SOME ASPECTS OF CHEMOPROPHYLAXIS AGAINST *Trypanosoma congolense*

by

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June, 1987.

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CONTENTS

DECLARATION

I, Iain Ross Bell, do hereby declare that the following thesis is all my own work and original. Collaborative assistance was received from the following sources:

Dr. H. Hirumi provided cultured *Trypanosoma congolense* IL Nat 3.1 metacyclics. Dr. S.K. Moloo supplied all *Glossina morsitans* tsetse flies, Professor Max Murray assisted with the pathological investigations and Dr. D.D. Whitelaw acted as local supervisor at ILRAD, The International Laboratory for Animal Diseases, Nairobi, Kenya, and provided useful discussion.

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SUMMARY

This thesis is concerned with investigating two aspects of the use of isometamidium chloride (Samorin, May and Baker, Dagenham) as a chemoprophylactic drug.

Firstly, twentyfour Boran cattle were injected with 1 mg/kg isometamidium chloride to investigate the duration of drug-induced prophylaxis against infection by metacyclic forms of Trypanosome congolense, and to determine if specific antibody responses to the organisms were mounted by animals under chemoprophylactic cover. Complete protection against either single challenge by five tsetse infected with Trypanosoma congolense, or repeated challenge at monthly intervals by five tsetse, lasted for 148 days or approximately five months. Even at six months post treatment, two-thirds of the cattle were still resistant to challenge with either trypanosome-infected tsetse, or titrated doses of in vitro derived metacyclic forms of T. congolense (5×10^2 to 5×10^5 organisms), innoculated intradermally. No animal which resisted infection developed either detectable skin reactions at the site of the metacyclic innoculation or produced trypanosome-specific antibodies. It was concluded that drug levels in the skin were effective at preventing trypanosome multiplication, thus preventing the development of parasitaemia or priming of the hosts' immune system.

Secondly, the local tissue toxicity of isometamidium chloride and its dextran complex were investigated. Four Boran cattle/

cattle were injected at different sites with 2% and 4% isometamidium chloride or 2% isometamidium-dextran complex. Sequential slaughter at 7, 28 and 56 days post injection was carried out and the innoculation sites examined for changes in gross pathology and histology. Both 2% and 4% isometamidium chloride given at a dose of 1mg/kg/injection site resulted in severe tissue damage which progressed from a necrotic, oedematous, haemorrhagic lesion at day 7 to extensive fibrosis by day 56. Isometamidium-dextran complex produced a limited well encapsulated lesion and was safe to inject subcutaneously.

GENERAL INTRODUCTION

Disease:

Trypanosomiasis (or Nagana) of cattle is a parasitic disease caused by several species of extracellular haemaprotozoan flagellates which are normally cyclically transmitted by an insect vector, the tsetse fly (Genus Glossina). The tsetse fly has a wide distribution throughout the African continent affecting some 10 million square kilometers in 38 different countries between 15°N and 30°S.

The organisms, which cause a clinical disease resulting in anaemia, poor productivity and often death, render some parts of Africa totally unsuitable for the maintenance of domestic livestock. This results in increased pressure on those areas that are tsetse-free with resultant overgrazing, soil erosion and destruction of the environment.

It has been estimated that half of the world's population is either undernourished or suffering from malnutrition (Wright, 1961) and also that their daily intake of protein is insufficient for normal growth and mental development (Jasiorowski, 1972). However, in direct contrast to this, 70% of the world's livestock resources are located in underdeveloped countries (FAO, 1970). Hence, it is not the lack of animals which is responsible for the low protein intake of these countries but rather their poor productivity.

The importance of trypanosomiasis has often been stressed. For example, in 1976 the WHO stated 'Sleeping sickness (African trypanosomiasis) constitutes a permanent and serious risk to health and well-being of at least 35 million people and animal trypanosomiasis/

trypanosomiasis is the main obstacle to the development of the vast potential for livestock development in the African continent'.

This 'potential' for livestock development and hence the serious economic effect of trypanosomiasis has been the subject of many discussions. Lumsden (1968) estimated that in 1963 the 10.3 m km^2 devoid of domestic livestock would be capable of supporting 125 million cattle. Other estimates have ranged from 120 million cattle (Jasiorowski, 1972) to 145 million (Finelle, 1974).

Hence, it is clear that effective trypanosomiasis control would have far reaching social, economic and political effects throughout the African continent.

Aetiology:

Trypanosomiasis is caused by several species of protozoan haemoflagellates which belong to the genus Trypanosoma. These are blood-borne parasites which range from 8 to 39 μm in size (Mulligan, 1970). They are characteristically spindle-shaped with a centrally placed oval nucleus and a single elongated mitochondrion which extends along much of the cell. Towards the posterior end of the cell is situated a small densely staining structure known as the kinetoplast which is composed of DNA. In close proximity to the kinetoplast is a small pocket (flagellar pocket) from which emerges a single flagellum which is attached along the longitudinal axis of the cell. Also present is a prominant layer of microtubules which lie just beneath the plasma membrane and it is thought that these act to maintain the shape of the cell during locomotion.

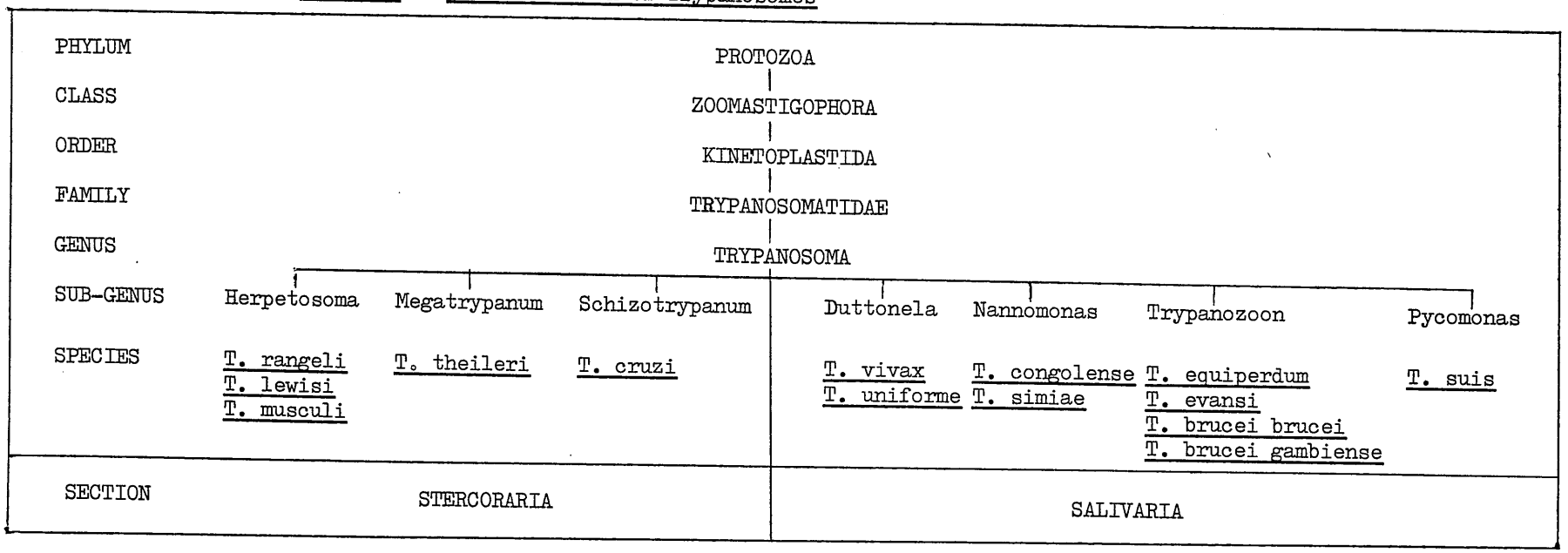
Perhaps/

Perhaps one of the most important structural features of the trypanosome is its possession of a surface coat (Vickerman, 1969). This coat which is 10 to 15 nm in depth is only visible by electron microscopy and uniformly covers the complete plasma membrane. It is, however, absent from the developmental stages of the trypanosome present within the tsetse fly which are non-infective but it is once again present when the trypanosomes invade the salivary gland and become infective metacyclic trypanosomes. Structurally it is composed predominantly of a single glycoprotein molecule of MW 55000 to 65000 K. It is this glycoprotein which changes its antigenic profile throughout an infection (Cross, 1978) and so allows the trypanosome to evade the host's immune response. This results in the classical waxing and waning parasitaemic profile which is debilitating and often fatal. This process is termed antigenic variation and is the most important factor to be overcome in the search for an effective vaccine.

Since 1895 when Bruce discovered the first pathogenic trypanosome, Trypanosoma brucei, more than 30 other pathogenic organisms have been isolated. Hoare (1966, 1970, 1972) subjected these to a thorough revision and classification (Fig. 1).

There are 3 species of trypanosome which are pathogenic to cattle, namely, Trypanosoma (Nannomonas) congolense, T. (Duttonela) vivax and T. brucei brucei which belongs to the Trypanozoon subgenus. All three species are found in cattle throughout tsetse-infested areas and often give rise to mixed infections. It is possible to distinguish between these three species by examination of a stained thin/

FIGURE 1: Classification of Trypanosomes



After Hoare, C.A. (1972)

thin blood smear, to determine differences in their morphology, and also by observing a wet blood film when differences in behaviour can be detected (Murray, Murray and McIntyre, 1977; Paris, Murray and Mcodimba, 1982).

Of the three species of trypanosome of veterinary importance found in cattle, T. congolense and T. vivax are the most prevalent and have always been considered to be more pathogenic than T. b. brucei. However, by subinoculation of blood into laboratory animals as a means of parasite detection and identification, the frequency of T. b. brucei infection would appear to be higher than previously thought. Various studies also exist which suggest that T. b. brucei can be pathogenic in its own right (Murray, Murray, Wallace, Morrison and McIntyre, 1979).

T.b. brucei is important for another reason, namely, because it is closely related to and morphologically indistinguishable from the causative agents of human sleeping sickness, i.e. T. b. gambiense and T. b. rhodesiense. Unlike T. b. brucei these two species are not found throughout the tsetse fly belt but are restricted to localised foci. These foci can lie dormant for many years and suddenly flare up giving rise to widespread infection in the local human population.

It is known that T. b. rhodesiense can infect cattle, while isolates with similar isoenzyme patterns to T. b. gambiense have been found in pigs and dogs (Gibson, Menlitz, Lanham and Godfrey, 1978). At present isolates with T. b. brucei characteristics are commonly subjected to a blood incubation infectivity test (B.I.I.T.). In the/

In the test, isolates are incubated with human sera which is known to be cytotoxic for T. b. brucei but not for T. b. rhodesiense and T. b. gambiense (Rickman and Robson, 1970). A good example of the use of this test was provided by Joshua, Magadi and Kayit (1983) when isolates potentially infective for man were derived from cattle in an area endemic for sleeping sickness caused by T. b. gambiense. It is also noteworthy that T. b. brucei can occasionally give rise to variants which are potentially infective for humans (Whitelaw, Moulton and Murray, 1983).

The Vector:

African trypanosomes are in the main cyclically transmitted by the insects of the genus Glossina (Mulligan, 1970). There are 22 species of Glossina, commonly termed tsetse flies, each potentially capable of transmitting trypanosomiasis. However, certain species are more important in the disease transmission than others since the various species have different habitat and feeding preferences, trypanosome infection rates and transmission efficency.

Tsetse flies can be broadly divided into three main groups according to their habitat:

(a) Savannah (Morsitans group);

(b) Riverine (Palpalis group);

(c) Forest (Fusca group).

Of these three groups, the first two, i.e. Savannah and Riverine, are the most important vectors of the disease due to their close proximity to both wild game and domestic livestock.

Mechanical/

Mechanical transmission can and does occur, although its importance in Africa is unknown. It is more important for T. vivax than for other species, a point well illustrated by the spread of T. vivax in S. America, where the tsetse fly does not exist. Intra-uterine transmission of trypanosomes also has been reported (Ruchel, 1975) and dogs can become infected after eating infected meat (Moloo, Losos and Kutuza, 1973).

Antigenic Variation:

The prospect of immunisation against African trypanosomiasis appears as remote today as it has ever been, and the reason for this lies in the phenomenon of antigenic variation. Throughout a natural infection distinct waves of parasitaemia occur in which the successive parasite populations differ antigenically from the preceding ones. It is this progressive antigenic variation which is the prime mechanism for parasite survival in an immunocompetent host.

The variable antigen which results in this phenomenon is known to be the surface coat of the trypanosome and it is termed the Variant Specific Surface Antigen (VSSA). It is the predominant form of this antigen to which the host responds during each parasitaemic wave, thus resulting in the following remission. However, not all of the parasites have the same VSSA and, as a result, those that have a different VSSA are able to multiply and give rise to the next parasitaemic wave.

The number of different antigenic types which can be produced by one strain of trypanosome is unknown. With one clone of T. equiperdum/

equiperdum at least 101 different antigenic types were noted (Capbern, Giroud Baltz and Mattern, 1977) and Gray (1965) concluded that for T. brucei at least there was no limit to the number of antigens which could be produced other than that imposed by the time for which the infected host survived.

There is, however, evidence that trypanosome 'strains' have a tendency to develop particular antigenic types. Broom and Brown (1940) showed that one 'strain' of trypanosome transmitted by different tsetse had antigens in common which were termed 'basic' antigens. Evidence also exists that the appearance of variable antigens occurs in a predictable order after infection (Gray, 1965), the so called predominant antigens. However, later in infection the emergency of new VSSAs was less predictable. More recent work (LeRay, Barry and Vickerman, 1978) has demonstrated that the tsetse fly inoculates a mixture of metacyclic VSSAs and it has been demonstrated that these are present in roughly constant proportions in the saliva of the tsetse, regardless of the VSSA originally ingested (Hajduk and Vickerman, 1981).

Immune Response:

Following natural infection in domestic animals, protective immunity to trypanosomiasis is an infrequent sequel due to the occurrence of antigenic variation (vide supra). However, using a number of methods, it is possible to induce immunity against specific antigenic variants (vide infra). The exact nature of this immune response has undergone intensive research for a number of years and the current view, supported by a large body of evidence, is that protection is dependent upon the production of serum antibodies.

Following/

Following infection the primary response is the production of IgM antibodies and this is followed later in the infection by the appearance of IgG antibodies. Throughout an infection the IgM antibody levels remain high, responding to the constantly altering surface antigens of the trypanosomes. This has been confirmed by Musoke, Nantulya, Barbet, Kironde and McGuire (1981), who demonstrated that most of the IgM antibody can be adsorbed if a sufficiently wide range of the VSSAs is used. Of the two types of antibody, it is considered that the IgM is more efficient than the IgG antibodies as determined by various in vitro tests and by in vivo protection.

The importance of the humoral response in the control of parasitaemia has been supported by a number of studies. By using X-irradiation or immunosuppressive drugs, it has been shown that the host, when infected, was unable to control the parasitaemia due to a failure of antibody production (Luckins, 1972). Passive immunisation studies using immune serum support the role of humoral antibodies in conferring protection (Takayanagi, Kambara and Enriques, 1973). Similarly, the transfer of spleen cells can also result in the transfer of immunity (Takayanagi et al., 1973) while the transfer of T cells does not (Campbell and Philips, 1976). In addition, if anti-μ chain antibody is administered to mice from birth then, due to the resulting B cell suppression, infected mice have a decreased survival time and are unable to respond to an irradiated vaccine (Campbell, Esser and Weinbaum, 1977). Hence an intact B cell response would appear to be essential for the development of an effective immune response against the African trypanosomes.

The/

The next question to be raised is how does the antibody result in the destruction and elimination of the parasites. It has been proposed that this could occur either by trypanolysis or by opsonisation and subsequent phagocytosis.

Antibody-dependent lysis of trypanosomes (trypanolysis) has been shown to occur readily in vitro (Lourie and Connor, 1936) provided a source of complement is added. The fact that complement is necessary would suggest that this process is not effective in vivo since it has been demonstrated that trypanosome infected animals suffer from a hypocomplementaemia (Fiennes, Jones and Laws, 1946). The most likely method by which antibody results in the removal of parasites from the circulation would therefore appear to be opsonisation and resulting phagocytosis by the cells of the mononuclear phagocytic series (MPS).

In vitro studies have shown that mouse and rat macrophages phagocytose T. b. brucei and T. b. gambiense when incubated along with immune serum (Lumsden and Herbert, 1967). In vivo studies of the role of antibody and macrophages in the control of parasitaemia have also been carried out (Holmes, MacAskill, Whitelaw, Jennings and Urquhart, 1979; MacAskill, Holmes, Whitelaw, McConnell, Jennings and Urquhart, 1980; MacAskill, Holmes, Jennings and Urquhart, 1981). In these experiments, involving the immune clearance of intravenously injected ^{75}Se-labelled trypanosomes, it was demonstrated that, while in non-immune animals most of the parasites remained in the circulation, in immune animals the parasites were rapidly removed by the liver. Within 15 minutes 50% of the radioactivity which was bound to the trypanosomes could be found in the liver.

Later/

Later in passive immunisation studies using radiolabelled trypanosomes, it was demonstrated that hepatic uptake was directly related to the quantity of circulating antibody and that hepatic clearance levels as high as those in immune animals could easily be achieved. Incubation of the trypanosomes in immune serum prior to intravenous injection resulted in a similar degree of hepatic uptake. That macrophage phagocytosis was dependent on the presence of antibody was reinforced by other experiments. Firstly, it was shown that suppression of antibody formation by prior irradiation at a level which did not affect macrophage function resulted in only a marginal increase in clearance values. It was also demonstrated that prior lysis was not a prerequisite to phagocytic function. Recent unpublished results confirm that hepatic clearance of radiolabelled trypanosomes can occur in goats and is similarly dependent on the presence of antibody (Bell, personal observation).

In trypanosome infected animals, it is well recognised that in spite of high levels of immunoglobulin (up to 20 times that of normal animals), immunosuppression occurs. Hence mice infected with T. brucei are almost completely incapable of mounting an antibody response to injections of sheep red blood cells (Goodwin, 1970).

Post-mortem examination of T. brucei infected mice shows generalised lymph node enlargement and marked splenomegaly (Murray, Jennings, Murray and Urquhart, 1974). The splenomegaly is so marked that it is easily visible in the live animal as gross abdominal distension. Exactly how the trypanosome infection with its resulting hypertrophy and hyperplasia of lymphoid tissue results in an immuno-suppressive/

suppressive state is unknown, although it is probable that there is some malfunction of cellular interactions between the T lymphocytes, macrophages and B lymphocytes.

Reduced antibody responses have been reported to occur in infected cattle. By using a mixed clostridial vaccine, Holmes, Mamo, Thompson, Knight, Luckins, Murray, Murray, Jennings and Urquhart (1974) demonstrated reduced antibody responses in cattle experimentally infected with T. congolense. Similarly a reduced response to louping-ill vaccine occurred in cattle which were infected with T. congolense, T. vivax and T. brucei (Whitelaw, Scott, Reid, Holmes, Jennings and Urquhart, 1979). However, studies using Foot and Mouse Disease vaccine, although demonstrating a considerably reduced antibody response, were less dramatic since all titres were above those required to give 95% protection (Scott, Pegram, Holmes, Pay, Knight, Jennings and Urquhart, 1977).

Since the host's immune response against trypanosomes essentially involves circulating antibody, various serological tests have been developed for diagnostic and experimental purposes. These include:- the infectivity neutralisation test; the in vitro agglutination test; the in vitro lysis test; the in vivo protection test and a solid phase radiometric binding assay.

The Chancre:

This is the name given to the local skin reaction which develops following a bite by a trypanosome-infected tsetse fly. The reaction is detected before the development of parasitaemia and

is/

is the first clinical indication of infection. They have been described as occurring in man (Fairbairn and Godfrey, 1957), rabbits (Luckins, Rae and Gray, 1983) and ruminants (Emery and Moloo, 1980, 1981; Emery, Akol, Murray, Morrison and Moloo, 1980; Akol and Murray, 1981, 1982). Characteristically chancres are of delayed onset and variable duration.

In cattle chancres have been demonstrated following feeds by tsetse infected with the three main species of trypanosome (Akol and Murray, 1981). The magnitude of the reaction was different between the three species:- T. brucei produced a more severe reaction than T. congolense, while the chancre induced by T. vivax was the smallest.

The chancre first appears as a small 2 to 3 mm nodule at the site of the tsetse fly feed and then quickly develops into a raised, indurated, hot, painful swelling that persists for a time before decreasing in size until it is no longer recognised at about one month post-inoculation (Akol and Murray, 1981).

Histologically the lesions are characterised by an intense inflammatory reaction. The initial reaction is due to numerous polymorphonuclear leucocytes but these are later replaced by a mononuclear cellular infiltrate consisting of small to medium lymphocytes (Akol and Murray, 1981).

Work has also demonstrated that reinfection of treated or infected animals with a homologous clone of trypanosome does not result in the development of a chancre, while reinfection using a heterologous clone results in both chancre development and parasitaemia (Akol/

(Akol, Murray and Morrison, 1981). The immunity to homologous cyclical rechallenge was shown to occur irrespective of the blood-stream VSSA ingested by the fly.

Clinical Signs and Pathology:

The clinical disease produced in cattle by African trypanosomes can vary from an acute fatal infection resulting in death within two weeks to a chronic wasting disease which extends over a period of many months. Which disease syndrome occurs depends on a number of factors including the level of challenge, the strain of trypanosome, the breed of cattle and the plane of nutrition (Morrison, Murray and McIntyre, 1982a).

Infection with various isolates of T. vivax can result in an acute septicaemic-like condition with the affected cattle showing a sustained high parasitaemia, increase in temperature and often exhibiting haemorrhages of mucosal membranes. These animals may die within two weeks of infection. Some isolates of T. congolense may result in death within 6 - 10 weeks of infection. However, it is more usual for a chronic infection to occur with all three species of trypanosome.

The chronic disease is characterised by a number of well-known clinical signs. Classically in the early stages of infection, as the parasitaemia becomes patent, there is an intermittent pyrexia and at this time the superficial lymph nodes become enlarged and are easily palpated. Gradually an anaemia develops and after 4 -6 weeks the packed cell volume (PCV) may have decreased by up to 50% of normal.

Even/

Even though the cattle continue to eat, there is a gradual loss of body condition until they become extremely emaciated. Affected animals appear wasted with a dull staring coat and a 'hunched-up' appearance. They are lethargic and eventually become so weak that they are unable to rise and forage for themselves. Eventually death occurs, often from congestive cardiac failure and the classical signs of this are often noted, i.e. subcutaneous oedema, pulsating distended jugular veins and a tachycardia which terminally is replaced by a bradycardia.

Decreased fertility is often stated to be a clinical sign of trypanosomiasis, with abortion in pregnant cows. This is consistent with a wasting, intermittently pyrexic disease.

Post-mortem examination of an animal which has died of trypanosomiasis will reveal a number of features depending on whether the animal was an acute or chronic case. In the chronic case the picture is that of an emaciated wet carcase which is pale with atrophy of body fat and muscle wastage. Distinct signs of congestive cardiac failure are often present with an enlarged dilated heart and a large liver showing chronic venous congestion. Serous fluid may be present in the body cavities and subcutaneous oedema is often present. In acute cases the lymph nodes may be enlarged, while in chronic cases they are normal or decreased in size. An almost constant feature is the presence of enlarged haemal lymph nodes.

In acute cases due to T. vivax infection, the main findings are vascular engorgement with haemorrhages of the mucous membranes. The carcase is normally in good condition since death occurs before emaciation can develop.

Current Control Methods:

The control of trypanosomiasis has, over the years, been divided into two main areas directed against either the vector or the parasite itself.

The control of the vector can be further subdivided into direct and indirect tsetse control methods. Direct control is carried out by the use of insecticides either to treat the cattle or the land. Insecticides applied to the cattle either by spraying or dipping results in the death of flies coming in contact with the animals. Similarly, insecticides can be used to spray selectively the resting and breeding sites favoured by the tsetse, so called 'ground spraying', or they can be sprayed by aircraft and helicopters over large areas of land.

Insecticides which have been used to date include the chlorinated hydrocarbons such as DDT, Dieldrin and Thiodane (endosulphan), as well as the organophosphates such as Fenthion.

All the available evidence suggests that the Glossina are especially susceptible to the organochloride insecticides, as compared with other insects. This is especially true for endosulphan which is claimed to act selectively (Burnett, 1970). It is however, extremely toxic to fish and great care is required to prevent contamination of rivers and lakes.

A problem with the use of organochlorides lies in their extreme persistence in the environment and their effect on wild life, especially birds. As a result, it has been proposed that preference should be given to the organophosphates as they decompose after some time and do not leave problematic residues (Seifert, 1973, cited by Ruchel, 1975).

The/

The use of fly traps has also been tried. In the past these have had only limited success. Recent developments in this area have been more successful and field trials are currently being conducted in Zimbabwe (Vale, 1982).

The main indirect tsetse control method is the clearance of bush (Ford, Nash and Welch, 1970) which results in the removal of the bioclimate necessary for the successful breeding of the tsetse with a resulting elimination of the flies. This method can be used to form a corridor to separate tsetse-free land from that which is infested. Land cleared of bush can be used for a number of purposes. Cattle can be safely grazed except at the peripheries where they are likely to come in contact with tsetse, or it can be used for crop cultivation. Once land is clear, it should be settled immediately and kept clear in order that the land so expensively freed from the tsetse fly remains so.

The culling of wild game has also been used (Ford, 1970) as these animals were considered to be an important reservoir of the disease and also the main food supply for tsetse flies. However this policy is now considered to be unacceptable. One alternative has been the suggestion that, since the game is relatively resistant to trypanosomiasis, they should be 'farmed' in tsetse infected areas instead of cattle (Ford, 1963) thus reducing the risk involved in meat production.

Another novel method of control being investigated is the sterile male technique (Dame, 1970). The rationale of this technique is that female flies of the genus Glossina mate only once in their lives/

lives and if they do so with a sterile male, they will not produce any offspring. However, the production of these sterile males in the numbers needed is prohibitively expensive.

Even in the unlikely event of complete tsetse control being achieved, it would not result in total freedom from the disease as mechanical transmission can and does occur. This is exemplified by the introduction and spread of T. vivax in South America where no flies of the genus Glossina occur.

As a result, even with vector control, the second method, i.e. control of the parasite, is necessary. This has been achieved mainly by the use of either chemotherapeutic or chemoprophylactic drugs (vide infra).

Chemotherapy involves the use of drugs to treat a clinically infected animal, the most commonly used ones being Diminazene aceturate (Berenil, Hoechst) and Homidium chloride (Novidium, May and Baker; Ethidium, Boots). In most cases response is good with the parasite being rapidly cleared from the blood. However, in longstanding infections the PCV may not recover as would be expected. After treatment reinfection can rapidly occur since these drugs do not have a prolonged prophylactic period. In cattle, Berenil at 7 mg/kg protects against infection with T. congolense for 6 days, and the pre-patent period is prolonged if infection occurs up to 18 days post-treatment (Wellde and Chumo, 1983).

Recently the efficacy of these curative drugs to sterilise the body of parasites has been brought into doubt. Jennings, Whitelaw/

Whitelaw and Urquhart (1977) demonstrated that during infection of mice with T. brucei, the timing of treatment after infection influenced whether or not relapse occurred. Whether a similar phenomenon occurs in cattle is not known but with some T. vivax infections relapses generally occurred 10 to 40 days after treatment (MacLennan and Na'isa, 1970; MacLennan, 1971).

