenkins, G.C.and Facer C.A.,(1985). Haematology of African trypanosomiasis.In: The immunology and pathogenesis of trypanosomiasis. Tizard,I.R ed CRC press Inc.Florida. U.S.A pp 13-44

Jennings, F.W. (1990). Future prospects for the chemotherapy of human trypanosomiasis. 2. Combination chemotherapy and African trypanosomiasis. Trans. Roy. Soc. Trop. Med. & Hyg. 84 (5), 618 - 621.

Jennings, F.W. (1991). Chemotherapy of trypanosomiasis: the potentiation of antimonial compounds by difluoromethylornithine (DFMO). Trop. Med. & Parasitol. 42, 135 - 138.

Jibbo, J.M.C., Durkin, J. Light, D.E, Murray M. Sonnes K. and Trail J.M.L. (1987). Chemoprophylaxis: Its successful use in the control of trypanosomiasis in Boran cattle at Mkwaja Ranch Tanzania. In proceeding of the African trypanotolerant Network held in Nairobi, Kenya, Nov. 1987 pp 23 - 27.

Kageruka, P., Kazadi, J.M.L., Molisho, S.D. and Jochems, M. (1993). Trypanosome prevalence and animal reservoir of T.b.gambiense in Zaire: In OAU/STRC 1993.

Kaggwa, E; Mungua, W.K. and Mugera, G.M., (1988). Relapse in dogs experimentally infected with T.brucei and treated with diminazene aceturate or isometamidium chloride. Vet. parasitol. 27, 199 - 208.

Katunguka-Rwakishaya, E., Murray, M., and Holmes, P.H. (1991). The pathophysiology of T.congolense infection in sheep Trans. Roy. Soc. Trop. Med. & Hyg. 85 (6), 841.

Kimeto, B.A.,Mugera. G.M.and Nyaga P.N.,(1990). Haemorrhagic pancarditis in cattle infected with Trypanosoma vivax Vet.Parasitol.34 (4),295-301.

Kobayakawa, T; Louis J, Izui S.and Lambert P.H. (1979). Autoimmune response to DNA, red blood cells and thymocyte antigens in association with polyclonal antibody synthesis during experimental African trypanosomiasis. J. Immunol. 122, 296-301.

Lee, C.W., Parker, J.D., Kultzer, H.,Baldry D.A.T., Bettany, B.W.and Tunstall, J.,(1980). The experimental application of insecticides from a helicopter for the control of riverine populations of Glossina tachinoides in W.Africa V11 Studies on physical properties of sprays of endosulfan and deltametrin applied to G. tachinoides habitats in the R. Komoe valley Upper Volta. Tropical Pest Management 26, 377-384

Luckins. A.G., Rae, P.F. and Gray A.R. (1986). Infection, Immunity and the development of local skin reactions in rabbits infected with cyclically transmitted stocks of T.congolense Annals. of Trop. Med. & Parasitol. 77, 569-571

Maillard, J.L., Congo, I., Bassinga, A and Cuviller, J.F., (1992). Immunogenetics of Bauole cattle in the Lobi area of Burkina Faso. Environment of this trypanotolerant population. Rev. d,elev. et de. Med. Vet. de pays trop. 45 (1), 63 - 68.

Maxie, M.G. and Valli, V.E.O. (1979). Haemodilution in bovine trypanosomiasis. In: Pathogenicity of Trypanosomes G.J. Losos G.J.and Chouinard A.ed IDRC 132 Ottawa, Canada pp 145 - 150.

Mellors, A. and Samad, A. (1989). The acquisition of lipids by African Trypanosomes. Parasitol. Today 5 (8), 239-244.

Misset, O, Bos, O.J.M. and Opperdoes, F.R., (1986). Glycolytic enzymes of T.brucei. Simultaneous purification, intraglycosomal concentrations and physical properties. Eur. J. of Biochem. 157, 441-453.

