TRYPANOCIDAL ACTIVITY OF SOME ORGANOTIN COMPOUNDS ON TRYPANOSOMA BRUCEI - INFECTED MICE

**A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,
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AHMADU BELLO UNIVERSITY,
ZARIA**

BY

**SHUAIBU, MOHAMMED NASIR
B.Sc(HONS),ABU.**

DECLARATION

I hereby declare that this thesis is a product of my own research work. No previous submission of it has been made for a degree at any University. The work of other researchers are fully acknowledged and referred to accordingly.

Shuwai

SHUAIBU, MOHAMMED NASIR

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DATE

DIGITIZED

DEDICATION

To my parents, Brothers, sisters, friends, colleagues and the rest of my family.

CERTIFICATION

This thesis entitled 'Trypanocidical activity of some organotin compounds on Trypanosoma brucei-infected mice' by SHUAIBU, Mohammed Nasir meets the regulations governing the award of the degree of Master in Science of Biochemistry of Ahmadu Bello University, and it is approved for its contribution to knowledge and literary presentation.

DR. D. A. AMEH — DATE 22/8/97
CHAIRMAN, SUPERVISORY COMMITTEE

DR. J. J. BONIRE. — DATE 21/10/97
MEMBER, SUPERVISORY COMMITTEE

DR. A. O. ADAUDI. — DATE 14-10-97
MEMBER, SUPERVISORY COMMITTEE

HEAD, BIOCHEMISTRY DEPARTMENT — DATE 22/8/97
AHMADU BELLO UNIVERSITY, ZARIA

DEAN POSTGRADUATE SCHOOL — DATE 24/2/99
AHMADU BELLO UNIVERSITY, ZARIA

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ABSTRACT

The organotin compounds dibutyltin dichloride (DBTC), diphenyltin dichioride (DPTC), tributyltin chloride (TBTC) and triphenyltin chloride (TPTC) were tested in Trypanosoma brucei-infected mice for trypanocidal activity. DBTC and DPTC where found to be trypanocidal. At a dose of 10mg/kg/day(l 32uM) and 15mg/kg/day(l 75u.M) DBTC and DPTC respectively for five consecutive days cleared the blood of the parasites.

Subinoculation of the healthy mice with the homogenates of liver, spleen, kidney and cerebrospinal fluid (CSF) from the mice considered cured; showed 33.3%, 66.7% and 33.3% relapse following the subinoculation with homogenate of liver, spleen and CSF from the DBTC-treated mice respectively while 50.0%, 50% and 33.3% relapses where seen following subinoculation with homogenate of liver spleen and CSF from the DPTC-treated mice respectively. No relapse was seen from the subinoculation of homogenate from the kidney of both DBTC- and DPTC-treated mice

Histopathological analysis of the liver and kidney revealed abnormalities associated with destruction of the organ integrity. No significant damage was seen in all the spleens.

These compounds (DBTC and DPTC) may be chemotherapeutically useful in the treatment of trypanosomiasis but more is needed to be explored on the toxic effects and none complete clearance of the T. b. brucei from all the bodily tissues of the infected mice.

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CHAPTER ONE

1.0 INTRODUCTION

African trypanosomiasis or sleeping sickness, also known as Nagana in cattle, is a severe animal and human disease caused by trypanosomes, transmitted by tsetse flies and it is often fatal if untreated. The disease occurs in scattered foci throughout the sub-saharan tsetse belts of Africa - an area of some 10 million square kilometres (TDR, 1990). About 50 million people in around 200 foci in the 37 affected countries are at risk with 25,000 new cases reported annually but this is considered an underestimate (Service, 1992).

Animal trypanosomiasis is of tremendous economic importance to man in terms of food production vis-a-vis the feeding of ever-growing population. In the sub-saharan Africa, the disease is transmitted to domestic livestock by the tsetse fly.The presence of this disease inpedes livestock production (directly) and crop production (indirectly) (ILRAD, 1989) in the endemic regions. Most small-scale farmers in developing countries do not have access to motorized vehicles. Thus, on small farms in many part of Africa where both crops and livestock are grown, cattle are the only means of power, for pulling ploughs to prepare land for cultivation, for transporting farm produce to market and for providing manure to maintain and enhance soil fertility (ILRAD, 1991). The magnitude of the risk posed by trypanosomiasis in limiting efforts to raise cattle and make milk and meat available in Africa needs no emphasis (Marsden, 1982). According to ILRAD report 1990, the losses in meat production attributed to trypanosomiasis is estimated to be US $5 million annually in the tsetse-infested areas of Africa.

The South American trypanosomiasis (known as Chaga's disease) is caused by a protozoan parasite called Trypanosoma cruzi, transmitted by blood sucking "assassin" bugs' (Triatoma infestans). The disease is endemic in all of Central and South America (occasional cases in South USA) with 90 million people at risk and about 16-18 million clincal cases reported annually (TDR, 1990).

The current control methods of animal trypanosomiasis largely relies on treating the infected

livestock with trypanocidal drugs, destruction of the tsetse fly breeding by bush clearing and sterile insect release method; and the use of trypanotolerant livestock breeds that are relatively resistant to the pathogenic effects of the disease. These control strategies are frequently used in isolation. However, it is becoming increasingly apparent that a sustainable control of trypanosomiasis is more likely to be achieved by integrating the different control strategies (Andrew, 1993).

The absence of an effective vaccine and lack of a coherent strategy for controlling the insect vectors has rendered the livestock industry highly dependent on the small number of drugs currently available for the treatment and prevention of the disease (Holmes et al , 1988). Some of the drugs currently in use act by interfering with certain components of the metabolism of the parasites. But the protozoan parasites (to which trypanosomes belong) unlike bacteria present fewer biochemical features for selective attack and hence most trypanocides have toxic side effects. Some at times produce toxic side effects that may be more severe than those of the disease. The problem of devising ways to kill these pathogens without also killing the cells of the host has prompted most research workers to focus their attention towards the development of vaccine, which do not attack the parasites but rather stimulates the host's immune system to forestall infection. Because of the antigenic variation in the variant surface of glycoprotein (VSG) of the trypanosomes, this has not been practicable till now (Zielgelbauer and Overath, 1993).

Drug resistance, which is due to both host- and parasite- related factors as well as protracted treatment protocols (ILRAD, 1984) have made combined therapy sometimes useful at certain stage of the disease (Jennings, 1991b, Egwu et al, 1993, Arowolo and Uche, 1988).

It is in the light of the aforementioned and owing to the current interest in the development of new, potent and less toxic trypanocides, that some organotin compounds are tested in vivo for trypanocidal activity. This work therefore is intended to

i) assess the trypanocidal properties of selected organotin compounds;

ii) study the toxic effects of the selected organotin compounds that are found to be active against trypanosomes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 Biology of Trypanosomes

2.1.1 *Trypanosomes*

Trypanosomes are unicellular protozoan parasites responsible for widespread acute and chronic disease in human and domestic animals. They live extracellularly in the blood and tissues of their mammalian hosts (Ziegelbauer and Overath, 1993). Various species of these organisms belonging to the genus Trypanosoma are responsible for sleeping sickness in human in tropical Africa and a very similar disease called Nagana in cattle.

2.1.2 **Morphology and Motility**

In Giemsa stained blood smear, the sub-genera or species of Trypanosomes are distinguished by their size, shape, location and size of the kinetoplast, position of nucleus and the attachment and length of the flagellum. In wet mount, the type of motility and locomotion are also useful in differentiating subgenera. For example Trypanosoma vivax has a fluttering movement, resulting in a rapid darting linear locomotion. Trypanosoma congolense has a slow intermittent twisting movement, while T. brucei and other species in the subgenera Trypanozoon have a rapid twisting motion with a relatively slow forward motion.

Morphologically different species of trypanosomes vary considerably in shape and size, even in the same host. The principal structural features of a Trypanosome is depicted in figure 1, while some of the morphological shapes that may be found during the different developmental stages of this parasite is shown in figure 2. There is a short broad form without a flagellum, a long slender form with a long flagellum, and an intermediate form (Fig 2). In some of the broad or stumpy forms the nucleus is displaced posteriorly especially when the parasite is growing. The blood stream forms of the mammalian trypanosomes are basically lanceolate in shape, their body having the form of an elongated flattened blade, while its ends taper to a point. The parasitic cells average 22 microns long, but they may vary from 12-35 microns long. They characteristically possess kinetoplast, a body of deeply staining nucleoprotein (DNA) situated near the base of the flagellum (Fig 1).

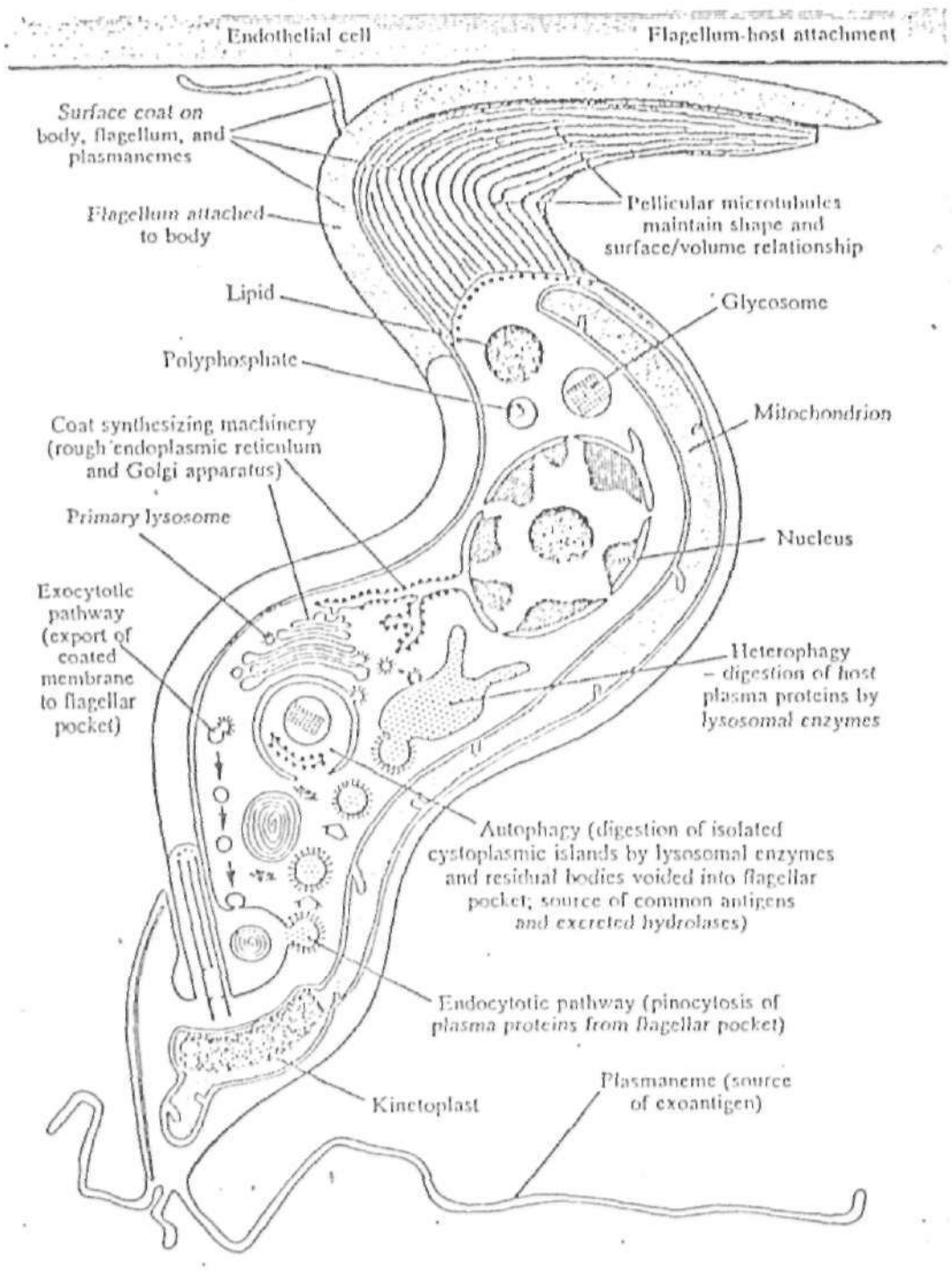


Fig. 1. Principal structures of a salivarian trypanosome and their functions in relation to pathogenesis.

Source: Vickerman, K (1969).