In areas where trypanosomiasis is endemic and cattle populations are stable a better approach to control is the use of chemoprophylaxis. This involves administering the drug at a regular interval to prevent the cattle becoming infected. In this case the drug of choice is Isometamidium chloride (Samorin, May and Baker Ltd.).

The ideal method for the control of trypanosomiasis is immunisation. However, no satisfactory field vaccine is available and the possibility of one being produced remains remote. The major constraint is the ability of the trypanosome to undergo seemingly limitless antigenic variation (vide supra).

Over the years numerous attempts have been made to vaccinate animals against trypanosomiasis using a variety of prepared antigens. Koch (1901) used live attenuated trypanosomes, while Schilling (1935) tried killed T. brucei and T. vivax, both without success. More recently, irradiated trypanosomes have been used with limited success to produce resistance in cattle against T. rhodesiense (Wellde, Duxbury, Sadun, Langbehn, Letzsch, Deindl and Warui, 1973). These results were improved upon by Morrison, Black, Paris, Hinson and Wells (1982) using trypanosomes and purified VSSA. However, protection/

SUMMARY OF CURATIVE AND PROPHYLACTIC DRUGS CURRENTLY AVAILABLE FOR AFRICAN BOVINE TRYPANOSOMIASIS

Trypanocide	Type	Dose mg/kg	Comments
QUINAPYRAMINE:			
(1) Dimethosulphate Trypacide (May & Baker Limited)	C	4.4 - 5 s.c.	May cause curare-like systemic toxicity. Aggravated by stress. Can cause kidney damage.
(2) Prosalt Dimethosulphate : chloride (3:2) Trypacide Prosalt (May & Baker Ltd.)	P	4.4 - 5 s.c. of the active dimethosulphate	Prophylaxis of about two months depending on the level of challenge.
DIMINAZENE ACETURATE: Berenil (Fabwerke Hoechst AG.)	C	3.0 - 7.0 i.m.	Rapidly excreted, therefore difficult to induce drug resistance. Prophylactic period of about 10 days.
HOMIDIUM BROMIDE: Ethidium (Boots Pure Drug Co. Ltd.)	C	1.0 i.m.	At high doses may cause toxic liver damage. At normal doses will cause local tissue damage. Chloride salt (Novidium) soluble in cold water. Bromide only soluble in hot water.
ISOMETAMIDIUM CHLORIDE: Samorin (May & Baker Limited)	C P	0.25 - 0.5 i.m. 0.5 - 1.0 i.m.	Causes local tissue damage at the site of inoculation. At high doses causes toxic liver damage. Soluble in cold water. Heat sensitive. Use only when made fresh.

C = Curative drug. P = Prophylactic drug.

protection is only achieved against the specific variants which were present in the vaccine. The easiest method to demonstrate this is by infection, treatment and subsequent reinfection using homologous and heterologous strains of trypanosomes. Animals are protected against homologous challenge but not heterologous ones (Akol, Murray and Morrison, 1981).

A more promising method of control currently being considered is the exploitation of the genetic resistance which certain breeds of cattle and small ruminants have been shown to possess.

This resistance or 'trypanotolerance' is generally attributed to the taurine breeds of cattle in West and Central Africa, namely, the N'Dama and the West African Shorthorn, as well as to various native breeds of sheep and goats (Murray, Trail and Grootenhuis, 1984). These authors point out that, even though the ability of these breeds to survive in tsetse infested areas was recognised as early as the beginning of this century, they have not been fully exploited, probably because of the misguided impression that their productivity was low.

Recently it has been demonstrated conclusively that trypanotolerance is an innate characteristic and is not due to an acquired resistance (Murray, Clifford, Gettinby, Snow and McIntyre, 1981). These workers exposed equal numbers of N'Dama and Zebu cattle, which had never before experienced trypanosomiasis, to a natural challenge. All of the Zebu cattle died of trypanosomiasis while, although the N'Dama became infected, the majority remained in good condition.

This/

This superior survival in the face of challenge is thought to be due to a more efficient immune response.

In view of the poor prospects of new methods of control being developed in the foreseeable future, it is likely that heavy reliance will continue to be placed on chemotherapy and chemo-prophylaxis.

Historical Background to Chemotherapy and Chemoprophylaxis:

Since 1895 when Bruce first recognised the causative agent of trypanosomiasis, numerous compounds have been synthesised and tested to determine their trypanocidal activity. In spite of intensive research in the first half of this century, the number of effective drugs available is extremely limited and no new drug has been marketed since the early 1960s. The few active 'cattle' drugs which have become established in the field or have survived preliminary drug trials are: tartar emetic, quinapyramine (Antrycide), homidium bromide (Ethidium), pyrithidium bromide (Prothidium), isometamidium chloride (Samorin) and diminazene aceturate (Berenil).

The situation is in fact worse than this might suggest since tartar emetic, the only drug not to cause drug resistance, is no longer used and Antrycide ceased to be manufactured in 1976, although it has very recently been remarketed as Trypacide (May and Baker Ltd., England) and Noroquin (Norbrook Laboratories (GB) Ltd.).

The possibility that a new drug will be introduced must be considered remote since the potential profits are too low for drug companies to invest large sums of money in the necessary research and development.

Williamson (1970) described the evolution of trypanocidal drugs for both man and animals in his authoritative review of the chemotherapeutic and chemoprophylactic agents and pointed out that the initial development of modern anti-infective chemotherapy owed much to the preoccupation around the turn of the century with the control of trypanosomiasis. Initial drug development was directed against sleeping sickness in man and it was not until later that agents directed against animal trypanosomiasis were developed.

One scientist intimately involved with the development of anti-trypanosomal drugs was Paul Ehrlich (1854 - 1915) who was studying the use of the newly introduced synthetic dyes as histological and haematological stains. The selective uptake of these stains by certain cells led Ehrlich to try them as therapeutic agents. Thus the therapeutic activity of methylene blue in human tertian malaria was discovered. However, due to the lack of an experimental model for this disease, progress faltered until after the first World War. Ehrlich adopted a similar approach in his search for an agent effective against trypanosomes. Using a mouse model infected with T. equinum he demonstrated weak trypanocidal activity in the dye, Benzopurpurin, a member of the benzidine azo dyes. By introducing an extra water-solubilising sulphonic acid substituent in the molecules, the resulting sodium salt, Trypan Red, turned out to have both curative and prophylactic properties. For the first time an experimentally produced disease had been cured by a synthetic organic compound of known chemical composition (Williamson, 1970).

Two/

Two years prior to this it has been shown that sodium arsenite was effective in treating T. brucei infections in rats and mice (Laveran and Mensil, 1902, cited by Williamson, 1970). Thus the stage was set for research along two different lines: (1) examination of synthetic dyes; and (2) examination of organic arsenicals and antimonials. The culmination of these two lines of development were Suramin and Tryparsamide, both directed against the human disease.

Tartar emetic (sodium antinomyl tartrate), a trivalent antimony complex and the mainstay in the treatment of bovine trypanosomiasis for over forty years, was first shown to have trypanocidal activity by Plimmer and Thompson (1908) who demonstrated its activity against nagana and surra infection in rats. Bevan, impressed by the currative action of intravenous (i.v.) tartar emetic in sleeping sickness, was led to try it against trypanosome infections in cattle. Over the next 18 years he successfully treated several thousand cattle in Southern Rhodesia showing its efficacy against both T.congolense and T. vivax infections (Bevan, 1928). However, because of the severe tissue reactions it induced, tartar emetic had to be administered by i.v. injection which restricted its administration to veterinarians and trained lay staff. Despite this, and the discovery of other related antimony preparations such as Antimosan which could be injected intramuscularly or subcutaneously, tartar emetic continued to be used until the early 1950s. The low therapeutic index of the drug with up to 6% mortality at normal dosage rates was tolerated because of the high mortality in untreated cattle (50%) (Wilson, 1958).

The modern phenanthridine drugs are all based on the later work of Ehrlich with acaridine-based dyes. This work progressed rapidly following the discovery that the activity of the triphenylmethane dye, Tryparosan, was due to an acaridine compound present as an impurity. This resulted in the development of trypaflavine (acriflavine) by Ehrlich and Benda in 1908. Although it never progressed past the trial stage of development, Browning and Gilmour (1913) demonstrated that it had good antiseptic properties even in the presence of serum and these properties were put to good use in the first World War. Work continued looking at segments of the acaridine molecule and resulted in an examination of the simpler anil- and styrylquinolines. Some of these were shown to be powerful antiseptics and certain amino derivatives turned out to be active trypanocides (Browning, Cohen, Ellingworth and Gulbransen, 1926).

Continued research produced Styryl 245, a compound showing long prophylaxis and trypanocidal activity (Browning and Gulbransen, 1934). The antimalarial Atebrin was also discovered.

Trypanocidal activity was found to be linked in particular with the quaternary phenylphenanthridines with two amino groups. This resulted in the discovery of phenidium chloride by Browning, Morgan, Robb and Walls (1938) which was active against the Trypanozoon subgenus. Field trials in Africa confirmed its activity but poor solubility and a narrow therapeutic index indicated that further research was required. As a result, dimidium bromide (Dimidium) was introduced. It was readily soluble and active against T. congolense

in/

in the field (Carmichael and Bell, 1944). Mass treatment was carried out in East Africa using this drug after its introduction in 1943 - 45 but widespread resistance developed by 1952 (Leach and Roberts, 1981). A resulting increase in dosage caused severe toxic effects previously described by Randall and Beveridge (1946). The next significant development came with the synthesis of homidium bromide (Ethidium) from dimidium bromide (Watkins and Woolfe, 1952). This proved to be as effective against T. congolense and T. vivax as Dimidium and considerably less toxic.

At the same time as the discovery of the phenanthridines in Britain, Jench (1937) reported that a 4-aminoquinoline derivative, Surfen C, had marked activity against T. congolense. Unfortunately, Surfen C did not prove satisfactory when tested in the field due to severe toxicity reactions. Some animals collapsed and died from shock within 15 minutes after a dose of 2 mg/kg body weight (Le Roux, 1936), while even at 1 mg/kg body weight systemic toxicity and severe local reactions were noted (van Rensberg, 1938).

However, further research led to the synthesis of quinapyramine by Barrett, Cord and Hepworth (1953). Quinapyramine was used either as the soluble dimethosulphate salt (Antrycide) or as a mixture of the dimethosulphate and the weakly soluble chloride (Antrycide Prosalt) which was used as a prophylactic. From its introduction into the field in the 1950s, it was increasingly used as it held the unique distinction of being the first prophylactic drug available against T. congolense and T. vivax. The introduction of Antrycide coinciding with that of Ethidium had a profound effect on cattle treatment in Africa./

Africa. The number of animals treated increased dramatically; in Northern Nigeria treatments rose from 45,000 in 1951 - 52 to 641,000 in 1957 - 58 (Wilson, 1958). Other figures given by Williamson (1961) place the number of cattle treated with Ethidium at 400,000 to 600,000. Meanwhile, the number of doses of trypanocidal drug administered in Kenya doubled in the period between 1960 and 1963 from 468,000 to 836,000 (Fairclough and Parsons, 1964). However, associated with this massive treatment, Ethidium resistant strains of T. congolense soon became identified and by 1966 Jones-Davies and Folkers reported that they were becoming widespread in Northern Nigeria. Production of cattle in Africa increased as a direct result of this intensive drug use (Ford, 1965).

Following the development of Ethidium from Dimidium, the next phenanthridine to be introduced was pyrithidium bromide (Prothidium). This was synthesised by Watkins and Woolfe (1956) who substituted part of the Anthrycide molecule into a phenidium-like compound. The resulting compound had both good curative and prophylactic activity (Watkins, 1958). A dose of 0.2 - 0.4 mg/kg was curative for T. congolense (Whiteside, Fairclough and Bax, 1960), while one of 2 mg/kg protected for 59 days (Kirkby, 1964) to 71 days (Robson and Cawdery, 1958). At 4 mg/kg Prothidium protected for about 120 days (Robson and Cawdery, 1958). Smith (1959), however, determined prophylactic periods of 73 days for 2 mg/kg and 92 days for 4 mg/kg.

These results were all obtained from limited field studies and relied on the development of a patent parasitaemia as indicating the end of prophylaxis. As a result their significance is of doubtful value.

The/

The main problem with Prothidium, as with other phenanthridines, was the generation of a severe local reaction at the injection site (Cawdery, 1963; Robson and Cawdery, 1958; Smith, 1959; Stephen, 1962).

Metamidium was the next derivative of the phenanthridine series to be developed (Wragg, Washbourn, Brown and Hill, 1958). In this case a moiety of the Berenil molecule was attached to the Ethidium molecule giving rise to a mixture of two isomers. This mixture was termed Metamidium and was both curative and prophylactic. Under field conditions at a dose rate of 4 mg/kg protection of 18 weeks was achieved (Smith and Brown, 1960), while at 5 mg/kg protection lasted for 29 weeks (Stephen, 1960). Robson (1962) achieved 226 days (30 weeks) protection using 2 mg/kg. Meanwhile using a dose of 0.5 mg/kg Fairclough (1963a) achieved 29 weeks protection but even at this low dose, there was evidence of severe tissue damage at the site of injection. Further work revealed that the red isomer was more soluble and more active than its purple counterpart. Berg (1960) was able to identify its structure and isolate it in sufficient quantities for experimental purposes. In 1961 Isometamidium chloride (Samorin) appeared on the market with the following dosage recommendations:

0.5 mg/kg body weight - curative treatment

1 - 2 mg/kg body weight - curative for resistant strains

2 mg/kg body weight - as a prophylactic

In all cases it was to be injected intramuscularly. Since then the recommendations have changed to the following

0.25/

0.25 - 0.5 mg/kg body weight - curative treatment - for resistant strains use up to 1 mg/kg.

0.5 - 1.0 mg/kg body weight - prophylactic treatment - treat animals 2 - 6 times a year. Dose level and frequency of treatment depends on level of challenge (May and Baker Ltd., Drug Data Sheet).

An effective curative dose of Samorin can be as low as 0.1 mg/kg (Kirkby, 1963) depending on the sensitivity of the strain of trypanosome involved. A dose of 0.25 mg/kg, although basically curative, has been used to protect cattle from infection while they were trecked through tsetse infested areas to slaughter (Na'isa, 1969). Prophylaxis achieved with this drug under field conditions seems to be subject to some variation. Fairclough (1963a) using 0.5 mg/kg achieved 14 weeks prophylaxis, while Kirkby (1964) using 1 mg/kg achieved 97 days (14 weeks) or using 2 mg/kg 122 days (17 weeks) protection. Later Wiesenhutter, Turner and Kristensen (1968) reported 30 weeks protection with 1 mg/kg. Robson (1962), on the other hand, reported 256 days (36 weeks) protection with 2 mg/kg and 351 days (50 weeks) with 4 mg/kg. A possible reason for the differences in protective periods given by prophylactic drugs was postulated by Whiteside (1962) who suggested that the length of prophylaxis was inversely proportional to the intensity of challenge. This has never been demonstrated under carefully controlled experimental conditions. It is also noteworthy that, as in previous studies on the/

the duration of chemoprophylaxis, the period of prophylaxis was judged under field conditions and depended solely upon the time of development of a patent parasitaemia following drug administration.

Local reactions at the injection site again surfaced as a problem (Cawdery, 1963; Wilson, Le Roux, Paris, Davidson and Gray, 1975a), although Wiesenhutter et al. (1968), in a trial using Antrycide Pro Salt RF, Prothidium and Samorin, reported local reactions in 10%, 50% and 4% respectively of the treated animals.

Unfortunately, however, Antrycide and the group of newer aminophenanthridine drugs, Ethidium, Prothidium and Metamidium were all capable of giving rise to mutual cross-resistant strains in cattle (Whiteside, 1962). This cross-resistance was a result of inclusion in the new drugs of moieties of other active drugs.

Berenil (diminazene aceturate), first developed by Jensch (1955), could for a long time be used successfully in all cases of resistance in both East and West Africa (Whiteside, 1962; MacLennan, 1968). However, reports of drug resistance to Berenil have been noted (Jones-Davies, 1968; MacLennan and Na'isa, 1970; MacLennan, 1971). Berenil, an aromatic diamidine similar to the human preparation pentamidine, was developed as a result of the investigations into the Surfen C molecule (Williamson, 1970). Thus it is related to acriflavine initially developed by Ehrlich and Benda. Its successful use in the treatment of T. congolense infection in Zebu cattle was described by Milne, Robson and Lwebandiza (1955) when 2 mg/kg was used. Fussganger and Bauer (1958) determined that a dose of/

of 3.5 mg/kg was effective against both T. vivax and T. congolense but that 5 mg/kg was required for T. brucei. Increasingly Berenil was used as a therapeutic trypanocide in all parts of Africa. In 1957 2,000 doses were administered in Kenya but by 1961 this had risen to 190,000 (Fairclough, 1963a). Meanwhile in Northern Nigeria, because of widespread resistance to Antrycide and Ethidium, Berenil was introduced as the drug of first choice (MacLennan, 1968).

The presence of local tissue toxicity has always been a problem, especially with the prophylactic drugs of the phenanthridine group of compounds. Their use has often resulted in the down-grading or total condemnation of animal carcases at slaughter due to extensive tissue damage at the injection site. As a result, their use in trade cattle has tended to be discontinued in favour of treatment with Berenil which does not cause a severe local reaction (Fairclough and Parsons, 1964). However, even treatment with Berenil prior to slaughter can cause sufficient damage to result in a substantial decrease in profitability (Wilson et al. 1975a).

In 1956 Williamson and Desowitz reported on the prophylactic activity of Suramin complexes in animal trypanosomiasis. This was aimed primarily at increasing the length of prophylaxis achieved with each treatment but it was also recognised that it resulted in a decrease in tissue toxicity. Numerous papers have since been published demonstrating the reduction in tissue toxicity and the increase in prophylaxis achieved with these complexes (Berg, Brown and Lucas, 1961;/

1961; Gray and Stephen, 1962; Smith, 1959; Smith and Brown, 1960; Stephen, 1958, 1960, 1962). Ethidium suraminate was considered to be the most promising of the complexes. However, the local toxicity still proved to be a problem and, combined with high costs, hindered further development. Other complexing agents have been tried with varying degrees of success. Laminarin sulphate was used by Groves and Wilmshurst (1964) and recently James (1978) demonstrated in mice that a complex of Isometamidium chloride with Dextran sulphate resulted in both increased prophylaxis and decreased tissue damage.

Another problem associated with trypanocidal drug usage is the presence and spread of drug resistant strains of trypanosomes. The problem of drug resistance is compounded by the limited range of drugs available and the fact that no new drug has been introduced into the field for the last quarter of a century.

The first phenanthridine compounds, Phenidium and Dimidium, were introduced into Africa in 1943 - 1945 and by 1952 resistance was a problem in Rhodesia (Williamson, 1961). The next phenanthridine to be introduced, Ethidium, again resulted in the development of resistant strains in both West and East Africa (Jones-Davies and Folkers, 1966; Scott and Pegram, 1974).

The close structural relationships between certain drugs has resulted in the phenomenon of cross-resistance. Hence Prothidium, which is composed of Ethidium with a moiety of the Antrycide molecule attached to it, resulted in cross-resistance between these drugs (Williamson, 1961). The full extent of cross-resistance between various drugs was reviewed by Williamson (1970).

The/

The veterinary impact of cross-resistance has been reduced by the apparent inability of the two most commonly used drugs, Samorin and Berenil, to induce cross-resistance to each other (Williamson, 1970). Berenil itself was long considered to be an ideal drug incapable of inducing resistance due to its rapid excretion from the body, effectively preventing trypanosomes becoming exposed to sub-optimal concentrations of the drug. However, reports soon appeared demonstrating strains of both T. vivax and T. congolense which were resistant to normal doses of Berenil (MacLennan and Jones-Davies, 1967; Jones-Davies, 1967a, 1968; MacLennan, 1971; MacLennan and Na'isa, 1970) although the extent of this resistance appears to be limited.

Another problem associated with the use of drugs is the presence of residues in the meat and milk. This is particularly the case with the phenanthridine compounds. Braide and Eghianruwa (1980) demonstrated that isometamidium could still be detected in the liver and kidneys four weeks after treatment designed to protect cattle en route to slaughter. The long term effects of human consumption of trypanocidal-containing products have not yet been investigated.

The present studies are concerned with two particular aspects of chemoprophylaxis against T. congolense using isometamidium chloride, first, the duration of chemoprophylaxis and its relationship with the development of immunity and secondly the local tissue reactions following the injection of isometamidium and its dextran complex.

CHAPTER 1

Duration of Chemoprophylaxis and its Relationship to the Development of Immunity

Introduction:

In 1962 Whiteside discussed the effect of the weight of trypanosomiasis challenge in the field on the length of prophylaxis achieved by the use of drugs. This had been prompted by the observation that after a number of years of field use of Antrycide Prosalt, it was not known what duration of protection it gave. In different areas with different workers it produced periods of prophylaxis varying from three or four months to less than one month. This he attributed to differences in the level of challenge in different areas. In areas with a high incidence of the disease the length of prophylaxis afforded by the drug was apparently reduced. This interesting and important phenomenon has, so far as is known, never been examined under carefully controlled experimental conditions, though it continues to form the basis for the frequency and level of drug dosage in the field. How the level of challenge may alter the length of prophylaxis is unknown, although it has been suggested that the drug may be absorbed by inoculated trypanosomes following repeated tsetse challenge (Davey, 1957).

The variation in protection noted with the use of Antrycide Prosalt was not restricted to that class of drug alone. As previously described, prophylaxis provided by drugs of the phenanthridine series gave equally variable results in the field. For example, the following results have been reported following the use of Samorin.

Dose mg/kg	Length of Prophylaxis	Author
0.5	14 weeks	Fairclough, 1963
1.0	14 weeks	Kirkby, 1964
"	30 weeks	Weisenhutter et al. 1968
"	36 weeks	Robson, 1962
2.0	17 weeks	Kirkby, 1964
4.0	50 weeks	Robson, 1962

Although mentioned previously, it is worth repeating that all of the above information was derived from field studies where the duration of prophylaxis was judged by the development of a patent parasitaemia. Some authors judged the mean time to infection as being the effective period of prophylaxis, while others considered the prophylactic period to end only when the final animal had become parasitaemic. The correct prophylactic period should, in fact, be the time till the first infected tsetse bite which results in infection.

The development of immunity to trypanosomiasis following the use of drugs was first suggested by Bevan (1928, 1936). While working in Southern Rhodesia, he observed that clinical cases of bovine trypanosomiasis which recovered after treatment frequently remained in good health despite the presence of trypanosomes in their bloodstream. This type of immunity, characterised by a healthy state despite evidence of parasitaemia, was described by Bevan as 'tolerance'/

'tolerance'. Furthermore, he described a method of artificially inducing this tolerant state by 'vaccinating' cattle with strains of T. congolense which had been passaged in sheep followed, when necessary, by treatment with arsenicals.

In practice this tolerant state was found to break down due to infection with other trypanosome species, starvation, intercurrent disease or any other 'stress' inducing condition.

In comparison with this evidence, the development of a sterile immunity following prophylactic drug treatment was presented by Soltys (1955). He demonstrated that groups of Zebu cattle which had undergone prophylactic drug treatment with Antrycide every two months for a period of twenty-eight months, could, on drug removal, survive in a tsetse area for a period of not less than eight months without developing a patent parasitaemia. Meanwhile five control animals introduced at the same time all contracted severe T. congolense infections within three weeks. However, the validity of this sterile immunity was brought into doubt in 1958 when Smith suggested that repeated doses of Antrycide may leave tissue residues of a sufficient level to confer prophylaxis for many months after drug withdrawal.

In 1962 Whiteside reported some observations made on three groups of Zebu cattle which were introduced at different times into a trypanosome endemic area near Lake Victoria in Kenya. On introduction into the area, each group of cattle contracted trypanosomiasis within a period of four weeks. This infection and all subsequent ones were treated with the therapeutic drug Berenil. Initially/

Initially reinfection became patent at approximately four weeks. However, gradually this interval began to extend so that after the fourth challenge it had reached a maximum of eight weeks. The extra four weeks protection was considered to be due to the development of an immune response.

More recently, a series of papers by Wilson and his colleagues (Wilson et al., 1975a, b; Wilson, Paris, Luckins, Dar and Gray, 1976) described the effect of strategic drug use on the development of immunity in cattle. These experiments were carried out over a period of two to three years and a variety of parameters, such as changes in trypanocidal drug requirement, development of parasitaemia, ability to maintain normal blood values, growth rate and response to challenge after withdrawal of the drugs were used to assess the development of immunity.

In one paper (Wilson et al., 1975b) they described the performance of a herd of breeding Zebu cattle maintained for two years in an area of high trypanosome challenge. The effects of trypanosomal infections were controlled by periodic treatments with Berenil at 7 mg/kg, the frequency of drug treatment being based on the appearance of clinical signs and/or a reduction in the packed cell volume to below 20%. During the two years of this experiment they found that resistance to trypanosomiasis did not develop. However, during the second year of the experiment there was a decrease in the number of abortions and calf mortality. They considered that the lack of any development of immunity may have been due to the frequent use of Berenil reducing the effective antigenic stimulus.

In/

In a later experiment involving the maintenance of a herd of beef cattle in an area of medium trypanosome challenge three different trypanocidal drug regimes were used and more promising results were obtained (Wilson et al., 1975a, 1976). Cattle treated individually with Berenil on the appearance of clinical disease developed a partial immunity to trypanosomiasis after two years. However, if Berenil was used to treat all the animals in a group when any one became parasitaemic, then immunity did not develop. The final group of cattle, which were treated with Samorin when any one animal became parasitaemic, were considered to have developed a limited degree of immunity to the disease. It was concluded that this final group was the most suitable of the three regimes examined for the maintenance of beef cattle in a tsetse infested area.

A further study on the strategic use of trypanocidal drugs was described by Bourn and Scott in 1978. This involved the introduction and maintenance of work oxen in an area of high tsetse challenge in Western Ethiopia. Initially, 40 oxen were introduced into the area in 1972. Gradually more animals were added so that by 1977 450 were present. These animals were maintained by the use of two drugs, Berenil and Samorin.

Initially the oxen were given block treatment with Berenil at 3.5 mg/kg whenever signs of trypanosomiasis appeared in several of them. This resulted in treatment being necessary every 28 days and, as a consequence, prophylactic treatment with Samorin at 1.0 mg/kg was introduced. Unfortunately complete reliance on this phenanthridine/

phenanthridine drug was not possible because of the emergence of drug-resistant strains of T. congolense. For the remainder of the study Samorin and Berenil were used, alternating at approximately 9-monthly intervals. As a direct result of this judicious drug usage, fewer than 20 oxen died from trypanosomiasis during the 5 year study. In 1982 Holmes and Scott, reviewing the successful use of chemoprophylactic schemes in the control of trypanosomiasis, introduced some new data on the maintenance of the oxen. During the later part of the experiment it was noted that, even though 50 - 69% of the animals showed a patent parasitaemia, only 20% needed treatment in any one month. This suggested that a degree of non-sterile immunity had developed.

Exactly how the use of a prophylactic drug can result in the development of a degree of immunity is not clear but there are at least two possible ways. First, the cattle may have been infected at the time of the treatment thus resulting in immunity to the particular VSSAs present. After a number of such treatments a degree of protective immunity could appear depending on the total number of serodemes present in that area. Secondly, it may be possible that the animal, even when under chemoprophylaxis may mount an effective immune response against the antigenic stimulus produced by the intradermal inoculation of metacyclic trypanosomes as a result of infected tsetse fly bites without the subsequent development of a patent parasitaemia.