Mitchels, P.A.M. (1989). The Glycosomes of trypanosomes: Properties and Biogenesis of a microbody. Exptl. Parasitol. 69, 310 - 315.

Mitchels, P.A.M. Hannaert, V., Ernest, I and Alberty S. (1990). Organisation structure and evolutionary aspects of genes in trypanosomes. Biochem. Soc. Trans. 18, 727-729.

Murray, M. (1974). The pathology of African trypanosomiasis. In: progress in immunology 11 Brent, L. and Holborow, J.ed North Holland publishing Company Amsterdam. p.181-182.

Murray, M. Morison, W.I., Whitelaw. D.D., (1982). Host susceptibility to African trypanosomiasis: trypanotolerance.

Advance parasit. 21, Baker J.R and Muller, R. ed New York Academic press.p.1-68

Nantulya, V.M., Lindquist, K.J., Stevenson, P., and Mwangi, E.K. (1992). Application of monoclonal antibody based antigen detection enzyme linked immunosorbent assay. (antigen ELISA) for field diagnosis of bovine trypanosomiasis at Nguruman, Kenya. Annals of Trop. Med. & Parasitol. 86 (3), 225 - 230.

Naylor, D.C. (1971). The haematology and histopathology of T.congolense infection in cattle part II. Haematology (including symptoms). Trop. Anim. Hlth. Prod. 3, 159-168.

Ndung'u, J.M., Eckersall, P.D., Jennings, F.W., Wrighr, N.G. and Murray M., (1991). Changes in lipid metabolism in dogs experimentally infected with T.brucei In: OAU/STRC 15 No. 7082 pp 304-314.

Nyeko, J.A.P., Ole-Moiyoi, O.K., Mayiwa P.A. O., Otiero, L.H. and Ociba, P.M., (1990). Characterization of trypanosomes isolate from cattle in Uganda using species specific DNA probes reveals predominance of mixed infections. Insect. Sci. and Its Application 11 (3), 271 - 280.

Opperdoes, F.R. and Borst, P. (1977). Localization of nine glycolytic enzymes in a microbody-like organelle in Trypanosoma brucei. The glycosome. FEBS. Lett. (Netherlands) 80, 360-364.

Owago, M.L. (1989). Efficiency of the biconical trap and biases in catches of female and Tenerals of Glossina pallidipes-Austen.

Insect. Sci. appli. 10 (5), 651 -659.

Pentreath, V.W. (1991). The search for primary events causing the pathology in African Sleeping Sickness. Trans. Roy. Soc. Trop. Med. & Hyg. 85, 145 - 147.

Pinder, M and Authie. E. (1984). The appearance of Isometamidium resistant. T.congolense in West Africa. Acta. Tropica 41, 231 -241.

Politzer, H., Cuisance D; Ferrier, J and Sellin, E., (1980). Releases of irradiated males in the control of Glossina palpalis gambiense behaviour of released insects (Abstracts) P 167 In: XVI International and congress of entomology Kyoto Japan.

Price C.P. and Spencer, K. (1977). Determination of serum Albumin using Bromocresol Green: A modification of the method of Doumas 1971. Ann. Clin. Biochem. 14 105.

Rifkin , M.R. (1978). Identification of the trypanocidal factor in normal human serum high density lipoprotein. proc. Natl. Acad. Sci. USA 75, 3450 - 3454.

Roberts C.J. (1975). Ruminant lipid metabolism in Trypanosomiasis. Trans. Roy. Soc. Trop. Med. & Hyg. 69 (2), 275.

Roberts C.J.and Gray, A.R. (1973b). Studies on trypanosome resistant cattle II. The effect of trypanosomiasis on N'dama, muturu and zebu cattle Trop. Anim. Hlth. & Prod. 5, 220-233.

