FACTORS INFLUENCING THE DURATION OF ISOMETAMIDIUM PROPHYLAXIS AGAINST BOVINE TRYPANOSOMIASIS

Thesis submitted for the degree of Doctor of Philosophy to the Faculty of Veterinary Medicine, University of Glasgow

by

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I speak of Africa and golden joys.

Henry IV, Act V, Sc. 3

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SUMMARY

Experiments have been conducted with Boran (Bos indicus) cattle to determine the factors that influence the duration of isometamidium (Samorin) prophylaxis against experimental challenge with metacyclic forms of Trypanosoma congolense and Trypanosoma vivax.

The duration of a single isometamidium prophylactic treatment against T.congolense ILNat 3.1 and T.congolense IL 2642 was examined in 24 Boran steers with regard to (i) the dose of drug, (ii) the level of metacyclic challenge, and (iii) the influence of infection at the time of treatment with an unrelated serodeme. The cattle were repeatedly challenged at monthly intervals between 2 and 7 months following treatment, either by 5 infected Glossina morsitans centralis or by intradermal inoculation of $5x10^3$ or $5x10^5$ in vitro-derived metacyclic trypanosomes. A dose of 1 mg kg^{-1} body weight afforded complete protection for 4 months and 0.5mg kg^{-1} for 3 months against the two T.congolense serodemes examined, irrespective of the method or weight of challenge. In another group of cattle, which had an established infection at the time of treatment, the duration of chemoprophylaxis against an unrelated serodeme was the same as the other groups which had no previous experience of trypanosome infection. Antibodies to metacyclics did not appear in any of the cattle as long as the chemoprophylaxis was effective. An exception to this was the group challenged with $5x10^5$ in vitro-derived metacyclic parasites, in which low antibody titres were

detected. In all cases these proved to be non-protective. It was therefore concluded that under the experimental conditions employed, (i) there was a direct relationship betwen drug dosage and the duration of chemoprophylaxis, (ii) the weight of metacyclic challenge did not affect the duration of chemoprophylaxis, and (iii) when used to treat an existing infection, isometamidium exerted the same degree of chemoprophylactic activity.

In experiments with T.vivax, twenty Boran steers were infected with T.vivax transmitted by G.m.centralis; five with a T.vivax clone from Zaria, Nigeria (IL 2968), five with a T.vivax clone from Kilifi, Kenya (IL 2969), five with a T.vivax population from Galana, Kenya (IL 2982) and five with a T.vivax population from Likoni, Kenya (IL 2986). Eleven days after infection all 20 animals were treated with 0.5mg kg^{-1} isometamidium chloride. All steers except one infected with the Zaria T.vivax were completely cured. When twenty different Boran steers were administered a single prophylactic dose of 0.5mg kg^{-1} isometamidium chloride and subjected to monthly tsetse-transmitted challenge with the same T.vivax populations, complete protection was afforded for two months against challenge with the Zaria T.vivax, for one month against the Likoni T.vivax and for less than one month against the Kilifi and Galana T.vivax. The findings indicated that the level of sensitivity of a T.vivax population to the prophylactic activity of isometamidium cannot be concluded from sensitivity studies based on the

therapeutic action of the drug.

In a similar manner to studies with T.congolense, acquisition of immunity to homologous T.vivax challenge did not appear to occur whilst animals were protected by isometamidium and did not contribute to the apparent prophylactic period.

Using a T.congolense in vitro culture system, a qualitative technique was developed to detect trypanocidal activity in sera from drug-administered cattle:

Trypanocidal activity, as detected by inhibition of infectivity and in vitro growth of T.congolense ILNat 3.1, was detected in sera, collected from cattle as long as 5 months after administration of 1mg kg^{-1} isometamidium chloride; activity that correlated with susceptibility of cattle to challenge with T.congolense ILNat 3.1.

The same technique was used to confirm the high level of resistance expressed by the Kilifi T.vivax to the prophylactic activity of isometamidium. Trypanocidal activity, against T.congolense ILNat 3.1, was detected in serum taken from cattle 1 and 2 months after administration of 0.5mg kg^{-1} isometamidium chloride, at a time when the cattle were susceptible to challenge with the Kilifi T.vivax.

Similar studies were conducted with a T.vivax in vitro culture system: Freshly isolated bloodstream forms of T.vivax IL 2968 (Zaria) expressed a similar level of sensitivity as T.congolense ILNat 3.1 bloodstream forms to trypanocidal activity in sera from drug-treated cattle.

Freshly isolated murine bloodstream forms were found to be better suited to the detection of trypanocidal activity in sera from drug-treated cattle than freshly isolated bovine bloodstream forms.

It was concluded from *in vitro* tests using *T.congolense* ILNat 3.1 and *T.vivax* IL 2968 that there was in general a positive correlation between the expression of trypanocidal activity of serum *in vitro* and the susceptibility of cattle to challenge with the same trypanosome populations. However, the reliability of the *in vitro* test was diminished by the observation that sera from untreated cattle occasionally possessed trypanocidal activity. The reason for this latter observation is, at present, unknown.

ABBREVIATIONS USED IN TEXT, TABLES AND FIGURES

BC	Buffy-coat phase-contrast technique
BSA	Bovine serum albumin
BSS	Balanced salt solution
bVAT	Bloodstream variable antigen type
CD_{50}	50% curative dose (dose which cured 50% of animals treated)
cm	Centimeter
cm^2	Square centimeter
CNS	Central nervous system
DDT	Dichlorodiphenyltrichloroethane
DEAE	Diethylamino-ethyl
dm^{-3}	Per liter (per cubic decimeter)
DNA	Deoxyribonucleic acid
ECGS	Endothelial cell growth supplement
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
FITC	Fluorescein isothiocyanate conjugate
g^{-1}	Per gram
IC_{50}	50% inhibitory concentration (drug concentration which inhibited in vitro growth by 50%)
IFA	Indirect immunofluorescent antibody
IgG	Immunoglobulin G
IgG_1	Immunoglobulin G_1
IgG_2	Immunoglobulin G_2
IgM	Immunoglobulin M
ILNat	ILRAD Nannomonas antigenic type
ILRAD	International Laboratory for Research on Animal Diseases
i.p.	Intraperitoneal
i.u.	International units
kg	Kilogram
kg^{-1}	Per kilogram
km	Kilometer
km^2	Square kilometer
km^{-2}	Per square kilometer
M	Moles per liter
M199	Medium 199
MEF	Microtus montanus embryonic fibroblasts
MEM	Minimum essential medium
mg	Milligram
mg^{-1}	Per milligram
ml	Milliliter
ml^{-1}	Per milliliter
mM	Millimoles per liter
mVAT	Metacyclic variable antigen type
NAD	Nicotinamide adenine dinucleotide
PBS	Phosphate buffered saline
PCV	Packed red cell volume
PMN	Polymorphonuclear leucocytes
PSG	Phosphate saline glucose
RNA	Ribonucleic acid
S.D.	Standard deviation
ug	Microgram

ul	Microliter
um	Micrometer
VAT	Variable antigen type
VSSA	Variant specific surface antigen
v/v	Volume per volume
w/v	Weight per volume

INTRODUCTION

Despite 65-70% of the world's livestock population residing within the developing countries, this only provides 30% of the world's meat output (FAO, 1975). Since the estimated production of animal protein from livestock farming per 1,000 hectares in 1975 was 542kg in Africa, 4,113kg in Latin America and 38,083kg in Europe (FAO, 1975), African livestock farming appears to produce less animal protein than livestock farming in any other continent. It seems likely that this situation may deteriorate further since Africa's human population increased by twice the rate of food production between 1980 and 1985, and will probably increase from an estimated 470 million in 1985 to 877 million in 2000 (Anon, 1984). An increase in the continent's food production is thus urgently required.

Probably the most significant factor limiting extension of the cattle producing areas of Africa is the tsetse fly, the vector of trypanosomiasis (FAO, 1961; ILCA, 1985). The disease is cyclically transmitted by tsetse which belong to the genus Glossina (Mulligan, 1970). As early as 1857, David Livingstone had indicated the great importance of the disease in African cattle and linked its presence with the tsetse fly distribution. Tsetse-born trypanosomiasis renders approximately 10,000,000km^2 of prime African land unsuitable for cattle production (Swynnerton, 1936; FAO, 1961; FAO/WHO/OIE, 1982) and tsetse infested areas encompass 37% of the continent, including 38 countries. If it were not for the tsetse, 7,000,000km^2 of

this land would otherwise be suitable for livestock or mixed agriculture and would support an extra 140 million cattle and at least the equivalent number of sheep and goats (FAO/WHO/OIE, 1982). In so doing, it would generate an additional yearly income of US$750 million at 1980 prices (Finelle, 1980).

Of the 147 million cattle living within the 38 countries in which tsetse occur, approximately 30% are exposed to infection (FAO/WHO/OIE, 1982; Murray and Gray, 1984). In 1960 the potential capital value lost through permanent infestations was estimated to be US$5,000,000,000 (FAO, 1982). The most severely affected countries are those in West and Central Africa. Thus, although 26% of Africa's human population live in 18 countries in the land mass from Senegal to Zaire, the same area contains only 9% of Africa's cattle, sheep and goats (ILCA, 1979). In this region the average livestock biomass per capita is 26kg, contrasting to 136 kg for the remainder of Africa south of the Sahara, and 79 kg for the continent as a whole (ILCA, 1979). If one could remove trypanosomiasis from this region, the average cattle carrying capacity would increase from the current 3.4 cattle km^{-2} to a potential of 20 km^{-2}. In addition, the small ruminant population would increase approximately five fold (FAO, 1974).

In Southern and Eastern Africa the situation in many areas is no better, with up to 70% of the land infested by tsetse (e.g., Tanzania (FAO, 1974)).

Tsetse, by virtue of trypanosomiasis transmission,

exclude cattle from considerable areas. Where cattle are kept in association with tsetse, severe losses occur in livestock production; poor growth, weight loss, lowered milk yield, reduced traction capability, infertility and abortion (FAO/WHO/OIE, 1963; McDowell, 1977). The annual meat production loss due to trypanosomiasis has been estimated to be US$5 billion (FAO/WHO/OIE, 1963).

During the 1890's the rinderpest panzootics removed tsetse food sources and thus produced a large scale regression in tsetse-infested areas. However, most areas from where they disappeared have subsequently been reinfested (MacLennan, 1980). Since the 1950's, widespread territorial expansions of tsetse infestation in savanna areas has occurred. Since 1952, Glossina morsitans has re-occupied at least 25,900km^2 of central and northern Nigeria. Since 1950, G.morsitans in central Cameroon has re-occupied 20,860km^2 (MacLennan, 1980) and in south west Zambia, G.morsitans has re-occupied 11,700km^2 since 1953 (MacLennan, 1975). Similar advances are also known to be occurring in South-East Angola (FAO, 1979; MacLennan, 1980).

Because of the expansion of tsetse-infested areas, increasing pressure is being exerted on tsetse-free pastures, resulting in pasture degradation, decreased output and increased production costs. To meet the needs of the increasing African population, and to alleviate the pressure on the tsetse-free regions, the African farmer will have to utilise the tsetse-infested regions (ILCA,

1985).

BOVINE TRYPANOSOMIASIS - THE DISEASE SYNDROME

As early as 1857 a disease syndrome associated with the bite of the tsetse fly was described by Livingstone. A few days after being bitten, cattle were observed to develop an oculonasal discharge, submandibular, and occassional ventral abdominal, swelling and progressive emaciation.

In the ensuing years three distinct species of trypanosomes belonging to different sub-genera, and later shown to be pathogenic for domestic ruminants (Fiennes, 1950; Mulligan, 1970), were identified: Trypanosoma brucei brucei (subgenus Trypanozoon) (Plimmer and Bradford, 1899), T.(Nannomonas) congolense (Broden, 1904), T.(Duttonella) vivax (Ziemann, 1905). All three trypanosome species are found throughout the tsetse-infested areas with mixed infections being common in the field (Stephen, 1970).

The two most commonly used techniques for identification of the three species in the field are either morphological differences on stained fixed blood smears or characteristic motility behaviour when observed in a fresh blood film (see table 1).

Because of the occurrence of mixed infections and also the wide variation in species virulence, clearly defined clinical syndromes are by no means the usual. However, studies on field and experimental infections have indicated three main types of disease syndrome in cattle (Morrison,

Table 1

CHARACTERISTICS OF TRYPANOSOMES PATHOGENIC FOR CATTLE

(After Morrison, Murray and McIntyre, 1981)

SPECIES	SITE OF DEVELOPMENT IN TSETSE FLY	BLOODSTREAM FORMS IN CATTLE			
		SIZE (um)	MORPHOLOGY		BEHAVIOUR
T.congolense	Midgut Proboscis	9-18	Kinetoplast- Posterior end- Undulating membrane- Flagellum-	medium size, marginal blunt poorly defined no free flagellum	Sluggish movement often attached to red cells
T.vivax	Proboscis	20-27	Kinetoplast- Posterior end- Undulating membrane- Flagellum-	large, terminal rounded usually poorly defined free	Very rapid movement across the microscopic field
T.brucei	Midgut Salivary glands	15-39*	Kinetoplast- Posterior end- Undulating membrane- Flagellum-	small, subterminal pointed well defined free	Rapid movement in confined areas

*Polymorphic - slender, intermediate and stumpy forms; no free flagellum on stumpy form.

Murray and Akol, 1985):

1) ANAEMIA AND EMACIATION / POOR GROWTH

The most common clinical syndrome associated with bovine trypanosomiasis is that of progressive anaemia, the "cardinal clinical sign" (Hornby, 1921; Murray, 1974), associated with a persistent fluctuating parasitaemia (Morrison et al., 1981; Murray, 1979). All three pathogenic trypanosome species may produce this syndrome.

The outcome of the disease depends on a number of factors; the level of challenge, species of trypanosome (Hornby, 1952), breed of cattle (Morrison et al., 1981), the species of tsetse fly (Harley and Wilson, 1968), the absence or presence of intercurrent helminth infection (Griffin, Allonby and Preston, 1981; Specht, 1982) and probably the level of nutrition (Morrison et al., 1981). Generally, the pack red cell volume per cent (PCV) undergoes a progressive decrease to approximately 20% over the first 4-6 weeks of infection. In some animals the PCV continues to decline until death occurs, in others it is maintained at this low level for a variable period of time. In some of these animals death eventually occurs, however in others, after the disappearance of parasitaemia, the PCV rises and the animal recovers (Morrison et al., 1981).

2) ACUTE *T.VIVAX* SYNDROME

An acute haemorrhagic syndrome in which death occurs 2 to 4 weeks after infection has been described in East Africa and is associated with certain isolates of T.vivax

(Hudson, 1944; Mwongela, Kovatch and Fazil, 1981). A hyperacute syndrome associated with T.vivax infection was first recorded in 1923 in Dar es Salaam, Tanganyika. Cattle in good body condition died after a brief illness (Anon, 1923). Cornell (1936) reported similar acute T.vivax infections in Mbulu district, Tanganyika. In Kenya, acute T.vivax haemorrhagic syndromes have been described in which death occurred after a short illness (Hudson, 1944; Lewis, 1949; Anon, 1966; Mwongela et al., 1981; Wellde, Chumo, Adoyo, Kovatch, Mwongela and Opiyo, 1983). An outbreak of acute T.vivax infections has also been described in Ethiopia (Roeder, Scott and Pegram, 1984).