The work described in this chapter was directed towards investigating two important aspects of prophylaxis following treatment with Samorin.

First/

First, the duration of chemoprophylaxis following treatment of cattle with a single dose of Samorin at 1 mg/kg was examined under carefully controlled experimental conditions. This was carried out using metacyclic challenge at fixed time intervals after drug treatment. The second aspect to be examined was the appearance and possible role of specific anti-trypanosomal antibodies following each cyclical challenge. From such studies it was hoped to obtain valuable evidence on the possible interactions between drug levels and antibody response in determining the duration of prophylaxis in cattle following treatment with Samorin.

A single experiment was conducted, divided into two phases (I and II). In Phase I Samorin-treated cattle were challenged at monthly intervals between 1 and 5 months post-treatment with infected tsetse flies.

Six months after Samorin treatment (Phase II) the cattle were reallocated into new groups and subjected to various levels of metacyclic challenge.

MATERIALS AND METHODS

1. Experimental Animals:

Cattle

Thirty four 18-month old Boran castrated males were obtained from Kapiti Plains in the Athi River area of Kenya. This region is free from trypanosomiasis. The animals were approximately 210 kg in weight at the start of the experiment.

Goats

Adult East African x Galla goats were obtained from an area of Kenya known to be free from trypanosomiasis.

Mice

There were inbred male AJ mice obtained from the ILRAD colony. They were originally derived from the OLAC 76 strain (Oxfordshire Laboratory Animals Colonies). The mice weighed between 15 and 20 g and were approximately 10 - 12 weeks old at the start of each experiment.

2. Feeding and Housing:

Cattle

These were housed in a loose shed in groups of 15 animals on concrete bedded with wood shavings. They were given a daily diet consisting of 4 kg hay per animal and 3 kg of concentrate (Unga pencils, Unga Feeds, Nairobi). A mineral mixture was added to this diet.

Various other managerial features are noteworthy, namely, spraying with acaracide was discontinued after December, 1983 following introduction to the new housing. At the same time all the animals were/

were drenched with fenbendazole (Panacur, Hoechst) to eliminate any worm burdens which may have been present. All the animals were vaccinated against Foot and Mouth Disease in November, 1983, March, 1984 and July, 1984.

Goats

The goats used in these experiments were introduced into a concrete floored building in December, 1983. They were treated with fenbendazole (Panacur, Hoechst) to eliminate any worm burden and a 5-day course of Amprolium (MSD AGVET) administered to eliminate coccidia. Foot and Mouth vaccination was carried out as described above for the cattle. Each animal was given a daily diet of 0.5 kg hay and 0.5 kg concentrates. No minerals were added to reduce the risk of urolithiasis.

Mice

All animals were housed in plastic cages with sawdust bedding changed twice weekly. The cages were held in a metal stand in an animal house. Mice were fed ad libitum on mouse pencils (Unga Feeds Ltd., Nairobi). Drinking water was constantly available to all mice from plastic water bottles. No antibiotics were administered.

3. Tsetse Flies:

These were teneral male Glossina morsitans centralis flies obtained from the ILRAD tsetse colony.

4. Trypanosomes:

These belonged to the subgenus Nannomonas T. congolense IL 1180 (ILNat 3.1). This was originally isolated from a lion (LR 11/C9) in the Serengeti National Park by Geiby and Kauffman (1973). IL 1180 was then derived as follows:

5 ml citrated lion blood i.p. into 5 rats

↓ 23 days

STIB 212

↓ Mice 3 days

↓ Mice 8 days

IL 20 E8

↓ Irradiated mice (500 rad) 10 days

↓ Cloned irradiated mice (500 rad) 11 days

↓ Irradiated mice (500 rad) 5 days

IL 933

↓ Irradiated mice 3 days

↓ Cloned irradiated mice (500 rad) 11 days

IL 968

↓ Irradiated mice (600 rad) 7 days

* IL 1180 *

5. Preparation of cultured IL 1180 metacyclics:

Cultured IL 1180 metacyclics were kindly supplied by Dr. H. Hirumi. These were cultured as described by Hirumi, Hirumi and Moloo (1982) and separated from the culture fluid according to the method of Lanham and Godfrey (1970) using DEAE cellulose chromatography (DE52/

(DE52, Watman Ltd.). Pre-swollen DEAE cellulose (diethylaminoethyl cellulose) was equilibrated in PBS (phosphate buffered saline) and the pH adjusted to pH 8.0 with orthophosphoric acid. A glass funnel was then filled with the suspension and ice cold phosphate buffered glucose saline (PBGS) pH 8.0 used to bed down the cellulose. The culture medium was layered on top of the cellulose bed and allowed to adsorb on to the column. The metacyclic trypanosomes were washed free from the column with ice cold PBGS, collected and concentrated in a temperature controlled centrifuge at 2000 r.p.m. for 20 min at 4°C. The resulting pellet was washed 3 times in ice cold PBGS.

The final number of metacyclic trypanosomes/ml was determined using an improved Neubauer haemocytometer (Hawksley and Sons Ltd., London) and the concentration adjusted to the required amount using PBGS.

6. Enumeration of Trypanosomes

Two basic methods were used to determine the number of trypanosomes per ml:

(a) Neubauer Haemocytometer. This method was used to determine the number of cultured metacyclics per ml in order to carry out antibody detection tests. The method was essentially that described by Lumsden, Herbert and McNeillage (1973).

(b) Dark Ground Microscopy of the Buffy Coat Border. This method was used to determine the level of parasitaemia in the blood of infected cattle. A full description of this technique is given later (16. Parasitaemia).

7. Drugs:

The drug used in these experiments was Isometamidium chloride (Samorin, May and Baker, England; Lot DP 3947). This was freshly prepared as a 2% w/v solution in sterile distilled water and used at a dose rate of 1 mg/kg body weight.

8. Treatment of Cattle:

Twentyfour of the 34 cattle were treated with isometamidium chloride (Samorin, May and Baker Ltd.). The drug was administered by deep intramuscular injection into the middle third of the side of the neck, as recommended by the manufacturers. An 18 gauge $1\frac{1}{2}$ inch needle was used and care taken that none of the drug was injected subcutaneously. The treated animals were divided into six groups of 4 animals each, designated A - F. The drug treatment was carried out on 20/12/83. The remaining untreated animals were used as challenge controls.

9. Infection:

Adult East African x Galla goats were injected intramuscularly with Trypanosoma congolense IL 1180. The goats were bled from an ear vein daily (except for Sundays) and thin blood films and the buffy coat were examined for parasites using phase contrast microscopy. When the infection had become patent, teneral male Glossina morsitans centralis were fed on clipped flanks of the infected goats every second day over a period of 25 days. Thereafter the tsetse were allowed to probe on to warm slides at $37^{o}C$ and the saliva examined for infective metacyclic trypanosomes by phase contrast microscopy. Those with positive salivary probes were used to challenge the cattle, 5 infected flies per animal.

For/

For cyclical challenge, cattle were clipped on the flank and localised areas (2 cm square) for tsetse fly feeds were shaved closely with a scalpel blade using no soap, etc. so that the tsetse flies were not discouraged from feeding. Single infected tsetse flies in Geigy 1 cages were placed on each shaved site and the tsetse allowed to feed until the fly had taken a full blood meal, usually one to two minutes. The point of entry of the proboscis (bite site) was marked in each case. Infected tsetse were only used once in order that their infections had not been influenced by feeding on a Samorin-treated animal.

10. Infectivity of in vitro derived metacyclic trypanosomes

The infectivity of cultured trypanosomes was determined in mice. Tenfold dilutions of trypanosomes ranging from 10^6/ml to 10^1/ml were prepared in culture medium (Hirumi, Hirumi and Moloo, 1982). Six groups of five mice were inoculated intraperitoneally, each with 0.1 ml of the appropriate dilution. The mice were monitored for 30 days for parasitaemia.

11. Intradermal Challenge with IL 1180 Metacyclics:

Intradermal inoculation of metacyclic trypanosomes was carried out using a 25 gauge 5/8 inch needle to inject a volume of 0.1 ml at each site.

12. Skin Thickness:

Daily measurements of skin thickness were carried out using vernier callipers up until day 20. Thereafter they were measured every two to three days until day 28. The skin at the marked sites was first palpated to detect any small nodules then held with/

with the fingers of one hand while the thickness was measured (Emery and Moloo, 1980).

The mean skin thickness of the bite/inoculation sites on each animal was obtained. With the exception of the challenge controls, the mean of these results for the animals in a group was then calculated and presented as the pooled mean, along with the pooled mean standard error.

13. Draining Lymph Node:

The size of the draining lymph node (pre-femoral) was measured by the use of vernier callipers at the same time as the skin thickness was measured. Both width and length were measured (Emery and Moloo, 1980). With the exception of the challenge controls, the mean width and standard error of the lymph nodes for each group of animals was calculated.

14. Blood Samples:

Jugular blood samples were collected into 10 ml heparin treated vacutainer tubes. This was carried out daily from day 0 - 20 and every 2 - 3 days thereafter until 30 days post-challenge.

All cattle were bled once per week into 10 ml plain vacutainer tubes in order that serum could be collected. Serum was separated after leaving the blood samples at room temperature for 3 - 4 hours and stored at $-80^{\circ}C$ until needed.

15. Packed Cell Volume:

This was measured using a microhaematocrit centrifuge (Hawksley, England). Care was taken to ensure that the heparinised blood samples were well mixed prior to filling the capillary tube by placing/

placing the blood samples on a rotary mixer. The microhaematocrit tubes were filled with 70 µl of blood and centrifuged for 5 minutes before reading the PCV. With the exception of the challenge controls, the mean PCV of each group of animals was calculated.

16. Parasitaemia:

Heparinised blood samples were tested for the presence of parasites by examination of the buffy coat by phase contrast microscopy (Murray et al., 1977) following measurement of the haematocrit. The capillary tube was cut with a diamond pointed pen 1 mm below the buffy coat in order to incorporate the uppermost layer of erythrocytes. Using a capillary tube holder the erythrocytes, buffy coat and 1 mm of the plasma were gently expressed on to a clean slide, mixed and covered with a 22 x 22 mm coverslip. The preparations were examined using a Leitz SM microscope with a combination of Phaco 2 NPL 25/0.50 objectives, a Zernicke 204 condenser and Periplan NF 10x eyepiece (Leitz Wetzlar, Germany). The level of parasitaemia was estimated as described by Paris et al. (1982). The system used and its relationship to the number of trypanosomes are shown below:

Phase contrast buffy coat parasitaemia scoring system

Score	Tryps/field	Estimated Tryps / ml
6+	> 100	$> 5 \times 10^6$
5+	> 10	$> 5 \times 10^5$
4+	1 - 10	$10^4 - 5 \times 10^5$
3+	1/2f - 1/10f	$5 \times 10^4 - 5 \times 10^5$
2+	1 - 10/prep	$10^3 - 10^4$
1+	1/prep	$10^2 - 10^3$

17. Test for Low Parasitaemia by Subinoculation:

This procedure was carried out by the subinoculation of 0.2 ml heparinised blood intraperitoneally into each of 4 AJ mice from each steer which failed to show a parasitaemia after challenge. The blood sample used was taken between 25 and 28 days post-challenge and was inoculated within 2 hours. These mice were then monitored for a 30 day period to detect any developing parasitaemia.

18. Test for Samorin Sensitivity:

Blood samples for drug sensitivity tests were taken from each bovine which developed a patent parasitaemia, and subinoculated into mice. Twenty mice were infected with blood from each bovine and each mouse received 0.1 ml by intraperitoneal injection. When the mice developed a parasitaemia of approximately 1×10^7 trypanosomes/ml, they were divided into four groups of five mice. Three groups were treated by intraperitoneal injection of Samorin at doses of 1, 2 or 4 mg/kg body weight. The remaining group remained untreated.

19. Antibody Tests:

Two tests were used in an attempt to demonstrate the development of specific antibody against T. congolense ILRAD 1180 (ILNat 3.1). These were:

(A) Infectivity neutralisation test against cultured metacyclic trypanosomes; and

(B) Immune lysis test against cultured metacyclic trypanosomes.

(A) Infectivity neutralisation test

In vitro derived ILNat 3.1 metacyclic trypanosomes were centrifuged/

centrifuged three times in phosphate saline glucose (PSG) before being finally resuspended in PSG with 10% foetal calf serum, to give a final concentration of 2 x 10^4 trypanosomes per ml.

Three hundred µl of the serum to be tested was placed in an Eppendorf micro test tube and 300 µl of the trypanosome suspension added. This was gently mixed before finally being incubated at $4^{\circ}C$ for one hour. Each of 4 female AJ mice was injected with 0.1 ml of the above suspension. These mice were then monitored twice weekly for 30 days to determine whether or not they developed a parasitaemia. If they were still negative at the end of this period, it was assumed that specific antitrypanosome antibody had been present.

(B) Immune lysis test

In vitro-derived ILNat 3.1 metacyclic trypanosomes were centrifuged twice in PSG and once in phsophate buffered saline (PBS) before diluting to 1 x 10^7 per ml. Ten µl of this suspension was added to 100 µl fresh guinea pig serum. To 20 µl aliquots of this suspension 5 µl of test serum was added. This was incubated at room temperature for 1 hour before being checked under a microscope to determine the degree of lysis.

In Phase II the sera were diluted (1:5, 1:25 and 1:125) prior to the addition of the trypanosome suspension. The endpoint with a positive sera was taken as the dilution of sera (expressed as the reciprocal) at which over 90% of the organisms were lysed.

Plasma/

Plasma from an uninfected isomatamidium-treated steer served as a drug control for the antibody assays.

During Phase I of the experiment, pooled plasma samples from the animals in groups A to F (see experimental design) were analysed at each time point, whereas individual plasma was used for challenge controls. In Phase II the sera of all the cattle which developed parasitaemias were analysed individually, whereas only grouped sera pools were used for resistant cattle.

PHASE I

Experimental Design:

The twenty-four cattle treated as described in the Materials and Methods were divided into six groups of four animals each, designated groups A, B, C, D, E and F. A further five untreated animals were used as challenge controls and these constituted group G.

Following drug treatment on 20/12/83 the cattle were subjected to cyclical challenge by five infected Glossina morsitans centralis tsetse flies, according to the protocol shown in Table 1.1.

Following challenge, the animals were subjected to various clinical measurements as described in the Materials and Methods. Thus daily measurements of skin thickness, draining lymph node size, PCV and parasitaemia were carried out. Tests were also conducted for the detection of low level parasitaemias and the development of specific anti-trypanosomal antibodies in the challenged cattle.

Results:

The results of this experiment can be summarised by considering all the cattle as belonging to two categories. The first consisted of all the animals which were treated with Samorin at 1 mg/kg on 20/12/83 and were subsequently challenged. None of these animals developed any local skin reactions (chancres) or became parasitaemic. Thus, under the experimental conditions described, Samorin protected against cyclical infection for a period not less than 148 days or approximately 5 months.

The/

TABLE 1.1: Duration of prophylaxis against monthly challenge with tsetse-transmitted T. congolense IL 1180 in Boran cattle treated with Samorin 1-5 months after treatments: Experimental design.

Challenge Date			16/1	16/2	16/3	16/4	16/5
Months post-Samorin			1	2	3	4	5
	No.	Treatment					
Group A	(4)	Treated	4	4*	4*	4*	4*
B	(4)	"	4	-	-	-	-
C	(4)	"	-	4	-	-	-
D	(4)	"	-	-	4	-	-
E	(4)	"	-	-	-	4	-
F	(4)	"	-	-	-	-	4
G	(5)	Untreated	1	1	1	1	1

* = group rechallenged

\- = group not challenged

Group G consisted of untreated challenge controls

The second category consisting of the challenge control animals, one of which was subjected to challenge at each time point, all developed five chancres, became parasitaemic, demonstrated an increase in the draining lymph node size and showed an eventual decrease in their PCV.

Month 1:

The results following cyclical challenge on 16/1/84 are presented in Fig. 1.1.

Skin thickness

Groups A and B did not show any increase in the skin thickness at the site of infected tsetse fly bites. With group A the pooled mean skin thickness varied between 1.22 cm and 1.35 cm, while group B varied between 1.34 cm and 1.55 cm. The challenge control (G1), however, demonstrated an increase from 1.16 cm to a peak of 1.78 cm on day 14, an increase of 57%.

Lymph node size

Groups A and B did not show any significant increase in the draining lymph node size. The mean lymph node size for the four animals in group A varied randomly between 1.53 cm and 1.85 cm, while that of group B varied between 1.5 cm and 1.9 cm. The challenge control (G1), however, increased from an initial minimum of 1.3 cm to a maximum of 4.8 cm 15 days post-challenge, an increase of 300%.

PCV

Groups A and B showed a slight decrease in the PCV values over the 28 days monitoring period. Group A decreased from a mean/

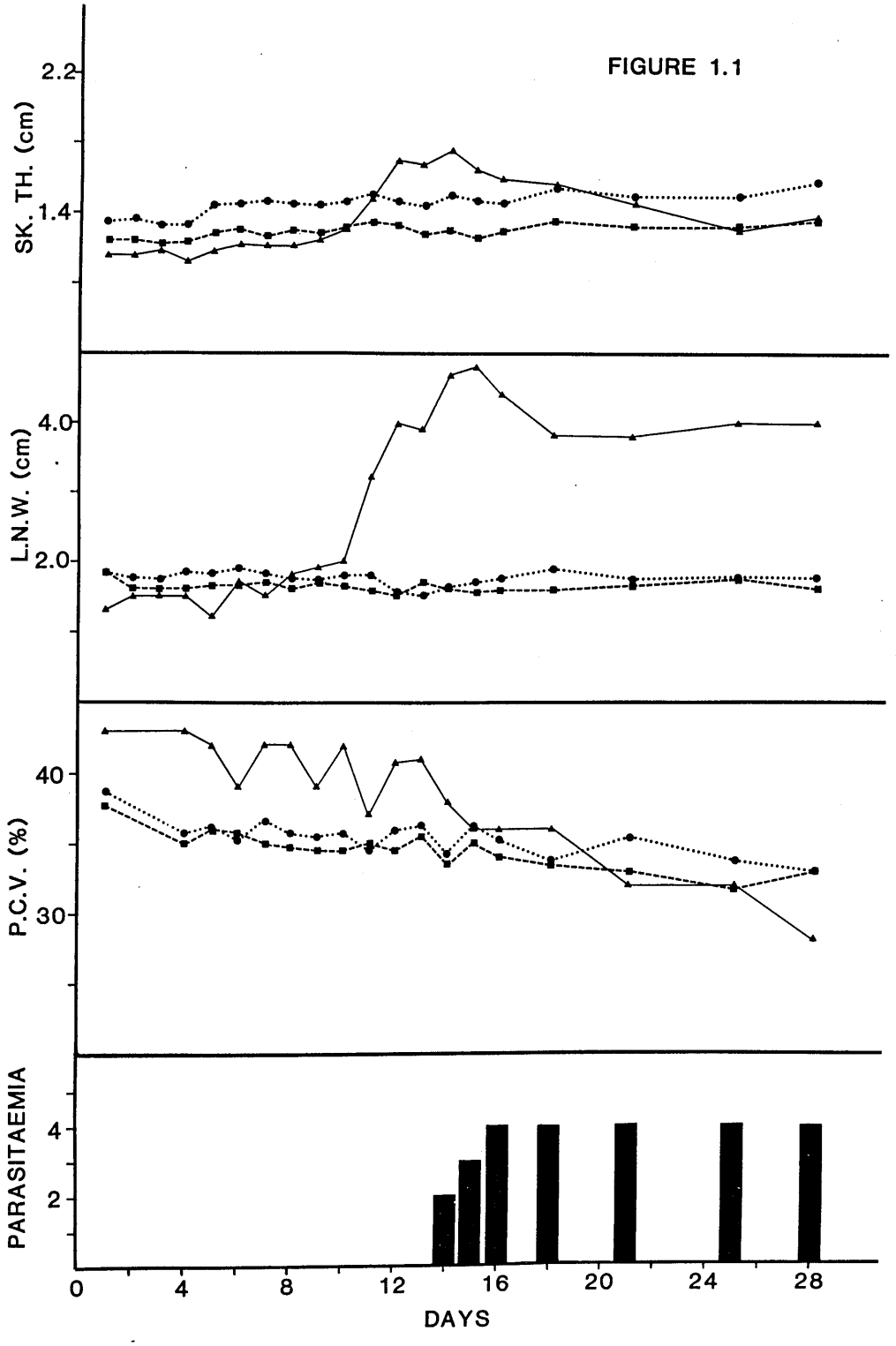


Figure 1.1: Fly challenge 1 month post-Samorin treatment. Change in skin thickness at tsetse bite sites (SK.TH.); width of draining lymph node (L.N.W.); change in packed red cell volume (P.C.V.); and the development of parasitaemia in the challenge control.

▲——▲ Challenge control; (G1) ■- - - -■ Group A; ●·····● Group B.

mean of 37.75% to 33.0%, while group B decreased from 38.75% to 33.0%. The challenge control (G1), however, demonstrated a large decrease in PCV from 43% to 28%. Thus the PCV decreased 37% from the initial value.

Parasitaemia

Only the challenge control (G1) developed a patent parasitaemia. This was first detected 14 days after challenge and reached its peak on day 16.

Month 2:

The results following cyclical challenge on 16/2/84 are presented in Fig. 1.2.

Skin thickness

Groups A and C did not show any significant increase in skin thickenss following infected tsetse fly bites. The pooled mean skin thickness from group A ranged between 1.36 and 1.52 cm, while group C ranged between 1.13 and 1.31 cm. The challenge control (G2), however, showed a large increase in the pooled mean skin thickness from 1.07 to 2.27 cm, an increase of 112%. This peak measurement occurred 13 days post-challenge.

Lymph node size

Groups A and C failed to show any increase in the breadth of the draining lymph node. Group A varied between 1.55 cm and 2.0 cm, while group C varied between 1.55 cm and 2.05 cm. In comparison, the challenge control increased from 1.8 cm to 5.0 cm, an increase of 178%. This peak was achieved 14 days post-challenge.

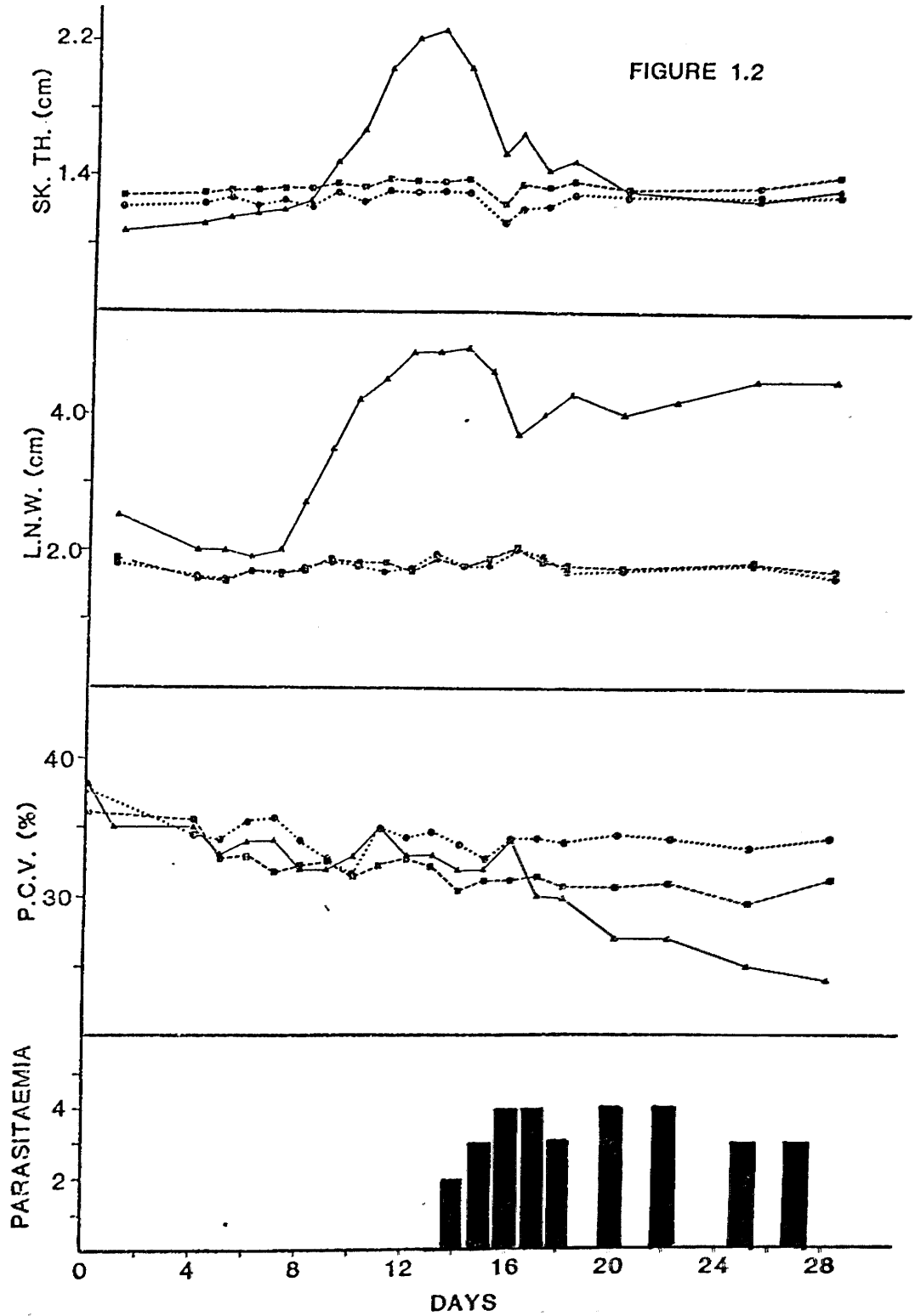


Figure 1.2: Fly challenge 2 months post-Samorin treatment. Change in skin thickness at tsetse bit sites (SK.TH.); width of draining lymph node (L.N.W.); change in packed red cell volume (P.C.V.); and the development of parasitaemia in the challenge control.

▲——▲ Challenge control; ■----■ Group A;
●.....● Group C

PCV

Groups A and C showed variation in the PCV measurement throughout the 28 day measurement period. Group A varied between 36% and 29.5%, while group C, on the other hand, varied between 37.75% and 31.75%. The challenge control (G2) however, fell from an initial reading of 38% to a minimum of 24% on day 28. Thus the PCV of the challenge control decreased by 36% over the experimental period.

Parasitaemia

Only the challenge control (G2) developed a patent parasitaemia. This was first detected 14 days after challenge and reached a peak on day 16.

Month 3:

The results following cyclical challenge on 16/3/84 are presented in Fig. 1.3.

Skin thickness

Groups A and D did not show any significant increase in the skin thickness at the five bite sites over the 28 day measurement period. The pooled mean skin thickness of group A ranged between 1.21 cm and 1.35 cm, while group D varied between 1.13 cm and 1.30 cm. In comparison, the challenge control (G3) rose from a mean of 0.97 cm to a peak of 1.97 cm by 12 days post-challenge, an increase of 103%.