Roberts.C.J; Tizard,I.R; Mellors,A; and Clarkson, A (1977) Lysophospholipases, lipid metabolism and the pathogenesis of

African trypanosomiasis. Lancet (England) 2 , 1187_ 1188

Rouzer, C.A and Cerami, A. (1980). Hypertriglyceridemia associated with T b. brucei infection in rabbits: role of defective triglyceride removal. Mol. & Biochem. Parasitol 2 31 - 38.

Schechter, P.j. and Sjoerdsma, A. (1986). Difluoromethylorinthine in the treatment of African Trypanosomiasis Parasitol. today 2 223 - 224.

Seed, J.R. and Sechelski, J. (1977). Tryptophol levels in mice infected with pharmacological doses of tryptophol and the effect of pyrazole and ethanol on these levels. Life Sci. 27 1603.

Shames, S.L., Fairlamb, A.H., Cerami, A. and Walsh, C.T. (1986). Lipid metabolism in trypanosomes. Biochem. 25 3519 - 3526.

Soloni, F.G. (1971). Simplified manual micromethod for determination of serum triglycerides. Clin. Chem. 17 529.

Stephen, L.E. (1970). Clinical manifestation of the trypanosomiases in livestock and other domestic animals. In: The African trypanosomiases. Mulligan H.W. ed George Allen and Unwin, London. pp 774 - 794.

Stibbs, H.H. and Seed, J.R. (1976). Elevated serum and hepatic tyrosine aminotransferase in voles chronically infected with T.b.gambiense Exptl. parasitol 29, 1-4.

Tizard, I.R., and Holmes, W.L. (1976). The generation of toxic activity from T.congolense. Experientia Basel 32, 1533-1534.

Tizard, I.R., Holmes, W.L., Nielsen, K.H. (1978a). Mechanism of anaemia in trypanosomiasis; studies on the role of hemolytic

fatty acids derived from T.congolense Tropenmed. Parasitol. 29, 108-114.

Turner, C.M.R., Aslam, N., Smith, E., Buchanan N. and Tait, A. (1991). The effect of genetic exchange on variable antigen expression in T.brucei. Parasitol. 103, 379-386.

Venkatsen, S. and Ormerod, W.E. (1976). Lipid content of the slender and stumpy forms of T.brucei rhodesiense. A comparative study. Comp. Biochem. Physiol. 53, 481-487.

Vickerman,K;(1962). The mechanism of cyclic development in trypanosomes of the Trypanosoma brucei subgroup an hypothesis based on ultrastructural observations. Trans. Roy. Soc. Trop.Med and Hyg. 56, 487-495.

Vickerman,K;(1965). Polymorphism and mitochondrial activity in sleeping sickness trypanosomes. Nature 208 No. 5012 pp 762-766.

Vickerman,K., Sless, F., Hoston, W. and Edwards, R.G. (1976). Some aspects of parasitaemic remission in experimental Trypanosoma brucei infections. In Van den Bossche, H., ed., Biochemistry of parasites and host parasite relationships. Amsterdam, North Holland Publishing Company, 401-408.

Weinberg, E. and Weiss, L. (1969). The structure of the spleen and haemolysis. Ann. Rev. of Med., 20 29 - 40.

Weitz, B.G.F. (1970). Infection and resistance. In the African trypanosomiases, Mulligan H.W. ed. George Allen. & Unwin. (London), pp 97 - 124.

WHO (1980). Environmental management for vector control. WHO technical report series No. 649 75 pp (Third report) of the WHO expert committee on vector Biology and control Geneva 13-19 November 1979.

Williamson, J. and Brown K.N. (1964). The chemical composition of trypanosome I protein amino acid and sugar analysis. Experimental parasitol. <u>11</u>, 161-175.

Zlatkis, A, Zak, B. and Boyle, A. (1953). A new method for the determination of serum cholesterol. J. Lab. Clin. Med. <u>41</u>, 486-492.