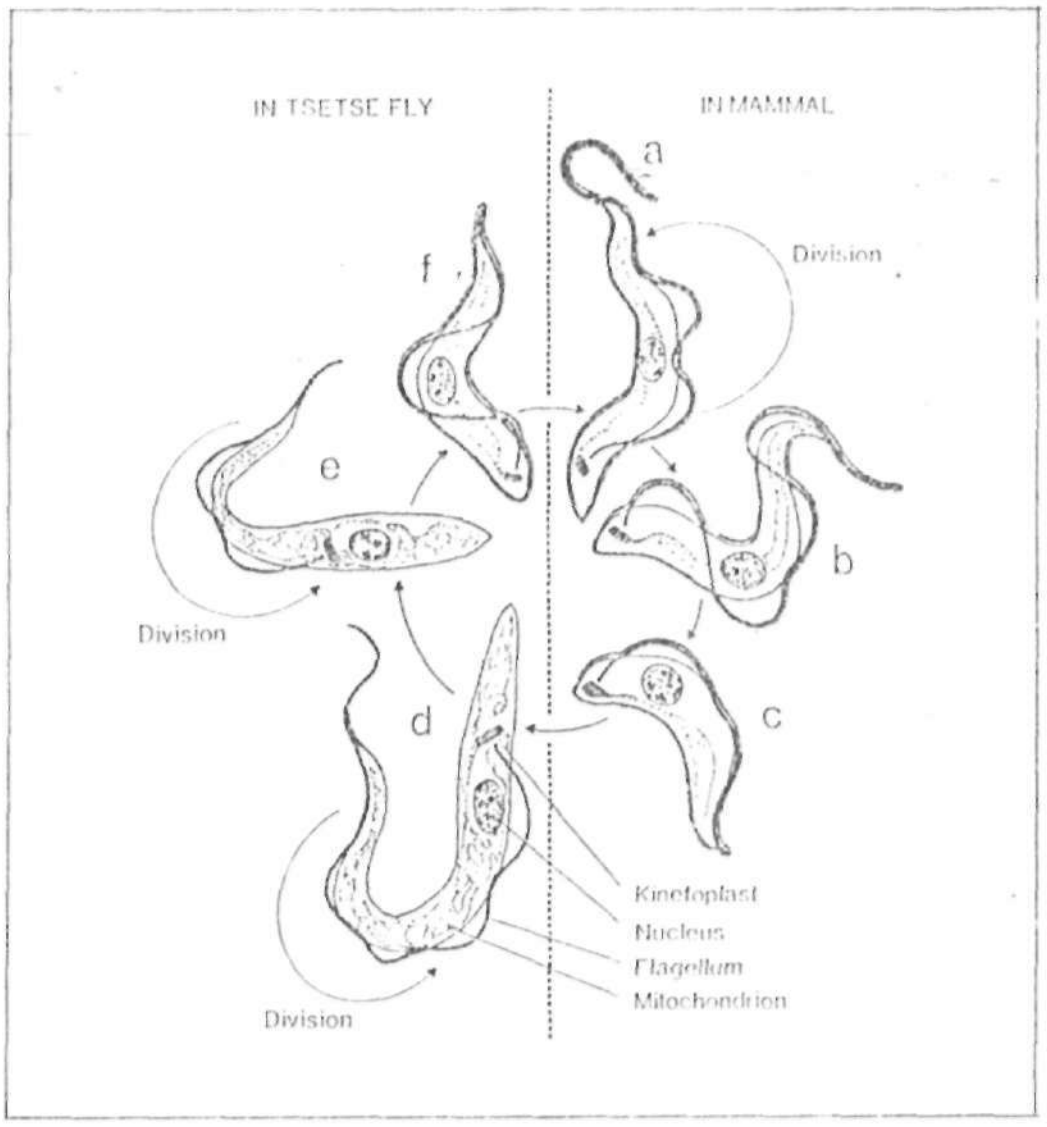


Figure 2: The life cycle of Trypanosoma brucei. In the animal host (a) is the slender form that multiply by binary fission, which transform into intermediate forms (b) and then into stumpy forms (c) which later infect tsetse flies.

In the tsetse fly midgut, (d) is the procyclic forms which enter proventriculus and assume epimastigote forms (e). Finally, metacyclic forms (f) which infect mammals and the life cycle repeated.

Source: ILRAD 1990 Annual Report.

2.1.3 **Transmission and Life cycle.**

The modes of transmission of trypanosomes depend on the subgenera and species, and includes cyclic and mechanical transmission by insect vectors, ingestion, transplacental infection and contact with infected mammalian host. Most species are spread by more than one method, but when insect vectors are involved they usually play the most important or dominant means of transmission.

Salivarian trypanosomes, except Trypanosoma evansi and T. equiperdum, are disseminated by at least 34 species and subspecies of tsetse flies belonging to the genus Glossina. These flies are divided on the basis of their evolution, geographic distribution and habitat into three groups: fusca palpalis and morsitans. Of the three groups, palpalis group dominates in West Africa and contain the most important subspecies of tsetse in West Africa G. tachinoides and G. fuscipes. The morsitans group has subspecies like G. pallidipes, G. longipalpis, G. morsitan morsitans while the Fusca group has G. fusca fusca, G. tarboniformis, G. medicorum, and G. nigrofusca (John, 1971).

Infection begins when trypanosomes are injected into the blood of a susceptible mammal by a tsetse fly as it feeds on the animal. In the animal host, slender forms of the parasites multiply by binary fission until large numbers build up in the blood. The trypanosomes then transform first into intermediate forms and then into stumpy forms, the latter of which are able to infect tsetse flies.

Stumpy forms of the parasites are ingested by a tsetse fly as it feeds on an infected animal. In the midgut of tsetse fly, procyclic forms arise and undergo division, after which the parasites enter the proventiculus and later the salivary glands of the fly, where they assume epimastigote forms and undergo further division. Finally, metacyclic forms arise in the salivary glands. Metacyclic forms are able to infect mammals, and the life cycle is repeated (Fig 2).

2.1.4 **Classification**

Pathogenic trypanosomes in the genus Trypanosoma are divided into two sections, salivaria and stercoraria, according to their pattern of development in the vector and mode of transmission by either the saliva or fecal contamination of the wound caused by the bite of the vector (Losos, 1986b).

Stercoraria, are trypanosomes in which the entire invertebrate cycle of development is confined to the alimentary tract or gut of the vector for example T. Lewisi, T. theileri and T. cruzi; while salivaria, are trypanosomes in which only initial stages of development take place in the gut of the vector while the final stages occur in the probocis. The salivarian trypanosomes unlike the non pathogenic stercorarian parasites, except T. cruzi are generally pathogenic.

There are several species of trapanosomes belonging to different sub-genera. Trypanosoma is in the class 'protozoa and their systematic position is shown in the revised classification below.

PHYLUM: Protozoa

SUB-PHYLUM: Sarcomatogophora

CLASS: Zomastigophora

ORDER: Kinetoplastida

SUB-ORDER: Trapanosomatina

FAMILY: Trypanosomatidae

GENUS: Trypanosoma

SUB-GENUS

SCHIZOTRYPANUM TRYPANOZOON HERPETOSOMA DUTONELLA NANNOMONAS MEGATYPANMUM PYCNOMONAS

The subgenera that contain the commonest special are:

TRYPAPANOZOON - T. brucei, T. rhodescience, T. Gambiense T. evansi, T. equiperdum

DUONELLA - T. vivax and T. unifome

NANNOMONAS - T. congolense and T. simiae

SCHIZOTRYPANUM: T. cruzi and T. vesereliens

2.1.5 **Infectivity**

Infectivity, defined as the ability of trypanosomes to invade and multiply in a host, is a variable depending on the species and strain of trypanosomes and also the species and nutritional state of the host. A species of trypanosomes is first of all, either infective or non-infective for a particular species of host. Trypanosoma brucei brucei is non-infectious to man due to the sensitivity of these parasites to the lytic activity of normal human serum (Tytler, et, al, 1995). Infectivity can be modified for a species of animal by serial passage of trypanosomes in the host.

2.1.6 ***Metabolism***

The compartmentalization of glycolytic enzymes into a specialized organelle, the glycosomes,

allows the blood stream form of trypanosome to rely solely on glycolysis for its energy production (Sommer et al, 1992). The protozoon parasite, T. brucei derives its metabolic energy exclusively from a unique type of glycolysis in which pyruvate, derived from glucose catabolism, is released into the host bloodstream (Barnard et, al, 1993). In trypanosomes, endogenous energy reserves are very low and without an exogenous carbohydrate supply, trypanosomes stop respiring and become immobile within a few minutes (Opperdoes et al, 1976).

Many of the metabolic pathways in trypanosomes have been defined and are used to direct the development of new chemotherapeutic agents (Losos, 1986a) which are specifically designed to exploit known biochemical differences between trypanosomes and their mammalian hosts (Arrick et al, 1981). For example, the elucidation of polyamine biosynthesis pathways in African trypanosomes (Pegg and McCann, 1982), has led to the development of eflornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase the first enzyme in their synthesis, as one of the drugs of choice for treatment of late stage sleeping sickness with central nervous system (CNS) involvement (Bitonti et al, 1990). The unusual gly colytic pathway in trypanosomes which is packaged into glycosomes within the parasites have been used for chemotherapentic intervention. For example, salicylhydroxamic acid (SHAM), is used as an inhibitor of L-α-glycerophosphate oxidase which block oxygen consumption (Gutleridge and Coombs. 1977) and simultaneous administration of glycerol and SHAM in vivo was shown to cure animals of salivarian trypanosomes (Kilgour, 1980). Klein et al in 1982 reported T. brucei to have unusually high intracellular concentration of carnitine and 0-bromoacetyl-α-Grnitine (BAC) has been found to inhibit carnitine acetyl transferase activity. Other chemotherapeutic strategies for the treatmnt of trypanosomiasis have exploited the use of buthionine sulfoximine (BSO), a potent and specific inhibitor of glutathione biosynthesis (Arrick, et al, 1981) and the use of organotins as inhibitors of phospholipases (Nok et al 1993).

These studies on trypanosomal metabolism also provide information as to how they injure the host. For example, one of the metabolic capabilities of the trypanosomes that contribute to the pathology of trypanosomiasis is the utilization of host tyrosine, phenylalanine and tryptophan, which lead to the depletion of these amino acids in the host with resultant lack of such important compounds like catecholamines and serotonin. The T. gambiense converts tryptophan mainly to tryptophol and indole acetic acid (Gutteridge & Coombs, 1977). These metabolites result in behavioural depression and alterations in sleep and patterns, activity, body temperature, blood pressure, glycogen and lipid metabolism (Gutteridge & Coombs, 1977). They may also have direct effect on the host; for instance tryptophol

interacts irreversibly with cell membrane leading to cell lysis (Tizzard et al, 1978) and phenylpyruvate is known to inhibit adrenaline synthesis. It is also known that trypanosomes are unable to synthesize fatty acids de novo and therefore depend on host lipids for their fatty acids synthesis (Mellors & Samad, 1989). This dependence of trypanosomes on host lipids and other host nutrients suggests that the survival of the parasite requires the appropriate concentration of the lipid in the host.

2.1.7 Antigenic Variation

The trypanosomes are normally transmitted cyclically by tsetse flies. The trypanosomes survive in the blood-stream of the mammalian host by periodically altering their antigenic profile so that the developing immune response of the host is rendered abortive (Turner, 1980).The African trypanosomes evade the humoral immune response by periodically changing the antigenic identity of their variant cell-surface glycoprotein (VSG) coat. The antigenic variation formation relies on DNA rearrangement events that can translocate a silent VSG gene to a telometrically located VSG gene expression site (Van der Ploeg et al, 1992). The disease is therefore characterized by a relapsing parasitaemia with each recrudescence representing the development of immunologically distinct variants (Turner, 1980). Therefore, according to Ziegelbauer and Overath (1993), since the repertoire for genes coding of antigenically different VSGs is very large, vaccination against trypanosomiasis based on the variant antigen is believed to be impossible as at the moment.

2.2.1 Trypanosomiasis

Trypanosomiasis is a group of diseases in man and animal caused by species of Trypanosoma, either one, two or more species invading the host simultaneously or sequentially to produce infections

(Losos, 1986a). The resulting disease, called sleeping sickness in people and nagana in animals, profoundly affect the public health of the rural populace and the pattern of agriculture in Africa (ILRAD, 1990). The disease causes anaemia, reduced productivity, reproductive disorder and death. The tsetse fly vector inhabits most of the humid and subhumid zones of the African continent, which make up about half of the non-desert area of Africa (ILRAD, 1990).

Human trypanosomiasis is caused by Trypanosoma brucei rhodesiense, carrying Rhodesian sleeping sickness and T. b. gambiense, carrying the Gambian sleeping sickness, while T. cruzi is responsible for the Chaga's disease in South America. The course of the disease is usually acute with T. rhodesiense but more chronic with T. gambiense.

Three trypanosome species are known to cause the disease in African animal trypanosomiasis. These include Trypanosoma congolense, T. b. brucei and T. vivax. The course of the infection may differ even for the same species, but in general T. congolense causes a more acute disease than T. vivax. The South American trypanosomiasis, also known as Chagas disease is caused by T. cruzi which is transmitted by the large blood-sucking 'assassin bugs' of the sub-family Triatominae. There are two phases of the disease: an early acute phase and a chronic phase. The chronic form of the disease is difficult to cure. Two drugs, nifurtimox and benznidazole, can be used for the very early infections, but early diagnosis is difficult. Adverse side-effects can occur following the use of these drugs (ILRAD, 1990).

2.2.2 Pathology

The basic mechanisms by which trypanosomes injure tissues are probably multifactorial for any given species of trypanosomes. The mechanism involves utilization and excretion of metabolites, excretion of toxic substances, mechanical disruption of host's tissues and immunologically mediated injury. These mechanisms, singularly or in combination are potentially harmful (Losos, 1986a).

The main pathological feature of trypanosome infections in susceptible animal is anaemia. Although much have been documented on the proposed mechanism of anaemia, there is no general agreement on its induction.

Immunologically mediated injury involves basically the destruction of erythrocytes (Kobayashi, et al, 1976). It has been postulated that trypanosomes attach themselves to the erythrocyte membrane, during acute trypanosomiasis with high parasitaemia, and produce sialidase (neuraminidase) which cleaves erythrocyte surface sialic acids, rendering the erythrocytes more prone to sequestration by the reticuloendothelial systems (Banks, 1980, Esievo et al, 1982).