Lymph node size

Groups A and D showed no significant change in the size of the draining lymph node following challenge. Group A measured between

1.48/

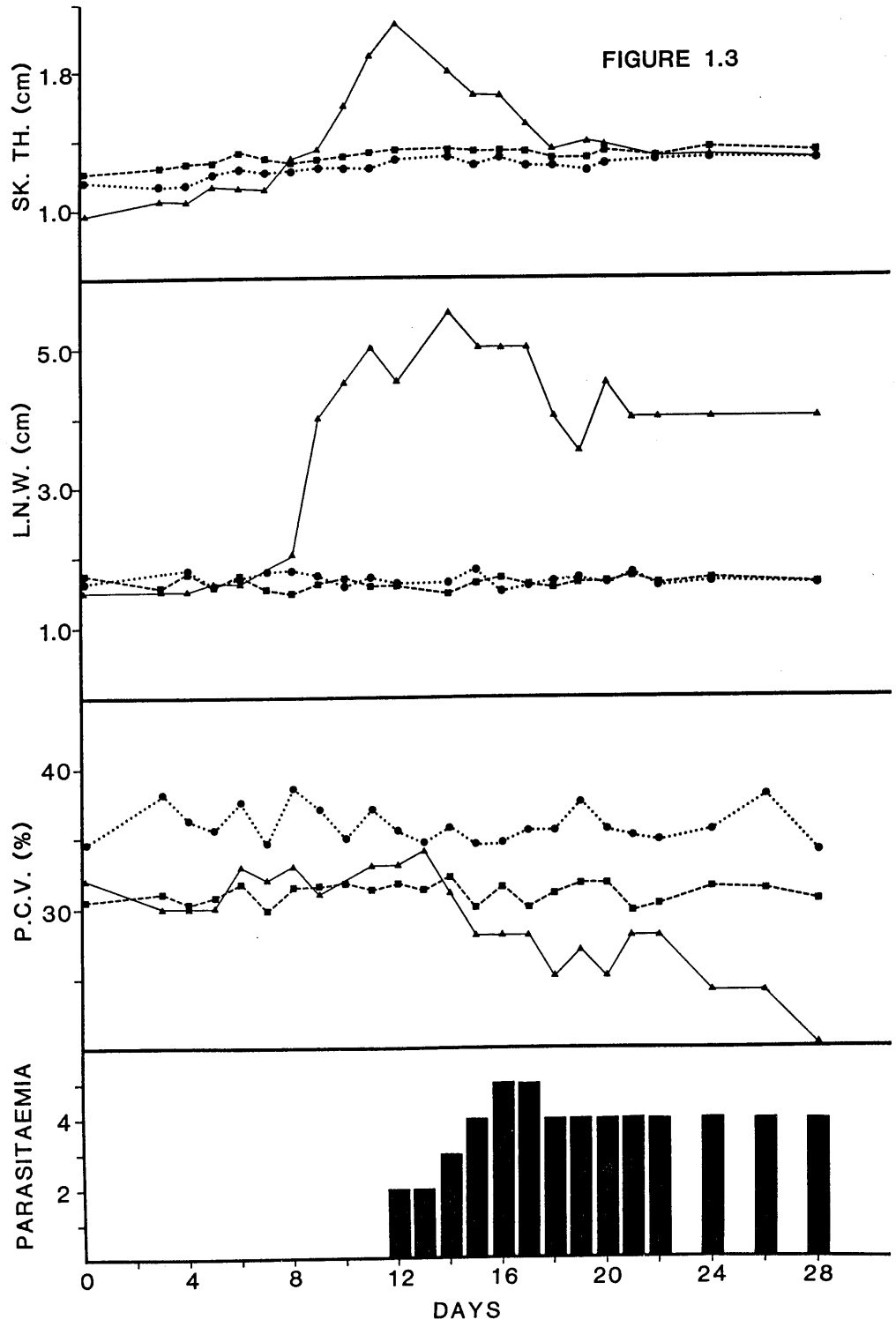


Figure 1.3: Fly challenge 3 months post-Samorin treatment. Change in skin thickness at tsetse bite site (SK.TH.); width of draining lymph node (L.N.W.); change in packed red cell volume (P.C.V.); and the development of parasitaemia in the challenge control.

▲——▲ Challenge control; ■- - - -■ Group A; ●·····● Group D.
(G3)

1.48 cm and 1.75 cm, while group D varied between 1.50 cm and 1.83 cm. Compared to these measurements, the challenge control (G3) showed a 266% increase in width from 1.5 cm to 5.0 cm.

PCV

Groups A and D showed an intermittent variation in the PCV measurements throughout the 28 day measurement period. Group A varied between 31.75% and 29.75%, while group D varied between 38.5% and 34.0%. The challenge control (G3) showed a significant decrease from 32.0% to 20%, a decrease of 44%.

Parasitaemia

Only the challenge control (G3) developed a patent parasitaemia. This was first detected 12 days after challenge and a peak value was reached on day 16.

Month 4:

The results following cyclical challenge on 16/4/84 are presented in Fig. 1.4.

Skin thickness

Groups A and E showed no significant change throughout the 28 day measurement period. With group A the pooled mean skin thickness varied between 1.06 cm and 1.20 cm, while group E varied between 1.21 cm and 1.38 cm. The challenge control (G4) however, demonstrated an 80% increase in the mean skin thickness measurement at the five tsetse bite sites, rising from 1.25 cm to 2.19 cm.

Lymph node size

Again groups A and E did not show any significant change in the size of the draining lymph node throughout the measuring period. The/

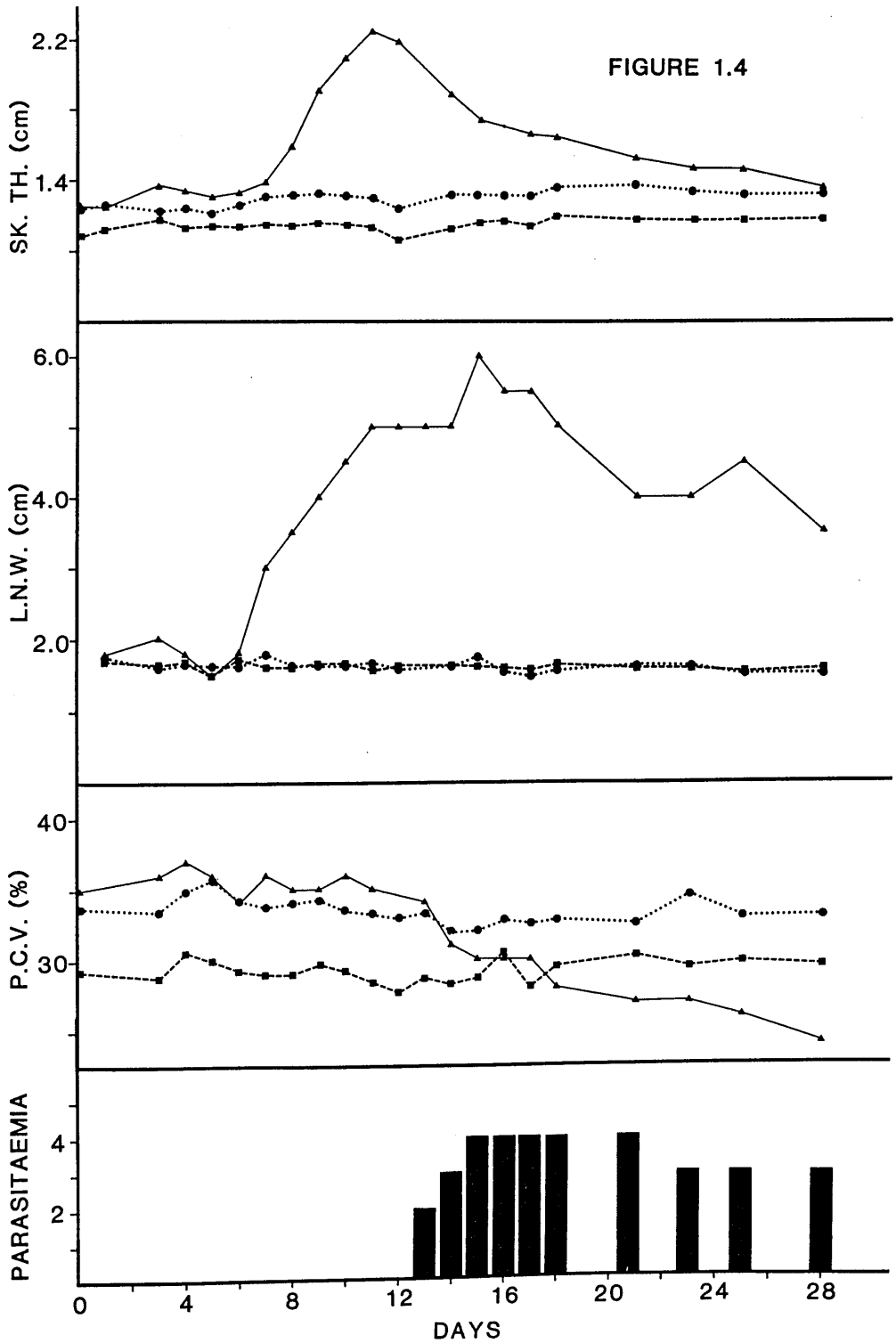


Figure 1.4: Fly challenge 4 months post-Samorin treatment. Change in skin thickness at tsetse bite sites (SK.TH.); width of draining lymph nodes (L.N.W.); change in packed red cell volume (P.C.V.); and the development of parasitaemia in the challenge control.

▲——▲ Challenge control; ■- - - -■ Group A; ●·····● Group B.

(G4)

The mean lymph node width for the four animals in group A varied between 1.5 cm and 1.7 cm, while group E varied between 1.48 cm and 1.8 cm. The challenge control, however, rose from an initial minimum of 1.5 cm to a peak value of 6.0 cm, a rise of 300%. This peak was achieved 15 days post-challenge.

PCV

Groups A and E showed no significant change throughout the monitoring period. Group A varied between 30.5% and 28.0%, while group E varied between 35.75% and 32.0%. The challenge control (G4), however, showed a 35% decrease in PCV over the 28 day monitoring period, decreasing from 37% to 24%.

Parasitaemia

As in the previous three months, only the challenge control (G4) demonstrated a patent parasitaemia. This first appeared on day 13 and reached a peak 2 days later on day 15.

Month 5:

The results following cyclical challenge on 16/5/84 are presented in Fig. 1.5.

Skin thickness

Groups A and F showed no change in the skin thickness at the sites of infected tsetse fly bites over the monitoring period. With group A the pooled mean skin thickness varied between 0.94 cm and 1.11 cm, while group F varied between 1.06 cm and 1.28 cm. The challenge control (G5) however, demonstrated an increase from 1.43 cm to 2.0 cm, a relative increase of 40%.

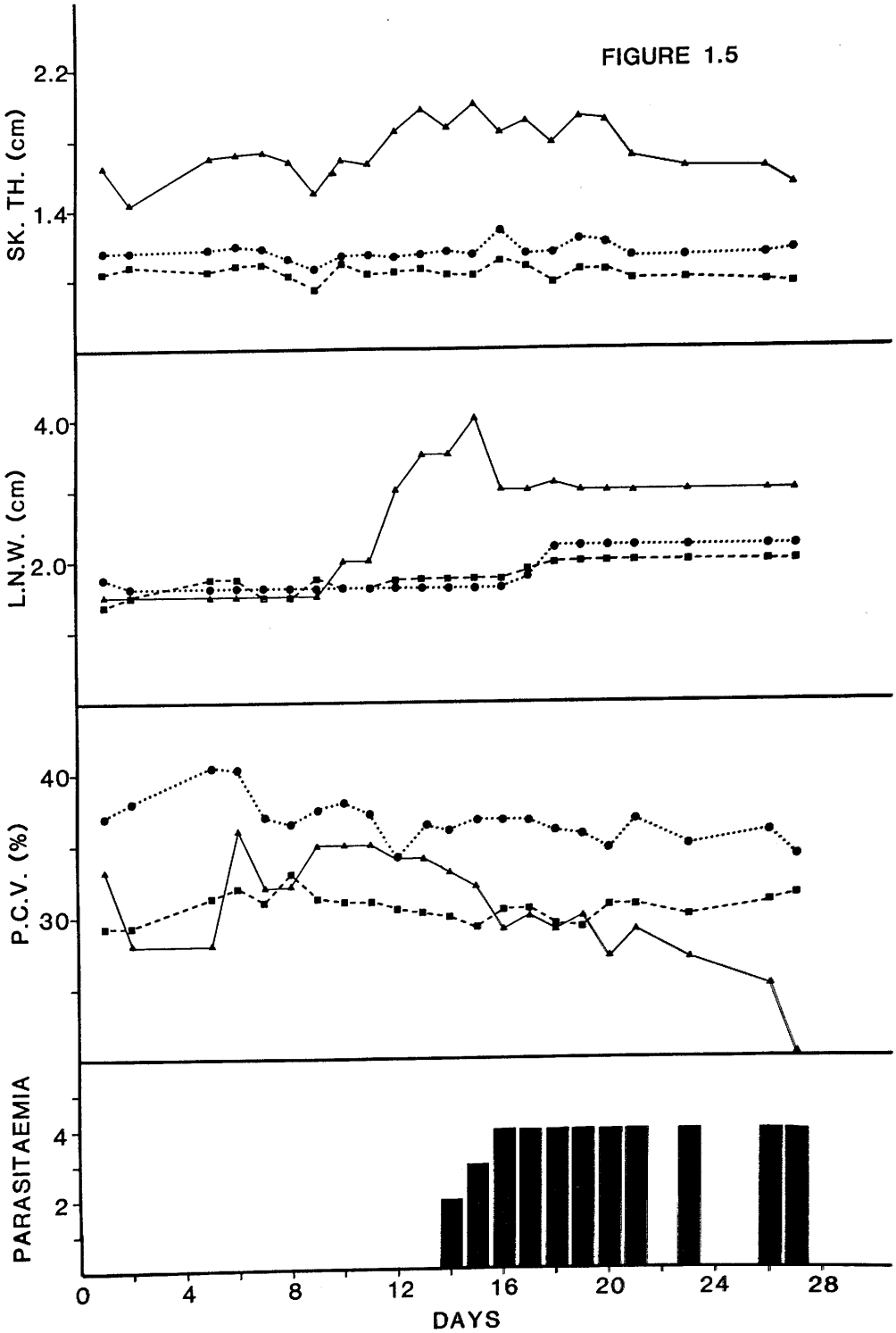


Figure 1.5: Fly challenge 5 months post-Samorin treatment. Change in skin thickness at tsetse bite sites (SK.TH.); width of draining lymph nodes (L.N.W.); change in packed red cell volume (P.C.V.); and the development of parasitaemia in the challenge control.

▲——▲ Challenge control; (G5) ■- - - -■ Group A; ●·····● Group F.

Lymph node size

Again the results mimic those seen in the previous four months. Groups A and F demonstrated no significant increase in the lymph node width over the monitoring period. Group A's measurements varied between 1.38 cm and 2.0 cm, while group F varied between 1.63 cm and 2.20 cm. The challenge control (G5), however, showed a significant increase from a minimum of 1.5 cm to a maximum of 4.0 cm. This increase of 166% was achieved 15 days post-challenge.

PCV

Once again only the challenge control showed any significant decrease in PCV value over the 28 day monitoring period. The PCV of group A varied between 29.25% and 33.0%, while that of group F varied between 34.0% and 40.5%. The challenge control (G5) from group G, however, showed a relative decrease of 44%, from 36% to 20%.

Parasitaemia

As with all the previous months, only the challenge control (G5) demonstrated a patent parasitaemia. This was first detected 14 days after challenge and a peak value was reached 2 days later.

Test for Low Parasitaemia by Sub-inoculation

These tests were carried out to determine whether a subpatent parasitaemia was present in those animals which failed to exhibit a patent infection. Between 25 and 28 days post-challenge 0.2 ml of blood was subinoculated into each of 4 female AJ mice from every animal which had failed to demonstrate a patent parasitaemia.

These/

These tests failed to demonstrate any subpatent parastiaemia in any animal.

Antibody Tests:

As described in the Materials and Methods, these were carried out by two methods, an infectivity neutralisation test and an immune lysis test. The results obtained from 16/1/84 to 11/6/84 are presented in Tables 1.2 and 1.3. These results can be summarised as follows: No antibody was detected in any of the animals in groups A, B, C, D, E and F. Antibody was detected in each of the challenge controls (group G), developing between 2 and 4 weeks post-challenge and coinciding with the development of a patent parasitaemia.

Sensitivity to Isometamidium:

Trypanosomes from all five control animals which developed bloodstream parasitaemias after challenge were screened for sensitivity to isometamidium in mice. All were completely sensitive to isometamidium at doses of 0.5 mg/kg.

TABLE 1.2: Infectivity neutralisation test against in vitro-derived metacyclics of ILNAT 3.1
(Summary of Results)

Date of serum sample	Group A	Group B	Challenge Control G1	Group C	Challenge Control G2	Group D	Challenge Control G3	Group E	Challenge Control G4	Group F	Challenge Control G5
16/1	*-	*-	*-								
30/1	-	-	-								
13/2	-	-	+								
16/2	*-	ND	ND	*-	*-						
20/2	-	-	ND	-	-						
27/2	-	ND	ND	-	-						
5/3	-	-	ND	-	-						
12/3	-	ND	ND	-	+						
19/3	*-	-	ND	-	+	*-	*-				
2/4	-	ND	ND	-	+	-	-				
9/4	ND	ND	ND	-	+	ND	ND				
16/4	*-	ND	ND	ND	ND	-	±	*-	*-		
30/4	-	ND	ND	ND	ND	-	+	-	-		
7/5	-	ND	ND	ND	ND	-	+	-	+		
16/5	*-	ND	ND	ND	ND	ND	ND	-	±	*-	*-
21/5	-	ND	ND	ND	ND	ND	ND	-	±	-	-
28/5	-	ND	ND	ND	ND	ND	ND	-	+	-	-
4/6	-	ND	ND	ND	ND	ND	ND	-	+	-	+
11/6	-	ND	ND	ND	ND	ND	ND	-	+	-	+

* = Day of Challenge ND = Sample not tested - therefore no data

- = absence of infectivity neutralising antibody + = presence of infectivity neutralising antibody

TABLE 1.3: Immune lysis test against in vitro-derived metacyclics of ILNAT 3.1
(Summary of Results)

Date of serum sample	Group A	Group C	Challenge Control G2	Group D	Challenge Control G3	Group E	Challenge Control G4	Group F	Challenge Control G5
16/2	* 0	* 0	* 0						
27/2	0	0	0						
12/3	0	0	50 **						
19/3	* 0	0	40	* 0	* 0				
26/3	0	0	20	0	0				
2/4	0	0	50	0	60				
9/4	0	0	40	0	80				
16/4	* 0	ND	ND	0	80	* 0	* 0		
24/4	0	ND	ND	0	90	0	0		
30/4	0	ND	ND	0	80	0	50		
7/5	0	ND	ND	0	50	0	90		
16/5	* 0	ND	ND	ND	ND	0	100	* 0	* 0
21/5	0	ND	ND	ND	ND	0	90	0	0
28/5	0	ND	ND	ND	ND	0	90	0	50
4/6	0	ND	ND	ND	ND	0	100	0	100
11/6	0	ND	ND	ND	ND	0	90	0	100

* Day of Challenge

** % lysis

Results from Group B + G, not shown

TABLE 1.4: Phase II: Reallocation of Boran cattle six months after Samorin treatment and exposure to varying levels of challenge with metacyclic T. congolense IL 1180.

Reallocated Group Phase II	Cattle number from Phase I				Challenge Controls	Challenge
1	A_1	A_2	A_3	A_4	G6	5 tsetse daily for 10 days
2	B_1	B_2	B_3	B_4	G7	5 tsetse on 1 day
3	C_1	D_1	E_1	F_1	G8	5×10^2 metacyclics
4	C_2	D_2	E_2	F_2	G9, G10	5×10^3 metacyclics
5	C_3	D_3	E_3	F_3		5×10^4 metacyclics
6	C_4	D_4	E_4	F_4		5×10^5 metacyclics

PHASE II

Experimental Design:

Following the failure of any of the animals treated with Samorin to develop a patent parasitaemia or detectable antibody responses following monthly challenge between 1 and 5 months post-treatment, the animals were reallocated into new groups and, along with new challenge controls (G6 - G10), were exposed to varying levels of metacyclic challenge at six months post-treatment. The allocation of the animals into the new groups is outlined in Table 1.4.

Five different levels of challenge were given: namely, five infected tsetse flies daily for 10 days; five infected flies on a single occasion; or 500, 5,000, 50,000, or 500,000 metacyclic trypanosomes by intradermal inoculation.

This challenge took place on 18/6/84 or 6 months after initial Samorin treatment and, in the case of group 1, occurred daily for ten days. Blood samples were collected regularly after challenge for PCV determination and parasite detection. Serum samples were collected once per week for antibody measurement. Due to the large number of animals involved and a lack of technical assistance, chancre and lymph node measurements could not be carried out daily.

Results:

Parasitaemia

The results obtained from Phase II of the study are presented in Table 1.5. All five challenge control animals became infected between/

TABLE 1.5: Result of challenge six months after Samorin treatment

Cattle Group	Treated Cattle				Controls
Group 1	R	23	R	R	14
Group 2	R	R	23	R	13
Group 3	16	20	R	R	14
Group 4	12	R	R	R	12, 14
Group 5	29	R	21	R	
Group 6	R	R	22	R	

The pattern in the grid corresponds to that in Table 1.4.

Number = day on which particular animal became parasitaemic

R = animal resistant to challenge

between 12 and 14 days post-challenge. Eight of the 24 Samorin-treated cattle became infected between 12 and 29 days post-challenge. However, there was no correlation between the animals which became parasitaemic and either weight or method of challenge (tsetse transmitted or cultured metacyclics) or the previous challenge history of the cattle, with the possible exception of animals from Group F in phase I which all resisted the six months challenge.

All breakthrough populations of trypanosomes were completely susceptible to Samorin when tested in mice.

Chancre development and lymph node changes

In this phase of the experiment it was not possible, for logistical reasons, to measure the skin thickness and lymph node changes on a daily basis. Nevertheless, only the animals which developed patent parasitaemias showed marked increases in skin thickness at the bite or inoculation site, and draining lymph node (Table 1.6). None of the Samorin-treated animals which were resistant to challenge showed any obvious increase in skin thickness or lymph node size.

Haematocrit changes

For logistical reasons PCV measurements were in general, terminated early. Thus results obtained are equivocal in nature (Table 1.7). The recorded PCV decreases are very low in both the challenge control group and those which developed a parasitaemia. Only in the cases of C1 and C2 were the measurements continued for a long enough period to give a significant decrease in PCV readings.

All the animals resistant to infection were measured for 39 days following challenge. Challenge controls were measured for/

TABLE 1.6. The number of bite or inoculation sites on each animal, the number which developed chancres and the prepatent period of cattle which became parasitaemic.

Group	Animal	No. of bite/ inoculation sites	No. of chancres	Prepatent period
1	A1	50	0	-
	A2	50	0	23
	A3	50	0	-
	A4	50	0	-
	G6	50	30	14
2	B1	5	0	-
	B2	5	0	-
	B3	5	3	23
	B4	5	0	-
	G7	5	5	13
3	C1	4	3	16
	D1	4	3	20
	E1	4	0	-
	F1	4	0	-
	G8	4	0	14
4	C2	6	6	12
	D2	6	0	-
	E2	6	0	-
	F2	6	0	-
	G9	6	5	12
	G12	6	6	12
5	C3	6	5	29
	D3	6	0	-
	E3	6	5	21
	F3	6	0	-
6	C4	6	0	-
	D4	6	0	-
	E4	6	5	22
	F4	6	0	-

TABLE 1.7. Changes in haematocrit values following challenge at month 6 (PCV as %)

	Test.Group	Days Following Challenge						Prepatent Period
		7	14	18	22	26	30	
Challenge Controls	Gp 1 (G6)	30	32	26				14
	Gp 2 (G7)	36	36	32				13
	Gp 3 (G8)	25	28	22				14
	Gp 4 (G9)	33	31	31				12
	Gp 4 (G10)	33	34	30				12
Resistant to Challenge	No change over 30 day period							
Susceptible to Challenge	Gp 1 (B3)	33	32	35	31	31	28	23
	Gp 2 (A2)	31	34	34	36	35	31	23
	Gp 3 (C1)	36	35	30	30	27	26	16
	Gp 4 (C2)	33	35	31	32	27	25	12
	Gp 5 (E3)	39	39	37	40	39	40	21
	Gp 6 (E4)	34	35	36	34	30	34	22

for 5 to 7 days after a parasitaemia was first detected. All the susceptible animals with the exception of C1 and C2 had measurements terminated seven days after the onset of parasitaemia.

Antibody detection

Infectivity neutralisation and immune lysis tests demonstrated the presence of antibody in all the animals which developed patent parasitaemias, at 2 - 4 weeks after challenge (Tables 1.8 and 1.9). In contrast, no antibodies could be detected in the Samorin-treated animals which resisted challenge.

Sensitivity to isometamidium

Trypanosomes from all five control cattle and the eight drug-treated cattle which developed a patent parasitaemia after challenge, were screened for sensitivity to isometamidium in mice. All were completely sensitive at doses of 0.5 mg/kg.

TABLE 1.8. Neutralisation test results against in vitro-derived metacyclic forms of Trypanosoma congolense following challenge at month 6.

	Test Group	Days Following Challenge									
		0		7		14		21		28	
		1/5	1/25	1/5	1/25	1/5	1/25	1/5	1/25	1/5	1/25
Challenge Controls	Gp 1 (G6)	+	+	+	+	-	3/4-	-	3/4-	-	-
	Gp 2 (G7)	+	+	+	+	+	+	-	+	-	+
	Gp 3 (G8)	+	+	+	+	+	+	+	+	-	+
	Gp 4 (G9, G10)	+	+	+	+	-	+	3/4-	+	-	-
Resistant to Challenge	Gp 1 (B1, B2, B4)	+	+	+	+	+	+	+	+	+	+
	Gp 2 (A1, A3, A4)	+	+	+	+	+	+	+	+	+	+
	Gp 3 (E1, F1)	+	+	+	+	+	+	+	+	+	+
	Gp 4 (D2, E2, F2)	+	+	+	+	+	+	+	+	+	+
	Gp 5 (C3, D3, F3) *	+	+	+	+	+	+	+	+	1/4-	+
	Gp 6 (C4, D4, F4)	+	+	+	+	+	+	+	+	+	+
Susceptible to Challenge	Gp 1 (B3)	+	+	+	+	+	+	1/4-	+	-	+
	Gp 2 (A2)	+	+	+	+	+	+	+	+	-	+
	Gp 3 (Ca)	+	+	+	+	+	+	2/4-	1/4-	3/4-	+
	Gp 3 (D1)	+	+	+	+	+	+	-	+	-	+
	Gp 4 (C2)	+	+	+	+	-	+	-	3/4-	-	-
	Gp 6 (E3)	+	+	+	+	3/4-	+	-	+	1/4-	+
	Gp 6 (E4)	+	+	+	+	-	+	3/4-	+	-	3/4-

* = > One animal became parasitaemic on day 29 after challenge

\+ = > Recipient mice parasitaemic therefore no neutralisation

\- = > Recipient mice negative therefore nutralisation occurred.

4 mice present per group

Plasma pools were assayed for resistant animals within a group. Breakthrough and challenge controls were assayed as an individual animal plasma.

Table 1.9. Immune lysis titres against *in vitro*-derived metacyclic forms of *Trypanosoma congolense* following challenge at 6 months after treatment.