APPENDIX

PREPARATION OF REAGENTS

For free fatty acid assay

1. Extraction solvent contained chloroform heptane and methanol (5:5:1).
2. Phosphate buffer PH 6.4 33mM was prepared by mixing 2 volumes $KH_2PO_4 \cdot 2H_2O$ (4.539g 12) and 1 volume (5.9g 12) and the pH adjusted to 64.
3. Stock buffer solution, 500mm 12.07g Cu $(NO_3)_2 \cdot 3H_2O$ was dissolved with 100ml water.
4. Triethanolamine solution 1M. 10ml triethanolamine was diluted to 100ml with water.
5. NaOH solution 1M.
6. Copper reagent: 10ml copper solution, 10ml triethanolamine solution, 6ml NaOH solution were diluted to 100ml with water and 33g NaCl was added and the pH adjusted to 8.1. This reagent was prepared daily.
7. 1,5-Diphenylcarbazide solution 48g/L in ethanol. This was prepared by adding 40mg in 10ml ethanol to 0.01ml triethanolamine solution.
8. Stock standard palmitic acid solution 2mM was prepared by dissolving 51.2mg palmitic acid in the extraction solvent and made up to 100ml. This was stored in tightly stoppered container.
9. Working standard solution were prepared by diluting 5ml stock to 20ml with extraction solvent to give a solution containing

500μM to put up with each batch; 2, 4, 6, 8, 10ml of stock standard were diluted to 20ml. These are equivalent to 200, 400, 600, 800, 1000μM. These were used for the standard curve.

Preparation of reagents for Albumin Determination

1. Stock succinate buffer, pH, 410, 0.05M Dissolve 10g NaOH and 56g succinic acid in 800ml water. Adjusted to pH 40 with 1M NaOH and made up to 1 litre with water and stored at 4^0C.
2. Stock BCG dye solution, 10M was prepared by dissolving 1.75g BCG (indicator grade) in 5ml 1M NaOH and made up to 250ml with water.
3. Stock sodium azide solution was prepared by dissolving 4g of sodium azide in 100ml of water.
4. Stock Brij 35. To 5g solid Brij 35 was added 8ml of water. This was warmed to dissolve and made up to 20ml with water.
5. To two litres of water in a 5 litres volumetric flask was added.
   i. Five hundred mill litre of stock succinate buffer.
   ii. 40ml stock dy solution, removing all traces of the dye from the pipette walls by washing with water.
   iii. 12.5ml stock azide.
   iv. 12.5ml stock Brij 35 was added and made up to volume with water. This was mixed thoroughly. The pH was adjusted to 4.5 and the absorbance at 615nm in a 1cm cell was 0.230.

6. Stock standard albumin solution, 100g/L was prepared by dissolving 10g Bovine serum albumin and 50mg sodium azide in water and made up to 100ml.
7. Working albumin standards 20, 30, 40, 50, and 60g/L were prepared by diluting the stock standard with a 50mg/L solution of sodium azide in water. These standards were stored at 4^0C.

Reagents for Cholesterol determination

1. 10% $FeCl_2$: Acetic acid (1:2 v/v)
2. Concentrated Sulphuric acid.

Reagents for Triglyceride determination

1. Isopropanol.
2. Alumina. activity grade 1 was washed with water until all fines were removed and dried in an oven overnight.
3. Saponifying agent. 50g KoH was dissolved in 600ml water and 40ml isopropanol was added to it.
4. Sodium metaperiodate reagent: 77g anhydrous aminonium acetate was dissolved in 700ml of water, 60ml glacial acetic acid and 65mg $NaIO_4$ were added.
5. Acetylacetone reagent: 7.5ml acetylacetone was added to 200ml isopropanol and mixed. 700ml water was added and mixed well.
6. Standard of triolein. As stock standard 10ml/L was prepared by dissolving 9g in 100ml of isopropanol, 4ml of this was diluted to 10ml for use this was tightly sealed and kept at 4^0C.