Another theory on the etiology of anaemia implicates phospholipases. Tizzard et al (1978) observed that Trypanosoma congolense possessed a haemolytic material which is composed of mixture of free fatty acids and lysolecithin produced by the action of phospholipases on host phospholipids. The products of this reaction are shown to be haemolytic due to their detergent-like properties which make them to be powerful surface agents thereby membranolytic.

The general consensus amongst investigators in trypanosome anaemia is haemolysis which is a central factor. They have enunciated the general features of haemolytic anaemias which include accelerated red blood cells (RBC) destruction, compensatory erythropoietic hyperplasia, RBC destruction, and shortened RBC survival. Additional evidence include erythrophagocytosis and haemosiderosis seen in spleen, liver and other organs. All these features are reported to be most marked when parasitaemia is high (Anosa, 1988).

Other factors which contribute to anaemia in trypanosomiasis are haemodilution (increase in total blood volume) and haemorrhage (produced by haemorrhagic trypanosomes). These factors were shown to be minor and most likely do not contribute significantly to the anaemia (Anosa, 1988).

Dyshaemopoiesis was also shown to contribute to trypanosome anaemia and several workers demonstrated the following mechanisms to be responsible for dyshaemopoiesis

(a) stem cell injury with reduction in numbers due to trypanosome toxins and competition between different stem cell lines for space and nutrition

(b) phagocytosis of erythroid cells in spleen and

(c) the trapping of iron in macrophages of the mononuclear phagocyte system with resultant reduced availability for erythropoiesis. This effect is more marked during chronic than acute crisis.

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2.3 Clinical Manifestation/Symptoms

Clinical manifestations are produced following the bite of an infected tsetse fly which deposits trypanosomes in sub-cutaneous tissues of the host giving rise to a hard painful red nodule called the chancre which appears in the skin between four to ten days after the tsetse bite (Fiennes, 1953). The trypanosomes then invade the blood and lymph vessels leading initially to malaise, lassitude and irregular fevers in humans. This is then followed by a range of symptoms including headache, anaemia, joint pains and swollen tissues (spleen, liver and lymph nodes), progressing, as the parasites invade the central nervous, to mental deterioration, coma and death (TDR, 1990). Trypanosoma b. rhodesiense infection is usually acute, causing severe symptoms and death within a few days or weeks; T. b. gambiense infection tends to progress more slowly (chronic) (TDR, 1990).

In the case of Chaga's disease, often, there is a small sore at the site of bite where the parasites entered the body. Within a few days, fever and swollen lymph nodes may develop, sometimes making early stages of infection look like malaria. This early acute phase of infection can be fatal, but usually the patient survives to enter a symptomless phase which may last many months or years. During this period, however, the parasites are invading most organs of the body, so that chronic symptoms eventually develop - often involving irreversible damage to heart and intestine and the patient becomes progressively weaker and may die from heart failure (TDR, 1990).

2.3.0 Control of the Disease

In the absence of an effective vaccine, control of animal trypanosomiasis relies on three methods: treating the livestock with trypanocidal drugs, using insecticides, traps or targets to reduce tsetse fly population and keeping indigenous livestock breeds that tolerate infection with parasites (ILRAD, 1990).

2.3.1 Vector Control

Despite the application of insecticides, traps or targets, the distribution of tsetse flies in Africa has remained virtually unchanged over the past 50 years. Any population reductions have usually been temporary, with either resurgence or reinvasion from surrounding areas (Dransfield, 1987) and this is

compounded by the movement of traditional herders into tsetse-infested humid and sub-humid zones in response to drought and political upheaval (Tacher, et al, 1992). This has resulted in increased competition for land, increased trypanosomiasis risk and practical difficulties in tsetse control.

In the case of chaga's disease, control relies on the use of residual insecticides to kill the triatomine bugs in houses together with health education and low cost housing improvements to render houses unsuitable for colonization by the bugs (TDR, 1990).

2.3.2 **Trypanotolerance**

This is the genetic ability to resist the pathogenic effects of trypanosome infection. N'Dama (Bos taurus) and other cattle breeds indigenous in West Africa are trypanotolerant, being able to tolerate infection with trypanosomes (ILRAD, 1990). Many species of wildlife, some strains of rats and possibly human are also trypanotolerant (Murray et al, 1984).

It is believed that mechanisms to resist the disease evolved in trypanotolerant breeds because of long exposure to the parasites; having been probably introduced to the continent as far back as five to seven thousand years ago. (ILRAD, 1990).

Rearing of trypanotolerant livestock is possible in tsetse-affected areas, but has two main drawbacks. First, many farmers believe these breeds are less productive than others, relatively poor milk production and typically small in size hence not ideal for draught work. Second, few of such animals are available - constituting only 5% of the total cattle population in the 37 countries where tsetse occur (ILRAD, 1990).

2.4.1 **Chemotherapy and Chemoprophylaxis**

Trypanocide used in the treatment and protection are the most common single method employed for the control of animal trypanosomiasis. Effective application of chemotherapy and chemoprophylaxis in the field depends on several factors which include the species of the trypanosomes causing the infection, severity of the challenge, species of animal and lastly occurrence of resistance strain (Losos, 1986a).

Despite the advances in chemotherapy made in the first part of this century, trypanosomes and their insect vector, the tsetse fly, continue to pose a serious threat to the health and economic well-being of millions of Africans. Recent advances in the elucidation of trypanosome metabolism have encouraged the development of new chemotherapeutic agents specifically designed to exploit known biochemical differences between trypanosomes and their mammalian hosts (Arrick et al, 1981). Recently, eflornithine or α-difluoromethylornithine; ornidyl (DFMO); originally developed as an

anticancer agent, is an irreversible inhibitor of ornithine decarboxylase, the first enzyme in the biosynthesis of polyamine, putrescine and spermine; has been found effective in the treatment of both early and late stage Gambian sleeping sickness in human patients (Bitonti et al, 1990).

Generally, antitrypanosomal drugs can be used in two distinct modes:- prophylaxis and treatment. For human African trypanosomiasis, pentamidine is used for prophylaxis,suramin for treatment of early stages of the diseases and melarsoprol for the treatment of late stages when trypanosomes are present in central nervous system (Gutteridge, 1985). For animal trypanosomiasis, quinapyramine, isometamedium and pyrithidium are used for chemoprophylaxis while diminazene aceturate, homidium, pyrithidium, isometamedium and quinapyramine are used for treatment purposes (Anthony, 1986).

2.4.2 **Toxicity and Resistance**

The chemotherapy of trypanosomiasis is beset with many problems - including a limited repertoire of compounds, resistance to drugs, drug toxicity, and sometimes the need for a protracted treatment protocol (Nok et al, 1993).

The problems of toxicity impose severe restrictions on the choice and use of trypanocides. An ideal drug for the treatment of either the African or the American trypanosomiasis should have the following properties:

i) active on administration in a single dose (or a small number of doses over a short period);

ii) have minimal side effects; and

iii) active against all stages of the disease causing parasites by all strains and species of trypanosomes (Gutteridge, 1985).

Presently, none of the drugs in use for either African trypanosomiasis or Chaga's disease meets all these criteria. Melarsoprol and eflornithine, drugs of choice for the treatment of late stage sleeping sickness, have always resulted in complicated treatment by inducing a post-treatment meningoencephalitis that may be fatal (Hunter et al, 1992). Although eflornithine is less toxic than melarsoprol, eflornithine may not be the first-choice therapy in rural Africa for economic and logistic reasons. But for the vast majority of patients who relapse after melarsoprol treatment, eflornithine

becomes a useful replacement (Milord et al, 1992).

The term 'drug resistance' is frequently used to explain any therapeutic or prophylactic failure (Holmes et al, 1988). Resistance to quinapyrine, homidium and pyrithidium develops so rapidly that in many African countries, the widely employed trypanocide remain isometamidium (curative and prophylaxis) and diminazene aceturate (curative) (Anthony, 1986). Although resistance of trypanosomes to diminazene aceturate and isometamidium chloride is reported (Arowolo et al, 1988) it is not as prevalent as others.

Drug resistance complicates treatment of many infectious diseases and this term 'drug resistance' covers both host- and parasite-related factors.

Host-related factors include poor distribution of drug to infected tissues or intracellular sites, variation in drug metabolism between individuals, and a diminished activity of a drug in animals with suppressed immune systems (ILRAD, 1990).

The origins of parasite-related resistance may be natural, as in non-susceptible strain with no previous exposure to drugs, or it may be due to drugs exposure or to cross resistance or mutagen induced. Parasite-related factors that contribute to resistance include reduced drug accumulation in the parasite, a change in enzyme target through increase in enzyme levels, changes in enzyme affinity, alteration in drug metabolism,

increased metabolic production or retention, and use of alternative pathways to bye-pass the site of inhibition (ILRAD, 1990).

2.5.0 Organotins

Organotins are compounds which possess one or more direct tin-carbon covalent bond(s) that are responsible for the specific properties of such molecules. They started to get commercial significance in the 1950s and a larger number of organic compounds of tin than any other element are presently in commercial use (Klause et al, 1989).

The current worldwide annual rate of production of the organotins is of the order of 60,000 tons, their main end-uses being, polyvinyl chloride (PVC) stabilizers, wood preservatives and fungicides, marine antifouling agents, agrochemical fungicides and miticides and in disinfectants (Crowe, 1987). The general structure of the organotin is as follows

$$R_y Sn X_{4-y} \quad (y = 1 - 4)$$

where R = an alkyl or aryl group

Sn = the central tin atom in the oxidation state of +4

X = a singly charged anion or an anionic organic group.

The biological properties are affected by both the number of carbon-tin bonds in the molecule and the nature of the group(s) directly bound to the tin atom.

2.5.1 **Classification**

Organotin compounds are classified as mono-organotin ($RSnX_3$), diorganotin (R_2 Sn X_2), triorganotin (R_3 Sn X) and tetraorganotin (R_4 Sn). Maximum biological activity is obtained in triorganotins (Van der kerk et al, 1956) and tetraorganotins behave as precursors for the more toxic triorganotins. Thus when a tetraorganotin is administered, there is a time lag while one of the organic groups, R is lost by metabolism to the corresponding triorganotin, which produces the toxic effect (Blunden et al, 1985). The toxic phenomenon arise by the inhibition of mitochondrial oxidative phosphorylation (Aldridge, 1976), while the degree of toxic action is believed to be controlled by the ease with which the triorganotin compound interact with amino acids at certain active sites (Hall et al, 1977) with imidazole N-H of histidine residue and/or S-H group being the most likely binding sites (Smith, 1978b).

Diorganotins are the largest group of tin compounds studied for anti-tumour activity. They tend to be less toxic than the triorganotin, and also act by inhibiting mitochondrial oxidative phosphorylation. However, the means by which this effect is brought about, is different from that of R_3SnX, in that they inhibit α-keto acid oxidation. They act probably by combining with enzymes or coenzymes which possess vicinal dithiol group (Aldridge, 1976), for example reduced lipoic acid.

The monoorganotins, RSnX, are the least toxic of the organotins and consequently do not show any toxic action in mammals (Smith, 1978a)

2.5.2 **Chemotherapeutic and other Application**

In the early 1950s Van der Kerk and Luijten laid the foundation of bioorganotin chemistry by systematically exploring the biological properties of organotin compounds. The rapid growth of this new field was assisted by the discovery of anticancer activity in platinum compounds in 1969.Despite their poor solubility in water, which is a major obstacle to the improvement of their activity (Attassi, 1985), organotins have found application in a very broad spectrum of human endeavour, and the day is not far away when the application may only be limited by the imagination of chemists (Saxena, 1986).

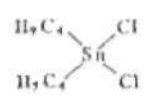
The majority of organotin compounds are toxic to biological systems. However, selective toxicity of a compound is the basis for its chemotherapeutic and other biocidal application (Saxena, 1986).

2.5.2a **Antitumour Activity**

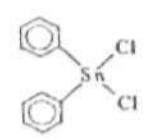
One of the major developments in the field of bioorganotin chemistry in the 1980s was the finding that organotin compounds can play an important role as antitumour agents (Saxena, 1986). Though organotin compounds have been extensively studied as fungicides, bactericides and acaricides, little information was available on the organotin as antitumour agents. A large number of organotin compounds have now been tested and many show reproducible antitumour activity in mice (Briddle and Gray, 1989).

In 1972, Brown, demonstrated that tumour growth in mice was retarded by triphenyltin acetate, but not by the chloride, when administered either in the food or by injection. A wide variety of organotin compounds were screened as antitumour agents, during 1973 - 1977 period by the Institute for Organic Chemistry TNO, Utretch, the Netherlands, sponsored by the International Tin Research Institute, London. Of these only few were found to be active and were not of sufficient activity to warrant further tests (Crowe, 1987). Of the four classes of organotin compounds, diorganotins, R_2SnX_2 are the largest group of tin compounds studied for antitumour activity (Crowe, 1989). Diorganotin dichloride complexes, $R_2SnX_2L_2$ (R=ethyl, butyl or phenyl; L_2 = bidentate ligand) were shown to have activity against different murine transplantable tumours (Barbieri et al, 1982, Crowe et al, 1980, Attasi, 1985.).