Test Group	Days after Challenge				
	0	7	14	21	28
Challenge controls					
Group 1 (G6)*	-	-	±	125	125
Group 2 (G7)	-	-	125	125	125
Group 3 (G8)	-	-	-	5	25
Group 4 (G9, G10)	-	-	±	5	25
Isometamidium-treated Cattle Resistant to Challenge					
Group 1 (B1, B2, B4)	-	-	-	-	-
Group 2 (A1, A3, A4)	-	-	-	-	-
Group 3 (E1, F1)	-	-	-	-	-
Group 4 (D2, E2, F2)	-	-	-	-	-
Group 5† (C3†, D3, F3)	-	-	-	-	±
Group 6 (C4, D4, F4)	-	-	-	-	-
Susceptible to Challenge					
Group 1 (B3)	-	-	±	5	25
Group 2 (A2)	-	-	±	5	25
Group 3 (C1)	-	-	±	5	25
Group 3 (D1)	-	-	-	5	25
Group 4 (C2)	-	-	125	125	125
Group 5 (E3)	-	-	±	5	25
Group 6 (E4)	-	-	-	-	25

* Phase I group and number of animal

† One animal became parasitaemic on day 29 after challenge. Plasma pools were assayed for the resistant animals within a group: breakthroughs and controls were assayed on individual animal plasma.

± Slight antibody response detected but 90 per cent lysis not achieved at 1.5 dilution.

DISCUSSION

The present experiment was successful in providing information on the length of prophylaxis afforded by isometamidium chloride against challenge by metacyclic forms of T. congolense IL Nat 3.1 under strictly controlled experimental conditions.

It was established that the treatment of cattle with 1 mg/kg of isometamidium chloride resulted in protection against cyclical challenge with metacyclic T. congolense IL Nat 3.1, delivered by 5 infected tsetse fly bites for at least 148 days, or approximately 5 months. In addition two thirds of the cattle (16 out of 24) successfully resisted challenge at 6 months, even when subjected to multiple fly challenge (5 infected flies daily for 10 days), or intradermal inoculation of up to 500,000 in vitro derived metacyclics. In addition the study also provides useful information on the relationship between chemoprophylaxis and the development of an immune response following challenge with T. congolense.

Only those cattle which became parasitaemic following challenge demonstrated an antibody response indicating that no antigenic priming of the immune system occured while the animals were chemoprophylactically protected by isometamidium.

The six isometamidium treated cattle which became infected following the challenge on month 6 in general demonstrated a prolonged prepatent period. This probably indicates a residual effect of the isometamidium slowing down the development of the trypanosomes at the inoculation site and so retarding the development of a patent parasitaemia.

The/

The protection achieved during this study can only be attributed to the presence of effective drug concentrations at the level of the skin and not the development of antitrypanosome antibody.

The demonstration that isometamidium can confer prophylaxis for up to 5 months demonstrates the important value of such agents. It also provides experimental confirmation of observations from the field that prophylactic cover, as judged by the development of parasitaemia, may extend to 30 weeks (Weisenhutter et al., 1968).

In 1962 Whiteside suggested that the duration of chemoprophylaxis may be directly related to the level of challenge and in this context it is of some importance to try and equate, if possible, the level of challenge in the present study with that in field situations.

Initially the cattle were subjected to a maximum of 5 infected fly bites per animal per month. Logistically any greater number of flies would have proved difficult to obtain. Exactly where this level of challenge would fit into the often discussed 'low, medium and high' levels of challenge is not clear.

Under field conditions the relationship between tsetse fly challenge and trypanosome risk are complex. Many factors determine whether an animal is likely to be bitten and subsequently become parasitaemic (Moloo, Steiger and Brian, 1973, 1980). Consequently, the efficacy of a drug like isometamidium can only be assessed by testing it against a known number of infective trypanosomes delivered at the level of the skin.

In/

In these experiments it was found that a single cultured metacyclic trypanosome was capable of infecting a mouse. Furthermore, when a tsetse fly infected with IL Nat 3.1 feeds, the average inoculum is 100 metacyclics (S.K. Mollo, unpublished data). Thus 5 infected flies per month is equivalent to challenge with 500 metacyclic trypanosomes.

In East Africa this could be expected to be the level of challenge experienced by cattle in areas of medium to high tsetse density (S.K. Mollo, personal communication).

At month six the maximum level of challenge was 5 x 10/5 in vitro-derived metacyclics which is equivalent to the bites of 5000 infected tsetse flies, a one thousand fold increase to the previous challenge level. However three out of four cattle in group six which were challenged by this number still resisted infection.

In a previous study (Kirkby, 1964), in an area designated 'high challenge', the infection rate of the wild tsetse fly population was assessed to be 11%. Assuming that all of these flies were infective to challenge the cattle with 5 x 10/5 metacyclics would be equivalent to an attack of 45000 tsetse flies per month or 215 per day.

Nevertheless it is likely that there are differences in susceptibility between animals exposed to a high number of metacyclic trypanosomes on a single occasion, as in most groups in this experiment, and cattle exposed in the field where challenge probably occurs more frequently at a lower intensity.

This/

This study not only provides information on the length of prophylaxis afforded by isometamidium but also illustrates the relationship between chemoprophylaxis and the development of an immune response. As has been previously stated no detectable antibody response occured in cattle which failed to become infected after challenge, indicating that priming of the immune system failed to occur. This would indicate that the development and multiplication of metacyclic trypanosomes was arrested at the level of the skin, that is at the site of inoculation. This is supported by studies using irradiated trypanosomes (Morrison et al., 1982b). They demonstrated that 10^7 irradiated bloodstream trypanosomes were required to elicit an antibody response. A similar number is also required to induce a detectable skin response (Dwinger, personal communication).

The fact that priming of the immune system failed to occur and the inference that immunity had no part to play in the length of prophylaxis achieved is of particular interest when considering the findings of previous research. A number of field studies by various workers have all suggested the existence of a state of non-sterile immunity following trypanocidal drug treatments.

Bevan (1928, 1936) was the first to suggest that the use of drugs could result in the development of immunity against trypanosomiasis. This was later supported by a number of studies carried out in East Africa by Wilson and his colleagues (1975a, 1976). Using various drug dosage regimes they demonstrated that cattle treated individually with Berenil on the appearance of clinical disease, developed a

partial/

immunity to trypanosomiasis after two years. Of more significance to this study was the group of cattle which were all treated with isometamidium when any one animal became parasitaemic. This group was considered to have developed a limited degree of immunity as judged by a reduction in the need for further treatments.

More recently still Bourn and Scott (1978) demonstrated the development of a non-sterile immunity following strategic drug usage in work oxen in Ethiopia.

All of these field observations should be looked at in the light of experimental studies which show that it is reasonably easy to induce a sterile immunity to homologous challenge following infection and treatment (Akol and Murray, 1983).

In comparing the results of this present study with the development of immunity under field conditions it is important to consider a number of factors. During field trials many of the cattle undergoing treatment may already be harbouring a patent infection. As such following treatment immunity against homologous infection would be conferred. Provided there was a limited number of antigenic variants in an area it is possible to conceive that immunity may develop after a number of such treatments. It should also be remembered that under field conditions specific antibody responses due to natural challenge could not be measured. As such 'immunity' could only be measured by indirect methods such as a reduction in the necessity for treatment, a decrease in clinically diagnosed trypanosomiasis cases, a reduced abortion rate etc.

These/

These parameters could in fact be due to a true sterile immunity resulting from infection and treatment.

The question of drug residues is also worthy of consideration. Because no sensitive test is available to measure drug levels in the various tissues it is impossible to measure the effect of drug residues in the situations where immunity is considered to have developed. This is demonstrated by the work of Soltys (1955) who concluded that repeated doses of Antrycide over a period of time resulted in a sterile immunity. This work was questioned in 1958 by Smith who suggested that the extended period of prophylaxis was in fact due to a persistant presence of drug residues.

Under field conditions the prophylaxis afforded by the use of isometamidium is often of a considerably shorter duration than that achieved in this study. Kirkby (1964) determined a prophylactic period of only 14 weeks. A number of reasons could account for this occurrence.

During this study only one specific antigenic type of Trypanosoma congolense was examined, namely IL Nat 3.1. This was known to be especially sensitive to isometamidium (Pinder and Authie, 1984) and for this reason was selected for the present study. In field situations a variety of T. congolense antigenic types could be present, along with T. vivax. Different antigenic types of both species can show different levels of sensitivity to isometamidium as judged by 'infection and treatment' regimens (Whiteside, 1962; Pinder and Authie, 1984) and this may reduce the prophylactic period conferred by the drug.

A/

A reduced prophylactic period could also be the result of relapse infections from privileged sites within the body, to which the drug has no access, (Jennings, Whitelaw and Urquhart, 1977; Whitelaw, Moulton, Morrison and Murray, 1985). Similarly a relative underdosing of the drug in the field situation where no apparatus for weighing animals is available could easily result in a reduction of prophylaxis.

Finally the often quoted importance of the weight of challenge as discussed by Whiteside (1962) could be responsible for the variation in length of prophylaxis. Davey (1957) suggested that this may be due to the inoculated trypanosomes absorbing a finite amount of the drug.

This study differs from the situation in the field in yet another factor. All the cattle which took part in the present studies were in good body condition, free from any concurrent infection and their nutritional status was sufficient to result in an approximate weight gain of 60 kg over the first 5 months. Under field conditions this is unlikly to be the case. The influence of these poorly understood factors on the length of prophylaxis is worthy of further investigation. Similarly the effect of prior infection should also be studied.

Because of the importance of drug residues active at the level of the skin any future studies should utilise cyclical challenge if at all possible. Intravenous inoculation of trypanosomes represents an artificial route of infection and bypasses a potentially important site of drug activity.

It is therefore proposed that future investigations should utilize challenge with metacyclic forms of different serodemes of T. congolense and examination should be made of various factors which might influence the duration of chemoprophylaxis, namely dose of drug, the level of challenge, the nutritional status of the host and the effect of treatment of an established infection on the subsequent duration of prophylaxis. An understanding of the importance of these factors is central to the proper use of prophylactic drugs in the field.

CHAPTER 2

Studies on the Local Tissue Toxicity of Isometamidium and its Dextran Complex

Introduction:

As discussed in the general introduction, one of the factors which is considered to limit the use of antitrypanosome drugs is the severe toxic reactions they induce (Holmes and Scott, 1982). This is especially true for the phenanthridine group of drugs.

Dimidium when used in African cattle caused a high incidence of delayed toxicity. This was characterised by signs resembling photosensitisation frequently appearing approximately six weeks after administration (Bell, 1945; Randall and Beveridge, 1946). The basis of this systemic toxicity appeared to be damage to the liver parenchyma caused by the drug.

The compounds which succeeded Dimidium, i.e. Ethidium, Novidium, Prothidium, Metamidium and Isometamidium, were also capable of causing this systemic toxicity but only at considerably higher doses than would be used therapeutically (Williamson, 1970).

With these drugs it is the local reaction and not the systemic toxicity which limits the dosage rate (Cawdery, 1963). Prothidium and Ethidium when injected subcutaneously give rise to large, firm, plaque-like oedematous swellings. These swellings often rupture discharging dead neutrophils and drug stained fluid (Smith, 1959; Robson and Cawdery, 1958). Healing of this painful lesion occurs slowly. Both of the previous authors also showed that Ethidium suriminate caused local reactions which were more severe than that cuased by Ethidium or Prothidium. In comparison to this, Metamidium suriminate caused only a slight reaction at the point of injection, while/

while Metamidium alone caused reactions similar to Ethidium and Prothidium (Gray and Stephen 1962).

In order to circumvent the problem of sterile abscess formation with resulting rupture and loss of the drug depot, it was recommended by the manufacturers that the phenanthridine products be given by deep intramuscular injection. This resulted in a sterile abscess deep within the muscle which was unable to discharge to the outside.

The intramuscular use of Isometamidium was investigated by Cawdery (1963). He concluded that 'the use of intramuscular injections in a prophylactic required are contra-indicated with the very irritant phenthridine derivatives'. This was mainly due to the inconvenience and pain they must have caused to the animal and also the loss of revenue due to the condemnation of part of the carcase.

The local toxicity is underlined by the following description made by Dr. W.P. Boyt 'Repeated treatments lead to a massive fibrosis and the cervical musculature becomes virtually non-functional. Contraction of the scar tissue leads to the nose being tilted dorsally and the animal has to kneel to graze', (quoted by Williamson, 1979).

Wilson et al., (1975a) also noted severe reactions with the use of Isometamidium characterised by large intramuscular swellings which left permanently indurated tissue and made it necessary to change the site of the injection to a different part of the neck at each drug treatment.

In an effort to reduce the local toxicity of Isometamidium, other methods of administering the drug have been examined. Toure (1973)/

(1973) administered 0.5 mg/kg Isometamidium intravenously without any systemic toxicity in both cattle and goats. More importantly, no local tissue damage resulted. However, the prophylactic period afforded by therapy of this type would appear to be less than an intramuscular or subcutaneous injection (Hill and McFadzean, 1963) due to the absence of the drug depot which is normally bound to protein at the site of the injection. This protein binding is thought to be essential for prolonged prophylaxis. The therapeutic index for the drug administered by this route, i.e. intravenously, is also low as a dose of 1 mg/kg resulted in the death of 3 goats within 4 hours of administration (Toure, 1973).

Cawdery and Knight (1961) described a method of administering Homidium suriminate suspended in a highly viscous oily base which was injected subcutaneously by means of a modified lever grease gun. This method showed promise in that it partly reduced the local reaction, although it was unable to prevent rupture of the drug depot to the surface. The hypothesis behind this was to achieve a depot using a suspension of a soluble salt in a vehicle from which the drug could be dissolved slowly, while at the same time protecting the surrounding tissue from high concentrations of the irritant cations.

A similar hypothesis, along with an attempt to achieve prolonged prophylaxis, was also behind the development of the sparingly soluble salts of the cationic 'cattle drugs' with a suitably large anion, such as suramin (Williamson and Desowitz, 1956; Williamson, 1957/

1957; Stephen, 1958). The systemic and local tolerance to these preparations was considerably greater than to the uncomplexed drug, while prophylactic activity was in a few cases enhanced. The suramin complexes were, however, still sufficiently toxic to prevent their development for routine use. A contributory factor was also the expense and difficulty in obtaining suramin (Williamson, 1976).

Suramin itself induces a severe local reaction if adminstered intramuscularly (Rollo, 1970), so it was thought that other complexing agents might yield better results. Groves and Wilmshurst (1964) used Laminarins sulphate, a degraded polysaccharide derivative from seaweed, to form complexes with the phenanthridine trypanocides. James (1978) continued this work using Dextran sulphate and showed in mice and rats that the complex formed between Isometamidium and Dextran sulphate exhibited both reduced toxicity and increased prophylaxis. Similar results were obtained by Aliu and Chineume (1980), also in mice.

Despite the clear recognition that local toxicity reactions are a serious handicap in the administration of trypanocidal drugs, the pathological basis of the local tissue reaction is very poorly documented. Thus, due to this dearth of information, it was decided to carry out a limited experiment to examine the lesions induced by intramuscular inoculations of both Samorin and Samorin Dextran. The effect of subcutaneous injections of Samorin Dextran were also studied but not of Samorin because of previous reports of severe lesions being caused by this method of administration of the phenanthridine compounds and the resulting distress to the animal involved.

MATERIALS AND METHODS

1. Experimental Animals:

Cattle

These consisted of four 18 month old castrated male Boran cattle weighing approximately 230 kg. They had been obtained from the Kapiti Plains in the Athi river area of Kenya.

2. Management:

The management of the cattle was the same as described in Chapter 1.

3. Drugs

Isometamidium chloride (Samorin, May and Baker, Lot DP 3946) was freshly prepared as both 2% and 4% w/v solutions in sterile distilled water and used at a dose rate of 1 mg/kg body weight.

Dextran complex of isometamidium chloride was freshly prepared before use from isometamidium chloride and Dextran sulphate (Pharmacia, Sweden). Fresh solutions of equal concentrations of isometamidium chloride and Dextran (4% w/v) were prepared in sterile distilled water. An equal volume of the Dextran solution was added slowly to the isometamidium chloride solution with vigorous mixing until precipitation occurred. This precipitate could be resuspended either by vigorous hand mixing or by the use of a vortex mixer. The resulting suspension was injected using an 18 gauge 1½" needle. The final suspension contained 2% w/v isometamidium chloride.

4. Experimental Protocol:

The four animals were inoculated with the drug at a dose rate of 1 mg/kg/injection site, as indicated in Table 2.1.

After/

TABLE 2.1: Post-mortem day; Route of administration; Concentration of drug; Site of inoculation and drug used.

Post-Mortem Day	Route	%	Vol. ml.	Site	Animal No.
Samorin:					
7	i.m. i.m.	2 4	9.5 4.75	a, c b	1
28	i.m. i.m.	2 4	11.0 5.5	a, c b	2
56	i.m. i.m.	2 4	11.8 5.9	a, c b	3
Samorin Dextran:					
7*	i.m. s.c.	2 2	11.0 11.0	d e	2
28**	s.c.	2	14.1	b	4
56	i.m. s.c. s.c.	2 2 2	14.1 14.1 14.1	a c d	4

KEY:

a = Middle third of the right hand side of the neck
b = Middle third of the left hand side of the neck
c = Middle of the right hand gluteal muscle mass
d = Middle of the left hand gluteal muscle mass
e = Middle of the right hand thigh area

i.m. = intramuscular inoculation
s.c. = subcutaneous inoculation

* Samorin Dextran inoculated on day 21, i.e. seven days before animal No. 2 was due to be slaughtered.

** Biopsy taken at 28 days after inoculation.

After injection on day 0 the following parameters were regularly measured:

(1) Temperature;

(2) Pulse rate;

(3) Respiration rate;

(4) Draining lymph node size; and

(5) Clinical description of lesion.

The animals were slaughtered for post-mortem examination of the inoculation sites according to the timetable described in Table 2.1. Samples were also taken for histological preparation. After fixing in corrosive formyl, samples were embedded in paraffin blocks and 5 μm sections obtained. Three methods of staining were used:

(1) Haematoxylin and Eosin (H & E) (Stevens, 1982a);

(2) Martius, Scarlett, Blue (MSB) (Bradbury and Gordon, 1982);

(3) Perl's Prussion Blue (PPB) (Stevens, 1982b).

Corrosive formyl is composed of a saturated mercuric chloride solution containing 10% formalin.

RESULTS

1. Clinical Findings:

Following drug inoculation no significant changes were noted in the various clinical parameters measured. The visual appearance of lesions at the inoculation sites developed as follows:

(a) Samorin inoculation sites (Animals 1, 2 and 3)

Initially no lesion was detected at these sites. However, on day 5 a muscular swelling was noted at all of the Samorin inoculation sites. No difference was detected between those sites injected with 2% or 4% Samorin. On day 1, prior to the detection of a visible swelling, a distinct lameness was noted in the right hind leg of animal (2), and this persisted until it was slaughtered on day 28. A slight lameness was detected in animal (3) between 7 and 14 days post-inoculation.

The muscular swellings first noted on day 5 persisted until each animal was slaughtered. The size of the lesions were impossible to measure due to their intramuscular position.

(b) Samorin-Dextran inoculation sites (Animal 4)

The intramuscular (i.m.) inoculations of Samorin-Dextran resulted in a slight muscular swelling which was first noted on day 7 and persisted until termination of the experiment on day 56. The exact size of the lesion cannot be stated due to its intramuscular position.

Subcutaneous (s.c.) inoculation of Samorin-Dextran resulted in the development of distinct lesions. In animal (4) on the left-hand side (LHS) of the neck, a large fluid filled subcutaneous swelling/

swelling was present on day 1. It was approximately 7 x 5 x 3 cm in size. By day 3 it had increased to 10 x 5 cm and it maintained this size until day 10, becoming firm in nature. Thereafter it gradually decreased in size so that by day 28 when it was removed for biopsy it measured 6 x 5 x 5 cm.

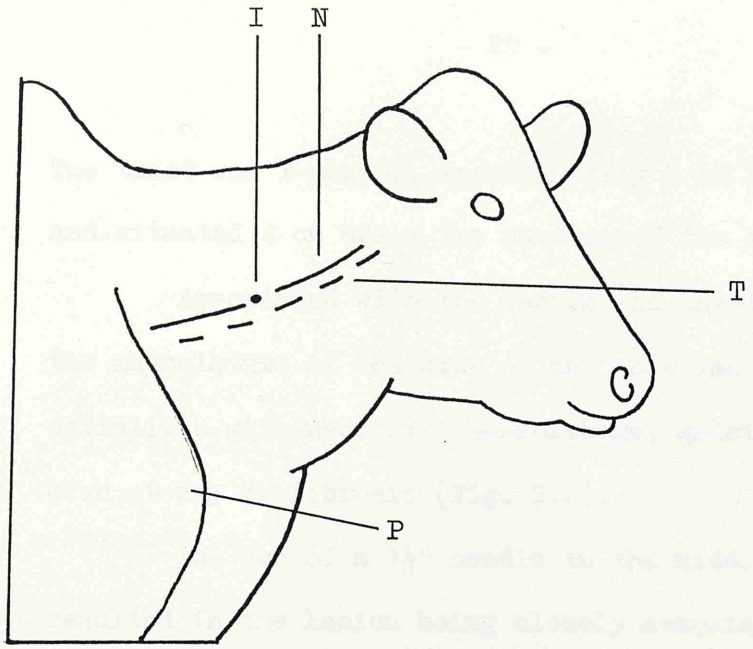
Inoculation into the subcutaneous tissues of the RHS gluteal area and the left-hand side (LHS) gluteal area in the same animal also resulted in distinct lesions. Due to the thickness and tension of the skin at these sites it was not possible to measure the size of the lesions accurately. An approximate size was obtained by measuring the lesion's horizontal diameter. On day 2 they were approximately 8 cm in diameter and this increased to 11 cm by day 7. This size was maintained till day 14, then gradually decreased to 9 cm by day 34. The lesion present on the LHS gluteal area continued this decrease so that by day 56 its diameter was 7 cm. The lesion present on the RHS gluteal area, however, developed a large fluctuating swelling of 16 cm diameter on day 35. Puncture of this swelling allowed aspiration of frank blood indicating that a haematoma had developed. This gradually decreased in size so that by day 56 it measured only 9 cm in diameter.

2. Gross Pathology:

Day 7

(a) 2% Samorin (RHS Neck)

At the site of inoculation there was a haemorrhagic necrotic lesion from which extended a haemorrhagic necrotic tract for a distance of 10 cm on either side of the inoculation site (Fig. 2.1). The/



I = Inoculation site
N = Necrotic Tract
T = Transverse Processes
P = Point of the shoulder

Figure 2.1: Day 7. Lesion due to 2% Samorin.
Diagram demonstrates the position of the inoculation site and the haemorrhagic necrotic tract in relation to the transverse process of the cervical vertebrae.

Figure 2.2: Day 7. Neck muscle following 2% Samorin inoculation. Shows the severe haemorrhagic necrotic tract (Left Hand Side) and the cellulitic reaction present in the intramuscular connective tissue (Right Hand Side).

The tract was Y-shaped, approximately 2 cm broad by 2 cm deep and situated 4 cm below the surface of the skin.

Associated with the lesion and involving the majority of the musculature of the side of the neck was a severe myositis and cellulitis with necrosis, haemorrhage, gelatinous oedema and, even at day 7, fibrosis (Fig. 2.2).

The use of a 1½" needle in the middle third of the neck resulted in the lesion being closely associated with the transverse processes of the cervical vertebrae. The main necrotic tract was situated above the transverse processes but the surrounding extensive muscle damage enclosed the processes.

(b) 4% Samorin (LHS Neck)

The inoculation site and lesion was associated with the transverse processes of the cervical vertebrae as in the previous description. It had resulted in a severe haemorrhagic necrotic lesion several centimeters in diameter from which extended a necrotic sinus traversing the neck, as illustrated in Fig. 2.3. The rest of the neck muscles resembled the RHS of the neck with the majority showing a severe myositis and cellulitis accompanied by necrosis, gelatinous oedema and the beginning of fibrosis.

Furthermore, on both the right and left sides of the neck the affected muscle had a yellow green atrophic appearance.

(c) 2% Samorin (RHS Gluteal)

Compared to the inoculation sites described in (a) and (b), this reaction was well sited in that it was deep into the muscle with no involvement of bony tissue. At the inoculation site there was/

I = Inoculation site

N = Necrotic Tract

T = Transverse Processes

P = Point of the shoulder

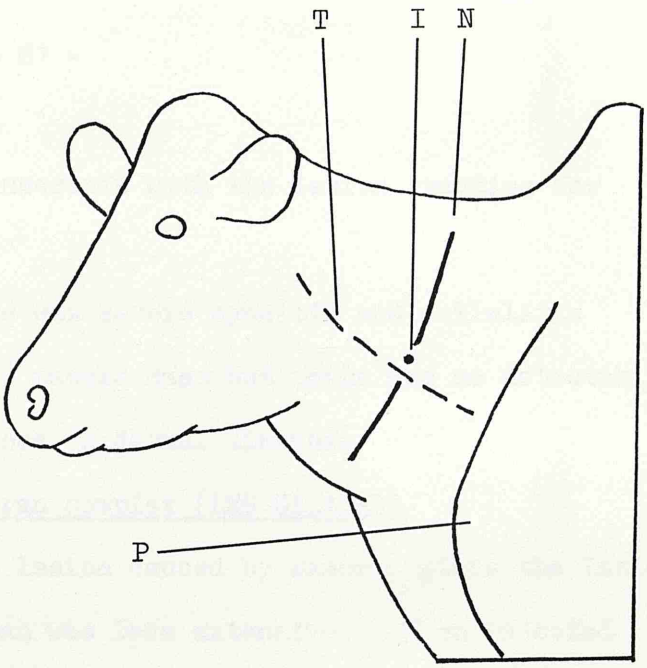


Figure 2.3: Day 7. Lesion due to 4% Samorin.
Diagram demonstrates the position of the inoculation site and the necrotic tissues in relation to the transverse process of the cervical vertebrae.

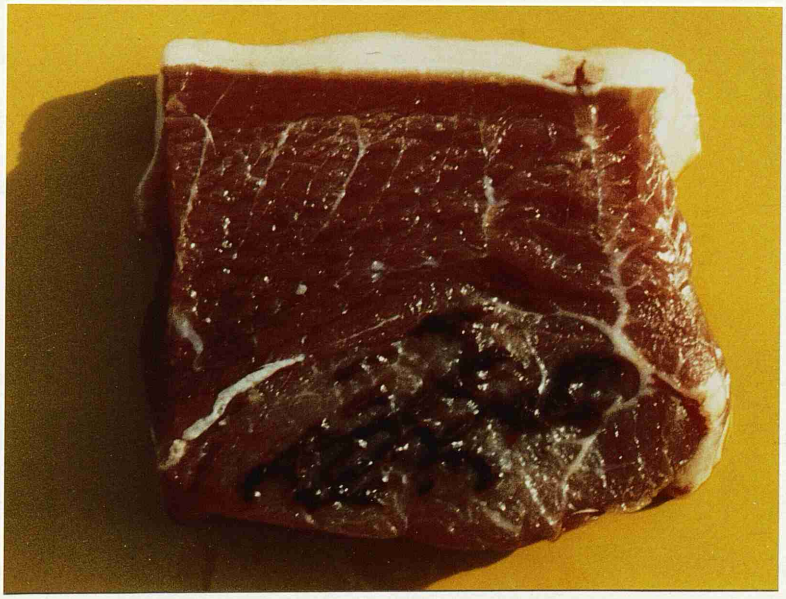


Figure 2.4: Day 7. Gluteal muscle following Samorin-Dextran inoculation. Shows the small red/black tracts resulting from Samorin-Dextran.

was a focus of haemorrhagic necrosis with the lesion tracking for 10 cm on either side.