The syndrome is characterised by pronounced pyrexia, persistent high levels of parasitaemia and, in the haemorrhagic form, blood-stained diarrhoea (Hudson, 1944; Lewis, 1949; Anon, 1966; Mwongela et al., 1981; Wellde et al., 1983). Gross pathological changes described for the haemorrhagic form include diffuse petechial or ecchymotic haemorrhages on all visceral surfaces, and haemorrhage into the alimentary tract (Hudson, 1944; Anon, 1966; Mwongela et al., 1981).

3) MENINGOENCEPHALITIS

Early studies on human African trypanosomiasis ("sleeping sickness") described a meningoencephalitic stage (Thomas and Breinl, 1905; Mott, 1906) associated with infections by both T.brucei rhodesiense and T.brucei

gambiense. This, so called stage II form of the disease, is conventionally defined by the presence of trypanosomes in the central nervous system (CNS) as assessed by lumbar puncture (WHO, 1979). Compared to infections with T.congolense and T.vivax in cattle, T.brucei is considered to be the least pathogenic, based on the severity of anaemia produced and the level of parasitaemia (Fiennes, 1970; Killick-Kendrick, 1971). However, clinical abnormalities of the central nervous system are observed when cattle are infected with certain serodemes of T.brucei (Losos and Ikede, 1972; Morrison, Murray, Whitelaw and Sayer, 1983). Affected animals showed a gradual loss of body condition over 2-6 weeks prior to death. Signs of CNS involvement were always observed: depression, unsteady gait, head pressing and in some, circling. One to three days before death the animals became recumbent and exhibited opisthotonus and paddling of the limbs. In those cattle that developed CNS disease the pathological changes were very similar to those described for fatal cases of human trypanosomiasis (Mott, 1907; Manuelidis, Robertson, Amberson, Polak and Haymaker, 1965; Morrison et al., 1983). In both cases an extensive infiltrate, composed of lymphocytes, plasma cells and macrophages, occurred in the meninges and perivascularly in the Virchow-Robin spaces. A bovine CNS syndrome has been described in two situations; firstly as a primary infection with certain serodemes of T.brucei and, secondly, varying periods after cattle infected with T.brucei were treated with the trypanocidal

drug Berenil (diminazene aceturate, Hoechst, Frankfurt, W. Germany) (Morrison et al., 1981). A similar disease syndrome has been described in cattle experimentally infected with T.b.rhodesiense (Wellde, Kovatch, Hockmeyer, Owiti, Masaba and Arap Siongok, 1980). It therefore has to be questioned as to whether some putative populations of T.b.brucei are in reality T.b.rhodesiense. Morrison et al. (1983) described one cow, which developed CNS disease. A population of trypanosomes was found which was resistant to lysis by human serum in the blood incubation infectivity test, despite the fact that the population (GUTat 3.1) used to infect the animal was sensitive to human serum. The authors suggested that a clear distinction between the two subspecies T.brucei brucei and T.b.rhodesiense, did not exist, but instead that each serodeme can elaborate variants that are, or are not, infective for man.

PATHOGENESIS

THE CHANCRE - The local skin reaction

Subsequent to the successful bite of an infected tsetse fly a local skin reaction of variable intensity develops several days after challenge in susceptible domestic animals and man. This occurs in association with the establishment of metacyclic trypanosomes (Emery, Akol, Murray, Morrison and Moloo, 1980a). Since the lesion in domestic livestock is analogous to that considered pathognomonic of primary syphilis in man it has been termed the "chancre" (Stargardt, 1914). Localised skin swellings

associated with the bite of tsetse have been described both in man (Manson, 1903; Ringenbach, 1913) and cattle (Livingstone, 1857). However, it was not until Kleine (1909) demonstrated that the tsetse fly was the vector of trypanosomiasis that the development of a chancre was realised to be the first sign of infection.

That the skin reaction represents a response towards the trypanosome was shown by the failure of bites from uninfected tsetse flies to elicit any such reaction (Emery and Moloo, 1980; Akol and Murray, 1982). Immediate hypersensitivity reactions associated with the bites of uninfected tsetse have been described in goats (Nash, 1970), cattle (Akol and Murray, 1983) and humans (Lester and Lloyd, 1928; Gordon and Crewe, 1948) though these were assumed to be hypersensitivity reactions to tsetse saliva since they only occurred in animals previously bitten. A similar immediate wheal and flare reaction, occurring 2-3 minutes post tsetse-feed, also occurred in cattle receiving several bites from infected tsetse. The response developed independently of any subsequent chancre reaction (Akol and Murray, 1983). This is in contrast to the chancre which begins as a nodular lesion 5-10 days after an infected bite (Luckins and Gray, 1978; Emery and Moloo, 1980).

Chancres have been reported to occur in all domestic ruminants following tsetse-transmitted infection with the three pathogenic trypanosome species.

In cattle, chancres have been described following experimental infection with T.congolense (Roberts, Gray and

Gray, 1969; Bolton, 1970; Luckins and Gray, 1978; Gray and Luckins, 1979, 1980a,b; Murray, Barry, Morrison, Williams, Hirumi and Rovis, 1980; Emery et al., 1980a; Akol and Murray, 1981, 1982, 1983), T.vivax (Emery et al., 1980a; Akol and Murray, 1981, 1983) and T.b.brucei (Emery et al., 1980a; Akol and Murray, 1981, 1983).

In goats, chancres have occurred following experimental infection with T.congolense (Emery and Moloo, 1981); T.vivax (Emery and Moloo, 1981) and T.b.brucei (Emery and Moloo, 1980; Barry and Emery, 1984).

In sheep, chancres have also been experimentally produced following fly infection with T.congolense (Uilenberg, Maillot and Giret, 1973; Gray and Luckins, 1979, 1980a,b).

In addition to tsetse inoculation of metacyclic trypanosomes, intradermal syringe inoculation has also been shown to elicit chancre development (Bolton, 1970; Luckins, Rae and Gray, 1981; Akol and Murray, 1982).

The development of a chancre is not indicative of subsequent systemic infection since chemoprophylactic studies in rabbits have shown that chancres can occur without the subsequent development of parasitaemia (Silayo, 1984).

Trypanosomes may be found in fluid aspirated from the skin lesions several days before detection in the peripheral blood (Willet and Gordon, 1957; Seed and Gam, 1967; Bolton, 1970; Luckins and Gray, 1978; Emery et al., 1980a). Thus the chancre appears to initially restrict

systemic migration of the trypanosomes. The ability of the skin to restrict the immediate spread of trypanosomes has been suggested to be associated with a physiological response of the skin and also with the skin thickness (Emery and Moloo, 1980) since chancres are not a feature of the thin-skinned smaller laboratory animals; namely mouse, rat and guinea pig (Willet and Gordon, 1957; Emery and Moloo, 1980). In these animals, trypanosomes appear to be deposited subcutaneously and rapidly enter the bloodstream (Willet and Gordon, 1957).

In domestic livestock, after proliferation at the level of the skin, trypanosomes reach the systemic circulation via the lymph stream (Emery, Barry and Moloo, 1980b; Barry and Emery, 1984).

The development and degree of skin reaction can vary greatly. Intrinsic differences occur between serodemes of T.congolense, with some regularly inducing reactions and others consistently failing to do so (Gray and Luckins, 1980a). The degree of skin reaction is also dependent on the number of trypanosomes inoculated (Willet and Gordon, 1957; Emery et al., 1980a; Emery and Moloo, 1981; Luckins et al., 1981). Similar conclusions can be drawn from studies on the lesion elicited by each trypanosome species. Based on daily examination of saliva, Otieno and Darji (1979) suggested that tsetse extrude fewer T.vivax metacyclics than T.congolense metacyclics, whilst tsetse infected with T.brucei extrude much greater numbers of metacyclic

trypanosomes than flies infected with T.congolense (Moloo, S.K., unpublished data). In a comparison of the skin reactions elicited in goats by metacyclic forms of T.vivax, T.congolense and T.brucei, that induced by T.vivax was the least dramatic and the chancre produced by T.brucei appeared 3-4 days earlier than with the other two species (Emery and Moloo, 1980, 1981).

Histologically the skin at the site of the tsetse bite undergoes marked changes in cellularity and structure. Generally, between approximately day 8 and 15 there is progressive disorganisation and degeneration of dermal collagen, during which a large cellular infiltrate occurs. Between day 14 and 21 the inflammation subsides. The trypanosomes appeared most numerous in reactions up to approximately day 12 but were still detected in low numbers until at least day 30 in calves (Gray and Luckins, 1979, 1980b).

In goats, the cellular infiltrate elicited by T.brucei was characterised initially by a pronounced influx of polymorphonuclear leucocytes (PMN) which were replaced by plasma cells and macrophages during regression of the lesion (Emery and Moloo, 1980). That produced by T.congolense was approximately similar. In contrast, the cellular infiltrate in response to T.vivax was composed mainly of lymphocytes and macrophages with a minimal PMN contribution (Emery and Moloo, 1981). The involvement of PMN appeared to parallel the level of inflammation and tissue oedema. Gray and Luckins (1979, 1980b) demonstrated

that the cellular infiltrate associated with T.congolense infection in cattle consisted principally of lymphocytes and macrophages. Akol and Murray (1982) described a similar constructed experiment in which the initial intense inflammatory reaction contained numerous PMN which were later replaced by lymphocytes and plasma cells. Differences in virulence or antigenicity of the different trypanosome stocks used in the two reports, as well as the timing of the skin biopsy, may account for the differences in cellular infiltrate described (Akol and Murray, 1982). The initial cellular infiltrate is probably a response to chemotactic factors (Cook, 1980) as well as other factors generated by parasites that increase vascular permeability (Tizard and Holmes, 1977). Subsequent cellular infiltrates probably occur in association with an immune response to surface antigens of the parasite, although the exact nature of this response is as yet unknown.

ANAEMIA

The development of anaemia is a characteristic occurrence in cattle infected with both T.congolense (Hornby, 1921, 1929, 1952; French and Hornby, 1935; Fiennes, 1950, 1954) and T.vivax (Johnson, 1941; Hudson, 1944; Hornby, 1952; Stephen, 1966a; Godfrey, Leach, Roberts and Killick-Kendrick, 1968; Fiennes, 1970) so much so that it has been termed the "cardinal sign" and "major disease-promoting factor" of bovine African trypanosomiasis (Hornby, 1921; Murray, 1974).

Murray (1979) divided the disease into three stages according to the absence or presence of trypanosomes and on clinical and pathological findings:

During the first stage, which may last anything from 3 to 12 weeks (Murray, 1979) the onset and severity of anaemia correlates with the level and duration of parasitaemia (Jennings, Murray, Murray and Urquhart, 1974; Holmes and Mamo, 1975; Holmes and Jennings, 1976; Dargie, Murray, Murray and McIntyre, 1979b). Numerous reports have described the anaemia during this stage as haemolytic (Fiennes, 1954; Naylor, 1971; Woodruff, Ziegler, Hathaway and Gwata, 1973; Jennings et al., 1974; Murray M., Murray, Jennings, Fisher and Urquhart, 1974; Mamo and Holmes, 1975; Holmes and Jennings, 1976; Valli, Forsberg and McSherry, 1978a; Valli and Mills, 1980) and being intravascular in origin (Fiennes, 1954; Esievo, Saror and Adegoke, 1984); although extravascular haemolysis has been described (Naylor, 1971).

The haemolytic nature of the anaemia appears to be multifactorial in origin (Murray, 1979) and depends on the presence of trypanosomes. The production of a haemolysin by trypanosomes was postulated by Fiennes (1950). Studies in vitro with T.brucei have lead to the characterisation and purification of a haemolytic pronase-sensitive factor (Chi, Webb, Lambert and Miescher, 1975, 1976). In addition, free fatty-acids and phospholipase A, exhibiting haemolytic activity, have been shown to be released by T.congolense undergoing autolysis (Tizard and Holmes, 1976;

Tizard, Holmes and Nielsen, 1978a,b). Work conducted with T.brucei-infected rats indicated that haemolytic factors were also operative in vitro, although the activity was generated by dying trypanosomes (Murray, 1979). Finally, workers have postulated that at high levels of parasitaemia in T.vivax infections of cattle, trypanosomes produce neuraminidase which cleaves erythrocyte surface sialic acid, predisposing the cells to physiological damage and phagocytosis by the mononuclear phagocytic system (Esievo, Saror, Ilemobade and Hallaway, 1982).

A state of pyrexia often occurs during trypanosome infections. Since red blood cells exposed to temperatures a few degrees above normal have a shortened half life in vivo, associated with increased osmotic fragility (Karle, 1969), pyrexia may contribute towards red cell damage and destruction (Murray, 1979).

An association between parasite destruction and haemolysis has been reported (Fiennes, 1970; Kobayashi, Tizard and Woo, 1976). A phenomenon observed in many immune-complex diseases is that preformed antigen-antibody-complement complexes may be adsorbed onto erythrocytes (Worledge, 1973). In trypanosomiasis, the occurrence of glomerulonephritis (Lambert and Houba, 1974), high immunoconglutinin levels (Ingram and Soltys, 1960) and rheumatoid factors (Klein, Mattern and Meuwissen, 1971) indicate that immune complexes are produced. Woo and Kobayashi (1975) have shown that trypanosome antigens can be adsorbed onto the surface of rabbit red blood cells

which, in the presence of homologous antibody and complement are lysed. In T.congolense-infected calves, trypanosome specific IgG and IgM have been eluted from the surface of red blood cells (Kobayashi et al., 1976). Antigen-antibody complexes adherent to red blood cells have also been described in cattle infected with T.vivax but not with T.congolense (Maxie, Losos and Tabel, 1976).

In stage 1 of the anaemia, splenomegaly is a consistent finding (Murray, 1979) and is due to a combination of lymphoid hyperplasia and erythrophagocytosis (MacKenzie and Cruickshank, 1973; Jennings et al., 1974; Murray M. et al., 1974).

The exact mechanism initiating red blood cell removal from the circulation is unclear, though there is much evidence for the operation of immune mechanisms predisposing cells to phagocytosis (Zoutendyk and Gear, 1951; Ingram and Soltys, 1960; Woodruff, 1973; Woo and Kobayashi, 1975; Kobayashi et al., 1976; Maxie et al., 1976).

Haemodilution also appears to play a significant part in the aetiology of the anaemia in cattle infected with either T.congolense or T.vivax (Fiennes, 1954; Clarkson, 1968; Naylor, 1971; Holmes and Mamo, 1975; Valli et al., 1978a) although subsequent work with both T.congolense and T.brucei in N'Dama and Zebu cattle has shed doubt on this finding (Dargie, Murray, Murray, Grimshaw and McIntyre, 1979a; Dargie et al., 1979b).