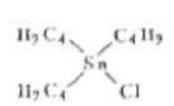
Dibutyltin dichlorides exhibited activity in vivo against Ehrlich ascites tumour and IMC carcinoma, but was inactive against P.388 leukaemia (Crowe et al,1984). Attasi in 1985, reported the activity of diphenyltin dichloride 3,4,7,8 tetramethyl (-1,10-) phenanthroline (Ph_2SnCl_2. $T_m$phen) against p.388 leukaemia and renal carcinoma.



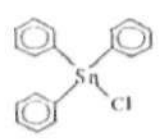
Dibutyltin dichloride



Diphenyltin dichloride



Tributyltin chloride



Triphenyltin chloride

2.5.2b **Biocidal organotin compounds of Agricultural Importance**

After the report of Van der Kerk and Luijten, in 1956, that various di-and triorganotin acetate showed activity against Aspergillus niger and Botrytis allii, the first organotin to be used commercially was triphenyltin acetate (Ph_3SnOA_c) which is used against Cercospora beticola in sugar beets and Phytophthera infestans in potatoes (Hiirtel, 1958).

Organotin compounds have found application in forestry for wood preservation, and tributyltin compounds are the most important of them. Enhanced fungicidal activity of tributyltin oxide, $(Bu_3Sn)_2O$ against Cercospora puteana in pine wood was reported by Cox in 1981.

Other application of organotin compounds include chemotherapy of leshmaniasis where dioctyltin maleate was found active (Peters et al, 1980),in in vitro tests against Entoamoeba histolytica where tributyltin compounds where found to have greater activity than the drug emetine (Saxena et al ,1982) and in vitro and in vivo effects of tributyltin oxide $(Bu_3Sn)_20$ in the treatment of Trypanosoma brucei brucei infection in mice (Nok et al, 1993).

2.5.3 **Toxicity**

The great majority of organotin compounds are toxic to biological systems and there are various factors which determine the toxicity of organotin compounds. These factors are enumerated below.

i. The nature of organic group; where alkyl groups are shown to be more toxic than aryl groups. For example, maximum mammalian toxicity is given by triethyltins. The trend of toxicity rises to a maximum and then steadily decreases as the number of carbon atom increases.

ii. The number of organic groups; where the maximum toxicity appears in triorganotin compounds.

iii. Method of administration, like any other toxic agent, organotin compounds injected into the body are more toxic than the same compound ingested orally.

iv. Nature of the inorganic group, where variation of the inorganic group X, within any particular

series is usually found to have no significant effect on the toxicity, unless if itself is biologically active.

Other factors which contribute to the toxicity are organisms tested and frequency of doses (Saxena 1986).

2.5.3a **Mechanism of Toxicity**

A number of mechanisms have been proposed to explain the toxicity of organotin compounds and the major ones are discussed below.

i) Inactivation of Enzymes. The majority of organotin compounds are toxic because they combine with some enzymes, and thereby inactivate them. The metal interacts with amino acids at certain active sites and forms bond that is too strong to be readily broken, thereby preventing the enzyme from reacting with its substrate (Saxena, 1986). The most likely binding sites being the imidazole N-H of a histidine residue and/or thiol S-H group (Smith, 1978b). Active sites for biomolecules (present in organism) may be blocked by binding to an organotin compound. Binding of some organotin compounds to the cysteine and histidine residues of protein through pentacoordination of tin atom has been suggested (Smith, 1978b). For example, Taketa et al (1980) reported that triethyltin compounds bind to cat haemoglobin through thiol group of residue Cys-13 and imidazole group of residue His-20 of the a-chain.

One of the three basic modes of action of triorganotins on mitochondria, is the direct inhibition of ATPase complex which manifests itself in the loss of ATPase and ATP synthase activities. This effect appears to be the result of an interacion with the Fo component of the F_1F_o ATPase, causing the inhibition of proton(H^+) conduction through the system (Connerton and Griffiths, 1989).

ii) Effects on central nervous system. Certain organotin compounds are toxic because they cause oedema in the brain and CNS. Many of them weaken or destroy myelin and proteins, which coat nerve fibres.

iii) Physiological Disorders: Organotin compounds cause a number of physiological disorders. Triphenyltin fluoride has been reported to induce hyperglycaemia and hyperglyceradaemia in rabbit plasma (Wada et al, 1981). Certain organotin compounds, upon oral administration inhibited gastric emptying, thereby causing fluid retention. Triphenyltin has been shown to provoke 'all or none' K^+ loss pattern from the cell (Borst-Pauwels et al, 1983).

iv) Cross-linking with DNA: The ultimate fate of the active R_2Sn moiety is possible that it cross-links with DNA in a manner similar to that of cisplatin. The metal coordinates with suitably orientated nitrogenous bases on DNA. The cross-linking with DNA was shown to be on the N-7 nitrogen atoms on two adjacent guanine rings of the same chain - the d(pGpG) segment which contains deoxyguanosine units (Crowe, 1987), thus disrup ti6ng thc DNA helix and so interfere with replication.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.1 Animals and Parasites.

Trypanosoma brucei brucei was obtained from the department of parasitology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The parasites were maintained in mice and when inoculated into into mice produced death at a dose of 10^2 parasites in laboratory mice in 23-33 days (chronic infection). The animals (mice) used were purchased from the department of Pharmacology, Ahmadu Bello University Zaria. These were fed ad libitum with Sanders grower mash.

3.1.2 Chemicals

Ethanol, chloroform, acetone and formaldehyde were purchased from May and Baker Ltd, Dagenham, England. All other reagents were of analytical grade and purchased from BDH, UK and Sigma Chemical Company St. Louis Missouri, USA.

Diminazene aceturate (dimenaphen) was purchased from Phenix Pharmaceutical (N.V. Belgium). The organotin compounds were given by Dr. J.J. Bonire of Chemistry Department, Ahmadu Bello University, Zaria.

3.1.3 Inoculation of the Animals

The mice in each group were intraperitoneally inoculated with about 10_2 of T. b brucei obtained from a donor mouse's blood. The blood was diluted with phosphate saline glucose (PSG) and 0.1ml of each was inoculated into an apparently healthy mouse using 1ml syringe with 26 gauge needle. The level of parasitaemia was routinely monitored in blood obtained from the tail vein by wet film preparation on a slide and mounted on microscope (x40 objective lens).

3.1.4 **Counting of the Parasites**

About 5µl each of fresh tail blood from the infected mouse was obtained and diluted with about 5 ul of PSG on a slide. It was then covered with a slide coverslip and examined at x400 magnification. A minimum of 20 fields were examined to observe and count the motile parasites as follows:

Dilution 5µl:5µl

Dilution factor (D_f) = 2

Total number of parasites in 20 fields = T_p

∴ *Number of parasites per ml of blood*

$T_p \times D_f \times 10^3$

3.2.0 **Administration of the organotins/Treatments**

3.2.1 **Dibutyl tin dichloride (DBTC).**

An initial dose-dependent trial was conducted using 5, 10, 20 and 50mgDBTC/kg body weight twenty four hourly. Therefore 10mgDBTC/kg/day(33µmoles/kg/day) was used because it produced maximum cure with minimum toxicity symptoms.

Twenty mice were divided into three groups; group I of twelve mice and group II and III four mice each. The members of group I and II were intraperitoneally infected with approximately 10^2 trypanosomes and group III members were inoculated with 0.1ml PSG to serve as negative control. Treatment commenced six days post-infection and members of group I were treated with 10mgDBTC/kg/day(33µmoles/kg/day) *until the parasitemia disappeared. The group II members were* treated with placebo to serve as positive control. The trypanocidal effect of DBTC was assessed by determining the level of parasitemia every twenty four hour after treatment. The treated group members were observed periodically for any relapse in parasitemia.

3.2.2 **Diphenyltin dichloride (DPTC)**

Dose dependent treatment trial was conducted as described in section 3.2.1 above. Pilot studies showed a dose of 10mgDPTC/kg/day and 20mg/kg/day to have maximum parasiticidal effect with 10mg/kg/day having relatively longer treatment protocol. For this treatment protocol, 15mgDPTC/kg/day (44µmole/kg/day) was used and followed the same procedure as in section 3.2.1 above.

3.2.3 Tributyltin chloride (TBTC)

A preliminary studies was carried out as in 3.2.1 above. The doses were found to be toxic. But 5mgTBTC/kg/day (15.4µmole/kg/day) dose only showed mild toxic effects, and was used for the treatment.

Sixteen mice were divided into three groups. Group I was made of eight mice while groups II and III were made up of four each. GroupI and II were each inoculated intraperitoneally with 10^2 trypanosomes. The third group members were inoculated with 0.1ml PBSG to serve as negative control. Treatment commenced six days post-infection and group I members were treated with 5mgTBTC/kg/day while group II members were administered with placebo to serve as positive control. The trypanocidal effect of TBTC was assessed by determining the level of parasitemia twenty four hourly after treatment.

3.2.4 Triphenyltin chloride (TPTC)

The same preliminary study was carried out as in section 3.2.1 above and 7.5mgTPTC/kg/day(19.5µmole/kg/day) was used. The same procedure was followed as in 3.2.3 above.

3.2.5 Diminazene aceturate (Dimenaphen)

The curative dose of diminazene aceturate 7mg/kg was used (Arowolo et al, 1988). The trypanocidal effects of all the organotins were compared to the standard diminazene aceturate-treated positive control group.

3.3.1 Subinoculation Experiment

Three mice each from the DBTC-, DPTC- and dimenaphen-treated groups were sacrificed three weeks post-treatment and subinoculation was carried out as described by (Nok et al, 1993) but with slight modification. About 0.3ml of blood was recovered from each animal and used to inoculate 2 healthy mice. All the tissues (liver, spleen and kidney) were removed, washed, weighed and macerated separately in PSBG. The same concentration of each organ homogenate in PBSG was inoculated into healthy mice (0.1ml). The parasitemia was then routinely checked for four weeks.

3.3.2 Cerebrospinal fluid (CSF)

The CSF was withdrawn and inoculated into healthy mice as described by Nok et al, 1993. The hair at the back of the mouse was shaved. The animal was positioned with its head touching its hind limbs, a posture making the vertebrae conspicuous. A needle was used to puncture the vertebrae and 0.1ml of the CSF was inoculated into each mouse interperiteneally. The parasitemia was routinely checked for four weeks.

3.4.1 Histopathological Tests

Formalin fixed sections of the kidney, liver and spleen from both control and treated mice were taken for histopathological examination at the Ahmadu Bello University, Teaching Hospital, Zaria. A 3-4cm thick tissue slices were cut from each organ for processing. The cut tissues were transferred to the automatic tissue processor where they were further fixed in 10 percent buffered formalin-saline for 2 hours, and then dehydrated for 2 hours in each of the ascending grades of alcohol (85%, 90% and 100% v/v). The dehydrated tissues were then cleared in toluene for 2 hours and the tissues impregnated in molten paraffin for 2 hours, after which the tissue slices were embedded in paraffin wax and left to cool. The blocks were then trimmed and sectioned on the microtome at 5 microns. The ribbon of sections were then floated in a

warm water bath, suitable sections were selected and attached to slides, dried on hot plate and stained.

The sections were dewaxed in xylene and rehydrated in descending grades of alcohol: 100%, 90% and 70%. They were then stained in haematoxylin for about 5 minutes, differentiated in 1% acid alcohol, blued in Scott's tap water and stained in eosin for 3 minutes. They were then rinsed and dehydrated in ascending grades of alcohol solution 70%, 90% and 100% and finally cleared in xylene and mounted in pox.

The slides were then examined microscopically for lesions.

3.5.1 Toxicity Studies

An initial rough dose-range finding trial was conducted to determine the minimum dose that will give 100% death and the maximum dose that will not produce any death.

Five different doses were selected and administered intraperitoneally. The animals in each group were observed for twenty four hours. The LD_{50} was calculated using Arithmetic Method of Karber (Karber et al 1931). I n this method the difference between two consecutive doses and the mean of the number of the animals dying at the dose levels are calculated. The sum of the products of the mean dead and the dose difference is divided by the animals in each groups. The LD_{50} is then calculated as shown in appendix B.

4.0 RESULTS

The results of studies on the trypanocidal properties as well as the toxic effects of selected organotin compounds are shown in figures 4.1 to 4.5; in tables 4.1 to 4.7 and plates 4.1 to 4.2.