As in the neck, there was severe myositis and cellulitis involving most of the gluteal muscle mass but there was no detectable involvement of the subcutaneous or dermal tissues.

(d) 2% Samorin-Dextran complex (LHS Gluteal)

In comparison to the lesion caused by Samorin alone the lesion resulting from Samorin-Dextran was less extensive. When injected into the gluteal muscles, it resulted in a small well-circumscribed (6 x 5 x 3 cm) firm lesion. On sectioning, its cut surface was stippled with small dark red/black tracks approximately 1 mm in diameter. The muscle immediately surrounding these tracks was pale in colour, while outside this lesion there was no apparent muscle involvement (Fig. 2.4).

Thus, in contrast to Samorin alone, Samorin-Dextran did not cause generalised myositis, necrosis, gelatinous oedema and cellulitis.

(e) 2% Samorin-Dextran complex (RHS Thigh)

Due to a poor inoculation technique, this injection did not go subcutaneously as intended but resulted in an intramuscular lesion similar to that described in (d) above. The lesion was a well-circumscribed tube, 2 cm in diameter and 7 cm long. In cross-section it was stippled with small 1 mm diameter necrotic tracts and/or drug deposits while on longitudinal section they appeared as thin dark branching lines. Outside this immediate lesion there was very little muscle involvement and no muscle discolouration

Day 28

(a) 2% Samorin (RHS Neck)

A massive lesion was present around the transverse processes of the cervical vertebrae, extending to 20 cm in length by 15 cm in width and approximately 8 cm in depth. Centrally a large haemorrhagic necrotic tract was present. This was surrounded by extensive diffuse myositis, cellulitis, gelatinous oedema and extensive fibrosis. The lesion when compared to that present at day 7 appeared to be more extensive and still very active but containing large amounts of fibrosis.

(b) 4% Samorin (LHS Neck)

This lesion was similar to that described above, although it was not as large. It was approximately 15 cm long by 10 cm wide and 8 cm deep with a centrally placed necrotic tract surrounded by myositis, cellulitis and fibrosis. The surrounding muscle appeared to be pale yellow/green in colour. Again the lesion surrounded the transverse processes of the cervical vertebrae.

(c) 2% Samorin (RHS Gluteal)

This inoculation resulted in a massive necrotic tract surrounded by myositis, cellulitis and fibrosis involving the majority of the gluteal muscle mass. The areas of fibrosis gave the lesion a firm consistency and resulted in it being very easy to cut (Fig. 2.5).

(d) 2% Samorin-Dextran complex (LHS Neck)

A large subcutaneous mass consisting of nodules 10 mm in diameter of dark red/black firm material was present. These lay in the loose subcutaneous tissue and had also migrated into the underlying/

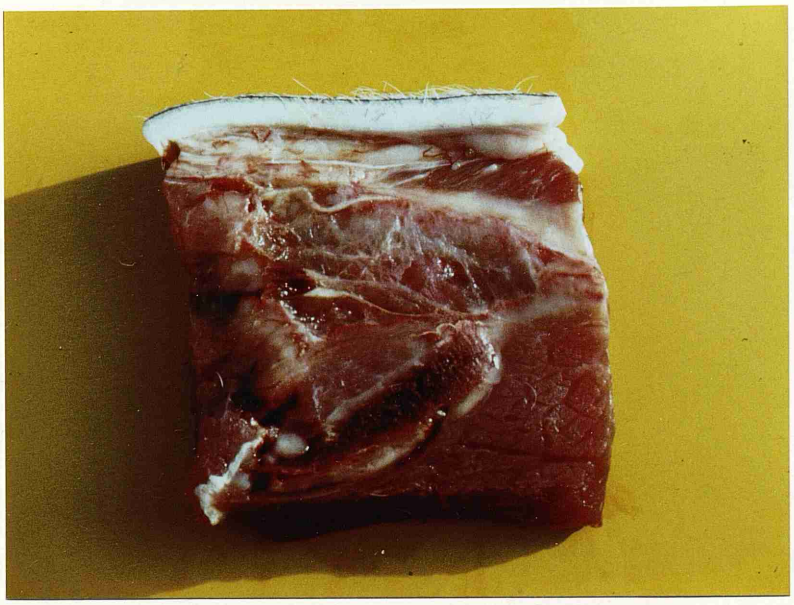


Figure 2.5: Day 28. Gluteal muscle followng 2% Samorin inoculation. Shows the massive extent of the lesion with a centrally placed necrotic tract surrounded by cellulitic type reaction, oedema and fibrosis.

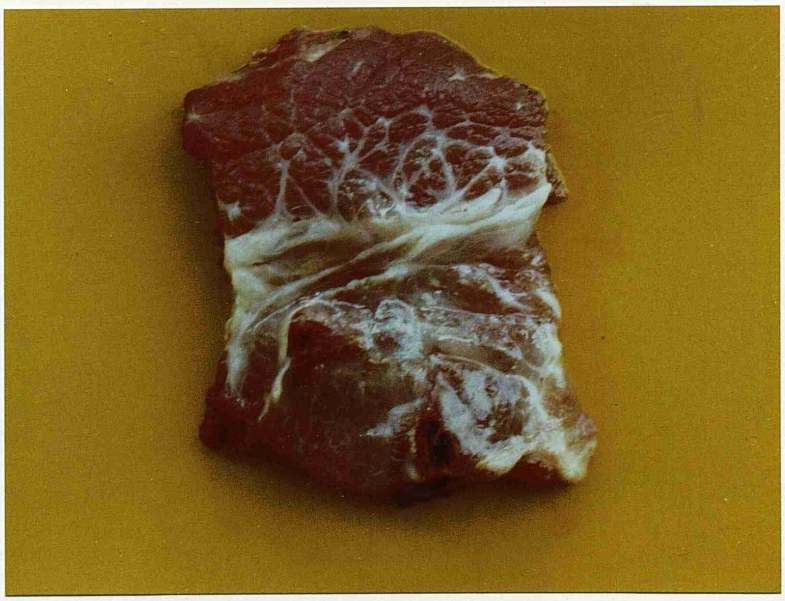


Figure 2.6: Day 56. Gluteal muscle following 2% Samorin inoculation. Shows the extensive fibrosis which is prominent at this stage.

underlying muscle. Numerous blood vessels were present throughout the drug deposits which resulted in the biopsy being very bloody.

Day 56

(a) 2% Samorin (RHS Neck)

At day 56 the majority of the side of the neck was involved in this lesion which was 28 cm in length. There was a central area of haemorrhagic necrosis 4 cm long, 4 cm deep and 2 cm wide. This was dark red/black in colour and was surrounded by a greenish white area of fibrosis approximately 3 cm wide. The muscle surrounding this was pale yellow in colour with white streaks of fibrous tissue running through it. Between the muscle bundles the connective tissue was very gelatinous and fluid in appearance due to the presence of extensive oedema.

(b) 4% Samorin (LHS Neck)

In this case the lesion was similar to that described above except that it was not as extensive. The central necrotic tract was 3 cm long by 2 cm wide and 1 cm deep and this was surrounded by muscle discolouration and fibrosis involving a large proportion of the neck. The lesion surrounded by transverse processes of the cervical vertebrae and extended from the trachea to the ligamentum nuchae. Connective tissue oedema was again a feature along with numerous fibrous tracts in the muscle.

(c) 2% Samorin (RHS Gluteal)

A 20 cm necrotic/cellulitic tract was present in an inter-muscular connective tissue band. This band was 8 cm wide and 1 cm deep./

deep. The surround muscle was pale, yellow green in colour for a distance of up to 6 cm. Running through the muscle were white fibrous tissue bands, while other connective tissue was oedematous in nature. Overall the large amount of fibrosis resulted in the lesion having a firm consistency (Fig. 2.6).

(d) 2% Samorin-Dextran complex (RHS Neck i.m.)

Eight weeks after the initial inoculation, the lesion caused by the Samorin-Dextran complex was well-circumscribed and considerably smaller than that caused by Samorin alone. The lesion was tube shaped 8 cm long by 3 cm in diameter, and was situated above the transverse processes of the cervical vertebrae. The main lesion consisted of muscle interspersed with red/black streaks of drug deposit and surrounded by a connective tissue reaction. Outside this immediate area the muscle was discoloured for a distance of 3 - 4 cm.

(e) 2% Samorin-Dextran complex (RHS Gluteal s.c.)

The subcutaneous inoculation of Samorin-Dextran resulted in a firm drug deposit which had no adverse effect on the overlying skin and was contained within the subcutaneous tissues (Fig. 2.7).

The main part of the lesion was approximately 8 cm in diameter to a depth of 2 cm. It was composed of dark red/black nodules of drug deposit which was surrounded by a firm fibrous tissue reaction. The lesion extended approximately 5 cm on all sides of this main area and was composed of a connective tissue proliferation with petechial haemorrhages throughout. The area directly below the drug depot was slightly discoloured yellow to a maximum depth of 3 cm.

Figure 2.7: Day 56. Subcutaneous reaction following Samorin-Dextran inoculation.

(f) 2% Samorin-Dextran complex (LHS Gluteal s.c.)

As above - except a small nodule of drug was found in the underlying muscle.

2. Histopathology:

Examination of histological sections revealed no real difference between the effects of 2% and 4% Samorin. Hence the following descriptions apply to both.

(a) Samorin - Day 7 - i.m. (Figs. 2.8, 2.9, 2.10 and 2.11)

A severe diffuse necrotising myositis was present with a massive neutrophil infiltration, severe congestion and marked exudation of fluid containing numerous fibrin deposits. Thrombi were present in both blood vessels and lymphatics while numerous lymphocytes were present in the infiltrate. A granulomatous reaction was present surrounding the Samorin deposits and included multinucleated giant cells, the cytoplasm of which contained Samorin. Samorin deposits were also visible in the cytoplasm of macrophages.

(b) Samorin - Day 28 - i.m. (Figs. 2.12, 2.13, 2.14 and 2.15)

As at day 7 a severe reaction with muscle degeneration and necrosis was present at the inoculation site. Interstitial fibrosis was more prominant and in some areas quite extensive, while Samorin deposits were easily visible in both extracellular areas and in the cytoplasm of macrophages. Numerous giant cells were also present with Samorin in their cytoplasm and fibrinous oedema remained a prominant feature.

(c) Samorin - Day 56 - i.m. (Figs. 2.16, 2.17 and 2.18)

At 56 days post-inoculation large areas of necrosis were still present. At this stage interstitial fibrosis was extensive, although/

although some areas still showed an acute inflammatory reaction. Again numerous macrophages and giant cells were present with deposits of Samorin in their cytoplasm. At this stage muscle regeneration also appeared to be a feature.

(d) Samorin-Dextran complex - Day 7 - i.m. (Figs. 2.19 and 2.20)

The inoculation of Samorin-Dextran has resulted in a much more limited reaction than that caused by Samorin. Haemorrhagic tracts were present and were surrounded by an interstitial myositis. The reaction was much less severe than that caused by Samorin, with more fibrous tissue and fewer neutrophils.

The basic lesion consisted of a chronic granulomatous reaction with a large number of macrophages surrounding the deposits of Samorin-Dextran. Outside this lesion an interstitial myositis was present. Some areas showed large numbers of neutrophils surrounding the Samorin-Dextran deposits which gave it an abscess-like appearance.

(e) Samorin-Dextran complex - Day 28 - s.c. (Fig. 2.21)

The lesion present at day 28, following a subcutaneous inoculation, was less severe in that the only notable feature was a large number of macrophages and fibrous tissue surrounding the Samorin-Dextran deposits. Very few neutrophils were present in this lesion.

(f) Samorin-Dextran complex - Day 56 - i.m. (Fig. 2.22)

At 56 days post-inoculation, the reaction had progressed to a stage of advanced fibrosis with the presence of dense fibrous tissue. Large amounts of Samorin-Dextran were still present in the/

the centre of the granulomatous-type reaction and in the cytoplasm of macrophages and giant cells.

(g) Samorin-Dextran complex - Day 56 - s.c. (Figs 2.23 and 2.24)

As with the intramuscular injection, the prime feature of this lesion was the presence of advanced fibrosis. At high magnification the presence of drug deposits in the cytoplasm of both macrophages and giant cells was visible. Such cells also surrounded extracellular deposits of Samorin-Dextran.

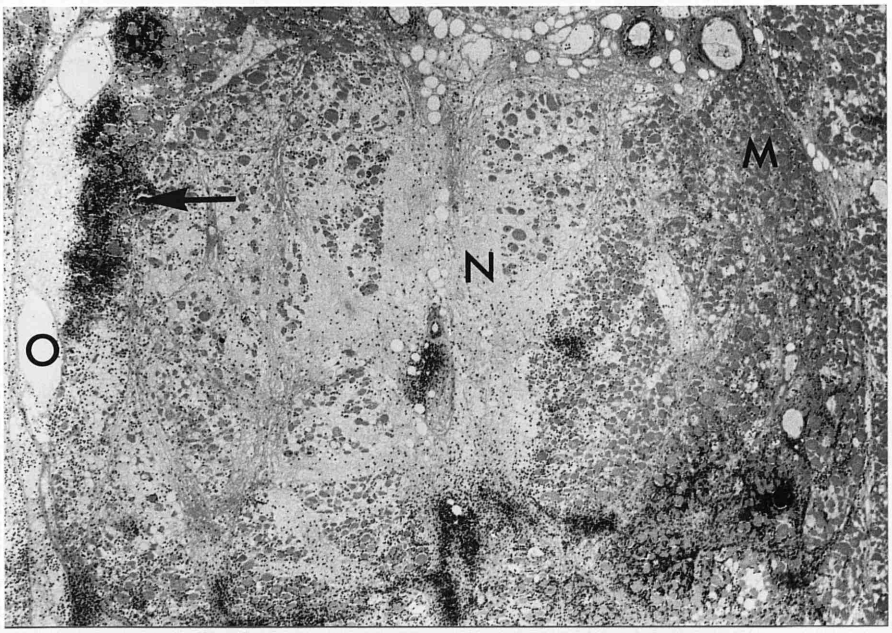


Figure 2.8: Day 7. Neck muscle following 2% Samorin inoculation (x 34 H & E)
Severe necrotising myositis (M) with muscle necrosis and destruction (N) accompanied by intensive congestion, fibrinous oedema (O) and a massive neutrophil infiltrate (arrow).

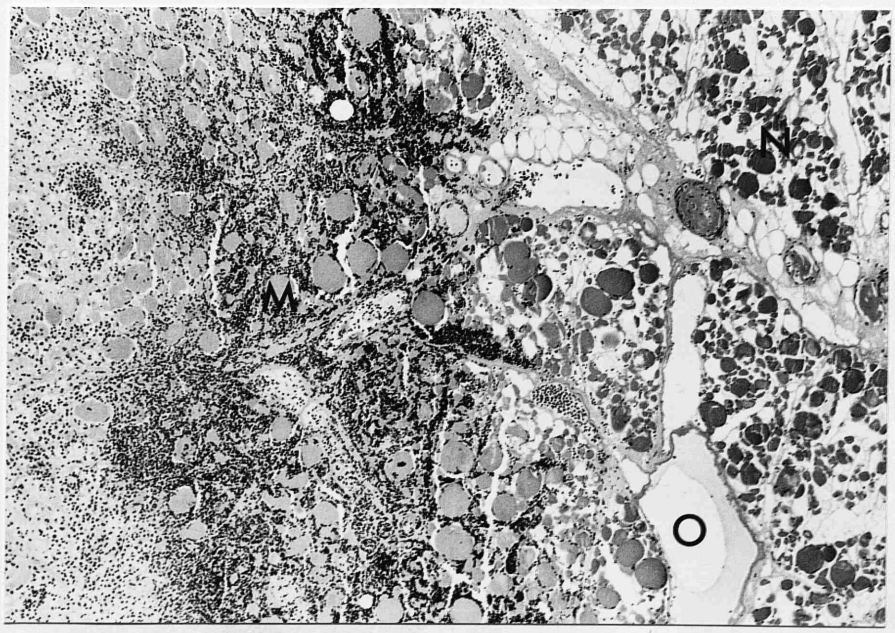


Figure 2.9: Day 7. Neck muscle following 4% Samorin inoculation (x 54 H & E)
Severe muscle necrosis (N), extensive myositis (M) and severe oedema (O).

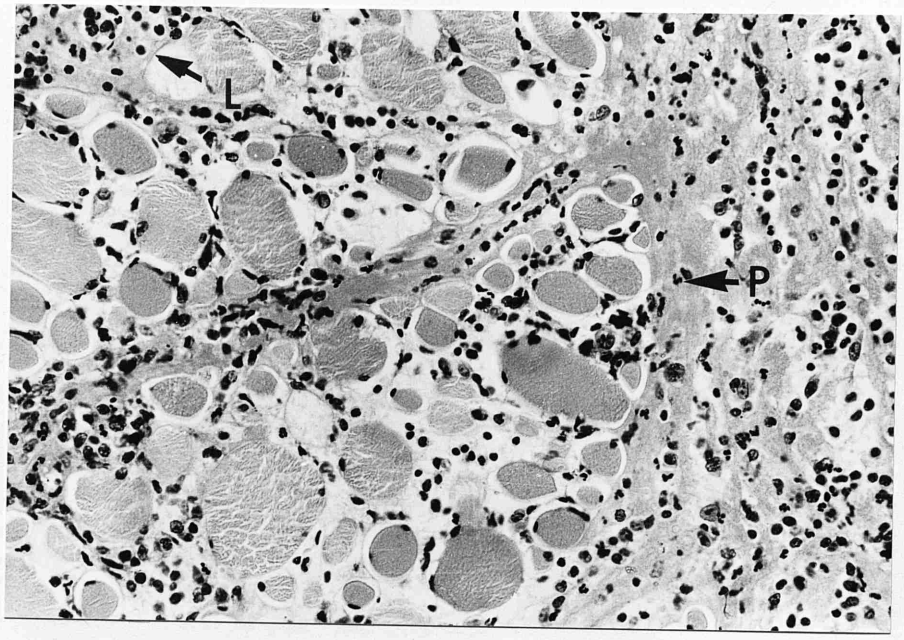


Figure 2.10: Day 7. Neck muscle following 2% Samorin inoculation (x340 H & E)
Myositis with muscle necrosis and degeneration. Numerous neutrophils (P) are present and a few lymphocytes (L).

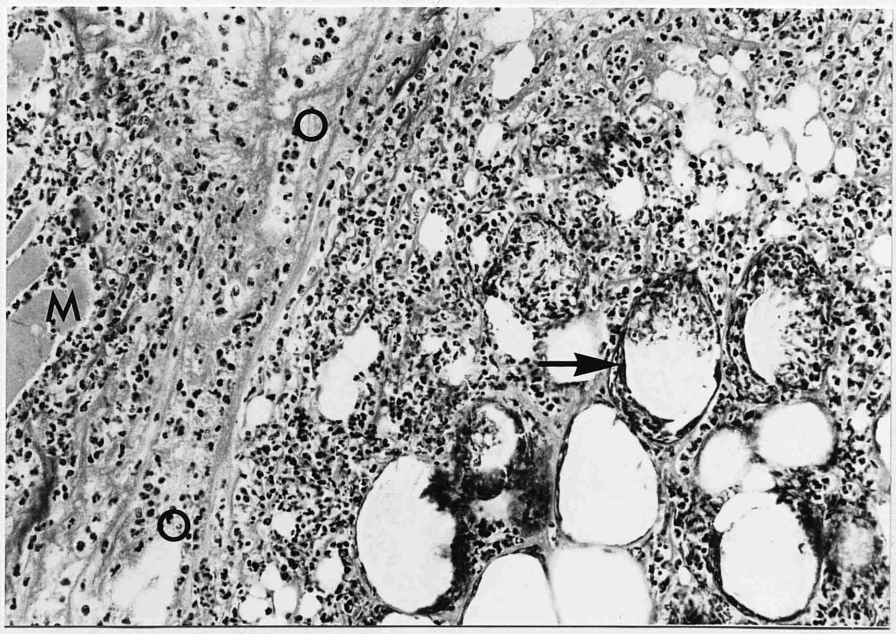


Figure 2.11: Day 7. Neck muscle following 2% Samorin inoculation (x 213 MSB)
Samorin depositions surrounded by multi-nucleated giant cells (arrow) and the presence of myositis (M). These two areas are divided by interstitial oedema with fibrin deposits (O).

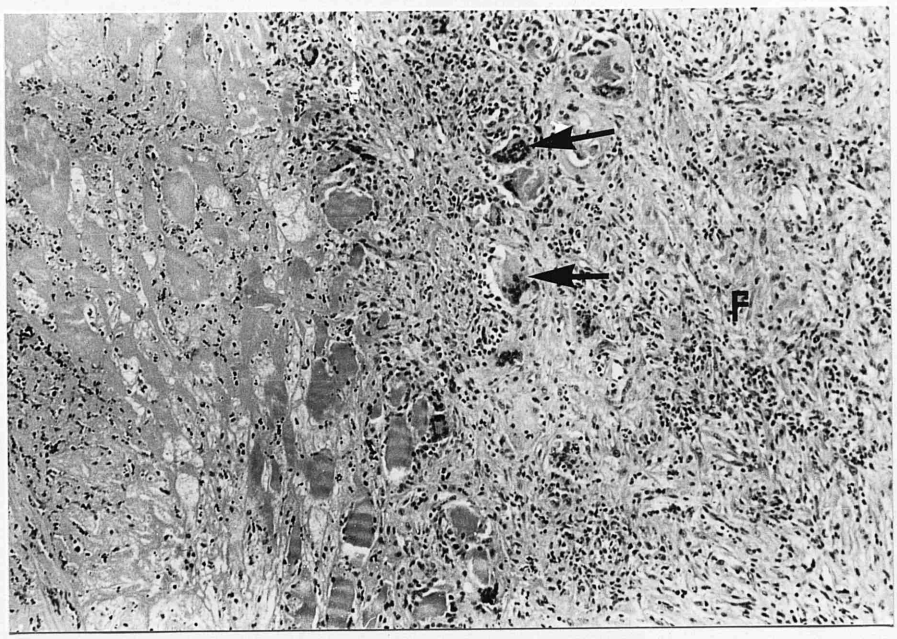


Figure 2.12: Day 28. Neck muscle following 4% Samorin inoculation (x 86 H & E)
Area of severe destructive myositis. The reaction is still acute with muscle necrosis, increased number of neutrophils and fibrinous oedema. By day 28 the active areas have been surrounded by a fibrous tissue reaction (F) including multinucleated giant cells (arrow).

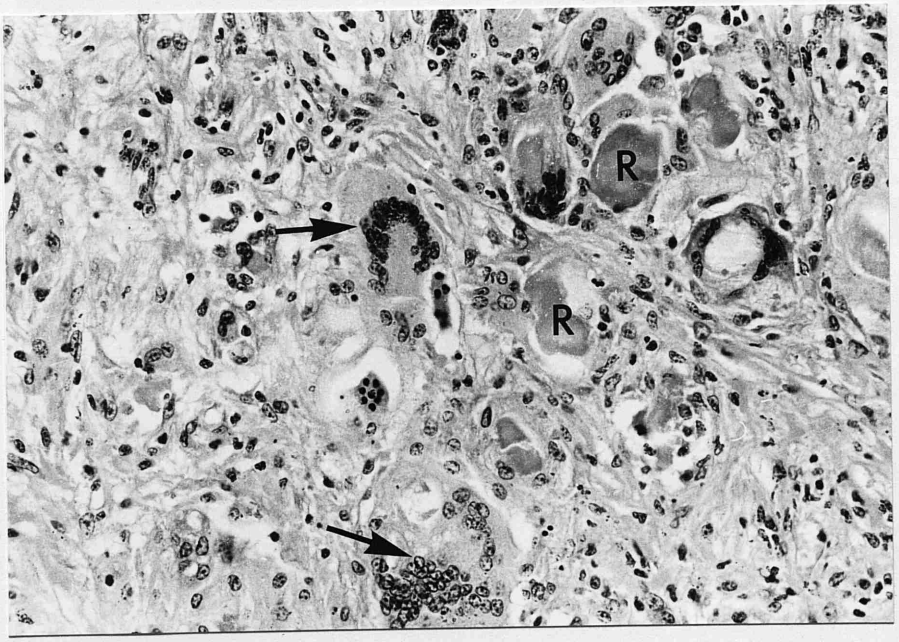


Figure 2.13: Day 28. Neck muscle following 4% Samorin inoculation (x 213 H & E)
Prominent multinucleated giant cells (arrow) in the connective tissue reaction.
Remnants of muscle fibre are still present (R).

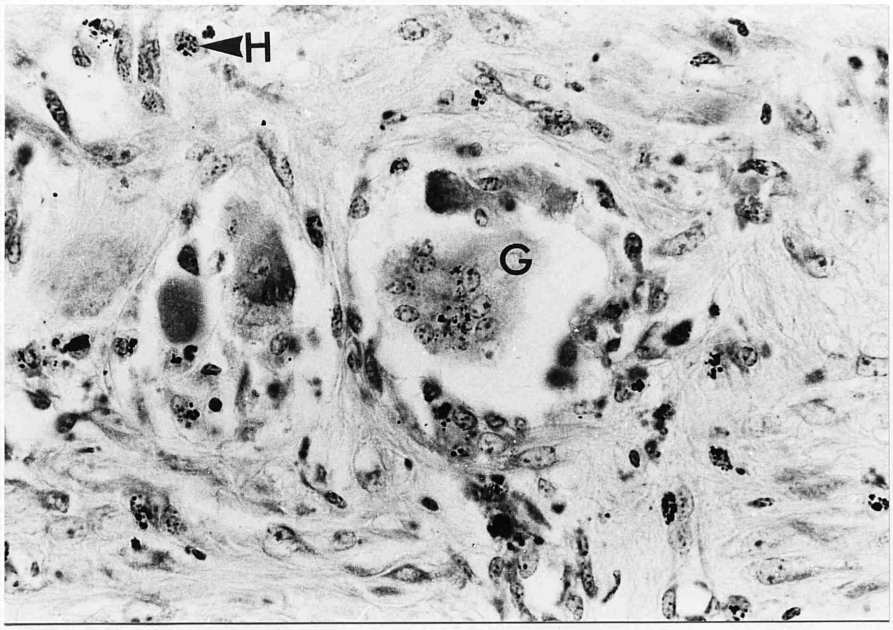


Figure 2.14: Day 28. Neck muscle following 4% Samorin inoculation (x 340 PPB)
Giant cells(G) and macrophages (H) with Samorin present in their cytoplasm.