During stage 1 of the anaemia in rabbits and cattle,

erythropoiesis is either unaffected or increased (Boreham and Goodwin, 1966; Holmes and Mamo, 1975; Holmes and Jennings, 1976; Dargie et al., 1979b).

With both T.vivax and T.congolense infections in cattle a macrocytic normochromic anaemia occurs. However, as the disease progresses to stage 2 and 3 the anaemia becomes normocytic normochromic (Fiennes, 1950, 1954; Naylor, 1971; Maxie et al., 1976; Valli et al., 1978a). Mamo and Holmes (1975) descibed the anaemia as being normocytic normochromic in stages 1. In many animals death occurs at this stage. However, in some animals a further two stages are observed:

Stage 2 of the anaemia is characterised by intermittent low level parasitaemia and by a persistent low level anaemia. Haemolysis continues, and is associated with sustained hyperactivity of the mononuclear phagocytic system (Murray, 1979). Despite this fact, splenomegaly is no longer prominent and the level of haemosiderin deposition throughout the body is increased.

Stage 3 is characterised by the absence of trypanosomes both in the blood and tissues and animals may either remain anaemic or recover.

Cattle may die in stage 2 or stage 3 of the disease (Murray, 1979).

PYREXIA

In T.congolense infections, persistent low level pyrexia has been described in acute infections,

intermittent low level pyrexia in subacute disease and occasional pyrexia in chronic disease (Fiennes, 1970; Hornby, 1952; Valli, Forsberg and Robinson, 1978b). In contrast, T.vivax infections in cattle are commonly characterised by pyrexia (Hudson, 1944; Hill and Esuruoso, 1976; Van den Ingh, Zwart, Van Miert and Schotman, 1976; Esuruoso, 1977; Maxie, Losos and Tabel, 1979; Obi and Anosa, 1980; Mwongela et al., 1981). Furthermore, Maxie et al. (1979) showed that throughout the entire infection there was a significant positive correlation between the daily level of parasitaemia and the temperature elevation in a group of cattle infected with T.vivax but not in a group of cattle infected with T.congolense.

During infections with both T.vivax and T.congolense periods of pyrexia have been associated with periods of parasitaemia (Hudson, 1944; Fiennes, 1950, 1970; Van den Ingh et al., 1976), while normal temperatures occurred during periods of aparasitaemia. The highest temperatures observed in bovine trypanosomiasis (104+°F) have been described in association with the severest clinical syndrome; haemorrhagic T.vivax (Hudson, 1944).

Reports on the pathogenesis of pyrexia in trypanosomiasis are lacking. Presumably, in response to contact with trypanosome antigens, endogenous pyrogen is released from phagocytic cells resulting in the hypothalamic thermoregulatory set point being elevated (Kluger, 1981).

HAEMORRHAGIC *T.VIVAX*

Since haemorrhagic T.vivax often presents as a hyperacture syndrome, with animals dying as little as 14 days post infection (Hudson, 1944), dead animals are often found in good bodily condition. The main pathological findings are generalised petechial and ecchymotic haemorrhages on mucosae and serosae. Often, frank intestinal haemorrhages also occur (Hudson, 1944; Morrison et al., 1981; Mwongela et al., 1981; Wellde et al., 1983).

The development of severe thrombocytopaenia has been the major factor implicated in the development of the haemorrhages (Van den Ingh et al., 1976; Wellde et al., 1983). Prolonged bleeding and prothrombin times, increased levels of fibrinogin (Wellde et al., 1983) and the presence of fibrin degradation products (Van den Ingh et al., 1976; Wellde et al., 1983) indicate that disseminated intravascular coagulation may be responsible for the thrombocytopaenia.

IMMUNOLOGICAL MECHANISMS ASSOCIATED WITH BOVINE-TRYPANOSOMIASIS

ANTIGENIC VARIATION

The entire trypanosome plasma membrane of the body and flagellum is covered in a surface coat, 12-15nm thick (Vickerman, 1969; Vickerman and Luckins, 1969). When bloodstream forms of T.brucei are ingested by the tsetse fly the coat is shed. Only, when the trypanosome has

transformed through to the metacyclic stage, in the salivary gland, is the coat reacquired (Barry and Vickerman, 1979). The major constituent of the surface coat, isolated from T.brucei, is a glycoprotein (molecular weight approximately 65,000, as determined by sodium dodecyl sulphate gel electrophoresis) (Cross, 1975). Immunisation of mice with purified surface glycoprotein conferred protection against challenge with a small number of homologous, but not heterologous, trypanosomes.

The identity of any bloodstream or metacyclic trypanosome is based on the antigen expressed on its surface; the "variable antigen type" or VAT. Thus, an mVAT denotes the antigenic type expressed by a particular metacyclic trypanosome and bVAT the antigenic type expressed by a particular bloodstream trypanosome. A VAT repertoire consists of all the VATs that can potentially be expressed by a cloned trypanosome, and a serodeme refers to a clonally derived population expressing a certain VAT repertoire, as determined serologically (W.H.O., 1978).

The number of serologically different variant specific surface antigens (VSSAs) that can be expressed in a population derived from one trypanosome is unknown. Serological studies with T.b.equiperdum have shown there to be at least 101 (Capbern, Giroud, Baltz and Mattern, 1977). Until very recently variant specific antibody was thought to be responsible for inducing antigenic variation (Vickerman, 1978). However, in vitro, in the absence of any immune response mechanisms, antigenic variation still

occurred (Doyle, Hirumi, Hirumi, Lupton and Cross, 1980) and thus "nailed this particular theory in its coffin" (Vickerman, Barry, Hajduk and Tetley, 1980).

Regardless of the T.congolense or T.brucei bVAT ingested by the fly the mVAT repertoire, consisting of several different VATs (LeRay, Barry and Vickerman, 1978; Nantulya, Musoke, Moloo and Ngaira, 1983), is constant for a given serodeme (Jenni, 1977a,b; Vickerman et al., 1980; Esser, Schoenbechler, Gingrich and Diggs, 1981).

The mVAT repertoire appears to be more limited than the bVAT repertoire (Nantulya et al., 1983). Thus, Crowe, Barry, Luckins, Ross and Vickerman (1983) described the mVAT repertoire of a T.congolense stock as consisting of 12 different VATs.

Subsequent to an infected fly biting an animal, the VATs of the extruded T.brucei metacyclic trypanosomes continue to be expressed in the bloodstream trypanosome population present during the first parasitaemic wave (Barry, Hajduk, Vickerman and LeRay, 1979; Hajduk and Vickerman, 1981; Esser et al., 1981; Barry and Emery, 1984). It appears that all mVATs of T.brucei and T.congolense are expressed as bVATs (Nantulya, Musoke, Rurangirwa and Moloo, 1984). These findings explain the finding of Gray (1965); after cyclic transmission by tsetse there were certain VAT(s) that appeared in the first parasitaemic wave, termed the basic antigen(s), characteristic for each trypanosome line.

Thus, a combination of spontaneous antigenic variation

and immune selection appear to be responsible for the parasitaemia cycling observed in animals (Esser, Schoenbechler and Gingrich, 1982).

Finally, although a VAT may have been expressed it does not prevent its re-expression (Nantulya, Musoke, Barbet and Roelants, 1979; Nantulya et al., 1984). This fact may explain the self-cure phenomenon (Nantulya et al., 1984).

IMMUNOLOGICAL CHANGES DURING TRYPANOSOMIASIS

Work in mice has shown much evidence that antibody is the most important effector mechanism in immunity to trypanosomiasis. Immunity to homologous variant was passively transferred via serum from an immunised mouse (Campbell and Phillips, 1976). The transfer of splenic cells from an immunised mouse also conferred immunity (Luckins, 1972a; Campbell and Phillips, 1976) and of this cell population, a B-cell rich fraction rather than a T-cell rich fraction was shown to confer the immunity (Campbell and Phillips, 1976). B-cell deficient mice, to which anti-u-serum was administered from birth, could not be immunised with irradiated trypanosomes and were highly susceptible to infection (Campbell, Esser and Weinbaum, 1977). In contrast, congenitally athymic mice could be readily immunised with irradiated trypanosomes (Campbell, Esser and Phillips, 1978). Parasite VAT-specific antibody responses appear to be responsible for elimination of trypanosomes (Murray and Urquhart, 1977). For example, in

cattle infected with T.congolense, weekly collection of trypanosomes and serum showed that trypanosome populations were only neutralised in serum collected one or more weeks after collection of the trypanosome population (Wilson and Cunningham, 1972). In addition, a close correlation has been demonstrated between the degree of protection and the neutralising antibody titre (Morrison, Black, Paris, Hinson and Wells, 1982a; Wells, Emery, Hinson, Morrison and Murray, 1982).

Hypergammaglobulinaemia is a consistent finding in bovine trypanosomiasis, and soon after infection is largely due to elevated IgM levels (Luckins, 1972b,c, 1976; Clarkson and Penhale, 1973; Clarkson, Penhale, Edwards and Farrell, 1975; Kobayashi and Tizard, 1976; Nielsen, Sheppard, Holmes and Tizard, 1978a). In fact, elevated IgM levels have been said to possibly be indicative of trypanosome infection (Luckins, 1972c). Only slight alterations occurred in IgG_1 and IgG_2 levels during infection (Luckins, 1976; Nielsen et al., 1978a) although elevated IgG levels have been described (Luckins, 1972c). Since the rates of catabolism of IgG_1, IgG_2 and IgM increased 9 fold, 13 fold and 5 fold, respectively, there was a large increase in production of all three antibody isotypes. Trypanosome neutralising antibody activity in the first part of the infection occurred in these fractions, especially IgM (Luckins, 1976; Musoke, Nantulya, Barbet, Kironde and McGuire, 1981). However, from the second parasitaemic peak onwards the IgG fraction exhibited

the principle neutralising activity (Seed, 1972; Musoke et al., 1981).

In laboratory animals IgM appears to be the important isotype in controlling parasitaemia and conferring protection (Campbell et al., 1978) and there is evidence for the same in cattle (Luckins, 1976). Small animal studies have shown that some of the immunoglobulin is not directed towards trypanosome antigen and consists of heterophile antibodies, in addition to rheumatoid factors and a range of autoantibodies. Workers have suggested that this is associated with polyclonal B-cell activation (Houba, Brown, and Allison, 1969; Hudson, Byner, Freeman and Terry, 1976; Corsini, Clayton, Askonas and Ogilvie, 1977; Morrison, Roelants, Mayor-Withey and Murray, 1978), thereby resulting in exhaustion of B-lymphocyte potential, as observed in chronic infections. However, this may not occur in cattle since absorption with VATs isolated during the entire infection showed all the IgM and IgG_1 produced during the first 3 weeks of infection to be VAT specific (Musoke et al., 1981).

Immunoglobulin changes in cattle accompany a hypocomplementaemia (Nielsen, Sheppard, Holmes and Tizard, 1978b; Kobayashi and Tizard, 1976) which may be associated with trypanosomes producing complement activating substances or with increased complement catabolism. The presence of complement binding antigen-antibody complexes along with increased levels of immunoconglutinin, in laboratory animal infections, indicates widespread

utilisation and fixation of complement (Murray, P., Jennings, Murray and Urquhart, 1974).

Goodwin (1970) and Goodwin, Green, Guy and Voller (1972) reported on the immunodepressive effect of trypanosomiasis; they demonstrated depression of humoral responses to non-trypanosomal antigens during trypanosome infections of small animals. Altered macrophage activity (Grosskinsky and Askonas, 1981) and generation of suppressor T-cells (Eardley and Jayawardena, 1977) have both been described in small animal infections, and, in addition to a decreased half life of serum immunoglobulins (Nielsen et al., 1978a), may act together or separately to bring about immunodepression.

Suppression of antibody responses in infected cattle to several viral and bacterial vaccines have been examined; polyvalent clostridial vaccine (Holmes, Mammo, Thomson, Knight, Lucken, Murray, Murray, Jennings and Urquhart, 1974) Vibrio foetus antigen (MacKenzie, Boyt, Emslie, Lander and Swanepoel, 1975), foot and mouth disease vaccine (Scott, Pegram, Holmes, Pay, Knight, Jennings and Urquhart, 1977), louping-ill vaccine (Whitelaw, Scott, Reid, Holmes, Jennings and Urquhart, 1979) and Brucella abortus vaccine (Rurangirwa, Musoke, Nantulya and Tabel, 1983). Humoral immune responses to these vaccines appeared to be suppressed.

Simultaneous administration of a trypanocide on the day of vaccination produced a large improvement in the immune response to the louping-ill vaccine (Whitelaw et

al., 1979).

In contrast to the above findings, infections with T.congolense or T.vivax did not significantly depress the neutralising antibody response to live rinderpest vaccine when vaccination was carried out 8 or 25 days after infection (Rurangirwa, Mushi, Tabel, Tizard and Losos, 1980).

IMMUNITY ASSOCIATED WITH CYCLICAL INFECTION

Cattle infected by tsetse harbouring a cloned derivative of T.congolense, and treated with diminazene aceturate 3-4 weeks following infection, were immune when challenged with tsetse infected with homologous clones 3-5 weeks later. Skin reactions at the site of tsetse bites, and development of parasitaemia did not occur. However, if the second challenge was conducted with a heterologous clone, cattle developed chancres and became parasitaemic (Emery et al., 1980a; Akol and Murray, 1983). Similar findings have been made in goats fed on by T.brucei-infected tsetse (Emery et al., 1980a). The protective immunity appeared to be effected either at the level of the skin or the drainage lymph node since a skin reaction, indicative of trypanosome proliferation, did not occur (Emery et al., 1980a; Akol and Murray, 1983).

Immunity to cyclical challenge also occurred with uncloned stocks provided the same stock was used for rechallenge and that it only consisted of one serodeme (Akol and Murray, 1983).

The situation following tsetse-transmitted T.vivax infection appears to be very different to that described for T.congolense and T.brucei. Even when animals were repeatedly fly infected with the same serodeme and treated 3 to 4 weeks after each infection they continued to be susceptible both to homologous and heterologous challenge (De Gee, 1980). Possibly T.vivax, after cyclical transformation, does not revert to a constant mVAT repertoire. Secondly, since very few metacyclics are extruded at each feed, not all the mVATs may be inoculated. Thirdly, rapid antigenic variation of the metacyclic population may occur so that there is insufficient expansion of certain mVATs to stimulate protective antibody responses.

STRATEGIES FOR THE CONTROL OF TRYPANOSOMIASIS

In the absence of a vaccine for bovine trypanosomiasis, practical control measures include eradication and control of tsetse populations, the exploitation of trypanotolerant cattle and the administration of antitrypanosomal drugs to cattle (Morrison et al., 1981).

TSETSE CONTROL

Strategies for the control of tsetse can broadly be put under two major headings; chemical and non-chemical.

Chemical control consists of the application of

insecticides, either from the ground or from the air. Only three compounds have been used in large-scale field programmes: dichlorodiphenyltrichloroethane (DDT), dieldrin and endosulfan.

DDT was the first of the synthetic insecticides to be widely used. Because of its residual effect it is usually used on a single administration basis since deposits can remain lethal for tsetse up to one year after application (Baldry, 1963).