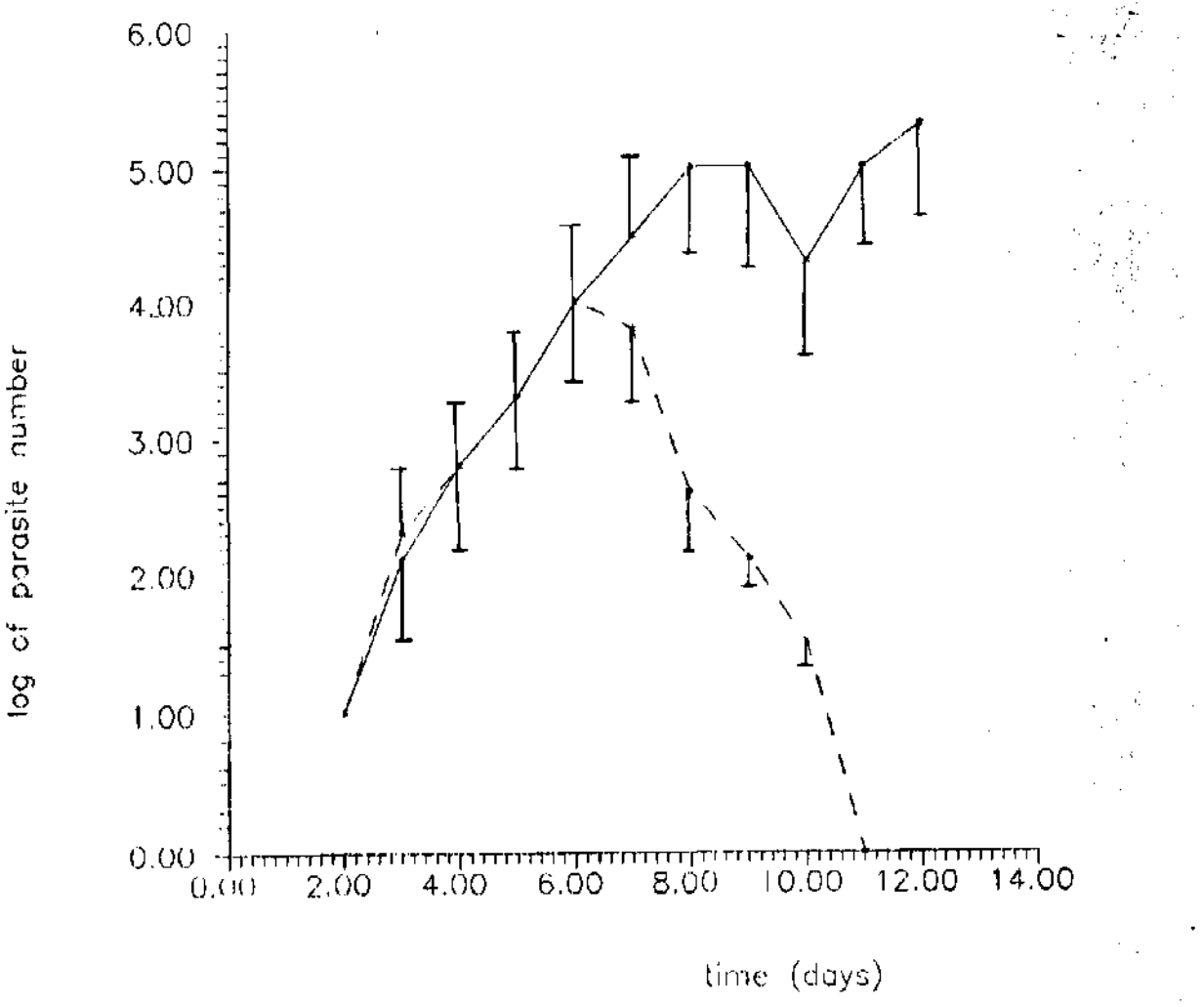


Figure 4.1 A graph of logarithm of parasite number against time of development of parasitaemia (days) following administration of 10mg (33μmoles) of dibutyltin dichloride/kg body weight.

Key:

_____ Positive control (infected not treated)

——— : Infected and treated with DBTC

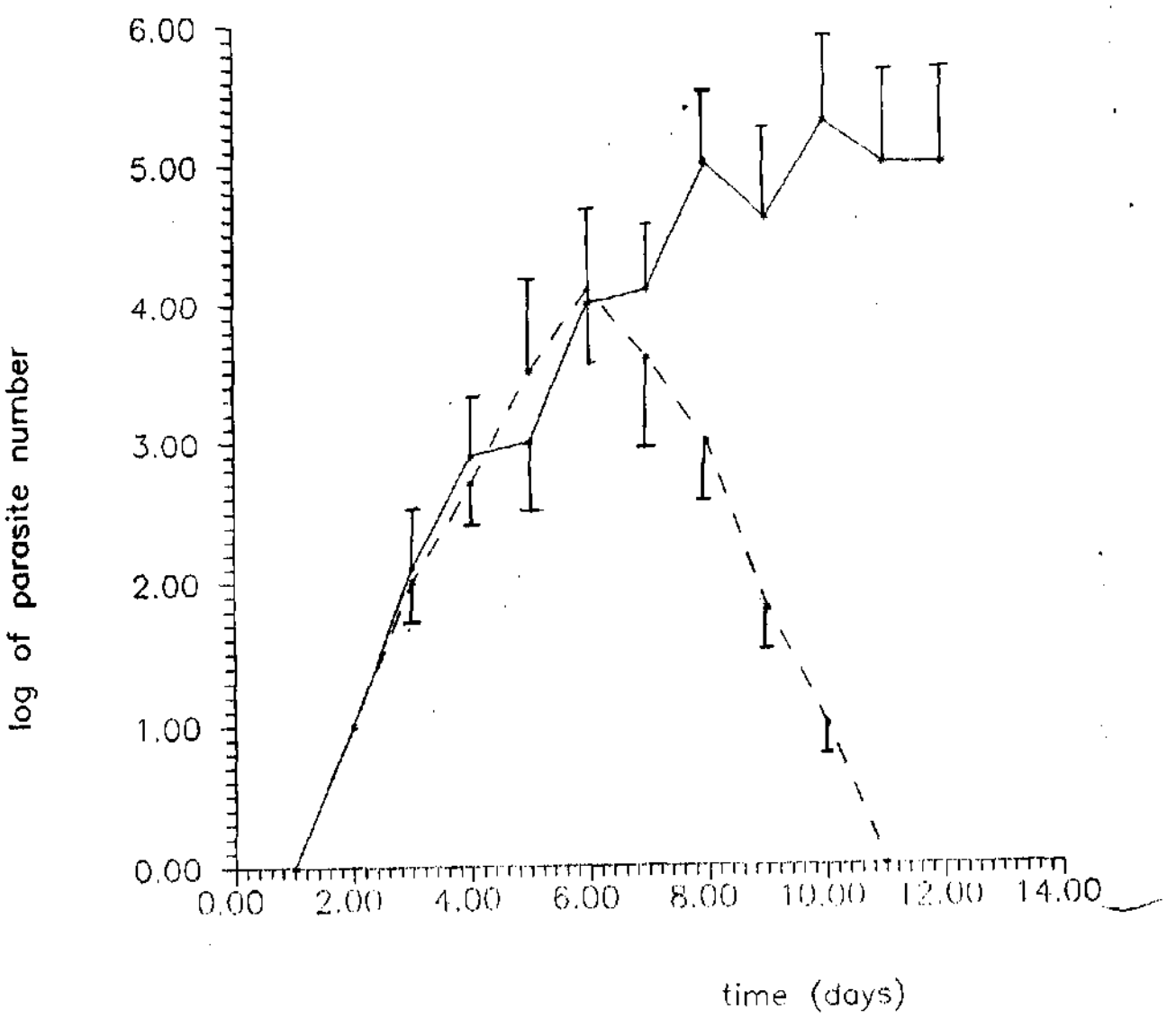


Figure 4.2 A graph of logarithm of parasite number against time of development of parasitaemia (days) following administration of 15mg (44μmoles) of diphenyltin dichloride/kg body weight.

Key:

_____ : Positive control (infected not treated)

------- : Infected and treated with DPTC

Figures 4.1 and 4.2 show graphs of logarithm of parasite number against time of development of parasitaemia (in days) following administration of 10mg (33 μ moles) dibutyltin dichloride and 15mg (44 μmoles) diphenyltin dichloride per kilogram body weights respectively.

Six days after infection with T. b. brucei, parasitaemia in the mice developed to a level of 10^4 parasites per millilitre of blood. Following the commencement of treatment with the dibutyltin dichloride and diphenyltin dichloride on the sixth day post-infection, there was a gradual decrease in the level of parasitaemia until the fifth day of treatment when no parasites were observed in the blood as could be seen in figures 4.1 and 4.2 respectively.

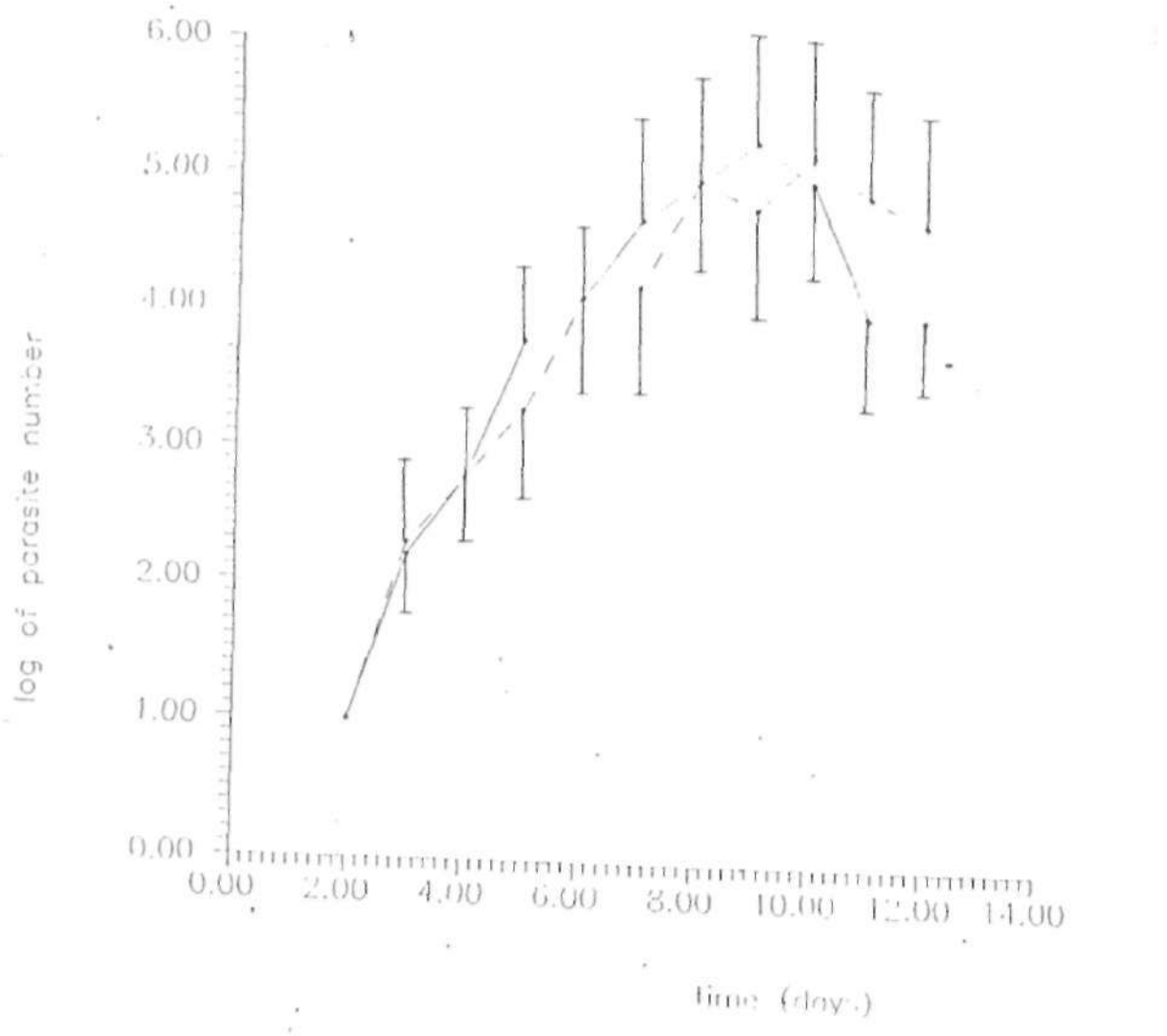


Figure: 4.3 *A graph of logarithm of parasite number against time of development of parasitaemia (in days)* following administration of 7.5mg(19.5μmoles) of triphenyltin chloride/kg body weight.