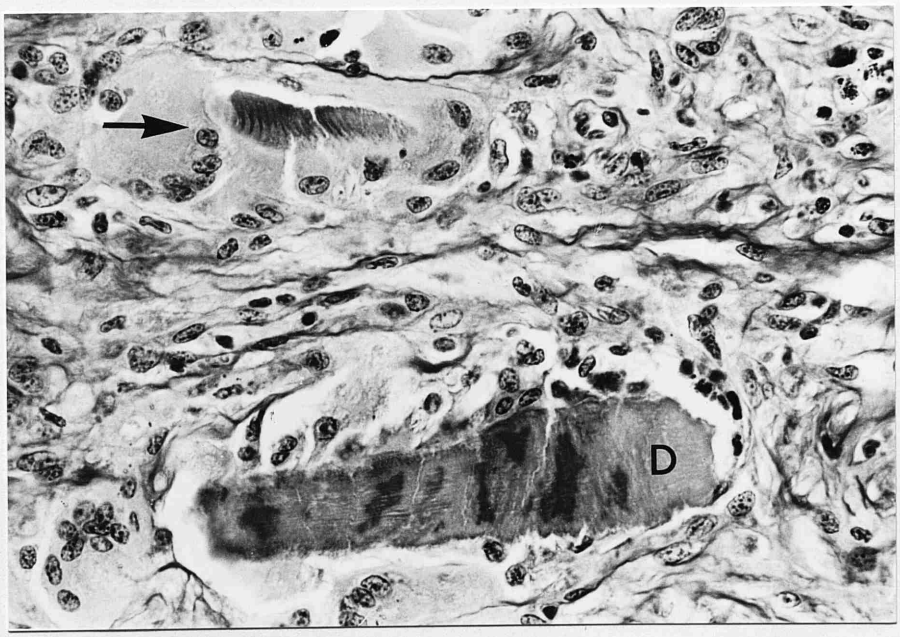


Figure 2.15: Day 28. Neck muscle following 4% Samorin inoculation (x 340 MSB)
Muscle degeneration (D) with sarcolemal cell proliferation (arrow) surrounded by interstitial fibrosis.

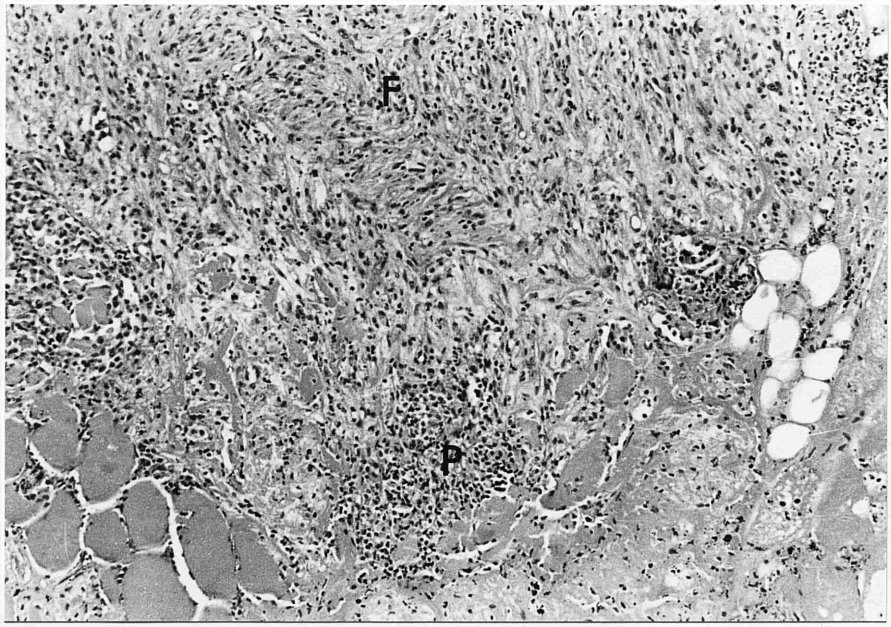


Figure 2.16: Day 56. Neck muscle following 2% Samorin inoculation (x 86 H & E)
Extensive fibrosis (F) present although muscle necrosis and neutrophil infiltration is still present (P).

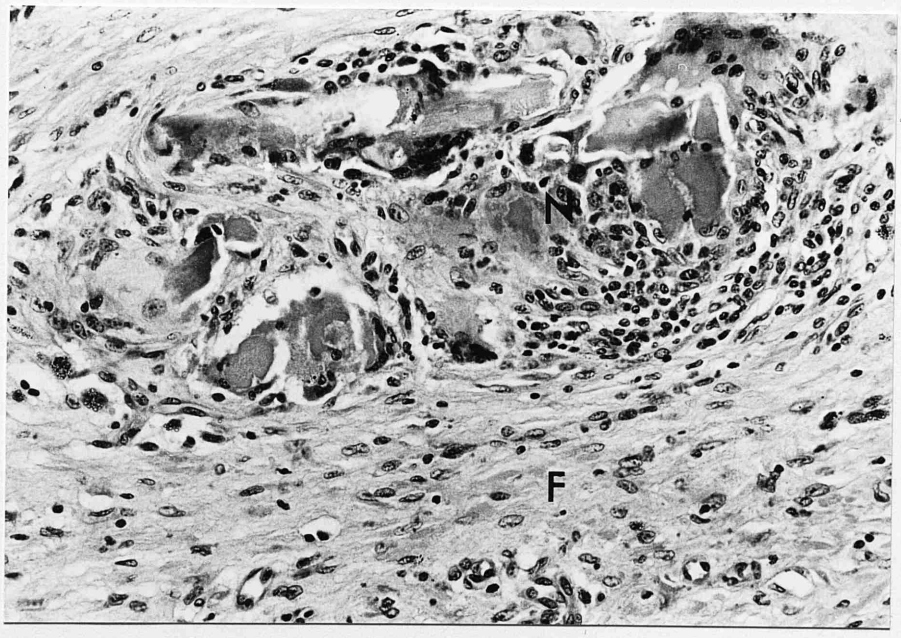


Figure 2.17: Day 56. Neck muscle following 2% Samorin inoculation (x 213 H & E)
Areas of muscle degeneration and necrosis (N) surrounded by fibrosis (F).

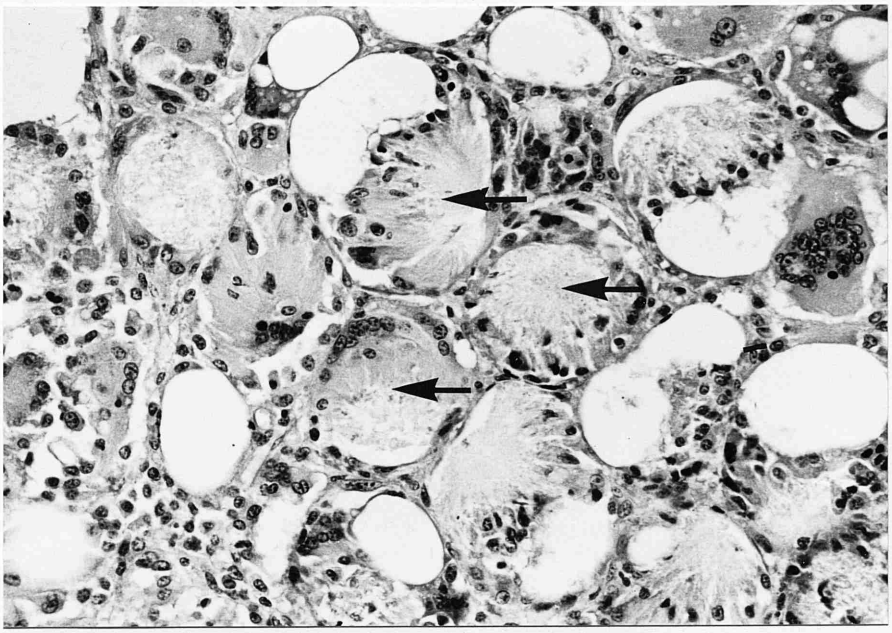


Figure 2.18: Day 56. Gluteal muscles following 2% Samorin inoculation (x 213 H & E)
Presence of Samorin at 56 days post-inoculation in the cytoplasm of giant cells (arrow).

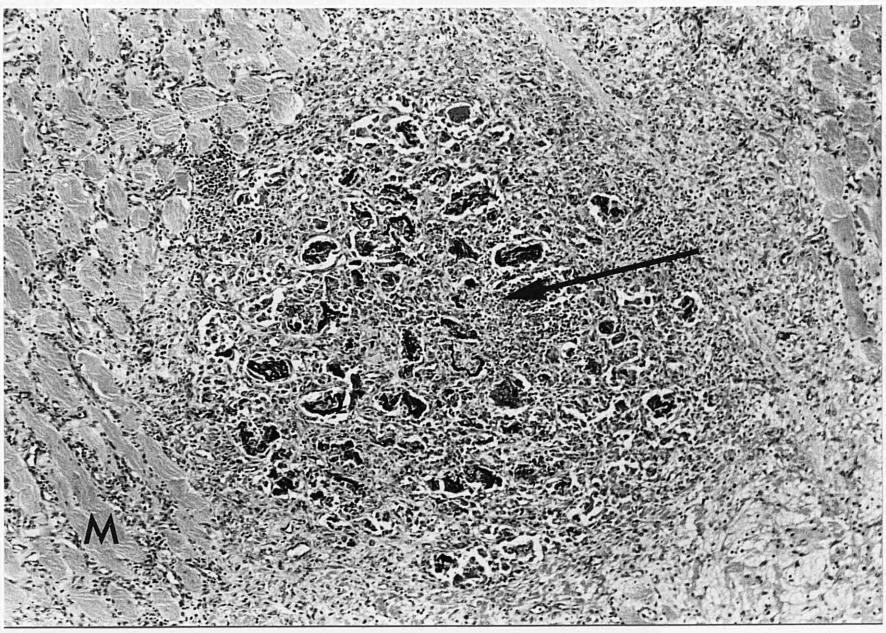


Figure 2.19: Day 7. Neck muscles following Samorin-Dextran inoculation (x 213 H & E)
Samorin-Dextran induced granulomatous reaction (arrow) surrounded by a severe interstitial myositis (M).

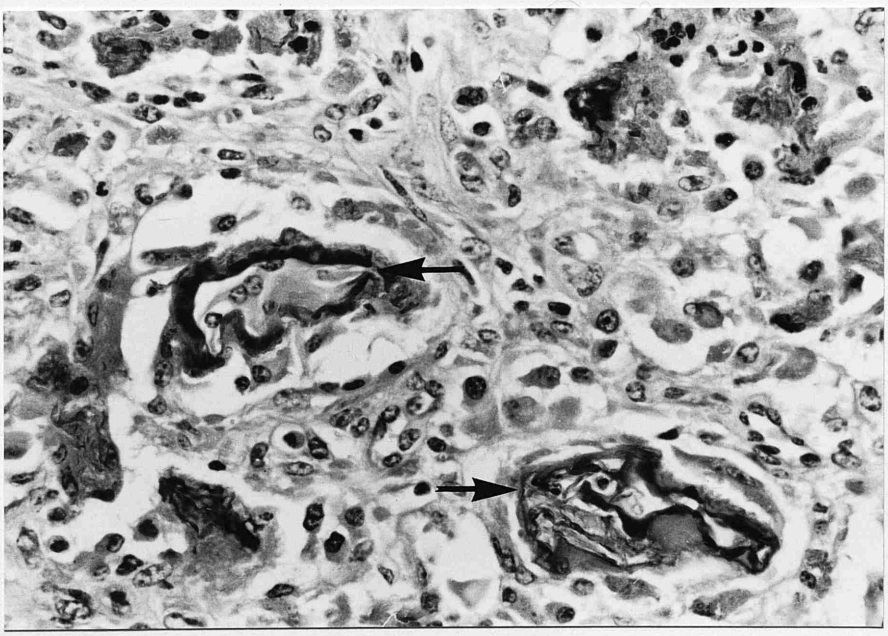


Figure 2.20: Day 7. Neck muscles following Samorin-Dextran inoculation (x 340 H & E)
Multiple deposits of Samorin-Dextran (arrow) surrounded by a granulomatous response composed of activated macrophages, multi-nucleated giant cells, small lymphocytes and connective tissue.

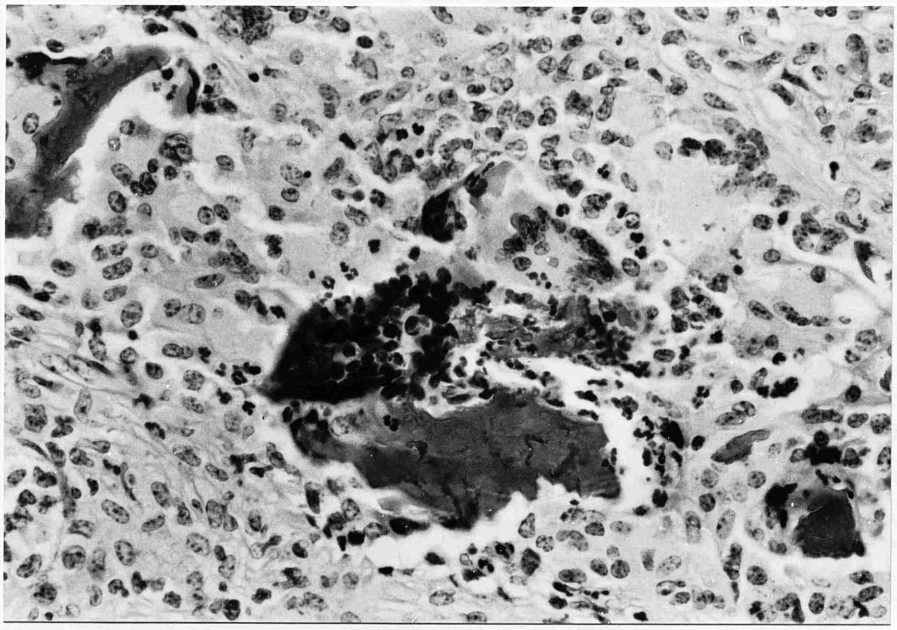


Figure 2.21: Day 28. Subcutaneous inoculation of Samorin-Dextran (x 340 H & E)
Samorin-Dextran surrounded by macrophages, giant cells, a few neutrophils and lymphocytes and fibrous tissue.

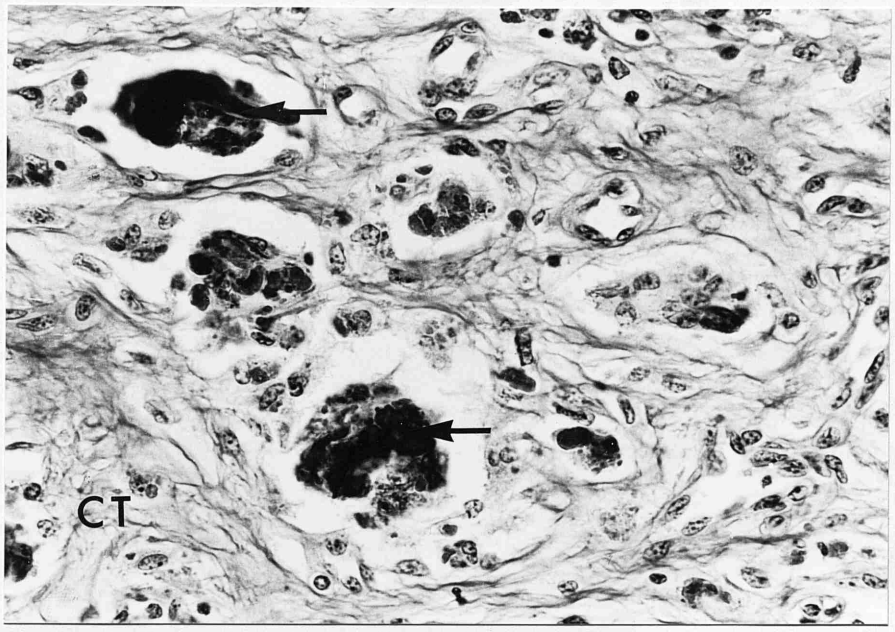


Figure 2.22: Day 56. Neck muscle following Samorin-Dextran inoculation (x 340 MSB)
Samorin-Dextran surrounded by macrophages (arrow) and dense connective tissue (CT).

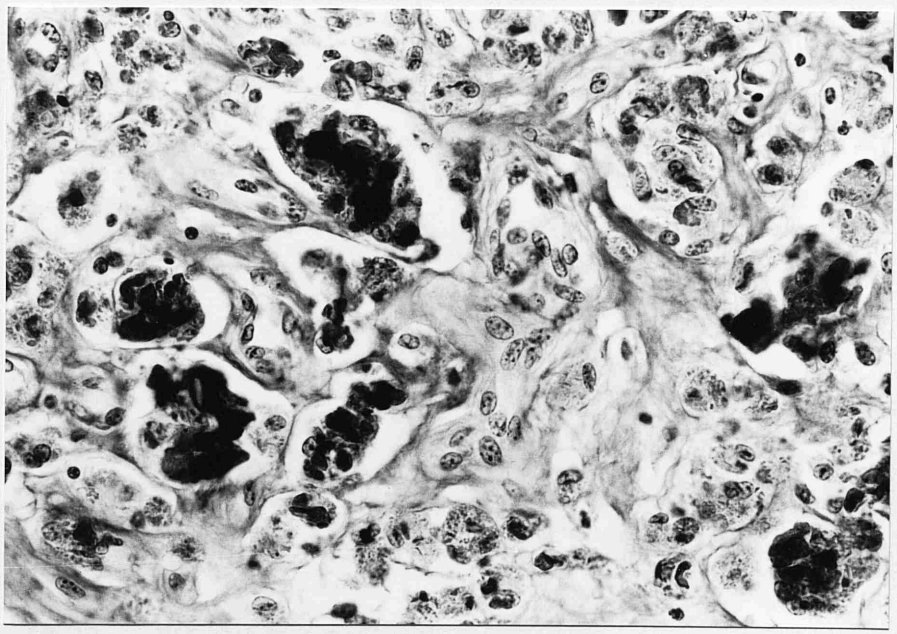


Figure 2.23: Day 56. Subcutaneous inoculation of Samorin-Dextran (x 340 MSB)
No obvious difference can be seen between this reaction and the lesion induced by intramuscular inoculation shown above.

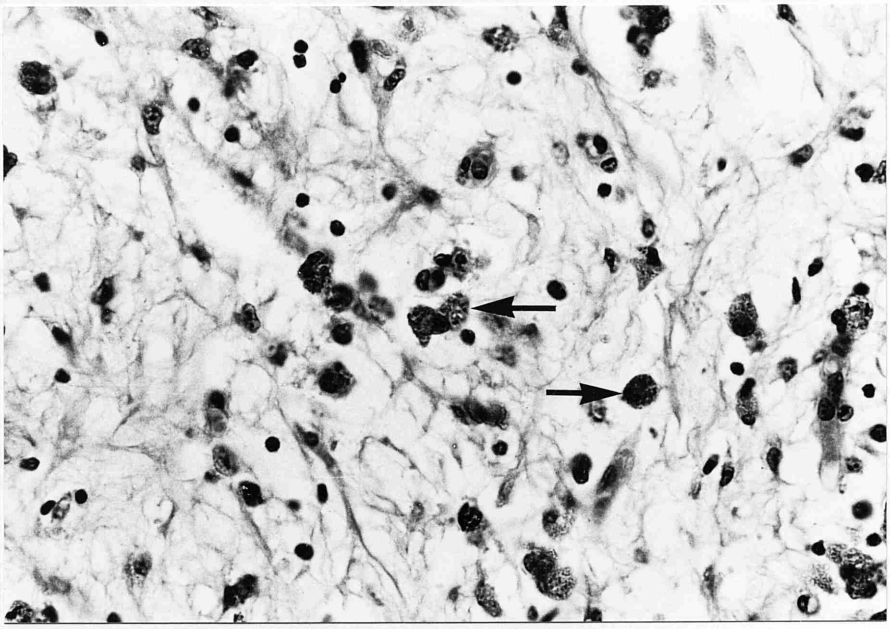


Figure 2.24: Day 56. Subcutaneous inoculation of Samorin-Dextran (x 340 H & E)
Samorin-Dextran deposits (arrow) visible in macrophages scattered throughout the interstitial reaction.

DISCUSSION

The results obtained from this limited trial confirm the severe nature of the tissue damage caused by the intramuscular inoculation of Samorin and demonstrate the great reduction in the local toxicity which occurs when the drug is complexed with Dextran sulphate.

Information on the gross pathology of the lesions is presented along with valuable new data on the histopathological nature of the lesion.

At day 7, following Samorin inoculation, there was a severe haemorrhagic necrotic lesion surrounded by myositis, cellulitis and severe oedema. This lesion appeared more extensive by day 28, although resolution had begun to occur with an increase in the fibrous tissue reaction. By day 56 this resolution was well in progress so that the major feature noted was extensive fibrosis. These results confirmed the extensive long lasting lesions noted by Cawdery (1963).

The histological changes were characterised by an initial severe necrotising myositis with a massive neutrophil infiltration, fibrinous oedema and marked congestion. This gradually progressed so that by day 56, although an acute inflammatory reaction was still present, interstitial fibrosis was the most prominant feature. A constant feature of the lesion was the presence of Samorin deposits in the cytoplasm of multinucleated giant cells and macrophages.

Compared/

Compared to the lesion caused by Samorin, Samorin-Dextran when inoculated intramuscularly resulted in a more limited reaction so that there was no generalised myositis, necrosis, oedema and cellulitis which was so prominent in the case of Samorin. Samorin-Dextran resulted in a small lesion well encapsulated by connective tissue.

The histopathological changes at day 7 were characterised by obvious deposits of Samorin-Dextran surrounded by macrophages and lymphocytes. This progressed so that by day 56 the Samorin-Dextran was surrounded by giant cells, macrophages and advanced fibrosis. This resulted in a distinct granulomatous appearance to the reaction.

The reaction to a subcutaneous inoculation was one of fibrosis, so that by day 56 it was difficult to distinguish between intramuscular and subcutaneous reactions on histological examination.

The severe tissue reaction caused by Samorin inoculation is considered to be one of the major factors limiting its use. Subcutaneous inoculation of the drug is contraindicated due to the severe nature of the reactions encountered and the manufacturers recommend that it is used by intramuscular inoculation only (Drug Data Sheet, May and Baker Ltd.). However, the extreme nature of the local tissue reaction which develops as a result of the drug inoculation has effectively restricted the sites used to the neck in the majority of cases. One exception to this is the case of draught oxen where a painful lesion on the side of the neck could seriously interfere with harnessing and so reduce the work capacity

of/

of the oxen. However, the alternative injection site of the gluteal area also has its drawbacks as it commonly results in a degree of lameness.

The preference for the use of the side of the neck stems from the poor quality of meat in that area of the carcase and so the effect on the final saleable product is kept to a minimum. Inoculation into the gluteal muscle would result in a major cut of meat being condemned and so would seriously decrease the profitability of meat production. Because of this, efforts to develop a formulation of the drug which does not cause these side effects is urgently required.

Samorin-Dextran shows promise in that the local reaction whether injected intramuscularly or subcutaneously is greatly reduced. The results obtained in this study confirm those of James (1978) and Aliu and Chineme (1980) who described the reduced toxicity when the drug was used in rodents. They also confirm the findings of Aliu and Sanusi (1979) who described a similar reduction in toxicity in cattle.

However, the local tissue toxicity reaction is of secondary importance to the trypanocidal activity of the drugs. The therapeutic activity of Samorin-Dextran compared to Samorin has been studied in both laboratory rodents (James, 1978; Aliu and Chineme, 1980) and cattle (Aliu and Sanusi, 1979). James concluded that there was no significant difference between the curative effect of Samorin and Samorin-Dextran. Aliu and Chineme (1980), however concluded/

concluded from rodent studies that Samorin-Dextran was not as efficacious. In cattle Aliu and Sanusi (1979) concluded that there was no significant difference between the curative effect of the soluble chloride salt and the insoluble dextran complex.

The prophylactic effect of Samorin is achieved by the presence of a drug depot at the inoculation site, from which the drug is constantly absorbed (Hill and McFadzean, 1963). Hence the local tissue toxicity may in fact be an integral part of the prophylactic action of the drug as it results in the presence of a local drug depot. It also binds selectively to other tissues, especially liver, kidney and spleen (Philips, Sternberg, Cronin, Sodergren and Vidal, 1967), a fact which could account for the prophylaxis, though greatly reduced, which occurs after intravenous administration (Toure, 1973). By complexing Samorin with Dextran an insoluble precipitate forms which, if left standing, separates to leave a clear supernatant. Resuspension and inoculation of this complex results in a foreign body granuloma reaction. Hence the drug depot is present as in insoluble precipitate and by releasing the active drug slowly may account for the increased length of prophylaxis achieved with this drug in rodents (James, 1978) while also preventing the high concentration of irritant cations which result in the severe local toxicity.

This, however, is only speculation as far as cattle are concerned, as no trials have yet been carried out in this species with/

with a view to determining the length of prophylaxis. Because of the great success in reducing the local toxicity to such a level that subcutaneous inoculations are possible, it is of prime importance that such experiments are conducted as soon as possible.

REFERENCES

Akol, G.W.O. and Murray, M. (1981). Early events in the pathogenesis of African trypanosomiasis in cattle: Localised skin reactions (chancres) which follow the bite of Glossina morsitans morsitans infected with Trypanosoma congolense, T. brucei and T. vivax. 17th Meeting ISCTRC, Arusha, Tanzania.

Akol, G.W.O. and Murray, M. (1982). Early events following challenge of cattle with tsetse infected with Trypanosoma congolense: Development of the local skin reaction. Veterinary Record, 110, 295 - 302.

Akol, G.W.O. and Murray, M. (1983). Trypanosoma congolense: Susceptibility of cattle to cyclical challenge. Experimental Parasitology, 55, 386 - 393.

Akol, G.W.O., Murray, M. and Morrison, W.I. (1981). Early events in the pathogenesis of African trypanosomiasis in cattle: cyclical rechallenge of cattle exposed to cloned isolates of Trypanosoma congolense. 17th Meeting ISCTRC, Arusha, Tanzania.

Aliu, Y.O. and Chineme, G.N. (1980). Isometamidium-Dextran complex: Toxicity and activity against Trypanosoma vivax and Trypanosoma congolense in rats and mice. Toxicology and Applied Pharmacology, 53, 196 - 203.

Aliu, Y.O. and Sannusi, A. (1979). Isometamidium-Dextran complex: therapeutic activity against Trypanosoma vivax infection in Zebu cattle. Journal of Veterinary Pharmacology and Therapy, 2, 265 - 274.

Barrett, P.A., Cord, F.H.S. and Hepworth, W. (1953). The synthesis of trypanocides. 1. Some pyrimidylaminoquinoline derivatives. Journal of Chemical Society, 50 - 58.

Bell, F.R. (1945). Further notes on the use of the Phenanthridine Compound 1553 in the treatment of Trypanosoma congolense infection in cattle. Veterinary Record, 57, 449.

Berg, S.S. (1960). Structure of Isometamidium (M & B 4180A). 7-m-amidinophenyldiazoamino-2-amino-10-ethyl-9-phenyl-phenanthridinium chloride hydrochloride, the red isomer present in metamidium. Nature (London), 188, 1106 - 1107.

Berg, S.S. Brown, K.N. and Lucas, J.M.S. (1961). The trypanocidal activity and local tolerance of sparingly soluble salts of metamidium. Annals of Tropical Medicine and Parasitology, 55, 298 - 304.

Bevan, LL.E.W. (1928). A method of inoculating cattle against trypanosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 22, 147 - 156.

Bevan, LL.E.W. (1936). Notes on immunity in trypanosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 30, 199 - 206.

Blaser, E., Jibo, J.M.C. and McIntyre, W.I.M. (1979). A field trial of the protective effect of Samorin and Berenil in Zebu cattle under ranching conditions in Tanzania. 15th Meeting ISCTRC, Banjul, The Gambia, 384 - 417.