Dieldrin is also a residual insecticide. Trials have shown the compound to be more effective than DDT (Burnett, Robinson and LeRoux, 1957) in that there was a better persistence of lethal deposits compared to DDT, especially in high rainfall areas.

Endosulfan is less persistent than DDT and dieldrin. Because of its good solubility in spray solvents it is especially suited to administration from aircraft (Hocking, Lee, Beesley and Matechi, 1966).

The use of insecticides in tsetse control programmes can produce environmental pollution and unwanted ecological effects (Koeman, Rijksen, Smies, Na'Isa and MacLennan, 1971). Of the three insecticides described, repeated aerial application of endosulfan appears to have less general adverse ecological impact (Gray, 1983). However, new highly active biodegradable insecticides are being developed which would decrease this problem (FAO, 1981).

The application of insecticides is not suited to all ecological zones. Areas of high rainfall and dense

vegetation tend to limit the feasibility and efficacy of insecticidal application (Jordan, 1974).

A variety of non-chemical methods have also been used to reduce tsetse in certain areas:

The clearance of vegetation and encouragement of human settlement and agricultural development, to create an adverse environment for tsetse, are long practiced successful techniques. In addition, workers have advocated the destruction of game to decrease the fly food source and thus the size of the natural infection reservoir (Jordan, 1974).

A wide variety of tsetse traps have been designed and used for ecological studies. In recent years, tsetse traps have been re-examined to see if their catching efficiency could be improved, so that, in some circumstances, they might be used to control tsetse populations. Workers have demonstrated that the efficiency of traps could be greatly improved if a source of natural host odour (Vale, 1974; Hargrove and Vale, 1979) or a mixture of carbon dioxide and acetone (a simulated host odour) (Vale, 1980) was associated with the trap. 1-Octen-3-ol has been identified as a potent olfactory stimulant for some Glossina spp. and in the field has been shown to be attractive by itself and also to enhance the attractiveness of carbon dioxide and acetone (Bursell, 1984; Hall, Beevor, Cork, Nesbitt and Vale, 1984). Possibly, these developments may play a role in future tsetse control programmes.

A sterile insect technique has also been investigated.

A small-scale trial was conducted with G.m.morsitans in Rhodesia. After releases, on an island in lake Kariba, of puparia which had been dipped in 5% tepa, 98% control was obtained in 9 months (Dame and Schmidt, 1970). Failure to achieve G.m.morsitans eradication with the same technique in other areas has been attributed to inadequate isolation barriers and the resultant immigration of flies (Williamson, Dame, Gates, Cobb, Bakuli and Warner, 1983).

TRYPANOTOLERANCE

The trypanotolerance trait was first described at the beginning of the twentieth century when certain indigenous taurine cattle breeds of West Africa were observed to survive and be productive in tsetse-infested areas (Pierre, 1906); in particular the N'Dama and West African shorthorn. Only these two breeds survive in large numbers, without the aid of trypanocides, in areas where significant tsetse risk exists (ILCA, 1979). In addition to these two breeds of cattle, many species of wild Bovidae have also been shown to exhibit innate resistance to trypanosomiasis (Murray, Morrison and Whitelaw, 1982).

N'Dama and West African shorthorn breeds constitute approximately 5% of the total cattle population in the 38 countries where tsetse occur (ILCA, 1979; FAO/WHO/OIE, 1982). In addition, it is estimated that without any additional control measures an area of about 2 million square kilometers exists in West and Central Africa that is suitable for trypanotolerant cattle (FAO, 1976). Their

failure to be exploited has been because of the breeds' small size and assumed low productivity. However, in areas of low or nil tsetse challenge, their productivity has been shown to be much higher than was originally believed (ILCA, 1979).

The trypanotolerance trait is not absolute since productivity appears to decrease as the level of challenge increases. Thus, N'Dama may exhibit stunting, wasting, abortion and even death, associated with trypanosomiasis (ILCA, 1979). That trypanotolerance is innate (genetically based) and not solely associated with acquired resistance to local trypanosome populations has been demonstrated (Murray et al., 1982). Using animals with no previous exposure to trypanosomiasis, N'Dama were significantly more resistant to trypanosomiasis than Zebu and imported exotic breeds in terms of productivity and survival. The prevalence, level, duration of parasitaemia and severity of anaemia were significantly less in trypanotolerant cattle. Because of the superior control of parasitaemia in these cattle, trypanotolerance was often attributed to differences in immune responsiveness. However, little data exists to support this theory. Murray et al. (1982) showed no significant difference in antibody levels in N'Dama and Zebu following challenge with T.brucei bloodstream forms. Desowitz (1959) found that N'Dama, previously infected with trypanosomes, eliminated a subsequent infection more rapidly than Zebu with a similar history. Using a respiratory inhibition test Desowitz demonstrated greater

activity in the N'Dama sera as compared to Zebu sera, and therefore suggested that the N'Dama's trypanotolerance lay in their ability to mount a superior secondary immune response. However, the antigenic composition of the trypanosome population used was unknown. Roberts and Gray (1973) showed little difference in susceptibility to primary challenge between the two breeds. However, following a second challenge the superior resistance of the N'Dama became apparent.

Using erythrokinetic studies, the superior resistance of N'Dama has been shown to be associated with their capacity to control parasitaemia rather than an ability to mount a more efficient erythropoietic response (Dargie et al., 1979a,b). The exact mechanism(s) underlying parasitaemia control in the bovine await further investigation.

Other factors that may play a role in trypanotolerance are the N'Dama's ability to forage, capacity for water conservation and heat tolerance, and skin physiology (Murray et al., 1980).

Finally, in addition to exhibiting trypanotolerance, N'Dama also appear to be more resistant to tick borne diseases; heartwater, anaplasmosis and babesiosis (Epstein, 1971). They also appear to be more resistant to streptothricosis (Stewart, 1937; Coleman, 1967).

CHEMOTHERAPY (GENERAL)

The demonstration by Plimmer and Thomson (1908) that

administration of tartar emetic (potassium antimonyl tartrate) to mice infected with T.brucei or T.evansi effected a cure, led to the first application of chemotherapy to bovine trypanosomiasis. In Tanganyika, Rhodesia and Zululand, regular intravenous application to cattle in the field demonstrated the drugs efficacy (Anon, 1923; Bevan, 1928).

Subsequent trypanocides belong to the chemical classes of acid naphthylamines, diamidines, quinaldines and phenanthridines.

No new trypanocide for domestic livestock has been marketed for over 25 years, primarily because of the prohibitive costs in developing a new drug (Hutner, 1977; Goodwin, 1978) and also the relatively small trypanocide market (Leach and Roberts, 1981). The large outlay ($20-30 million) required to make a new trypanocide commercially available (Williamson, 1980) may deter pharmaceutical companies unless they are encouraged financially by funds from non-commerical sources (Murray and Gray, 1984). There is certainly a need to develop drugs, even though they be uneconomic. The co-operation of universities with industry under the supervision of an independent "sponsor" may provide an answer (Ormerod, 1976).

In addition to the lack of new trypanocides, the effectiveness of the currently used trypanocides has been threatened due to the development of drug resistance. For further deterioration of the situation to be prevented, greater expenditure on testing and surveillance for drug

resistance is required (Leach and Roberts, 1981). However, because of logistical and financial problems associated with civil insurrection, drug costs, lack of funds to implement control programmes, the lack of an infrastructure required for drug administration, scarcities of fuel, vehicles and diagnostic services, erratic supply of drugs, syringes and needles, and the lack of well trained permanent staff, extreme limitations are imposed on the application of trypanocides in the field (Holmes and Scott, 1982; Murray and Gray, 1984). Because of these problems there is an increasing gap between treatment demand and the treatments actually administered (MacLennan, 1980).

Chemoprophylaxis and chemotherapy can be effective and economically justified if managed in an efficient manner (Blaser, Jibo and McIntyre, 1977; Leach and Roberts, 1981). The economic credibility of using trypanocides as opposed to tsetse control under certain conditions has been described (Jahnke, 1974). Unless the incidence of trypanosomiasis and stock carrying capacity are both high, the protection of cattle with trypanocides is preferable in an area where tsetse control costs are high (Finelle, 1976).

In areas where the tsetse population has been eliminated, chemotherapy, in its broadest sense, has a part to play in removing parasites from domestic livestock and decreasing mechanical transmission by other arthropod species (Gray, 1983).

Current veterinary chemotherapy uses five compounds to

combat trypanosomiasis; namely, suramin, quinapyramine dimethylsulphate +/- chloride, diminazene aceturate, ethidium chloride/bromide and isometamidium chloride.

CHEMOTHERAPY

Data regarding the generic name, compound class, trade name, dosage, route of administration, spectrum of activity and the species of animal on which the drug is used, for the five veterinary trypanocides is shown in table 2.

SURAMIN

Suramin was introduced in 1920 and is the oldest of the widely used trypanocides in animals. It is: hexa-sodium 3,3′-ureylene-bis [8-(3-benzamido-p-toluido)-1, 3,5-naphthalene sulphonate]; ($C_{51}H_{34}N_6Na_6O_{23}S_6$, molecular weight 1429).

Suramin is extremely anionic and binds readily to plasma proteins (Dewey and Wormall, 1946). This property may account for the drug's prophylactic activity.

Suramin has been shown to be ineffective against T.congolense and T.vivax infections of cattle (Ruchel, 1975), but does show some therapeutic activity towards T.simiae infections of pigs (Stephen, 1966b).

Suramin is efficaceous against infections with trypanosomes of the subgenus Trypanozoon, in horses,donkeys and camels (Leach, 1961; Leach and Roberts, 1981). It has been successfully used at 10mg kg^{-1} as a curative for surra in ponies, and at this dosage confers 30 days prophylaxis

Table 2

Chemotherapy of trypanosomiasis in domestic animals
(After Leach, 1973 and Kuttler and Kreier, 1986)

GENERIC NAME	COMPOUND CLASS	TRADE NAME	TYPE	DOSE AND ROUTE (mg/kg)	SUSCEPTIBLE TRYPANOSOMES	ANIMAL	COMMENTS
SURAMIN	SULPHONATED NAPHTHYLAMINE	NAGANOL[a],	C	7.0-10.0 i.v.	T.evansi, T.brucei, T.equiperdum	camels	Suramin resistant infections can be treated with quinapyramine
QUINAPYRAMINE DIMETHYLSULPHATE	QUINOLINE PYRAMIDINE	TRYPACIDE SULPHATE[b] NOROQUIN[c]	C	3.0-5.0 s.c. 3.0 s.c. 3.0-5.0 s.c.	T.evansi, T.equinum, T.equiperdum T.brucei (T.congolense, T.vivax)	camels horses cattle, small ruminants, pigs, dogs	Active against suramin-resistant strains. Dose should be administered in halves separated by a 6 hour interval. Infection in cattle involving T.vivax use 5mg kg^{-1}
QUINAPYRAMINE DIMETHYLSULPHATE: CHLORIDE 3:2 w/w	QUINOLINE PYRIMIDINE	TRYPACIDE PROSALT[b] NOROQUIN PROSALT[c]	P	As sulphate	As sulphate	horses, cales	As sulphate
DIMINAZENE ACETURATE	AROMATIC DIAMIDINE	BERENIL[d]	C	3.5-7.0 i.m.	T.vivax, T.congolense (T.brucei, T.evansi)	cattle, small ruminants, dogs	Active against homidium resistant infections Inactive against T.simiae
HOMIDIUM BROMIDE HOMIDIUM CHLORIDE	PHENANTHRIDINE	ETHIDIUM[e] NOVIDIUM[b]	C	1.0 i.m.	T.vivax, T.congolense	cattle, small ruminants, horses, pigs	Bromide soluble only in hot water. Chloride soluble in cold water. Either salt can be used as a sanative pair with diminazene aceturate
ISOMETAMIDIUM CHLORIDE	PHENANTHRIDINE AROMATIC AMIDINE	SAMORIN[b] TRYPAMIDIUM[f]	C P	0.25-0.5 i.m. 0.5-1.0 i.m.	T.vivax, T.congolense (T.brucei)	cattle	Soluble in water and is heat sensitive. Can be used for T.brucei in dogs - use 0.5-1.0mgkg^{-1}

a Bayer Co. Leverkusen, W. Germany
b May and Baker, Ltd. Dagenham, England
c Norbrook Lab. Ltd., Newry, N. Ireland
d Farbwerke Hoechst AG. Frankfurt, W. Germany
e FBC Ltd., Cambridge, England
f Rhone Merieux, Lyon, France

C = Curative
P = Prophylactic
i.m. = intramuscular
i.v. = intravenous
s.c. = subcutaneous
( ) = less susceptible

(Gill and Malhotra, 1971). Because of the lower toxicity for horses as compared to quinapyramine, suramin is particularly useful for the treatment of equine trypanosomiasis (Leach and Roberts, 1981). Inspite of this, its use has largely been replaced by that of quinapyramine.

Repeated administration of suramin to cattle may produce resistance to the drug, which can be treated with quinapyramine (Gill, 1971). The development of suramin resistance does not result in cross-resistance to other trypanocides (Gill, 1971).

Williamson and Desowitz (1956) showed that, unlike other trypanocides, suramin was acidic and could therefore be combined with basic substances, e.g., diminazene aceturate, the phenanthridines and quinapyramine dimethylsulphate, to form relatively insoluble complexes. The complexes with suramin were termed suraminates. Compared to their constituents the complexes exhibited decreased toxicity, and increased prophylactic activity (Williamson, 1957; Gill and Malhotra, 1963, 1971). The reduction in toxicity when given as a suraminate was most marked with quinapyramine dimethylsulphate and least with ethidium bromide (Desowitz, 1957).

Both ethidium bromide-suraminate and, to a lesser extent, quinapyramine dimethylsulphate-suraminate produced marked prophylaxis against fly-transmitted T.congolense and T.vivax when administered subcutaneously to cattle. Greater than 7 months prophylaxis was conferred with the

ethidium complex (Williamson and Desowitz, 1956). However, ethidium bromide-suraminate when given subcutaneously at 5mg kg^{-1} produced severe swellings (Stephen, 1958).

The quinapyramine dimethylsulphate-suraminate (Williamson, 1957) was very effective as a prophylactic against tsetse-transmitted T.simiae infections in pigs in West Africa (Stephen and Gray, 1960; Gray, 1961).

The complex is recommended as a prophylactic at 40mg kg^{-1} and at this dosage will protect growing pigs for 3 months and adult pigs for 6 months (Gray, 1961; Stephen, 1966b).

The same complex has been used to protect horses against T.evansi (Gill and Malhotra, 1971). Trials on quinapyramine dimethylsulphate-suraminate in cattle were stopped because of the formulation's high cost (Williamson, 1970).

MODE OF ACTION

Suramin enters trypanosomes complexed with serum protein via endocytosis (Fairlamb and Bowman, 1977, 1980). Several trypanosomal enzymes of T.b.brucei appear to be very sensitive to inhibition by suramin; glycerol-phosphate oxidase and NAD-linked glycerol-3-phosphate dehydrogenase (Fairlamb and Bowman, 1977; Fairlamb, 1981). Suramin may also interfere with ribosomal and lysosomal function of trypanosomes (Williamson and Macadam, 1965; Hart and Young, 1975).