KEY:

_____ : Positive control (infected not treated)

_ _ _ _ _ _ : Infected and treated with TPTC

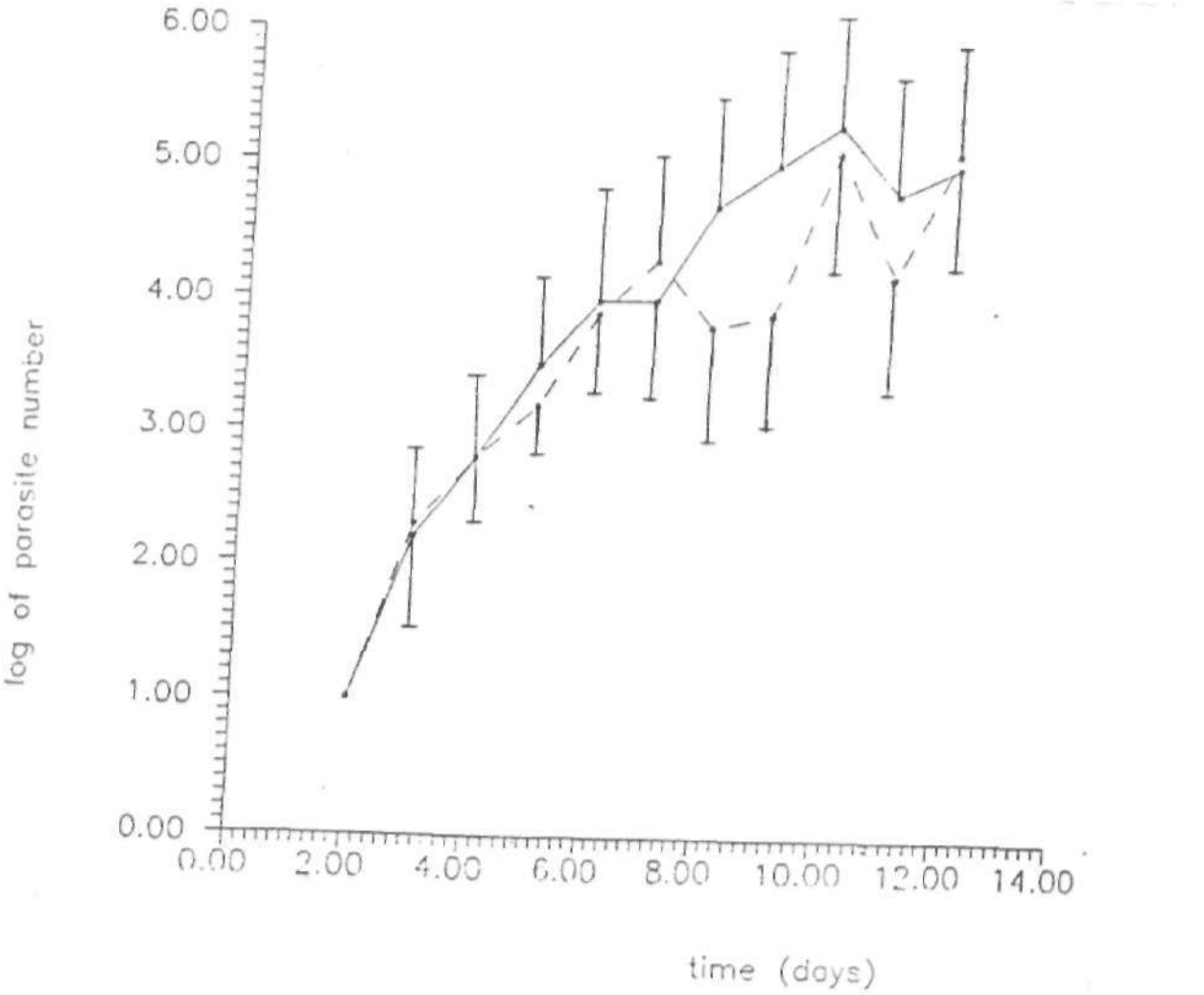


Figure 4.1 A graph of logarithm of parasite number against time of development of parasitaemia (days) following administration of 5mg (15.4μmoles) of tributyltin chloride/kg body weight.

Key:

_____ : Positive control (infected not treated)

------- Infected and treated with DPTC

Figures 4.3 and 4.4 show graphs of logarithm of parasite number against time of development of parasitaemia (in days) following administration of 7.5mg (19.5 μmoles) triphenyltin chloride and 5mg (15.4 μmoles) tributyltin chloride per kilogram body weights respectively.

Parasitaemia developed to a level of 10^4 parasites per millilitre of blood six days after infection of the mice with T. b. brucei. The treatment commenced on the sixth day post-infection. No decrease in the level of parasitaemia was observable in the triphenyltin chloride-treated (7.5mg/kg) and tributyltin chloride-treated (5mg/kg) mice as could be seen in the figures 4.3 and 4.4 respectively.

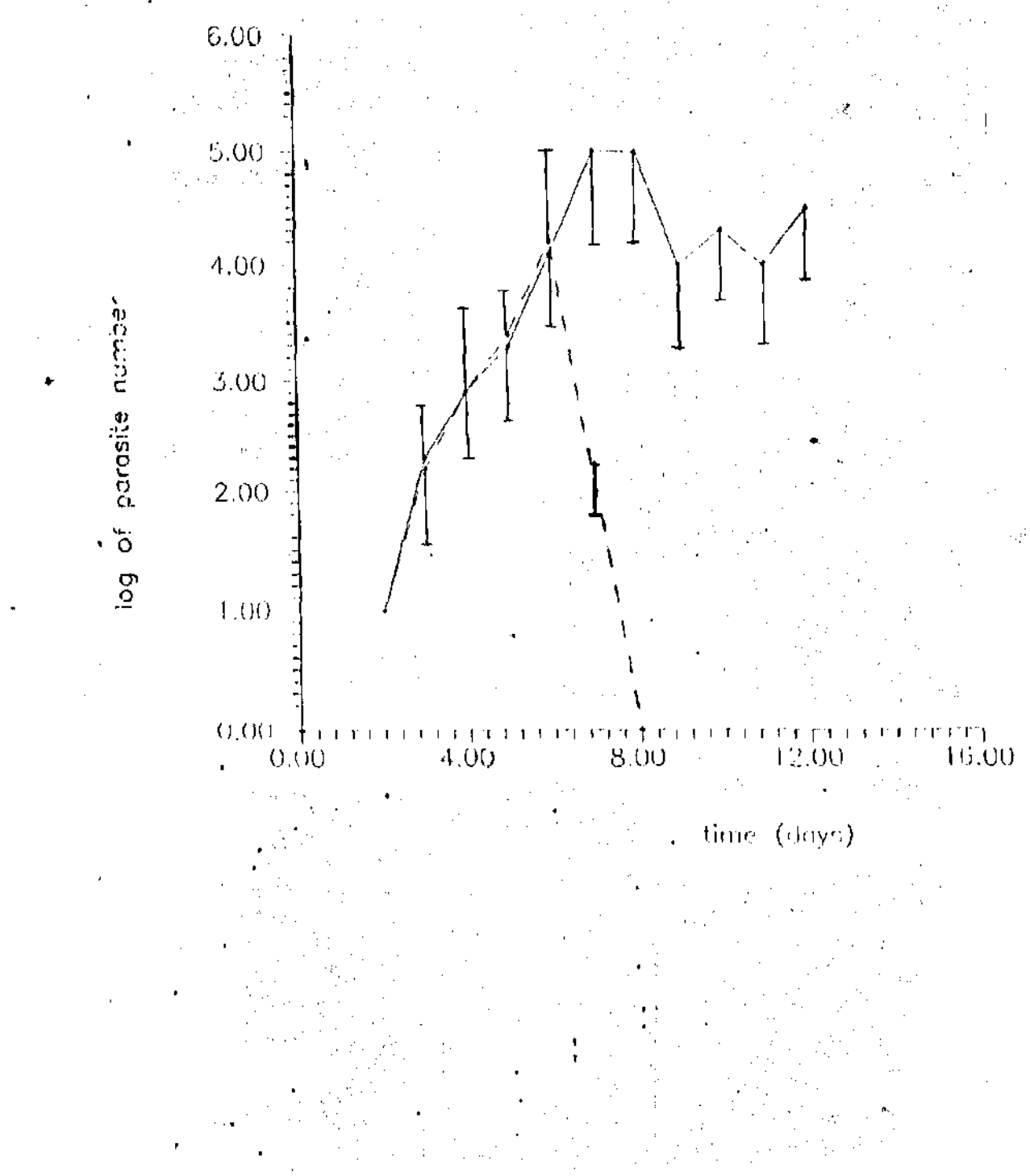


Figure: 4.5 A graph of logarithm of parasite number against time of development of parasitaemia (in days) following administration of 7.0mg diminazene accturate/kg body weight.

KEY:

_____ : Positive control (infected not treated)

_ _ _ _ _ _ : Infected and treated with diminazene accturate

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Figure 4.5 shows a graph of logarithm of parasite number against time of development of parasitaemia (in days) following administration of 7mg diminazene aceturate per kilogram body weight.

Parasitaemia developed to a level of 10^4 parasites per millilitre of blood six days post-infection. Two days of treatment at dose of 7mg diminazene aceturate per kilogram body weight sharply reduced the level of parasitaemia to zero as seen in the figure 4.5.

Table 4.1 **Result of studies of relapses in mice three weeks post-treatment with dibutyltin chloride**

Group	# of mice	% aparasitaemic after 2 weeks	% of relapses after 21 days	% of deaths
I	12	91.7	27.3	8.3
II	4	0.0	-	50.0
III	4	100	-	25.0
IV	4	100	-	0.0

Legend:
Group I. Mice infected and treated with DBTC
Group II. Mice infected and not treated with DBTC
Group III. Mice treated with DBTC but not infected
Group IV. Mice not infected and not treated with DBTC
- = not applicable

Table 4.1 shows the results of studies of relapse and death three weeks after treatment of the T. b. brucei-infected mice with 10mg dibutyltin dichloride per kilogram body weight. Comparing the percent aparasitaemic for all the groups two weeks after treatment, only mice that were infected and not treated with dibutyltin dichloride were zero percent aparasitaemic while 91.7% of mice that were infected and treated with dibutyltin dichloride were aparasitaemic. The two control groups of mice treated with dibutyltin but not infected and mice not infected and not treated with dibutyltin dichloride were 100% aparasitaemic.

From the table 27.3% of the treated mice were observed to relapse into the infection three weeks after treatment with 10mg dibutyltin dichloride /kg body weight. Eventhough the highest percentage of death (50.0%) was recorded in the group of mice infected and not treated compared with the mice that were infected and treated with dibutyltin dichloride and the mice that were not infected but treated with dibutyltin dichloride indicating beneficial effect of clearing the blood off the parasites, the dibutyltin dichloride-treated mice did not seem to recover fully from the side effects of the drug as compared to the control group.

Table 4.2: **Results of studies of relapses in mice three weeks post treatment with diphenyltin chloride**

Group	# of mice	% aparasitaemic after 2 weeks	% of relapses after 21 days	% of deaths
I	12	91.7	36.4	8.3
II	4	0.0	-	25.0
III	4	100	-	0.0
IV	4	100	-	0.0

Legend:
Group I. Mice infected and treated with DPTC
Group II. Mice infected and not treated with DPTC
Group III. Mice treated with DPTC but not infected
Group IV. Mice not infected and not treated with DPTC
- = not applicable

Table 4.2 shows the results of studies of relapse and death three weeks after treatment of the T. b. brucei-infected mice with 15mg diphenyltin dichloride per kilogram body weight. From the table, two weeks after treatment, 91.7% of the diphenyltin dichloride-treated mice (group I) were observed to be aparasitaemic compared with the mice that were infected and not treated with diphenyltin dichloride which had zero percent. The two control groups (III and IV) were observed to be 100% aparasitaemic each.

From the table 36.4% of the diphenyltin dichloride-treated mice were observed to relapse into the infection three weeks after treatment. The percentage deaths were observed to be 8.3% and 25.0% in the groups of mice that were infected and treated with diphenyltin dichloride and those that were infected and not treated and those that were infected and not treated with diphenyltin dichloride respectively. No death was recorded in control group IV. Comparing the diphenyltin dichloride-treated mice and those in the control group IV, the side effects of the drug on the mice such as decreased feeding pattern and ruffled fur did not seem to fully disappear.

Table 4.3 **Results of studies of relapses in mice two weeks post-treatment with triphenyltin chloride**

Group	# of mice	% aparasitaemic after 2 weeks	% of relapses after 21 days	% of deaths
I	8	0.0	100	50.0
II	4	0.0	-	25.0
III	4	100	-	25.0
IV	4	100	-	0.0

Legend:
Group I. Mice infected and treated with TPTC
Group II. Mice infected and not treated with TPTC
Group III. Mice treated with TPTC but not infected
Group IV. not infected and not treated with TPTC
- = not applicable

Table 4.3 shows the results of studies of relapse and death two weeks after treatment of the T. b. brucei-infected mice with 7.5mg triphenyltin chloride per kilogram body weight. From the table it is observed that the groups of mice infected and treated with triphenyltin chloride and those infected but not treated with triphenyltin chloride were all parasitaemic (0.0% aparasitaemic) two weeks after treatment. No trypanocidal effect was also observed in the preliminary studies. The control groups (III and IV) mice were each 100% aparasitaemic as none was infected with the parasites.

The percentage deaths of mice infected and treated with triphenyltin chloride (50.0%) was higher than that for mice infected but not treated with triphenyltin chloride which was 25.0%. It is also observed that 25.0% of the mice infected but not treated with triphenyltin chloride died as result of the trypanosomes infection as compared to control group (IV) with 0.0% death. The mice that survived the two weeks after treatment were observed to be in a state of chronic inactivity, decreased feeding pattern and sensitivity to touch and had ruffled fur.

Table 4.4 **Results of studies of relapses in mice post-treatment with tributyl chloride**

Group	# of mice	% aparasitaemic after 2 weeks	% of relapses after 21 days	% of deaths
I	8	0.0	100	87.5
II	4	0.0	-	50.0
III	4	100	-	50.0
IV	4	100	-	0.0

Legend:
Group I. Mice infected and treated with TBTC
Group II. Mice infected and not treated with TBTC
Group III. Mice treated with TBTC but not infected
Group IV. Mice not infected and not treated with TBTC
- = not applicable

Table 4.4 shows the results of studies of relapse and death two weeks after treatment of the T. b. brucei-infected mice with 5mg tributyltin chloride per kilogram body weight. No trypanocidal action was observed in the preliminary studies and as seen in figure 4.4; it is observed therefore that all the mice in the groups that were infected and treated with tributyltin chloride were all parasitaemic (0.0% aparasitaemic) two weeks after treatment. The two control groups of mice treated with tributyltin chloride but not infected and those not infected and not treated with tributyltin chloride were each 100% aparasitaemic as none of the mice was infected with the parasites.

The percentage deaths of mice infected and treated with TBTC (87.5%) was much higher than that for mice infected but not treated with TBTC which was 50.0%. It is also observed that 50% of the mice infected but not treated with tributyltin chloride died as a resuslt of the trypanosomes infection as compared to control group (IV) with 0.0% death. The mice that survived the two weeks after treatment were observed to be in a state of chronic inactivity, decreased feeding pattern decreased sensitivity to touch and sound and had ruffled fur.