Bourn, D. and Scott, J.M. (1978). The successful use of work oxen in agricultural development of tsetse infected land in Ethiopia. Tropical Animal Health and Production, 10, 191 - 203.

Bradbury, P. and Gordon, K.C. (1982). Connective tissues and stains. In: Theory and Practice of Histological Techniques. (Second Edition). (eds. John D. Bancroft and Alan Stevens), Churchill Livingston, Edinburgh, London, Melbourne and New York, Chpt. 8. 136 - 137.

Braide, V.B. and Eghianruwa, K.I. (1980). Isometamidium residues in goat tissues after parenteral administration. Research in Veterinary Science, 29, 111 - 113.

Broom, J.C. and Brown, H.C. (1940). Studies in trypanosomiasis. IV. Notes on the serological characters of Trypanosoma brucei after cyclical development in Glossina morsitans. Transactions of the Royal Society of Tropical Medicine and Hygiene, 34, 53 - 64.

Browning, C.H., Cohen, J.B., Ellingworth, S. and Gulbransern, R. (1926). Aminostyryl quinoline and aminoanil quinoline compounds. A new group of trypanocidal agents. Journal of Pathology and Bacteriology, 29, 317.

Browning and Gilmour (1913). Cited by Williamson (1970).

Browning, C.H. and Gulbransen, R. (1934). Prophylaxis of experimental trypanosome infections by chemotherapeutic agents. Journal of Pathology and Bacteriology, 39, 75 - 82.

Browning, C.H., Morgan, G.T., Robb, J.V.M. and Walls, L.P. (1938). The trypanocidal activity of certain phenanthridinium compounds. Journal of Pathology and Bacteriology, 46, 203 - 204.

Burnett, G.F. (1970). Control by insecticides. In: The African Trypanosomiases. (ed. H.W. Mulligan), George Allen and Unwin, Ltd., London. Chapt. 23.

Campbell, G.M., Esser, K.M. and Weinbawm, F.I. (1977). Trypanosoma rhodesiense infection in B cell deficient mice. Infection and Immunity, 18, 434 - 438.

Campbell, G.M. and Philips, S.M. (1976). Adoptive transfer of variant-specific resistance to Trypanosoma rhodesiense with β-Lymphocytes and serum. Infection and Immunity, 14, 1144 - 1150.

Capbern, A., Giroud, G., Baltz, T. and Mattern, P. (1977). Trypanosoma equiperdum étude des variations antigeniques au cours de la trypanosomose experimentale de lapin. Experimental Parasitology, 42, 6 - 13.

Carmichael, J. and Bell, F.R. (1944). The use of a new phenanthridinium compound 1553 in the treatment of Trypanosoma congolense infection in cattle. Veterinary Record, 56, 495 - 496.

Cawdery, M.J.H. (1963). Investigations in Uganda on the chemotherapy of bovine trypanosomiasis with particular reference to the local reactions of prophylactic compounds. 9th Meeting ISCTRC, Conakry, 87 - 99.

Cawdery, M.J.H. and Knight, R.H. (1961). A new method for the administration of trypanocidal drugs to animals. Veterinary Record, 73, 982 - 984.

Cross, G.A.M. (1978). Antigenic variation in trypanosomes. Proceedings of the Royal Society of London, 202, 55 - 72.

Dame, D.E. (1970). Control by sterilisation of Glossina. In: The African Trypanosomiases. (ed. H.W. Mulligan), George Allen and Unwin Ltd., London, Chpt. 26.

Davey, D.G. (1957). The chemotherapy of animal trypanosomiasis with particular reference to the trypanosomal diseases of domestic animals in Africa. Veterinary Review Annual, 3, 15 - 36.

Emery, D.L., Akol, G.W.O., Murray, M., Morrison, W.I. and Moloo, S.K. (1980). The chancre - early events in the pathogenesis of African trypanosomiasis in domestic livestock. In: The Host-Invader Interplay. (ed. H. Van den Borsche), Elsevier/North-Holland Biomedical Press, Amsterdam, New York, Oxford.

Emery, D.L. and Moloo, S.K. (1980). The sequential cellular changes in the local skin reaction produced in goats by Glossina morsitans morsitans infected with Trypanosoma (Trypanozoon) brucei. Acta Tropica, 37, 137 - 149.

Emery, D.L. and Moloo, S.K. (1981). The dynamics of the cellular reactions elicited in skin of goats by Glossina morsitans morsitans infected with Trypanosoma (Nannomonas) congolense or T. (Duttonella) vivax. Acta Tropica, 38, 15 - 28.

Fairbairn, H. and Godfrey, D.G. (1957). The local reaction in man at the site of infection with Trypanosoma rhodesiense. Annals of Tropical Medicine and Parasitology, 51, 464 - 470.

Fairclough, R. (1963a). A summary of the use of Berenil in Kenya. 9th Meeting ISCTRC, Conakry, 81 - 86.

Fairclough, R. (1963b). A comparison of metamidium, 'Samorin', 'Berenil' and ethidium bromide under field conditions in Kenya. Veterinary Record, 75, 855 - 858.

Fairclough, R. and Parsons, B.T. (1964). A note on the chemotherapy of trypanosomiasis in Kenya. Agricultural and Veterinary Chemicals, 5, 169 - 171.

F.A.O. (1970). Production Yearbook, 30, Rome.

Fiennes, R.N.T-W, Jones, E.R. and Laws, S.G. (1946). The course and pathology of Trypanosoma congolense (Broden) disease of cattle. Journal of Comparative Pathology and Therapy, 56, 1 - 27.

Finelle, P. (1974). African animal trypanosomiasis, Part IV. Economic Problems. World Animal Review, 10, 15 - 18.

Ford, E.J.H. (1962). Animal trypanosomiasis with special reference to chemotherapy. Research, London, 15, 11 - 16.

Ford, J. (1963). Game conservation and farming in relation to cattle farming and bovine trypanosomiasis. 9th Meeting ISCTRC, Conakry, 119 - 124.

Ford, J. (1965). Distribution of Glossina and epidemiological patterns in the African trypanosomiases. Journal of Tropical Medicine and Hygiene, 68, 211.

Ford, J. (1970). Control by destruction of the larger fauna. In: The African Trypanosomiases. (ed. H.W. Mulligan), George Allen and Unwin Ltd., London. Chpt. 28.

Ford, J., Nash, T.A.M. and Welch, J.R. (1970). Control by clearing of vegetation. In: The African Trypanosomiases. (ed. H.W. Mulligan), George Allen and Unwin Ltd., London, Chpt. 27.

Fussganger, R. and Bauer, F. (1958). Berenil, a new chemotherapeutic agent in veterinary medicine. Medical Chemistry, 6, 504.

Gibson, W., Mehlitz, D., Lanham, S.M. and Godfrey, D.G. (1978). The identification of Trypanosoma brucei gambiense in Liberian pigs and dogs by isoenzyme and by resistance to human plasma. Tropenmedizin und Parasitologie, 29, 335 - 345.

Goodwin, L.G. (1970). The pathology of African trypanosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 64, 797 - 817.

Gray, A.R. (1965). Antigenic variations in a strain of Trypanosoma brucei transmitted by Glossina morsitans and G. palpalis. Journal of General Microbiology, 41, 195 - 212.

Gray, A.R. and Stephen L.E. (1962). A comparative trial of the local toxicity and prophylactic activity against trypanosomiasis in west African zebu cattle, of metamidium chloride, suramin salt and embonate with antrycide pro-salt. Veterinary Record, 74, 80 - 84.

Groves, M.J. and Wilmshurst, E.C. (1964). Preparation and biological activity of some complexes of trypanocidal phenanthridinium compounds. Journal of Pharmacy and Pharmacology, 16 (Suppl.), 140T - 146T.

Hajduk, S.L. and Vickerman, K. (1981). Antigenic variation in cyclically-transmitted Trypanosoma brucei. Variable antigen type composition of the first parasitaemia in mice bitten by trypanosome-infected Glossina morsitans. Parasitology, 83, 609 - 620.

Hill, J. and McFadzean, J.A. (1963). Studies on isometamidium. Depots of isometamidium in mice and rats and their importance for prophylaxis against Trypanosoma congolense. Transactions of the Royal Society of Tropical Medicine and Hygiene, 57, 476 - 484.

Hirumi, H., Hirumi, K. and Moloo, S.K. (1982). Simplified methods for propagating Trypanosoma congolense metacyclics in vitro. Journal of Protozoology, 29, 480.

Hoare, C.A. (1966). The classification of mammalian trypanosomes. Advances in Parasitology, 5, 47 - 91.

Hoare, C.A. (1970). In: The African Trypanosomiases, (ed: H.W. Mulligan). George Allen and Unwin Ltd., London. Chpt. 2.

Hoare, C.A. (1972). In: The Trypanosomes of Mammals: A zoological monograph'. Blackwell Scientific Publications, Oxford and Edinburgh.

Holmes, P.H., MacAskill, J.A., Whitelaw, D.D., Jennings, F.W. and Urquhart, G.M. (1979). Immunological clearance of ^{75}Se-labelled Trypanosoma brucei in mice. I. Aspects of the radiolabelling technique. Immunology, 36, 415 - 420.

Holmes, P.H., Mamo, E., Thompson, A., Knight, P.A., Luckins, R., Murray, P.K., Murray, M., Jennings, F.W. and Urquhart, G.M. (1974). Immunosuppression in bovine trypanosomiasis. Veterinary Record, 95, 86 - 87.

Holmes, P.H. and Scott, J.M. (1982). Chemotherapy against animal trypanosomiasis. In: Perspectives in trypanosomiasis research , ed J.R. Baker. Research Studies Press, J. Wiley and Sons, Ltd. Chpt. 6.

James, D.M. (1978). Prophylactic activity in rodents of trypanocides complexed with dextran. Transactions of the Royal Society of Tropical Medicine and Hygiene, 72, 471 - 476.

Jasiorowski, A.H. (1972). FAO's activities in livestock development. World Animal Review, 1, 2 - 9.

Jennings, F.W., Whitelaw, D.D. and Urquhart, G.M. (1977). The relationship between duration of infection with Trypanosoma brucei in mice and the efficacy of chemotherapy. Parasitology, 75, 143 - 153.

Jensch, H. (1937). Neue Chemotherapeutika der 4-Amino-chinolin-reihe. Angewandte Chemie, 50, 891 - 895.

Jensch H. (1955). 4,4'-Diamidino-diazoaminobenzol, ein neues Mittel gegen Trypanosomen und Babesien - Infektionen. Arzneimittelforschung, 5, 634 - 635.

Jones-Davies, W.J. (1967a). A Berenil-resistant strain of T. vivax in cattle. Veterinary Record, 81, 567 - 568.

Jones-Davies, W.J. (1967b). The protection of a small group of Nigerian trade cattle from trypanosomiasis using 'Samorin'. Bulletin of Epizootic Diseases of Africa, 15, 323 - 335.

Jones-Davies, W.J. (1968). Berenil resistance in naturally occurring T. congolense. Bulletin of Epizootic Diseases of Africa, 16, 213 - 216.

Jones-Davies, W.J. and Folkers, C. (1966). Some observations on cross-resistance to 'Samorin' and 'Berenil' of homidium-resistant field strains of Trypanosoma congolense in Northern Nigerian cattle. 11th Meeting, ISCTRC, Nairobi, 35 - 40.

Joshua, R.A., Magaji, Y. and Kayit, Y.S. (1983). Isolation of human serum resistant Trypanozoon from cattle in Nigeria. Tropenmedizin und Parasitologie, 34, 201 - 202.

Kirkby, W.W. (1961). Prophylaxis under continuous exposure to the risk of natural infection with trypanosomiasis by tsetse flies: A comparative trial of metamidium chloride, antrycide prosalt and antrycide methylsulphate. Veterinary Record, 73, 1411 - 1415.

Kirkby, W.W. (1963). A trial to compare the therapeutic activity in cattle trypanosomiasis of Homidium and Isometamidium. Bulletin of Epizootic Diseases of Africa, 11, 299-301.

Kirkby, W.W. (1964). Prophylaxis and therapy under continuous exposure to the risk of natural infection with trypanosomiasis by tsetse flies. Bulletin of Epizootic Diseases of Africa, 12, 321 - 329.

Lanham, S.M. and Godfrey, D.G. (1970). Isolation of Salivarian trypanosomes from man and other mammals using DEAE-cellulose. Experimental Parasitology, 28, 521 - 534.

Leach, T.M. and Roberts, C.J. (1981). Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the Eastern hemisphere. Pharmacology and Therapeutics, 13, 91 - 147.

LeRay, D, Barry, J.D. and Vickerman, K. (1978). Antigenic heterogenecity of metacyclic forms of Trypanosoma brucei. Nature (London), 273, 300.

Le Roux, P.L. (1936). Trypanosomiasis. Annual Report for 1936. Veterinary Department of Northern Rhodesia. Appendix C64.

Lourie, E.M. and Connor, R.J. (1936). Trypanolysis in vitro by mouse immune serum. Annals of Tropical Medicine and Parasitology, 30 , 365 - 388.

Luckins, A.G. (1972). Effects of X-irradiation and cortisone treatment of Albino rats on infections with Brucei-complex trypanosomes. Transactions of the Royal Society of Tropical Medicine and Hygiene, 66, 130 - 139.

Luckins, A.G., Rae, P.F. and Gray, A.R. (1983). Infection, immunity and the development of local skin reaction in rabbits infected with cyclically-transmitted stocks of Trypanosoma congolense. Annals of Tropical Medicine and Parasitology, 77, 569 - 582.

Lumsden, W.H.R. and Wells, E.A. (1968). Trypanosomiasis. In: Infectious blood diseases of man and animals. Vol. II (ed. D. Weinman and M. Ristic). Academic Press, New York and London, 329 - 385.

Lumsden, W.H.R. and Herbert, W.J. (1967). Phagocytosis of trypanosomes by mouse peritoneal macrophages. Transactions of the Royal Society of Tropical Medicine and Hygiene, 61, 142.

Lumsden, W.H.R., Herbert, W.J. and McNeillage, G.J.C. (1973). Techniques with Trypanosomes. Churchill Livingstone, Edinburgh and London.

MacAskill, J.A., Holmes, P.H., Jennings, F.W. and Urquhart, G.M. (1981). Immunological clearance of ^{75}Se-labelled Trypanosoma brucei in mice. III Studies in animals with acute infections. Immunology, 43, 691 - 698.

MacAskill, J.A., Holmes, P.H., Whitelaw, D.D., McConnell, I., Jennings, F.W. and Urquhart, G.M. (1980). Immunological clearance of ^{75}Se labelled Trypanosoma brucei in mice. II. Mechanisms in immune animals. Immunology, 40, 629 - 635.

MacLennan, K.J.R. (1968). Some recent findings concerning the use of Berenil for the treatment of bovine trypanosomiasis in Northern Nigeria. Transactions of the Royal Society of Tropical Medicine and Hygiene, 62, 139 - 140.

MacLennan, K.J.R. (1971). The aparasitaemic interval following diaminazene aceturate therapy of a relapsing strain of T. vivax infecting cattle. Tropical Animal Health and Production, 3, 208 - 212.

MacLennan, K.J.R. and Jones-Davies, W.J. (1967). The occurrence of a Berenil-resistant Trypanosoma congolense strain in Northern Nigeria. Veterinary Record. 80, 389 - 390.

MacLennan, K.J.R. and Na'isa, B.K. (1970). Relapsing T. vivax infections in Nigerian Zebu cattle treated with diaminazene aceturate. Tropical Animal Health and Production, 2, 189 - 195.

Milne, A.H., Robson, J. and Lwebandiza, T. (1955). The efficacy of Berenil against T. congolense in Zebu cattle. Veterinary Record, 67, 280 - 281.

Moloo, S.K., Kutuza, S.B. and Boreham, D.F.L. (1980). Studies on Glossina pallipides, G. fuscipes fuscipes and G. brevipalpis in terms of the epidemiology and epizootiology of trypanosomiasis in south-eastern Uganda. Annals of Tropical Medicine and Parasitology, 74, 219 - 237.

Moloo, S.K., Losos, G.J. and Kutuza, S.B. (1973). Transmission of T. brucei to cats and dogs by feeding on infected goats. Transactions of the Royal Society of Tropical Medicine and Hygiene, 67, 287.

Moloo, S.K., Steiger, R.F. and Brian, R. (1973). Trypanosome infection rates in Glossina swynnertoni and G. pallidipes in Ikoma, Musama district, Tanzania. Parasitology, 66, 259 - 267.

Morrison, W.I., Murray, M. and McIntyre, W.I.M. (1982). Bovine trypanosomiasis. In: Diseases of cattle in the tropics. (eds. M. Ristic and W.I.M. McIntyre) Martinus Nijhoff, The Hague, Boston and London, Chpt. 36.

Morrison, W.I., Black, S.J., Paris, J. Hinson, C.A. and Wells, P.W. (1982). Protective immunity and specificity of antibody responses elicited in cattle by irradiated Trypanosoma brucei. Parasite Immunology, 4, 395 - 407.

Mulligan, H.W. (1970). The African trypanosomiasis. Ministry of Overseas Development, George Allen and Unwin.

Murray, M., Clifford, D.J., Gettinby, G., Snow, W.F. and McIntyre, W.I.M. (1981). Susceptibility to African trypanosomiasis of N'Dama and Zebu cattle in an area of Glossina morsitans submorsitans challenge. Veterinary Record, 109, 503 - 510.

Murray, P.K., Jennings, F.W., Murray, M. and Urquhart, G.M. (1974). The nature of immunosuppression in Trypanosoma brucei infections in mice. Immunology, 27, 825 - 840.

Murray, M., Murray, P.K. and McIntyre, W.I.M. (1977). An improved parasitological technique for the diagnosis of African trypanosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 71, 325 - 326.

Murray, P.K., Murray, M., Wallace, M., Morrison, W.I. and McIntyre, W.I.M. (1979). Trypanosomiasis in N'Dama and Zebu cattle I. An experimental investigation of susceptibility to Trypanosoma brucei, T. congolense and mixed infections. 15th Meeting ISCTRC, Banjul, The Gambia 476 - 481.

Murray, M., Trail, J.M.C. and Grootenhuis, J.G. (1984). Trypanotolerant livestock: potential and future exploitation. Outlook on Agriculture, 13, 43 - 51.

Musoke, A.J., Nantulya, V.M., Barbet, A.F., Kironde, F. and McGuire, T.C. (1981). Bovine immune response to African trypanosomiasis specific antibodies to variable surface glycoproteins of Trypanosoma brucei. Parasite Immunology, 3, 97 - 106.

Na'isa, B.K. (1969). The protection of 60 Nigerian trade cattle from trypanosomiasis using Samorin. Bulletin of Epizootic Diseases of Africa, 17, 45 - 54.

Paris, J., Murray, M. and McOdimba, F. (1982). A comparative evolution of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle. Acta Tropica, 39, 307 - 316.

Phillips, F.S., Sternberg, S.S., Cronin, A.P., Sodergren, J.E. and Vidal, P.M. (1967). Physiological disposition and intracellular localization of isometamidium. Cancer Research, 27, 333 - 346.

Pilmmer, H.G. and Thompson, J.D. (1908). Further results of the experimental treatment of trypanosomiasis in rats: being a progress report of a committee of the Royal Society. Proceedings of the Royal Society, Series B, 80 1 - 12.

Pinder, M. and Authie, E. (1984). The appearance of isometamidium resistant Trypanosoma congolense in West Africa. Acta Tropica, 41, 247 - 252.

Randall, J.A. and Beveridge, C.G.L. (1946). A note on the toxicity of Phenanthridinium 1553 in cattle. Veterinary Record, 58, 398 - 399.

Rickman, L.R. and Robson, J. (1970). The testing of proven Trypanosoma brucei and T. rhodesiense strains by the blood incubation infectivity test. Bulletin of the World Health Organisation, 42, 911 - 916.

Robson, J. (1962). Prophylaxis against trypanosomiasis in Zebu cattle. IV. A field trial of Metamidium and Isometamidium. Veterinary Record, 74, 913 - 917.

Robson, J. and Cawdery, M.J.H. (1958). Prophylaxis against trypanosomiasis in Zebu cattle. A comparison of Prothidium, the suraminates of ethidium and RD 2902, and Antrycide Prosalt. Veterinary Record, 70, 870 - 876.

Rollo, I.M. (1970). Miscellaneous drugs used in the treatment of protozoal infections. In: The pharmacological basis of therapeutics. (Fourth edition). (eds L.S. Goodman and A. Gilman). New York, MacMillan, 1145.

Ruchel, W.M. (1975). Chemoprophylaxis of bovine trypanosomiasis. Thesis, University of Gottingen.

Scott, J.M. and Pegram, R.G. (1974). A high incidence of Trypanosoma congolense strains resistant to Homidium Bromide in Ethiopia. Tropical Animal Health and Production, 6, 215 - 221.

Scott, J.M., Pegram, R.G., Holmes, P.H., Pay, T.W.F., Knight, P.A., Jennings, F.W. and Urquhart, G.M. (1977). Immunosuppression of bovine trypanosomiasis: field studies using foot and mouth disease vaccine and clostridial vaccine. Tropical Animal Health and Production, 9, 159 - 165.

Smith, I.M. (1959). Chemoprophylaxis against bovine trypanosomiasis. I. Duration of protection from prothidium and ethidium and R.D. 2902 suraminates in an area of high tsetse density. Journal of Comparative Pathology, 69, 105 - 115.

Smith, I.M. and Brown, K.H. (1960). Chemoprophylaxis against bovine trypanosomiasis. II. Duration of protection afforded by preparations of metamidium, prothidium and antrycide prosalt in an area of high tsetse density. Journal of Comparative Pathology, 70, 161 - 175.

Soltys, M.A. (1955). Studies on resistance to Trypanosoma congolense developed by Zebu cattle treated prophylactically with antrycide prosalt in an enzootic area of East Africa. Animals of Tropical Medicine and Parasitology, 49, 1 - 8.

Stephen, L.E. (1958). Suramin complexes: IV. Ethidium bromide complex. A large scale laboratory trial of its prophylactic activity in cattle. Annals of Tropical Medicine and Parasitology, 52, 417 - 426.

Stephen, L.E. (1960). The prophylactic and therapeutic activity of metamidium and its suramin salt against trypanosomiasis in cattle. Veterinary Record, 72, 80 - 84.

Stephen, L.E. (1962). Suramin complexes. X. Comparison of the prophylactic activity of Prothidium-suramin complex and Prothidiu bromide in West African Zebu cattle. Annals of Tropical Medicine and Parasitology, 56, 406 - 414.

Stevens, A. (1982a). The haematoxylins. In: Theory and Practice of Histological Techniques. (Second edition). (eds. D. Bancroft and A. Stevens), Churchill Livingston, Edinburgh, London, Chpt 7, 113 - 114.

Stevens, A. (1982b). Pigments and minerals. In: Theory and Practice of Histological Techniques. (Second edition). (eds J.D. Bancroft and A. Stevens, Churchill Livingston, Edinburgh, London. Chpt. 13, 258.

Takayanagi, T., Kambara, H. and Enriques, G.L. (1973). Immunity with spleen cell and antiserum transfer in mice. Experimental Parasitology, 33, 429 - 432.

Toure, S.M. (1973). Notes on the trypanocidal activity of isometamidium administered intravenously. Bulletin of Epizootic Diseases of Africa, 21, 1.

Vale, G.A. (1982). The improvememt of traps for tsetse flies (Diptera:Glossinidae). Bulletin of Entomological Research, 72, 95 - 106.

van Rensberg, S.W.J. (1938). Surfen C therapy in Trypanosoma congolense infection in bovines and ovines. Onderstepoort Journal of Veterinary Science and Animal Industry, 10, 13 - 20.

Vickerman, K. (1969). On the surface coat and flagellar adhesions in trypanosomes. Journal of Cell Science, 5, 163 - 193.

Watkins, T.J. (1958). Trypanocidal pyrinidylphenanthridine derivatives. British Patent.

Watkins, T.J. and Woolfe, G. (1956). Effect of changing the quaternizing group on the trypanocidal activity of dimidium bromide. Nature, London, 178, 368.

Wellde B.T. and Chumo, D.A. (1983). Persistence of Berenil in cattle. Tropical Animal Health and Production, 15, 149 - 150.

Whitelaw, D.D., Moulton, J.E., Morrison, W.I. and Murray, M. (1985). Central nervous system involvement in goats undergoing primary infections with Trypanosoma brucei and relapse infections after chemotherapy. Parasitology, 90, 255 - 268.

Whitelaw, D.D., Scott, J.M., Reid, H.W., Holmes, P.H., Jennings, F.W. and Urquhart, G.M. (1979). Immunosuppression in bovine trypanosomiasis: studies with louping-ill vaccine. Research in Veterinary Science, 26, 102 - 107.

Whiteside, E.F. (1962). Interactions between drugs, trypanosomes and cattle in the field. In: Drugs, parasites and hosts. (eds. L.G. Goodwin and R.H. Nimmo-Smith). Churchill, London, 116 - 141.

Wiesenhutter, E., Turner, D.B. and Kristensen, K.A. (1968). Aspects of current bovine trypanosomiasis control in Tanzania - a comparative field trial of available chemoprophylactics under ranching conditions. Bulletin of Epizootic Diseases of Africa, 16, 419 - 424.

Williamson, J. (1957). Suramin complexes: I. Prophylactic activity against Trypanosoma congolense in small animals. Annals of Tropical Medicine and Parasitology, 51, 440 - 456.

Williamson, J. (1961). Some problems in trypanocidal drug resistance. 8th Meeting, ISCTRC, Jos, Nigeria, 163 - 169.

Williamson, J. (1970). Review of chemotherapeutic and chemoprophylactic agents. In: The African Trypanosomiasis. (ed. H.W. Mulligan), George Allen and Unwin Ltd., London. Chpt. 7.

Williamson, J. (1976). Chemotherapy of African trypanosomiasis. Tropical Diseases Bulletin, 73, 531 - 542.

Williamson, J. (1979). Chemoresistance in trypanosomes. F.A.O., Expert consultations on research on trypanosomiasis. AGA: 801.

Williamson, J. and Desowitz, R.S. (1956). Prophylactic activity of suramin complexes in animal trypanosomiasis. Nature, London, 117, 1074 - 1075.

Wilson, A.J., Le Roux, T.G., Paris, J., Davidson, C.R. and Gray, A.R. (1975a). Observations on a herd of beef cattle maintained in a tsetse area, I. Assessment of chemotherapy as a method of the control of trypanosomiasis. Tropical Animal Health and Production, 7, 187 - 199.

Wilson, A.J., Paris, J. and Dar, F.K. (1975b). Maintenance of a herd of breeding cattle in an area of high trypanosome challenge. Tropical Animal Health and Production, 7, 63 - 71.

Wilson, J., Paris, J., Luckins, A.G., Dar, F.K. and Gray, A.R. (1976). Observations on a herd of beef cattle maintained in a tsetse area. II. Assessment of the development of immunity in association with trypanocidal drug treatment. Tropical Animal Health and Production, 8, 1 - 12.

Wilson, S.G. (1958). Animal trypanosomiasis in Northern Nigeria. In: Symposium on Animal Trypanosomiasis, 37 - 52. London, CCTA Publ. No. 45.

Wragg, W.R., Washbourne, K., Brown, K.N. and Hill, J. (1958). Metamidium: a new trypanocidal drug. Nature, London, 182, 1005 -1006.

Wright, N.C. (1961). The current food supply situation and present trends. In: Hunger: can it be averted? British Association for the Advancement of Science. London, 1 - 14.