QUINAPYRAMINE DIMETHYLSULPHATE/CHLORIDE

Quinapyramine was first described by Curd and Davey (1949, 1950) and is 4-amino-6(2-amino-6-methylpyramid-4-ylamino)-2-methylquinoline-1,1'di-metho(methylsulphate); ($C_{19}H_{28}N_6O_6S_2$, molecular weight 532.6). The drug has a broad spectrum of activity, being active against T.evansi, T.equinum, T.equiperdum, T.b.brucei (Curd and Davey, 1950; Leach, 1961), T.congolense and T.vivax (Davey, 1950). It appears to be most active against T.congolense (Davey, 1957).

Two salts of quinapyramine are used as trypanocides; the chloride and the dimethylsulphate.

Subsequent to the work of Davey (1950) the recommended dosage of quinapyramine dimethylsulphate was 5mg kg^{-1} (Davey, 1957). At this dosage it was shown to be a good therapeutic in the field, throughout Africa (Davey, 1957; Aspinall, Ford, Steel, Steele and Swan, 1960).

Following subcutaneous administration of quinapyramine chloride the drug remained localised in the subcutaneous tissue (Spinks, 1950). Although sufficient quantities were released into the circulation to exert a trypanocidal effect, with regard to prophylaxis (Curd and Davey, 1949), the level was too low to be effective as a therapeutic (Curd and Davey, 1950). Thus, for prophylactic purposes, quinapyramine was originally used as a mixture containing three parts by weight quinapyramine dimethylsulphate to four parts by weight quinapyramine chloride (Davey, 1957). Since 1958, a revised cheaper formulation has been used,

containing dimethylsulphate and chloride in a ratio of 3:2 w/w. The new formulation was equally as effective; 7.4mg kg^{-1} protected Zebu cattle in an area of medium tsetse challenge in Tanganyika for an average of 144 days (Robson, 1962), and for 3 months in an area of medium tsetse challenge in Kenya (Whiteside, 1962b).

Systemic toxicity associated with curare-like properties sometimes occurred in cattle treated with 5mg kg^{-1} quinapyramine dimethylsulphate (Unsworth and Chandler, 1952; Davey, 1957). Unfortunately, the manufacturers (May and Baker Ltd.) recommended that 5mg kg^{-1} be used to treat T.vivax infections in cattle.

Toxicity in equidae may be prevented by dividing the dose in two and injecting the second half 5-6 hours after the first (Leach and Roberts, 1981).

MODE OF ACTION

When administered to cultures of Crithidia oncopelti, quinapyramine inhibited RNA synthesis at concentrations that did not affect DNA synthesis (Newton, 1964). The drug also caused aggregation of isolated ribosomes. Interference with polyamine function is an additional suggested mode of action (Bacchi, 1981).

DIMINAZENE ACETURATE

Diminazene aceturate, the derivation of which has been described by Jensch (1955, 1958), consists of two

amidinophenyl moieties joined by a triazene bridge: p, p'-diamidino diazoaminobenzene diaceturate tetrahydrate: N-1,3-diamidino-phenyltriazene diaceturate tetrahydrate; ($C_{22}H_{29}N_9O_6.4H_2O$, molecular weight 587.6).

Fussganger and Bauer (1958) reported trials conducted with the drug across Africa. A dosage of 3.5mg kg^{-1} was sufficient to cure infections with T.vivax and T.congolense, whilst 5mg kg^{-1} was required for infections with T.brucei. The drug appeared to be ineffective against T.simiae.

Use of diminazene aceturate in camels is contra-indicated since the therapeutic dosage required for T.evansi infections (7mg kg^{-1}) can produce severe side-effects and even death (Leach, 1961). The only other species where systemic toxicity is a problem is the dog. Extensive haemorrhagic malacia of the CNS has occurred following administration of 15mg kg^{-1} (Losos and Crockett, 1969).

Excretion of diminazene aceturate from the body appears to occur rapidly (Bauer, 1958; Fairclough, 1962), thereby lessening the risk of resistance development (Leach and Roberts, 1981). Because of the lack of widespread resistance occurring, diminazene has replaced ethidium and quinapyramine as the curative trypanocide of choice.

A small number of workers have reported on the period of prophylaxis conferred by the drug. Fairclough (1963b) described prophylaxis lasting up to 24 hours following drug administration, whilst trypanocidal activity (demonstrated

by inhibition of infectivity after incubation in cattle sera) against T.brucei was detected in sera collected for up to 3 weeks from cattle following administration of 7mg kg^{-1} (Van Hoeve and Cunningham, 1964a,b). However, Wellde and Chumo (1983), also using a dosage of 7mg kg^{-1}, described complete protection lasting 6 days against T.congolense challenge. One half of the animals described were protected for 12 days following drug-administration.

Because of the short period of prophylaxis following drug-administration, diminazene aceturate has been used to measure the "severity of challenge" in an area. The technique has been termed the "Berenil Index" and the index refers to the average time required between diminazene aceturate treatments to eliminate infections in animals under constant challenge (i.e., the treatments per head per annum) (Boyt, Lovemore, Pilson and Smith, 1962). Whiteside (1962b) rated the incidence as "very high", "high", "medium" or "low" when the number of diminazene treatments required per head per annum were, respectively, 12, 6.5, 3 or 1.

MODE OF ACTION

Diamidines appear to be actively transported into trypanosomes against a concentration gradient (Damper and Patton, 1976a,b). Diamidine sensitive populations appeared to accumulate drug more rapidly and were therefore more rapidly affected (Damper and Patton, 1976a,b).

The trypanocidal effect is thought to be due to the

drug's interaction with DNA. The amidine moieties of the drug probably associate with the DNA phosphate groups, via salt bands, thereby inserting into the minor groove of the DNA helix, adjacent to an adenine-thymine base-pair (Festy, Sturm and Daune, 1975). Diamidines may therefore interfere with DNA function (Dalbow and Hill, 1976).

PHENANTHRIDINES

One of the first compounds of the phenanthridine series to be described was phenidium chloride (Browning, Morgan, Robb and Walls, 1938). Therapeutic studies were conducted in cattle but because of poor cure rates (approximately 70%) and severe local side effects at the site of administration (Hornby, Evans and Wilde, 1943), there were obvious reservations as to its usage.

Dimidium bromide was subsequently synthesised and appeared to be a promising drug after trials in Uganda on cattle infected with T.congolense (Bell, 1945), using a dosage of 1mg kg^{-1}. When 2mg kg^{-1} was used in field trials, local reactions occurred in most animals and nearly all exhibited signs of photosensitisation (Randall and Beveridge, 1946). Subsequently, the standard dosage became 1mg kg^{-1}, administered intramuscularly (Davey, 1957).

HOMIDIUM

Homidium was derived from dimidium by substituting the methyl group of the quaternary nitrogen with an ethyl group and is 2,7-diamino-9-phenyl-10-ethylphenanthridinium

bromide anhydrate; ($C_{21}H_{20}BrN_3$; molecular weight 394.3) (Watkins and Woolfe, 1952). The compound was first produced as the bromide salt (Ethidium). Homidium chloride (Novidium) is equally as active and, unlike the bromide, is soluble in cold water (Wragg, 1955).

Work conducted in Uganda using ethidium indicated T.vivax to be more sensitive to the drug than T.congolense (Mwambu, 1966).

Subsequent to drug administration only limited prophylactic activity has been described; complete prophylaxis lasted less than 2 weeks after administration of 1mg kg^{-1} (Leach, Karib, Ford and Wilmshurst, 1955).

Using an ethidium group-treatment regime, in an endemic area of Uganda, Mwambu (1971) demonstrated the resultant increase in productivity associated with a decreased incidence of infection.

ISOMETAMIDIUM CHLORIDE

Combination of the diazotised p-aminobenzamide moiety of diminazene with homidium chloride produced two isomers, both with trypanocidal activity, the combination of which was termed metamidium (M&B 4404) (Wragg, Washbourn, Brown and Hill, 1958). Metamidium was shown to be active in cattle against field strains of trypanosomes from East Africa (Fairclough, 1958). The drug was reasonably well tolerated when administered subcutaneously (Smith and Brown, 1960). Metamidium, as noted above, consisted of two isomers, one red and one purple (Wragg et al., 1958). The

structure of the highly soluble red isomer was described by Berg (1960); 7-(m-amidinophenyldiazo-amino)-2-amino-10-ethyl-9-phenyl-phenanthridinium chloride hydrochloride; ($C_{28}H_{25}ClN_7HCl$; molecular weight 531.5). The red isomer (M&B 4180A) was termed isometamidium chloride and was marketed in 1961 under the trade name Samorin.

May and Baker Ltd. recommend that, for T.congolense and T.vivax infections of cattle, a therapeutic dosage of 0.25-0.50mg kg^{-1} be used, whilst as a prophylactic, 0.5-1.0mg kg^{-1}. Even when administered intramuscularly isometamidium chloride produced severe local reactions and extensive fibrosis (Boyt, 1971). These lesions have been described both in cattle and camels (Slack and Nineham, 1968; Toure, 1973; Wilson, LeRoux, Paris, Davidson and Gray, 1975b; Balis and Richard, 1977). May and Baker Ltd. thus advise that the drug be administered deep intramuscularly, in the middle third of the neck. Volumes greater than 15ml should not be injected into one site, but divided (Finelle, 1973b). Intramuscular administration of isometamidium chloride at 4mg kg^{-1} resulted in systemic toxic effects; staggering, recumbency and occasionally death (Robson, 1962).

In an attempt to decrease the toxicity associated with isometamidium administration, workers have complexed it with dextran sulphate (m.w.500,000) in a 1:1 w/w manner (James, 1978; Aliu and Sannusi, 1979; Otaru and Sannusi, 1979; Aliu and Chineme, 1980). Formation of the complex increased the therapeutic index for rodents at least

four-fold (Otaru and Sannusi, 1979; Aliu and Chineme, 1980). In cattle, subcutaneous administration of a 2% w/v solution, even at 2mg kg^{-1}, produced a granuloma but no severe reaction (Aliu and Sannusi, 1979; Otaru and Sannusi, 1979). Finally, the period of prophylaxis in rodents was greatly extended (James, 1978). This has yet to be demonstrated in cattle.

Isometamidium is especially useful because of its efficacy on trypanosomes resistant to other commonly used trypanocides (Finelle, 1973a), and is the drug of choice for T.congolense infections of dogs (Aliu, 1981). T.brucei infections in dogs respond well to isometamidium (Toure, 1970) though quinapyramine is the drug of choice for first-stage infections (Aliu, 1981).

T.evansi infections have been successfully treated in camels at 1mg kg^{-1} (Petrovski, 1974) and in donkeys and dogs at 2mg kg^{-1} (Chand and Singh, 1970).

MODE OF ACTION OF PHENANTHRIDINES

Of the phenanthridine series, most work has been conducted with ethidium. Ethidium irreversibly inhibited Crithidia oncopelti multiplication (Newton, 1964) but was not toxic to a non-dividing population (Newton, 1957). Rapid inhibition of C.oncopelti DNA synthesis occurred without RNA or protein synthesis being affected (Newton, 1957). Ethidium probably prevents DNA replication by intercalating between adjacent nucleotide base pairs, distorting the double helix structure and thereby

preventing DNA polymerases from functioning (Newton, 1974).

The phenanthridines may also interfere with polyamine function. If polyamines are administered along with isometamidium, in vitro, the trypanocidal activity of isometamidium is abolished (Bacchi, 1981).

CHEMOPROPHYLAXIS

Chemoprophylaxis can be highly effective provided that a strict treatment regime is used (WHO, 1979; Connor, 1984).

The first widely used chemoprophylactic was quinapyramine. When used in cattle in contact with tsetse, the interval between cattle treatments could not be extended beyond 2 months (Fiennes, 1953). Resistance to quinapyramine eventually appeared (Robson and Wilde, 1954) and became widespread (Cockbill, 1967) with drug-administration being required every 2-3 weeks.

Pyrithidium bromide (Prothidium) (2-amino-7-(2-amino-6-methylpyramid-4-ylamino)-9-p-aminophenylphenanthridine-10,1'-dimethobromide) was introduced by Watkins and Woolfe (1956) as a prophylactic against bovine trypanosomiasis.

Studies both in East and West Africa showed that intramuscular administration of 2mg kg^{-1} pyrithidium bromide would protect cattle for anything from 4-8 months (Marshall, 1958; Lyttle, 1960; Finelle and Lacotte, 1965) and that it was superior to quinapyramine as a prophylactic (Smith and Brown, 1960). Pyrithidium was used in Nigeria but was abandoned because of the high incidence of

hepatotoxicity and the resultant high mortality (Stephen, 1962b). Because of the toxicity problems and the advent of a superior prophylactic, metamidium (Smith and Brown, 1960), usage of the drug declined. Pyrithidium was eventually withdrawn from the market in the late 1970s.

Both metamidium and isometamidium chloride were shown to be highly effective prophylactic drugs when given at a dosage of 0.5mg kg^{-1} (Fairclough, 1963a). Since 1967, isometamidium has been the sole prophylactic used in areas of high tsetse challenge (Williamson, 1976), and of the three trypanocides commonly used today in cattle (homidium chloride, diminazene aceturate and isometamidium chloride), is the only one that can be considered a prophylactic (Connor, 1984). A 4 month programme may be used, where, every 4 months, all animals are administered isometamidium (Finelle, 1973b). If trypanosomes appear before the next prophylactic injection is due, the curative diminazene aceturate is given (Finelle, 1973b).

Improvement in the economic return associated with using isometamidium prophylaxis, as opposed to other control programmes, has been described in Nigerian trek cattle (Na'Isa, 1969), ranch cattle in Kenya (Wilson et al., 1975b; Wilson, Njogu, Gatuta, Mgutu, Alushula and Dolan, 1981), ranch cattle in Tanzania (Blaser et al., 1977), ranch cattle in Mali (Logan, Goodwin, Tembely and Craig, 1984) and work oxen in Ethiopia (Bourn and Scott, 1978).

Reports on the period of prophylaxis conferred to

cattle, following administration of isometamidium, vary widely, as shown below:

Dose ($mgkg^{-1}$)	Duration of prophylaxis	Author
0.5	14 weeks (x)	Fairclough, 1963a
"	24 weeks (10%)	Wiesenhutter, Turner and Kristensen, 1968
1.0	2 weeks (c)	Pinder and Authie, 1984
"	4 weeks (c)	Lewis and Thomson, 1974
"	8 weeks (c)	Kirkby, 1964
"	9 weeks (c)	Lwembandisa, 1970
"	14 weeks (c)	Omwero-Wafula and Mayende, 1979
"	30 weeks (10%)	Wiesenhutter et al., 1968
2.0	12 weeks (c)	Kirkby, 1964
"	36 weeks (x)	Robson, 1962
4.0	50 weeks (x)	Robson, 1962

Definitions of "duration of prophylaxis" vary between authors. Some define it as the average duration of prophylaxis within a group of cattle (x); others as the time taken for 10% of a group to become infected (10%); and others as the complete period of prophylaxis, i.e., the period until the first animal in a group becomes infected (c). The last definition gives an indication of the minimum period of prophylaxis to be expected within a group of animals and thus the frequency of drug-administration required to prevent trypanosome patency within the group;

thereby maximising productivity. However, even this last definition does not give an entirely accurate figure for the period of complete prophylaxis since the stated period consists of both the true prophylactic period and the false prophylactic period (Ercoli, 1978). During the true prophylactic period the animal is refractory to challenge, whilst during the false prophylactic period animals are not completely refractory and either infections are developing but non-patent or the time to onset of parasitaemia is extended, thereby giving a false indication of the prophylactic cover.