Table 4.5 **Results of studies of relapses in mice four weeks post-treatment with diminazene aceturate**

Group	# of mice	% aparasitaemic after 2 weeks	% of relapses after 21 days	% of deaths
I	8	100	0.0	0.0
II	4	0.0	-	50.0
III	4	100	-	0.0
IV	4	100	-	0.0

Legend:
Group I. Mice infected and treated with diminazene
Group II. Mice infected and not treated with diminazene
Group III. Mice treated with diminazene but not infected
Group IV. Mice not infected and not treated with diminazene
- = not applicable

Table 4.5 shows the result of studies of relapse and death four weeks after treatment of the T. b. brucei-infected mice with 7mg diminazene aceturate per kilogram body weight. From the table it is clear that two weeks after treatment, 100% of the mice infected and treated with diminazene aceturate were observed to be aparasitaemic as compared with the two control groups (Groups III and IV) which were also 100% aparasitaemic. The group II mice were zero percent aparasitaemic as no treatment was given to them. Three weeks after treatment there was not any mice that relapsed from the infection showing zero percent in the group that were infected and treated with diminazene aceturate. No death was observed in the group of mice infected and treated with diminazene aceturate as well as in the two control groups that were treated with diminazene aceturate but not infected and those that were not infected and not treated with diminazene aceturate, while 50.0% death was observed in the group infected and not treated with diminazene aceturate which was due to the trypanosome infection. The level of activity and feeding pattern of the mice infected and treated with diminazene aceturate and those treated with diminazene aceturate but not infected was almost comparable with those in the control group that were not infected and not treated with diminazene aceturate.

Table 4.6 **Results of subinoculation with macerated liver, kidney and spleen, cerebrospinal fluid and blood of the mice cleared of parasitemia by treatment with DBTC, DPTC and diminazene aceturate.**

Group treatmentprotocol	Development of parasitemia after inoculation with tissue homogenate from the treated mice.				
	% parasitaemic after subinoculation				
	Liver	Kidney	Spleen	CSF	Blood
DBTC-treated	33.3	0.0	66.7	33.3	0.0
DPTC-treated	50.0	0.0	50.0	33.3	16.7
Diminazene-treated	25.0	0.0	25.0	0.0	0.0

Number of animals subinoculated in each group is 6

Table 4.6 shows the development of parasitaemia following the subinoculation of healthy mice with tissue homogenates from the dibutyltin dichloride-, diphenyltin dichloride - and diminazene aceturate-treated mice. It is observed from the table that no parasites were detected in the healthy mice subinoculated with the kidney homogenates from three groups of mice that received the drug treatments. The groups of healthy mice subinoculated with cerebero spinal fluid from the dibutyltin dichloride- and diphenyltin dichloride - treated mice showed 33.3% to develop parasitaemia for the two drugs while 0.0% was observed to develop parasitaemia from the diminazene aceturate-treated mice. From the table, 25.0% of the healthy mice subinoculated with liver homogenate of the diminazene aceturate-treated mice were observed to develop parasitaemia three weeks after subinoculation while 33.3% and 50.0% were observed to develop parasitaemia from the dibutyltin dichloride- and diphenyltin dichloride - treated mice respectively. From the table, it is also observed that no

parasitaemic development was observed after subinoculation of healthy mice with CSF from the diminazene aceturate-treated mice compared with 33.3% each from the dibutyltin dichloride- and diphenyltin dichloride- treated mice.

Table 4.7: **Acute toxicity showing the LD_{50} for the different organotin compounds**

	Compound	LD_{50} (mg/kg)
1.	Dibutyltin dichloride (DBTC)	95
2.	Diphenyltin dichloride (DPTC)	64
3.	Tributyltin chloride (TBTC)	38
4.	Triphenyltin chloride (TPTC)	75

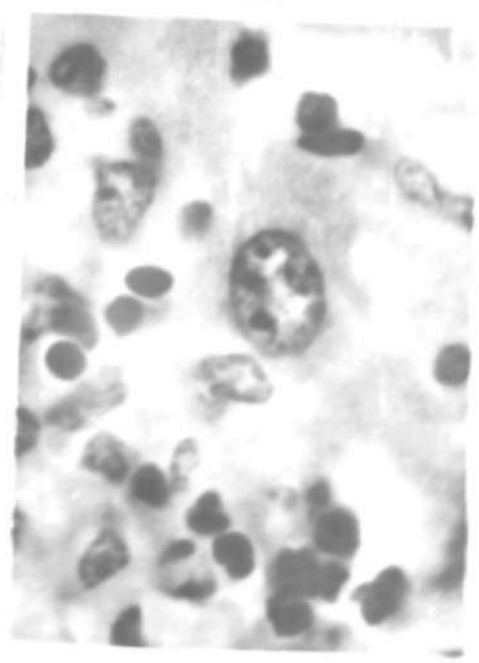


Plate 4.1. (H&E x 400)

The liver cells of a mouse treated with DPTC showing liver cell necrosis and leucocytic infiltration.

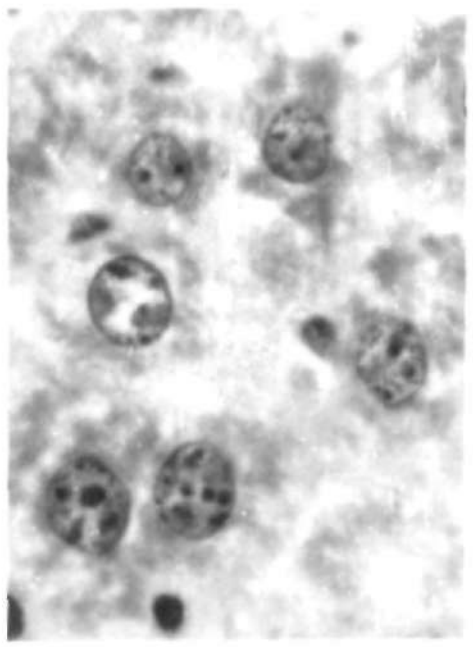


Plate 4.2. (H&C x 400)

The normal mouse liver cells showing regular nuclei and fairly distinct cytoplasmic borders.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Preliminary Results

The results show different effective doses for all the compounds screened. A treatment range of 5, 10, 20 and 50mg/kg for the selected organotin compounds used on the infected animals revealed that a dose of10mg/kg and 15mg/kg elicited maximum parasiticidal effect (75% and 60%) for dibutyltin and diphenyltin dichlorides respectively. It was also observed, that only the diorganotins showed trypanocidal activity toward the Trypanosoma brucei brucei - infected mice. The parasitaemia developed six days post-infections to 10^4 trypanosomes per millilitre of blood and treatment commenced immediately. The disappearance of the parasites from circulation indicates the trypanocidal action of DPTC and DBTC on the blood stream trypanosomes as compared to the persistence of parasitaemia in the positive control that were infected but not treated.

The triorganotins, tributyltin and triphenyltin chlorides did not show trypanocidal effect on the blood stream T. b. brucei-infected mice (Tables A.3 and A.4 and Figures 4.3 and 4.4). At dose below 5mg/kg no activity was observed and at dose above 10mg/kg the animals died depending on the dose administered. Brown, in 1972, demonstrated that tumour growth in mice was retarded by triphenyltin acetate(10mg/kg) but not by the chloride, when administered either in food or by injection. This observation therefore suggests similarly that triphenyltin and tributyltin chlorides did not retard the growth of T. b.brucei in the infected mice; they are toxic and apparently elicited no trypanocidal activity.

5.2 Trypanocidal Effects

Following the administration of 10mg(33μ moles)/kg, six days post-infection for five consecutive days, the parasites were apparently eliminated from the circulation. This is shown in Table A.1 and Figure 4.1. The persistence of the parasites in the blood of the members in the positive control group (infected but not treated) suggested that the disappearance of the trypanosomes in the treated

group was due to the activity of DBTC.

The organotin DPTC at a dose of 15mg(44mmoles)/kg body weight cleared the blood of the infected mice of the parasites within five days from the onset of the treatment (Table A.2 and figure 4.2). This also indicates the trypanocidal potential of the DPTC, since members in the positive control group died of massive parasitemia in an average of twenty eight days post-infection.

Concurrent use of diminazene aceturate as a standard drug at curative dose of 7mg/kg body weight (Arowolo et al 1988) threw more light on the trypanocidal potentials of these organotin compounds, as also reported by Nok et al, 1992 (Table A.5 and Figure 4.5).Diminazene aceturate and isometamedium chloride are the two most commonly used trypanocides in animal trypanosomiasis in Nigeria and diminazene aceturate has been found to clear parasites in the blood of the infected animal faster than isometamidium (Arowolo et al, 1988). It is well tolerated and the most prominent side effect appear to be a slight but persistent albuminuria (Gutteridge, 1985). Trypanosoma brucei was also reported to resist isometamidium treatment more than diminazene aceturate and that was why diminazene aceturate was choosen as the standard drug in this study. Dibutyltin dichloride at a dose lower than that of diphenyltin dichloride in five days rendered the infected mice aparasitaemic while diphenyltin dichloride produced its curative effect in the same number of days but at a higher dose (Tables A.1 & A.2 and figures 4.1 & 4.2). This observation supports the suggestion (Barbieri, et al, 1982, Crowe et al, 1984 and Huber et al 1985) that the activity of the diorganotin compounds (R_2SnX_2) in general is controlled by the R_2Sn moiety, and that the function of the X group is to deliver/transport the R_2Sn moiety to the site of action. Therefore, the variation in the activity of these two compounds (DBTC and DPTC) could be due to the nature of the R_2. moeity (Crowe, 1987).

The problems of toxicity and limitation of efficacy impose severe restrictions on the use of some trypanocides. Gutteridge (1985) described minimal side effects as one of the properties of an ideal antitrypanosomal drug. From figure 4.5, it is observed clearly that diminazene aceturate cleared the parasites from the blood of the T. b. brucei-infected mice faster than the DBTC and DPTC (figures

4.1 and 4.2). The 8.3% and zero percent deaths in the groups of mice infected and treated with DPTC and those treated with DPTC but not infected is an indication of the beneficial effect of the DPTC in clearing the parasites off the blood of the infected mice. Comparing the percentage deaths of group II (50.0%) and groups I, III and IV (0.0% each) in Table 4.5, it is observed that diminazene aceturate has a superior therapeutic effect with minimal side effects compared to the DBTC and DPTC that were also found to be active against the trypanosomes in the infected mice. This is because of the short period of treatment (two days) and minimal observable side effects in the diminazene aceturate-treated mice than in the DBTC and DPTC-treated mice with a relatively longer period of treatment (five days) and a more severe observable side effects. From Tables 4.1 and 4.2, 91.7% of all the DBTC- and DPTC-treated mice survived the average twenty eight days survival time of the T. b. brucei-infected but not treated mice while all the diminazene aceturate-treated mice were observed to survive four weeks after treatment. The mice considered cured after treatment with diminazene aceturate did recover from the side effect of the drug faster than the DBTC- and DPTC- treated mice which did not seem to recover fully from the side effect of the drugs three weeks after treatment.Such side effects include decreased feeding pattern,decresed activity and ruffled fur.

Most of the TBTC- and TPTC- treated mice did not survive up to the average twenty eight days survival time of the mice infected but not treated (Tables 4.3 and 4.4), probably because of the toxic effects of the drugs on the mice.The toxic effects may have resulted in lowered ability of the treated mice in fighting the infection. Comparing the percentage deaths of the mice infected and treated with TPTC and those treated with TPTC but not infected (50.0% and 25.0% respectively) in Table 4.3; with those of mice infected and treated with TBTC and those treated with TBTC but not infected (87.5% and 50.0% respectively) in Table 4.4, it is observed that there is a combined effect of the TPTC and T. b. brucei as well as that of TBTC and T. b. brucei in the mortality of the mice in group I of Tables 4.3 and 4.4. It can therefore be deduced that TBTC is more toxic than TPTC.

The findings that DBTC and DPTC did not clear off T. b. brucei from all the bodily tissues impose some constraints on them as possible trypanocides. It is however possible that a better trypanocidal activity could be obtained if DBTC and DPTC are used in combination with other judiciously selected compounds. It is also likely that change in route of administration and solvent types may influence their antitrypanosomal activity, as this may get the active component to the target site.

5.3 Relapses and Subinoculation

Although no trypanosomes were found in the blood after the five days teatment, relapses occur at about three weeks post-treatment. There was 27.3%, 36.4% and 0.00% relapses in the DBTC-, DPTC- and diminazene aceturate-treated groups respectively (Tables 4.1, 4.2 and 4.5). This indicates that the parasites were not completely eliminated from all the tissues.

Most important species of pathogenic African trypanosomes, namely T. vivax, T. congolense and trypanosomes in the brucei subgroup colonize different tissue microenvironments. The brucei subgroup - T. brucei, T. gambiense, T. rhodesiense and T. equiperdum - localize in solid tissues, particularly in the loose tissue stroma (Losos, 1978) and in the later stage cross the blood brain barrier to the cerebro-spinal fluid (Jennings, 1991a).