Even so, there is obviously a wide range in the apparent period of prophylaxis. There are a number of reasons that may account for the variation:

Firstly, working with quinapyramine chloride/sulphate, an almost linear relationship was demonstrated between the vector density, or trypanosomiasis risk, and the drug dosage-frequency required (Whiteside, 1955, 1962a,b). Other workers came to the same conclusion, stating that the prophylactic period depended on the virulence of the trypanosomes and the number of infective tsetse bites received per unit time (MacOwan, 1955; Lyttle, 1960).

Secondly, work in mice with T.b.brucei has shown that in animals apparently cured of infections, relapses may occur from drug privileged sites (Jennings, Whitelaw and Urquhart, 1977a,b; Jennings, Whitelaw, Holmes, Chizyuka and Urquhart, 1979), i.e., trypanosomes may re-emerge from tissues or organs, such as the brain, which are

inaccessible to most trypanocides.

Thirdly, acquisition of immunity following the usage of trypanocidal drugs has been suggested to contribute towards the prophylactic period (Bevan, 1928, 1936; Fiennes, 1953; Soltys, 1955; Wilson et al., 1975b; Wilson, Paris, Luckins, Dar and Gray, 1976; Bourn and Scott, 1978). As well as the acquisition of immunity following treatment of an infection, antigenic priming may occur in animals under chemoprophylactic cover. If there were a limited number of serodemes in a given area then, theoretically, there could be an immune contribution to the apparent prophylactic period.

Finally, differing levels of drug sensitivity between trypanosome populations may play a role in determining the duration of prophylaxis.

DRUG RESISTANCE

Resistance in parasitic nematodes is defined as a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species (Prichard, Hall, Kelly, Martin and Donald, 1980). In schistosomiasis chemotherapy, workers have indicated that resistance should be distinguished from tolerance (Coles, Bruce, Kinoti, Muthahi, Dias and Katz, 1986). If a significant change in response occurs in an area following widespread therapy then resistance is said to have occurred. However, unless data is available to confirm that resistance has occurred then isolates that

fail to respond to a schistosomicide are considered as tolerant, or innately resistant.

From its earliest days, the development of chemotherapy for bovine trypanosomiasis has been accompanied by the development of drug resistance (Williamson, 1979).

Drug resistance appears to be acquired by exposure of trypanosomes to sub-therapeutic levels, thereby facilitating selection of a drug-resistant subpopulation (Whiteside, 1962a; Leach and Roberts, 1981). Sub-therapeutic drug levels may occur because of incorrect dosage (directly or because of incorrect body weight estimation), a high incidence of trypanosomiasis, irregular treatment with prophylactics or stopping the usage of a prophylactic whilst animals are still at risk (Davey, 1950, 1957; Whiteside, 1960).

Since diminazene aceturate is quick acting, rapidly broken down and excreted (Bauer, 1958), subcurative concentrations exist for only a short period. This may account for the small number of reports describing resistance to the drug.

Resistance to diminazene aceturate to at least the level of 3.5mg kg^{-1} in cattle has been described. The majority of reports concern infections with West African T.vivax (Jones-Davies, 1967a,b; Graber, 1968; MacLennan and Na'Isa, 1970; Gray and Roberts, 1971; MacLennan, 1971, 1972) although resistance in East African T.vivax has been

reported (Mwambu and Mayende, 1971a,b). Reports of resistance to diminazene aceturate at 3.5mg kg^{-1} by T.congolense are much fewer (Jones-Davies, 1968; Gray and Roberts, 1971; Gitatha, 1979). Thus, T.vivax appears to express a higher level of innate resistance to diminazene aceturate than T.congolense (Williamson, 1960).

In contrast, resistance to homidium appears to occur more commonly in T.congolense than T.vivax. Widespread resistance to homidium has been described in Nigeria associated with T.congolense (Jones-Davies and Folkers, 1966b; Na'Isa, 1967; Folkers, Van Hoeve and Buys, 1968; Williamson, 1970). Homidium-resistant T.congolense have also been described in the Sudan (Gadir, Tahir, Razig and Osman, 1972), Ethiopia (Scott and Pegram, 1974) and Kenya (Gitatha, 1979). Reports of expression of homidium-resistance by T.vivax (Ilemobade and Buys, 1970) are lacking. This would suggest that T.congolense expresses a higher level of innate resistance to homidium than T.vivax (Williamson and Stephen, 1960).

Of the chemoprophylactics, widespread resistance to quinapyramine (Whiteside, 1960; Cockbill, 1967) lead to the withdrawal of its usage for bovine trypanosomiasis. Considerable resistance to quinapyramine was expressed by both T.congolense and T.vivax in cattle after a single treatment (Wilson, 1949) indicating how readily resistance could be acquired.

Folkers (1966) showed that resistance to isometamidium did not appear to develop readily when repeatedly using

0.25mg kg^{-1} isometamidium chloride under field conditions of high trypanosomiasis risk. In contrast, Lewis and Thomson (1974) in Rhodesia reported resistance to 1mg kg^{-1} after 4 years exposure to 1mg kg^{-1} isometamidium chloride administered every 12 weeks. Other workers have reported putative resistance in the field because of "early breakthroughs" when using 0.5-1.0mg kg^{-1} prophylactic regimes (Lewis and Thomson, 1974; Scott and Pegram, 1974; Kupper and Wolters, 1983; Pinder and Authie, 1984).

Elucidation of the level of resistance to isometamidium has been conducted by some of the above authors, and others, in cattle (see table 3), sheep (Arowolo and Ikede, 1980) and mice (Scott and Pegram, 1974; Arowolo and Ikede, 1977; Omwero-Wafula and Mayende, 1979; Pinder and Authie, 1984). Detection of resistant strains in small laboratory animals has its limitations because they are often not susceptible to infection with resistant strains (Williamson and Stephen, 1960). The same authors advocated the use of sheep as a homidium-resistance model. Sheep were infected by application of infected tsetse flies and then treated with a subcurative dose of homidium. The interval between treatment and relapse was taken as an index of the degree of homidium-resistance.

Extrapolation of results from sensitivity studies in mice and sheep to the cow cannot be directly made because of large differences in metabolic body weights (Arowolo and Ikede, 1977, 1980). Hawking (1963a) attempted to find a consistent relationship between the drug sensitivity of

Table 3

BOVINE STUDIES ON THE SENSITIVITY OF TRYPANOSOME POPULATIONS TO THE THERAPEUTIC ACTIVITY OF ISOMETAMIDIUM

LACK OF SENSITIVITY AT ($mgkg^{-1}$)	TRYPANOSOME SPECIES	REGION	AUTHORS	YEAR
0.25-0.80*	T.congolense	N. Nigeria	Jones-Davies and Folkers	1966a
0.8*	T.congolense	N. Nigeria	Na'Isa	1967
0.5	T.congolense	Nigeria	Gray and Roberts	1971
1.0	T.congolense	Rhodesia	Lewis and Thomson	1974
0.5-2.0	T.congolense	Kenya	Gitatha	1979
0.5	T.vivax(Y58)#	Nigeria	Ilemobade	1979
0.5-1.0+	T.congolense T.vivax	N.Ivory Coast	Kupper and Wolters	1983

* = Cross resistance studies.
# = Rodent adapted population.
+ = Field report.

trypanosome populations in mice and in cattle, but was unable to do so.

Sensitivity studies in mice have used various terms to define the sensitivity. The MED (minimal effective dose) denotes the minimal dose required to clear the blood of trypanosomes (Hawking, 1963a) and the subscript x in MED_x refers to the percentage of animals the MED value refers to. The MCD (minimal curative dose) is a more commonly used term (Mwambu, Mayende and Masinde, 1969; Walker and Watts, 1970; Scott and Pegram, 1974) and denotes the dosage of drug required to cure an infection. Again, the percentage of animals the term refers to is always stated.

Care must be taken in all therapeutic drug sensitivity studies to ensure that treatment occurs sufficiently soon after infection to rule out the possibility of relapses occurring from sites inaccessible to drug (Jennings et al., 1977a,b, 1979).

STABILITY OF DRUG RESISTANCE

The level of resistance of a T.congolense population resistant to quinapyramine decreased after 65 days repeated passage in rats, (Unsworth, 1954). In a similar manner, resistance to diminazene aceturate (expressed by a T.congolense population) appeared to be unstable during repeated bovine passages (Whiteside, 1963). In contrast, the sensitivity of a T.vivax population to quinapyramine after cyclical transmission through Glossina palpalis was stable. Gray and Roberts (1971) demonstrated the stability

of a drug-resistant T.congolense (resistant to diminazene aceturate at 7mg kg^{-1}, homidium chloride at 2mg kg^{-1} and isometamidium chloride at 0.5mg kg^{-1}) and T.vivax (resistant to diminazene aceturate at 7mg kg^{-1} and quinapyramine at 5mg kg^{-1}) after repeated cyclical transmission.

In the field, if cattle are withdrawn from an area and the use of the drug in question is stopped, drug-resistant strains of T.congolense and T.vivax tend to disappear from tsetse within 6-9 months (Whiteside, 1960).

CROSS RESISTANCE

With the appearance of resistance to trypanocides, the use of chemically unrelated trypanocides to treat relapses has not always effected a cure (Wilson, 1949; Karib, Ford and Wilmshurst, 1954; Whiteside, 1960). After extensive cross-resistance studies (1400 tests) in cattle, Whiteside (1960) formulated a cross-resistance table (see table 4). Most of the tests were conducted with T.congolense. The quinapyramine work was repeated with T.vivax and produced identical results. Of particular importance was the fact that trypanosomes resistant to quinapyramine were cross-resistant to diminazene. Thus, one reported case of diminazene resistance has been attributed to residual quinapyramine resistance (Mwambu and Mayende, 1971b).

Because of the cross-resistance problem, Whiteside (1958) introduced the term "sanative drug pair" to denote pairs of drugs that do not induce resistance to the other.

Table 4

CROSS RESISTANCE STUDIES

(after Whiteside, 1960)

	CROSS-RESISTANCE							
	CURATIVE DOSAGES				HIGHER DOSAGES			
TRYPANOSOMES RESISTANT TO	ANTRY-CIDE	HOMI-DIUM	META-MIDIUM	BERENIL	HOMI-DIUM 2*	META-MIDIUM 2	BERENIL 5 - 7	PROTH-IDIUM 2
QUINAPYRAMINE	R	+	+	+	+	0	+	0?
HOMIDIUM	+	R	+	0	R	0	0	0?
METAMIDIUM	+	+	R	0	+	R	0	0?
DIMINAZENE	0	0	0	R	(0)	0	R	(0)

* Figures = mg kg^{-1}.
R = Direct resistance.
0 = No cross-resistance.
+ = Cross-resistant.
0? = cross resistant to 1mg kg^{-1}, but not to 2mg kg^{-1}.
(0) = no observations.

On the basis of his work the only sanative pairs are homidium/diminazene and metamidium/diminazene. Thus, the use of an alternating programme of diminazene aceturate and isometamidium chloride has been recommended for the field (Finelle, 1974).

TRYPANOSOMIASIS CHEMOTHERAPY/IMMUNITY

Immunity associated with chemotherapy may either be non-sterile or sterile.

Bevan (1928, 1936) was the first to describe non-sterile immunity or "tolerance" following the use of trypanocidal drugs. A laboratory attenuated T.congolense, originating from the area experimental cattle were to be exposed, was inoculated into cattle and treated with potassium antimonyl tartrate. Cure was rarely effected, however the cattle remained in good health despite reinfection. Bevan termed this phenomenon "tolerance". If the trypanosomes were inoculated into fully susceptible cattle, full pathogenic effects were observed.

The tolerance was maintained by frequent challenge and if the tolerant cattle were "cured" they were fully susceptible to subsequent infection. Tolerance breakdown also occurred under the following circumstances; infection with a heterologous trypanosome species, starvation, excessive work, intercurrent infection and mineral or vitamin deficiences.

Other workers have also produced non-sterile immunity experimentally (Wilson, Cunningham and Harley, 1969;

Wilson, 1971). Bourn and Scott (1978) described a similar phenomenon in which a phenanthridinium-resistant T.congolense appeared in oxen. Despite the presence of parasitaemia the oxen remained in good condition whilst remaining on isometamidium prophylaxis. Whether this was true tolerance is questionable since trypanosome populations exhibiting resistance appeared to exhibit decreased pathogenicity (Goble, Ferrell and Stieglitz, 1959; Stephen, 1962a).

The production of non-sterile immunity in the field is not a favourable state since the cattle, by virtue of remaining parasitaemic, act as a constant source of infection for the vector (Ruchel, 1975).

Experimentally, sterile immunity to homologous T.congolense and T.brucei challenge has been readily conferred by a strategy of infection and cure (Wilson et al., 1969; Akol and Murray, 1983). The resultant protective antibody response has been shown to last up to 7 months following treatment (Wilson and Cunningham, 1972).

Development of immunity in cattle in the field has been assessed indirectly, by observing changes in (a) the need for trypanocidal drug treatment, (b) the ability to maintain normal blood values, (c) the ability to withstand prolonged periods of parasitaemia, (d) the acquired ability to gain weight in face of virulent trypanosome challenge when drug treatment is discontinued, (e) changes in serum immunoglobulin levels and (f) immunoflourescent antibody test in assessing the immune status on a herd basis

(Wilson, LeRoux, Dar, Paris, Davidson and Gray, 1975a; Wilson, 1976).

Wilson and his colleagues conducted a series of experiments to examine the development of immunity in the field, using different trypanocidal regimes. Indirect evidence for the development of immunity in calves, in contrast to adults, was presented by Wilson, Dar and Paris (1973). Zebu cattle were kept in an area of high tsetse-challenge in Uganda (South Busoga) for 2 years and treated on an individual basis with 7mg kg^{-1} diminazene aceturate when clinically ill. The adults required an average of 8.4 treatments per year whilst the calves, 5.5 per year.

In a second experiment, Zebu cattle were again maintained in an area of high tsetse-challenge in South Busoga. Cattle were treated on an individual basis, as in the first experiment. No significant resistance to trypanosomiasis developed in either the calves or the adults during 2 years of exposure. However, the number of abortions and calf mortality decreased (Wilson, Paris and Dar, 1975c).

Wilson et al. (1975b, 1976) compared three trypanocidal regimes; diminazene aceturate administered on an individual or group basis, and isometamidium chloride administered on a group basis. Boran steers were used and kept in a tsetse area of Kenya for $2^1/_2$ years. The challenge was medium or high for the first two years and low for the final 6 months.