In a supportive experiment, subinoculation of healthy mice was conducted with blood, cerebrospinal fluid and homogenate of liver, spleen and kidney of the apparently cured mice. A careful analysis of the results of the subinoculation studies of the healthy mice from the DBTC, DPTC and diminazene aceturate-treated mice (Table 4.6) provides an evidence as to the possible reason why diminazene aceturate and not DBTC or DPTC cured the mice of T. b. brucei infection. Both diminazene aceturate, DBTC and DPTC cleared the infected mice of blood and kidney parasites. This is an evidence that a drug may eliminate T. b. brucei from the blood and not cure the victim. The fact that there was 25% and 33.3% development of parasitaemia indicate that the complete clearance of the parasites from the liver may not be a very critical factor in the curative effects of diminazene aceturate and DBTC as in the case of DPTC with 50%. Diminazene aceturate-treated mice had 25% and 0% relapse from spleen and CSF subinoculation as against 66.7% and 33.3% for DBTC and 50% and 33.3% for DPTC. This provides an evidence that a poor or ineffective action of DBTC and DPTC in the CSF compartment and the spleen. In the light of the subinoculation results, it is possible that the parasites could have been in the liver and spleen during the aparasitaemic period and reappeared in the blood probably after plasma level of the drug has gone low (see table 4.6) or probably because of the induced suppression of the immune systems of the mice by the organotin (Seinen and Penninks, 1979; Vos et al, 1984; Vos et al

1985; Krajnc et al 1986; Attahiru et al 1991) is responsible for the non completion of the T. b. brucei clearance from the body of the mice. However this partial clearance of trypanosomes from the body of the mice is an indication of the trypanocidal potentials of the DBTC and DPTC, provided the active moieties of these compounds get to the target sites.

5.4 Acute toxicity and Histopathological Analysis

The acute toxicity study was carried out to detect the short term effect of high doses of these organotin compounds. It employed the determination of the median lethal dose (LD_{50}) of the compounds. This LD_{50} is the dose of any substance that will kill 50 percent of a given population within a specific period of time, usually 72 hours.

The LD_{50} values (Table 4.7 and Tables B.1 to B.4 in Appendix B) were obtained for the organotin compounds. The tributyltin chloride had the highest toxicity with the lowest LD_{50} of 38mg/kg. The others were moderately toxic with LD_{50} less than 100mg/kg. These values varied with those cited in literature. An explanation for these differences could be the variation in sensitivity between species and strains of the test animals and the type solvents in which the organotins were suspended.

The administration of different doses of the organotin compounds by intraperitoneal route to the mice, showed several signs of intoxication almost general to all the compounds. These include transient bizarre reaction (aimless wandering and jumping) followed by decreased activity, sensitivity to sound and touch, decreased feeding pattern, ruffling of fur, prostration and subsequent death within 72 hours from the time of administration.

The detoxification and excretion of foreign compounds (drug, toxic agents etc) in vertebrates is carried out by liver and kidney and that was the aim of choosing these organs (liver, kidney and spleen) for histopathological studies to assess the extent of damage, if any, in the organs.

In the present study, histopathological examination did not reveal any significant damage to the spleen but there were varying degrees of damage to the liver and kidney by the selected organotin compounds. The degree of damages caused to the liver and kidney varied, and depend on the type or

the class of the organotin and the nature of the R group.

The major damage to the liver were liver cell necrosis, leucocytic infiltration and fatty change in that order while in the case of kidney there were tubular casts, followed by leucocytic infiltration. The fatty liver degeration, leucocytic infiltrations and tubular casts formation observed in the liver and kidney are typical for toxic chemicals. Although the liver has a striking capacity for regeneration, the liver cell necrosis (death of hepatocytes) however small may not be favourable considering the effect of the parasite infection on the host liver (hepatomegaly) and the eventual implications of these on the vast metabolic activities carried out by the liver. The butyl derivatives (DBTC and TBTC) were more toxic than the phenyl derivatives (Kerk et al, 1954; Socaciu et al, 1994). Main damages were seen in kidney in the case of the butyl derivatives, with TBTC having maximal damage and a minimal to moderate damage to the liver. The phenyl derivatives (DPTC and TPTC) caused their main toxicity in the liver with DPTC (Plate 4.1 and 4.2) having maximal toxic effect, while a minimal renal toxicity was seen in all the two.

5.5 Conclusion and Suggestions

5.5.1 Conclusions

This work reveals the potentials of organotin compounds in the chemotherapy of trypanosomiasis, though the activity was low in the case of DBTC and DPTC as well as using these compounds as probes to study the physiology of trypanosomes.

The preliminary study has also shown the extent to which the organotin compound (DBTC, DPTC, TBTC, and TPTC) can affect the vital organs of the host - liver and kidney, but triorganotins (TBTC and TPTC) at the doses studied, rather were toxic on the test animals to make possible the observation of their trypanocidal activity or otherwise. The diorganotin compounds were less toxic on the host animals, making possible the observation of their activity on the trypanosomes, at the doses studied.

Therefore from these findings, DBTC and DPTC may be chemotherapeutically useful in the treatment of trypanosomiasis but because of their inherent toxic effects and non complete clearance of the T. b. brucei from all the bodily tissues of the infected mice at the doses studied, more is needed to be explore on these compounds with view of getting a better efficacy.

5.5.2 **Suggestions**

These compounds may have potentials for use in combined therapy with other compounds and therefore future research efforts should be directed towards this line. Also further studies would be important, on the synthesis of organotin derivatives with high activity and low toxicity probably the derivatives of amino acids and fatty acids, that are needed for vital life processes in trypanosomes. There is also a need to monitor the animal after treatment to see how long exactly does it take the animal to completely recover from the side effects of the drugs and also to find out whether specifically DBTC and DPTC are immunotoxic.

Change of solvent types and probably the use of doses lower than those used in this study may be tried with the view of finding a better trypanocidal action.

Finally, it is also important to try these compounds across species barrier so that ample information may be obtained on their trypanocidal action.

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APPENDIX A

te A.1 **Effects of intraperitoneal administration of dibutyltin dichloride (10mg/kg) on T. b. brucei-infected mice**

Group		Number of Parasites post-treatment							
	Period after treatment	24hr	48hr	72hr	96hr	120hr	144hr	168hr	192hr
I		(1.17±.13) 10^4	(4.01±4.41)10^3	(6.01±3.12)10 0^2	(6.71±2.5 2)10^1	(1.03±0.85)10	-	-	-
	Positive control	(1.01±.18) 10^1	(1.30±.35) 10^1	(1.02±0.32)10 0^5	(1.11±.34)10^5	(6.04±1.83)10 0^1	(2.04±0.5 2)10^5	(1.02±0.31)10^5	-
II	Negative control	-	-	-	-	-	-	-	-

The treatment commenced six-days post-infection.
Positive control = Infected but not treated
Negative control = Not infected, only administered with 0.1ml PSG
Values in group I are means±Sd of twelve determinations
Values in group II are means±Sd of four determinations
- = no parasitemia

Table A.2 **Effects of intraperitoneal administration of diphenyltin dichloride (15mg/kg) on T. b brucei - infected mice**

Group		Number of Parasites post-treatment							
	Period after treatment	24hr	48hr	72hr	96hr	120hr	144hr	168hr	192hr
I		$(1.03 \pm 0.81)\ 10^4$	$(7.02 \pm 2.32)\ 10^3$	$(1.33 \pm .49)\ 10^3$	$(1.17 \pm 2.52)\ 10^2$	$(3.32 \pm 1.34)\ 10$	-	-	-
II	Positive control	$(1.05 \pm 0.29)\ 10^5$	$(3.04 \pm 1.53)\ 10^4$	$(1.03 \pm 0.66)\ 10^5$	$(1.02 \pm .66)\ 10^5$	$(2.04 \pm .68)\ 10^4$	$(1.01 \pm 0.26)\ 10^5$	$(2.02 \pm 0.52)\ 10^5$	-
III	Negative control	-	-	-	-	-	-	-	-

The treatment commenced six days post-treatment.
Positive control = infected but treated with placebo.
Negative control = not infected, only administered with 0.1ml PSG.
Values in group I are means±SD of twelve determinations
Values in group II are means±SD of four determinations
- = no parasitemia.

Table A.3 **Effects of intraperitoneal administration of triphenyltin chloride (7.5mg/kg) on T. b. brucei-infected mice**

Group		Number of Parasites post-treatment							
	Period after treatment	24hr	48hr	72hr	96hr	120hr	144hr	168hr	192hr
I		(1.53 ± 0.52) 10^4	(1.52 ± .85) 10^3	(1.05 + .23) 10^5	(5.51 + 2.93) 10^3	(1.55 ± .56) 10^5	(8.52 ± 1.62) 10^4	(5.53 ± 4.84) 10^4	-
II	Positive control	(1.52 ± 0.18) 10^1	(5.03 ± 1.44) 10^3	(1.03 ± 0.12) 10^5	(2.04 ± .19) 10^5	(1.03 ± .12) 10^5	(1.02 ± 0.20) 10^5	(1.04 ± 0.16) 10^4	-
III	Negative control	-	-	-	-	-	-	-	-

The treatment commenced six-days post-infection.
Positive control = Infected but not treated
Negative control = Not infected, only administered with 0.1ml PSG
Values in group I are means±Sd of twelve determinations
Values in group II are means±Sd of four determinations
- = no parasitemia

Table A.4 **Effects of intraperitoneal administration of tributyltin chloride (5mg/kg) on T. b. brucei-infected mice.**

Group		Number of Parasites post-treatment							
	Period after treatment	24hr	48hr	72hr	96hr	120hr	144hr	168hr	192hr
I		(9.52 ± 1.75) 10^3	(2.02 ± 1.12) 10^3	(7.54 ± 2.81) 10^3	(8.54 ± 1.92) 10^3	(1.56 ± .50) 10^5	(1.53 ± .54) 10^5	(1.52 ± .47) 10^5	-
II	Positive control	(1.04 ± 0.25) 10^4	(1.02 ± .16) 10^4	(5.02 ± 2.33) 10^4	(1.03 ± .32) 10^5	(2.02 ± .52) 10^5	(6.03 ± 0.81) 10^4		-
III	Negative control	-	-	-	-	-	-	-	-

The treatment commenced six-days post-infection.
Positive control = Infected but not treated
Negative control = Not infected, only administered with 0.1ml PSG
Values in group I are means±Sd of twelve determinations
Values in group II are means±Sd of eight determinations
- = no parasitemia

Table A.5 **Effects of intraperitoneal administration of diminazene aceturate (7.0mg/kg) on T. b. brucei-infected mice.**

Group		*Number of Parasites post-treatment*							
	Period after treatment	24hr	48hr	72hr	96hr	120hr	144hr	168hr	192hr
I		$(1.54 \pm .43) 10^1$	$(1.03 \pm .17) 10^2$	-	-	-	-	-	-
II	Positive control	$(1.33 \pm 0.29) 10^1$	$(1.02 \pm .25) 10^5$	$(1.04 \pm .25) 10^5$	$(1.03 \pm .12) 10^5$	$(2.04 \pm .52) 10^4$	$(1.02 \pm 0.23) 10^4$	$(3.04 \pm 0.53) 10^4$	-
III	Negative control	-	-	-	-	-	-	-	-

The treatment commenced six-days post-infection.
Positive control = Infected but not treated
Negative control = Not infected, only administered with 0.1ml PSG
Values in group I are means±Sd of twelve determinations
Values in group II are means±Sd of four determinations
- = no parasitemia

APPENDIX B.

Table B.I **Arithmetic method of Karber for calculating the LD_{50} of DBTC.**

Group	Dose(mg/kg)	Dose Difference	Dead	#Survived	Mean Dead	Dose Difference x Mean Dead
1	40		0	2		
		40			0	0
2	80		1	1		
		40			0.5	20
3	120		2	0		
		40			1.0	40
4	160		2	0		60
		40				
5	180					

$$LD_{50} = 120 - \frac{60}{2} = 90\text{mg/kg}$$

$$LD_{20} = 18\text{mg/kg}$$

Table B.2 **Arithmetic method of Karber for calculating the LD_{50} of DPTC.**

Group	Dose(mg/kg)	Dose Difference	Dead	#Survived	Mean Dead	Dose Difference x Mean Dead
1	45		0	2		
		15			0	0
2	60		1	1		
		15			0.5	7.5
3	75		2	0		
		15			1	15
4	90		2	0		22.5
		15				
5	105					

$$LD_{50} = 75 - \frac{22.5}{2} = 64\text{mg/kg}$$

$LD_{20} = 12.8\text{mg/kg}$

Table B.3 **Arithmetic method of Karber for calculating the LD_{50} of TPTC.**

Group	Dose(mg/kg)	Dose Difference	Dead	#Survived	Mean Dead	Dose Difference x Mean Dead
1	25		0	2		
		25			**0**	0
2	50		1	1		
		25			**0.5**	12.5
3	75		1	1		
		25			0.5	12.5
4	**100**		2	0		
		25		**1**	25	
5	125					50

$$LD_{50} = 100 - \frac{50}{2} = 75\text{mg/kg}$$

$$LD_{20} = 15\text{mg/kg}$$

Table B.4 **Arithmetic method of Karber for calculating the LD_{50} of TBTC.**

Group	Dose(mg/kg)	Dose Difference	Dead	#Survived	Mean Dead	Dose Difference x Mean Dead
1	12.5		0	2		
		12.5			0	0
2	25		1	1		
		12.5			0.5	6.25
3	37.5		1	1		
		12.5			0.5	6.25
4	50		2	0		
		12.5			1	12.5
5	62.5					25

$LD_{50} = 50 - \frac{25}{2} = 37.5 \approx 38.mg/kg$

$LD_{20} = 7.6mg/kg$