Group 1 contained 20 cattle which were treated on an

individual basis with 5mg kg^{-1} diminazene aceturate either when severely affected by trypanosomiasis on a clinical basis, or when the PCV fell below 20%.

Group 2 contained 20 cattle which were treated on a group basis with 5mg kg^{-1} diminazene aceturate when trypanosomiasis was diagnosed in one animal.

Group 3 contained 20 cattle which were treated on a group basis with 0.5mg kg^{-1} isometamidium chloride when trypanosomiasis was diagnosed in one animal.

Group 1 showed evidence for acquisition of immunity; the period between diminazene aceturate treatments increased from a mean of 50-60 days after the first 3 treatments to 132 days after the ninth treatment and the period when trypanosomes were in the peripheral blood without side effect increased. However, of the three groups, group 1 had the lowest growth rate, emphasising the stunting effect of trypanosomiasis.

Group 2 showed no evidence for acquisition of immunity; there was no increase in time between drug treatments and no alteration in sensitivity to trypanosome pathogenicity after drug withdrawal. There were no anti-trypanosomal antibodies and only minor immunoglobulin changes. However, the group had a better growth rate compared to group 1 but worse than group 3.

Group 3 showed evidence for the acquisition of some immunity; the need for drug administration did not decrease, however if drug was removed, despite the presence of trypanosomes, the growth of cattle was the same in drug-

treated and untreated animals. No antibodies were detected and serum immunoglobulin changes were minimal. Group 3 had the best growth rate.

This work indicated that, provided a closely controlled drug regime was used, development of immunity was not a prerequisite for successful maintenance of cattle in a tsetse area (Leach and Roberts, 1981). Also, that for acquisition of immunity to occur, trypanosomes must be allowed to develop in the host before drug-treatment.

Investigations into the acquisition of immunity in cattle whilst under chemoprophylactic cover are lacking. Whiteside (1962a) stated that part of the observed prophylactic period was due to an immune response by the host and suggested that the animal was antigenically primed during part or all of the protective period. Cunningham (1966), in referring to this subject, stated that "even a slight immune response would probably enhance the action of a prophylactic drug". Little experimental evidence currently exists for these suppositions.

GENERAL MATERIALS AND METHODS

1) EXPERIMENTAL ANIMALS

(a) CATTLE

Yearling Boran steers were obtained from Kapiti Plains Ranch, situated in the Athi River area of Kenya. This region is free from tsetse, and trypanosomiasis is absent. On arrival at ILRAD all animals were administered orally 5mg kg^{-1} fenbendazole (Pancur; Hoechst). In addition, all cattle were screened serologically for antibodies to (a) Theileria parva schizonts and Theileria parva piroplasms using an IFA test (Goddeeris, Katende, Irvin and Chumo, 1982), (b) Anaplasma marginale and Babesia bigemina using an ELISA (Voller, Bidwell and Bartlett, 1975) and (c) T.congolense, T.vivax and T.brucei using an ELISA (Voller et al., 1975) and IFA test (Katende, Musoke, Nantulya and Goddeeris, 1987). The percentage of the cattle seropositive to each antigen were as follows:

T.parva schizonts	-	40%
T.parva piroplasms	-	46%
A.marginale	-	22%
B.bigemina	-	32%
T.congolense	-	0%
T.vivax	-	0%
T.brucei	-	0%

Rhipicephalus appendiculatus, the vector of T.parva parva, does not occur on Kapiti Ranch. The high antibody titres specific for T.parva schizonts and piroplasms are

thought to be due to cross-reactivity to a benign Theileria species, possibly T.taurotragi.

All cattle were walked through a spray race on a weekly basis and sprayed with 3% Delnav DFF (Cooper), a phosphatic insecticide, to eliminate ticks. Spraying of cattle was stopped for every 2 week period prior to being fed on by infected tsetse.

Prior to entering an experiment some control animals were kept in a field. Maintenance of cattle on this pasture had previously been shown to result in nutritional muscular dystrophy. Thus, all cattle maintained outside were intramuscularly administered 1ml MU-SE (Burns Biotec), containing the equivalent of 5mg selenium and 68 i.u. of vitamin E (as d-alpha-tocopheryl acetate), every 6 weeks.

Every 4 months, all cattle, were subcutaneously inoculated with 3ml of an inactivated quadrivalent (O/A/SATI/SAT2) foot and mouth disease vaccine (Wellcome Kenya Ltd., Cooper).

At the beginning of each experiment all cattle weighed approximately 200 kg.

For simplification of individual animal reference, each animal has been allocated a code indicating the animals' group and number, e.g., A3 denotes group A, animal number 3. Keys to the codes used for animals in each chapter are given in each chapter's appendix.

(b) GOATS

Adult East African x Galla goats were obtained from

the Naivasha/Nakuru region of the Kenyan rift valley, an area known to be free from trypanosomiasis. On arrival at ILRAD goats were administered 7.5mg kg^{-1} rafoxanide and 44mg kg^{-1} thiabendazole (Ranizole Drench; MSD Agvet) for helminths and nasal bots (Oestrus ovis), and 50mg powder of amprolium (Amprol 20%, MSD) daily for three days for coccidiosis. On entry into fly-proof isolation units all goats were administered 5mg kg^{-1} fenbendazole (Panacur 2.5%, Hoechst) and a second three day course of amprolium (50mg powder per day).

(c) MICE

Inbred male and female C57, C_3H, A/J, BALB/c, Swiss (derived from MF1) and C57Bl6XC_3H/He(F1) were obtained from the ILRAD breeding colonies. All were originally derived from O.L.A.C. (Oxfordshire Laboratory Animal Colonies) '76 strains. All mice weighed approximately 20g and were between 8 and 12 weeks old at the beginning of each experiment.

(d) GUINEA-PIGS

These were multicoloured, English type, obtained from the ILRAD breeding colony.

2) FEEDING AND HOUSING

(a) CATTLE

When housed for experiments, cattle were kept in groups of at most 16 animals on concrete, in a loose shed.

Cattle used in T.vivax experiments were housed in fly-proof isolation units. Cattle were bedded with wood shavings and cleaned out twice weekly. They were fed a diet of hay and commercial concentrate (2kg per day of young stock pencils; M/s Belfast Millers Ltd., Nairobi), supplemented on a daily basis with approximately 25g mineral mix per animal (Cooper, Wellcome Kenya Ltd). Cattle had free access to water.

All cattle were moved to insect-proof units for each experimental tsetse-fly challenge.

(b) GOATS

Goats were housed in fly-proof isolation units on a concrete floor and bedded with wood shavings. All were cleaned out twice weekly. They were fed hay and 200-300g of concentrate per day (Belfast Millers Ltd., Nairobi). Water was readily available at all times.

(c) MICE

All animals were housed in plastic cages with metal tops and kept in the mouse room of the ILRAD small animal unit. Sawdust bedding was changed twice weekly. Mice were fed on an ad libitum diet of maize-based mice pencils (Unga Feeds Ltd., Nairobi). Drinking water was continually available in plastic water bottles. No medication, of any type, was administered to the mice.

3) TSETSE FLIES

Tsetse flies were infected by allowing them to feed on infected goats or cattle with a patent parasitaemia. Except for the East African T.vivax populations which were inoculated into Boran calves, all other trypanosome populations were inoculated into goats for infecting tsetse. Approximately three days after trypanosomes were detected in the peripheral blood circulation, teneral male Glossina morsitans centralis, obtained from the ILRAD tsetse colony, were fed on them. The tsetse flies were maintained by daily feeding (except Sundays) on the infected animals. From day 25 following the first feed of tsetse on animals infected with T.congolense, flies were monitored for infection by microscopic examination of salivary probes (Burtt, 1946). Those flies extruding metacyclic trypanosomes in their saliva were used to challenge cattle.

Identification of tsetse infected with T.vivax by examination of salivary probes proved very inefficient since only a small number of infected flies extruded metacyclics in their probe. Thus, for challenge of cattle with T.vivax-infected tsetse, each fly that fed on an animal was dissected. Each non-infected fly was replaced with another until a total of ten infected flies had fed on each animal.

4) TRYPANOSOMES

The following populations were used:

(a) T.congolense ILNat 3.1 (IL 1180)

(b) T.congolense IL 2642

(c) T.vivax IL 2968 (Zaria, Nigeria)

(d) T.vivax IL 2969 (Kilifi, Kenya)

(e) T.vivax IL 2982 (Galana, Kenya)

(f) T.vivax IL 2986 (Likoni, Kenya)

(g) T.congolense IL 2856

T.congolense ILNat 3.1 is a homogeneous VAT population and has also been designated IL 1180. It is a doubly-cloned derivative of an isolate (LR 11/C9) made in the Serengeti National Park by Geigy and Kauffman (1973). The derivation of ILNat 3.1 from the original isolate is shown in figure 1, appendix V.

T.congolense IL 2642 is a doubly-cloned derivative of an isolate made from a cow in Busoga district, Uganda, 1962. Its derivation is shown in figure 2, appendix V.

T.vivax IL 2968 is a trebly-cloned derivative of an isolate made from a cow in Zaria, Nigeria, 1973 (Leeflang, Buys and Blotkamp, 1976). It is naturally rodent-infective. The derivation of IL 2968 is shown in figure 3, appendix V.

T.vivax IL 2969 is a non-rodent-infective T.vivax, originally isolated in Kilifi, Coastal Province, Kenya (figure 4, appendix V). It has been cloned once, in vitro.

T.vivax IL 2982 is a non-cloned derivative of an isolate made in Galana, Coastal Province, Kenya (figure 5, appendix V). It is non-infective for rodents.

T.vivax IL 2986 is a non-cloned derivative of an isolate made in Likoni, Coastal Province, Kenya (figure 6, appendix V). It is non-infective for rodents.

T.congolense IL 2856 is rodent infective and was originally isolated in Banankeladaga, Burkina Faso. The original isolate was termed Banankeladaga 83/CRTA/67 (Pinder and Authie, 1984) and the derivation of IL 2856 is shown in figure 7, appendix V.

5) ENUMERATION OF TRYPANOSOMES

Two methods were used to quantify the number of trypanosomes per millilitre of body fluids:

(a) Blood buffy-coat phase-contrast (BC) technique (Murray, Murray and McIntyre, 1977). This technique was used to determine the level of parasitaemia in cattle. Following measurement of the haematocrit in plain microhaematocrit capillary tubes (see section 14, Packed Cell Volume), using heparinised blood, the capillary tube was cut approximately 1mm below the lower end of the buffy coat with a diamond pointed pen, thereby incorporating the uppermost region of red blood cells. Using a microhaematocrit capillary tube holder, the erythrocyte layer, buffy coat, and approximately 3mm of plasma were gently expressed onto a clean microscope slide, mixed well and covered with a 22x22mm coverslip. The preparation was then examined with a Leitz (Leitz Wetzlar) SM Lux microscope with a combination of a periplan NF x10 eyepiece, a Phaco 2 NPF 25/0.50 objective and a Zernicke

402 condenser (Leitz Wetzlar, Germany).

The scoring system developed for correlating the number of trypanosomes counted to the level of parasitaemia (Paris, Murray and McOdimba, 1982) is shown below:

SCORE	TRYPANOSOMES/FIELD*	ESTIMATED PARASITAEMIA (TRYPANOSOMES ml^{-1})
6+	Swarming 100	$5x10^6$
5+	10	$5x10^5$
4+	1-10	10^4 - $5x10^5$
3+	1/2 fields to 1/10 fields	$5x10^3$ - $5x10^4$
2+	1-10 per preparation	10^3 - 10^4
1+	1 per preparation	10^2 - 10^3

* Magnification = x250

(b) NEUBAUER HAEMOCYTOMETER

Bovine and rodent-derived bloodstream forms, and *in vitro*-derived metacyclic trypanosomes were counted in a Neubauer haemocytometer, as described by Lumsden, Herbert and McNeillage (1973). Bloodstream forms were counted for subsequent isometamidium sensitivity studies in mice, and cultured metacyclics for intradermal inoculation into cattle and serological tests to detect the presence of anti-metacyclic antibodies. Only motile trypanosomes were counted, and dividing stages counted as one trypanosome.

6) DRUGS

All experiments described in this thesis were conducted with the drug isometamidium chloride (Samorin, May and Baker Ltd., Dagenham, England). Lot No. DP 3947 was used throughout the experiment described in chapter 1 with T.congolense ILNat 3.1 and T.congolense IL 2642, both in cattle and mice. Lot No. DM 4532 was used in all other experiments. The drug was freshly prepared in sterile distilled water. In cattle, a 4% w/v solution was used for a dosage of 1mg kg^{-1} and a 2% w/v solution for a dosage of 0.5mg kg^{-1}, as recommended by the manufacturers. The concentrations used for isometamidium sensitivity studies in mice on bovine breakthrough populations, and murine 50% curative dose (CD_{50}) determinations, were as described in section 16 and the materials and methods section of chapter 5.

Diminazene aceturate (Berenil, Farbwerke, Hoechst A.G., Frankfurt, W. Germany; Lot No. 095D575) was freshly prepared as a 7% w/v solution in sterile distilled water and administered to cattle at a dosage of 3.5mg kg^{-1}.

7) TREATMENT OF CATTLE

Isometamidium chloride was administered as a deep intramuscular injection in the middle third of the side of the neck using an 18 guage, $1^1/_2$ inch needle. Care was taken that none of the drug was deposited subcutaneously. Prior to injection the injection site was washed thoroughly with 70% methanol. Diminazene aceturate was administered

in a similar manner.

Cattle bodyweights were accurately determined prior to the administration of drug.

8) INFECTION OF TSETSE

For fly-pickups, goats were inoculated intramuscularly with T.congolense ILNat 3.1, T.congolense IL 2642 or T.vivax IL 2968, and Boran cattle intravenously inoculated with T.vivax IL 2969, T.vivax IL 2982 or T.vivax IL 2896. Both goats and cattle were bled daily (except on Sundays) from an ear vein. Thin blood films and the buffy-coat were examined for the presence of trypanosomes. Three to four days after trypanosomes were first detected, teneral male Glossina morsitans centralis from the ILRAD tsetse colony were allowed to feed on clipped flanks of the animals, in Geigy 1 cages. Tsetse were fed every day (except Saturdays and Sundays) over a period of 25 days. From day 25 onwards tsetse were monitored for infection by microscopic examination of salivary probes (Burtt, 1946) for the presence of metacyclic trypanosomes. With T.congolense populations and the Zaria T.vivax only those flies with positive salivary probes were used to challenge cattle.

Every challenge with T.congolense was conducted with 5 infective tsetse, whilst every challenge with T.vivax was conducted with 10 infected tsetse. When flies refused to feed they were replaced with flies that would. In contrast to a high infection rate obtained following dissection of tsetse infected with T.vivax populations, a very low

apparent infection rate was obtained (in the same fly populations) following examination of salivary probes. Thus, when cattle were challenged with T.vivax IL 2968, T.vivax IL 2969, T.vivax IL 2982 or T.vivax IL 2986, every fly that fed on an animal was dissected. Non-infected flies were replaced with others until a total of ten infected flies had fed on each animal.

9) CHALLENGE OF CATTLE

(a) FLY CHALLENGE

Cattle were clipped on one flank and for experiments where skin thickness changes were to be measured, localised areas (approximately $3cm^2$) were closely shaven with a scalpel blade to serve as tsetse feed sites (one tsetse per site). So as not to discourage tsetse from feeding, the shaved site was not washed with soap or any type of solvent. For experiments involving skin thickness observations, single infected tsetse, in 7x3cm transparent plastic tubes with netting at one end, were placed singly on each shaved site and allowed to feed until completely engorged. The point of entry of the probosis was marked with a large marker pen. Infected tsetse were only used once.

In experiments where skin thickness changes were not measured, tsetse were fed as a group in Geigy 1 cages.

Tsetse were starved for two days prior to being used to challenge cattle.

(b) *IN VITRO*-DERIVED METACYCLIC CHALLENGE

See materials and methods section of chapter 1.

10) SKIN THICKNESS

Measurement of the skin thickness at tsetse bite sites was conducted daily (except Sundays) until 30 days following each challenge. The skin was raised with the thumb and forefinger and the width measured with vernier calipers.

In contrast to the classical chancre skin reaction (a raised, hot indurated swelling) that occurred in non-treated control cattle following tsetse-transmission of both T.congolense ILNat 3.1 and T.congolense IL 2642, skin thickness changes in cattle administered isometamidium chloride, and susceptible to challenge, were in many cases much less pronounced. Skin thickness changes were therefore calculated by comparing the maximal skin thickness at the site of a tsetse bite with the average of the skin thickness measurements taken at the same site on days 0, 1 and 2. Skin thickness changes of between 0 and 20% have been taken as non-significant since variation in daily measurement lay within this range. A skin thickness change of 20-40% was termed a "slight" increase, 41-64% as a "moderate" increase and 65+% as a "large" increase.

11) DRAINAGE LYMPH NODE BREADTH

The breadth of the drainage prefemoral lymph node was measured daily, except Sundays. Because of the inability

in some animals to raise the prefemoral lymph node sufficiently to determine its breadth directly with calipers, the breadth was first determined with the thumb and forefinger. Keeping the two aforementioned digits in a fixed position, the distance between the two was measured with vernier calipers.

Changes in drainage prefemoral lymph node breadth were calculated in a similar manner to that described for the skin (section 10). Percentage increases in breadth below 30% were considered non-significant.

12) TEMPERATURE

The deep body temperature of cattle was measured daily, except Sundays. A Schot glass thermometer was inserted into the rectum, with the thermometer bulb adjacent to the rectal wall, and left in place for at least 30 seconds.

13) BLOOD SAMPLES

Jugular blood samples were collected, every 2-3 days, into 10ml ethylenediamine tetra-acetic acid (EDTA)-treated vacutainer tubes (Greiner) for determination of PCV and the level of parasitaemia.

All cattle were bled on a weekly basis into either 10ml EDTA-treated vacutainer tubes for plasma (T.congolense cattle experiment), or 10ml plain vacutainer tubes (Monoject) for collection of serum (all other experiments). Serum was collected after leaving the blood to stand for 6

hours at room temperature and then centrifugation at 1,200g for 10 minutes. Both serum and plasma were stored at -70°C until required for serological investigations.

14) PACKED CELL VOLUME

Tubes of blood were placed on a rotary mixer, prior to filling plain microhaematocrit capillary tubes (75mm x 1.5mm). Approximately 70ul of blood was added to capillary tubes which were then sealed at one end with Crystaseal (Hawksley, England). Capillary tubes were centrifuged for 5 minutes in an IEC MB microhaematocrit centrifuge and the PCV determined on a Hawksley micro-haematocrit reader.

15) DETERMINATION OF LOW LEVEL PARASITAEMIAS

In all cattle experiments conducted with rodent-infective trypanosomes, bovine blood was inoculated into mice in an attempt to detect trypanosomes not detected by the buffy-coat phase-contrast technique. Twelve and twenty-four days following every challenge, 0.2ml of EDTA-containing blood (from every cow challenged) was inoculated intraperitoneally (i.p.) into each of two mice. Mouse tailblood was examined twice weekly for a 30-day period to detect the presence of trypanosomes.

16) ISOMETAMIDIUM THERAPEUTIC SENSITIVITITY SCREENING

Determination of the isometamidium therapeutic sensitivity of bovine trypanosome populations was conducted in isometamidium prophylaxis experiments that used

rodent-infective trypanosome populations.

Subsequent to each challenge, trypanosome populations from control cattle, along with all breakthrough populations in drug-administered animals, were examined for sensitivity to isometamidium in mice. Each trypanosome population was inoculated into 2 series of mice consisting of 4 groups of 6 mice each, along with one group of 6 control mice. Series 1 was treated 6 hours after i.p. infection with 0.5, 1.0, 2.0 and 4.0mg kg^{-1} isometamidium chloride. Series 2 was treated in the same manner when the parasitaemia attained 10^7 trypanosomes per ml of mouse tail blood. Mice were monitored for 30 days after treatment for the presence of trypanosomes.

The quantity of isometamidium administered to mice was calculated by assuming the mouse weight to be 20g and the inoculation volume 0.2ml, i.e., 0.2ml of a solution containing 400mg dm^{-3} isometamidium chloride equated to a dosage of 4mg kg^{-1}.

17) ANTIBODY ASSAYS

Plasma, and serum inactivated at 56°C for 30 minutes, were assayed for anti-trypanosomal antibody.

For T.congolense serological investigations, in vitro-derived metacyclic trypanosomes were used.

For T.vivax studies, bovine bloodstream forms (from tsetse-infected cattle) were used.

(a) Infectivity neutralisation test (T.congolense)

Trypanosomes and plasma were diluted in phosphate saline glucose (PSG), 0.15M pH 8.0, containing 10% foetal bovine serum. Two-fold dilutions of each plasma sample in 0.75ml were added to an equal volume of a trypanosome suspension containing 7.5×10^3 organisms. After incubation for 30 minutes at 4°C, each plasma-trypanosome mixture was inoculated i.p. into a group of 6 mice, each receiving 0.2ml of the suspension. Mice tail-blood was examined twice weekly for the presence of trypanosomes for one month. If all mice within a group remained aparasitaemic throughout the observation period, complete neutralisation was said to have occurred.

Initial screening of plasma samples was conducted at a dilution of 1:2. If complete neutralisation occurred at this dilution, two-fold dilutions of the plasma sample were made until an end point dilution for complete neutralisation was determined.

(b) Trypanolysis test (T.congolense)

The trypanolysis test used was a modification of the technique described by Lumsden et al., 1973: Metacyclic trypanosomes were washed once in PSG and a metacyclic trypanosome suspension containing 2.5×10^7 trypanosomes per ml of PSG, pH 8.0, prepared. 10.8ml of fresh guinea-pig serum was added to 1.2ml of trypanosome suspension (resultant trypanosome suspension = 2.5×10^6 ml^{-1}) as a source of complement. 10ul of plasma, diluted in PSG, pH

8.0, was added to three different wells of a round-bottomed microtitre plate at dilutions of 1:1, 1:5 and 1:25. To each well was then added 40ul of trypanosome suspension ($1x10^5$ trypanosomes) thereby resulting in final plasma dilutions of 1:5, 1:25 and 1:125. After incubation at room temperature for one hour the contents of each well were expressed onto a microscope slide and examined under a coverslip using x400 phase-contrast optics. The percentage of lysed trypanosomes was determined and the end point of a positive plasma sample taken as the dilution of plasma at which over 90% of trypanosomes were lysed.

(c) Trypanolysis test (T.vivax)

Bovine-derived trypanosomes were used for antigen in the trypanolysis test. Parasites were collected for tests when the level of parasitaemia in a control steer (fly-transmitted infection) was such that approximately 10 trypanosomes were observed per field of a wet blood film (x400) (approximately $5x10^6$ ml^{-1}). Blood was collected into a 10ml Heparin-treated vacutainer tube (Greiner) and mixed with fresh guinea-pig serum in the ratio of 1 part blood to 10 parts guinea-pig serum. 50ul of trypanosome suspension was added to each 10ul aliquot of test serum in the well of a round-bottomed microtitre plate. After incubation at room temperature for 30 minutes the contents of each well were expressed onto a microscope slide, examined under x400 magnification, and the percentage of lysed trypanosomes determined.

(d) Indirect Immunofluorescent antibody test (T.vivax)

Separation of T.vivax bloodstream forms from bovine blood (collected as described in part (c)) was conducted as follows: 40ml of RPMI 1640, with the addition of 20mM Hepes, 10mM $NaHCO_3$ and 1% (v/v) L-Glutamine (29.2mg ml^{-1} RPMI 1640), was placed in a Falcon 50ml plastic centrifuge tube. 10ml of bovine blood, collected in heparin (Novo Industri A/S) was added (final concentration 10 i.u. ml^{-1}). After centrifugation at 330g for 15 minutes, 45ml was removed, leaving behind most erythrocytes and the buffy coat. The supernatant, in 50ml Falcon centrifuge tubes, was centrifuged at 1,300g for 15 minutes. After removal of the supernatant the pellet was resuspended in 1-2ml of PSG, pH 8.0, and pooled with the contents of all other centrifuge tubes. The trypanosome suspension was then placed onto 2cm depth of DEAE-cellulose (DE52, Whatman), newly packed in a small glass funnel with PSG, pH 8.0. Approximately 50ml of eluate was collected and centrifuged at 380g for 10 minutes. To prepare antigen to screen for variant specific antibody responses, the resultant pellet was resuspended in PSG, pH 8.0, containing 1% w/v formaldehyde. The mixture was left for $1/2$ hour to allow for complete fixation of trypanosomes and then centrifuged at 380g for 15 minutes. The resultant pellet was resuspended in phosphate buffered saline (PBS) containing 1% v/v rabbit serum so as to produce a trypanosome suspension of approximately $5x10^6$ ml^{-1}. "P.T.F.E.-coated multispot microscope slides" (Wellcome) were coated with

trypanosome antigen using a 100ul pipette and placed in a non-humidified 37°C incubator to dry out. Antigen-coated slides were wrapped in paper, then tin foil, and placed in polythene bags containing dessicated Silica gel, and stored at -70°C until required.

20ul of each serum sample was mixed with 180ul of lymphocyte lysate (Katende et al., 1987) and left for 30 minutes to absorb out non-specific binders. Initial screening was conducted at a dilution of 1:10. If antibody was detected at this dilution, further dilutions (1:50, 1:250 and 1:1250) were examined. The serum/lymphocyte lysate mixture was diluted with PBS containing 0.2% w/v bovine serum albumin (BSA) for non-specific reactants. 25ul of each serum dilution was added onto each antigen coated well. A positive and negative control serum sample were placed on each slide. The slides were placed over water in a Gelman moisturising chamber to decrease evaporation, and left at room temperature for 30 minutes.

Fluid was removed from over the slides (avoiding cross-contamination) and the slides then placed in PBS, 0.01M pH 7.2, including 0.2% w/v sodium azide. Two fifteen minute washes were carried out in this buffer, changing the buffer each time.

Sheep anti-bovine IgG_1, IgG_2 and IgM fluorescein isothiocyanate conjugate (FITC) was prepared as described by Goddeeris et al. (1982). A working dilution of conjugate was prepared by diluting it 1:50 in PBS, 0.01M, pH 7.2 + 0.2% BSA, containing Evans blue dye diluted 1:100

w/v for counter staining. After placing 20ul on each well the slides were placed over water in a moisturising chamber for 30 minutes.

After washing, slides were mounted using buffered glycerol (glycerol diluted 1:1 with PBS, 0.01M, pH 8.0) and 22mmx50mm coverslips.

Slides were viewed in an incident fluorescence optical system, using a x60 fluorescent oil phase-contrast objective N.A. 1.30 and a x6.3 periplan eyepiece. For fluorescence observation, light was supplied by an HBO 200 mercury arc lamp with a Leitz ploemopak 12/3 filter block.

An IFA test for anti-T.congolense common antigen antibody was conducted in an identical manner except that trypanosomes were fixed in normal saline containing 80% v/v acetone and 0.25% w/v formaldehyde (Katende et al., 1987) and the following serum dilutions used; 1:20, 1:100, 1:500 and 1:2,500.

IN VITRO MATERIALS AND METHODS

1) FEEDER LAYERS

(a) Bovine aorta endothelial cells

Bovine endothelial cultures were initiated as follows: The dorsal aorta was aseptically removed from a freshly killed cow and placed into Hanks' balanced salt solution (BSS) (GIBCO, U.K.) supplemented with penicillin (200 units ml^{-1})-streptomycin (200 units ml^{-1}). Using aseptic techniques, the outer layers of the aorta were removed with scissors and a scalpel. The aorta was then washed several times with Hanks' BSS, keeping the interior surface wet, and cut into pieces approximately $2cm^2$, avoiding areas where vessels enter the aorta. The internal surface of each piece was then placed on a large drop of collagenase solution (containing 1mg ml^{-1} of Type IV-S collagenase (168 units mg^{-1}, Sigma, London) in Dulbecco's PBS) on a sterile glass petri-dish and incubated for 10-15 minutes at 37°C. The loosened endothelial cells were detached by gently pipetting collagenase over the internal surface. The resultant suspensions of endothelial cells were pooled and collected by centrifugation at 160g for 5 minutes. The pellet was then resuspended in unsupplemented Medium 199 (M199) with Hanks' salt solution (GIBCO, U.K.) and centrifuged at 160g for 5 minutes. The pellet was resuspended in approximately 3ml of fresh M199 with Hanks' salt solution containing 10% (v/v) tryptose phosphate broth (GIBCO, U.K.) (2.9mg $100ml^{-1}$ M199), 20% (v/v)

heat-inactivated (56°C for 30 minutes) foetal bovine serum (Flow Laboratories, Scotland), 300ug ml^{-1} endothelial cell growth supplement (ECGS) (Collaborative Research, Waltham, Mass.), 1% (w/v) L-Glutamine, and penicillin (100 units ml^{-1})-streptomycin (100ug ml^{-1}). 0.5ml of culture medium of the same composition was placed into the central 8 wells of Tissue Cluster Plates (24 wells per plate; Costar, Cambridge, Mass.). 50ul of cell suspension was added to each well containing medium. The plates were placed in a humidified incubator gassed with 3-4% CO_2 in air at 37°C. On day 7 each well received an additional 0.5ml of medium. Medium was changed on a weekly basis for the following four weeks, by which time the cell layer had become confluent. Only those wells containing confluent endothelial cell layers, free of fibroblasts, were selected for further work.

Endothelial cells were removed from the wells by trypsin-EDTA treatment (one part of (0.05% (w/v) Trypsin (1:250) and 0.02% (w/v) EDTA (Flow Laboratories)) to two parts of (Ca^{2+} and Mg^{2+} - free Earle's BSS (GIBCO, U.K.)) at 37°C for 5 minutes and re-seeded into similar plates. Culture medium identical in composition to that already described was used, except that for passages 1 to 3 the ECGS was reduced to 200ug ml^{-1}. In passages beyond passage 3, ECGS was not included. Passages were conducted every 4 to 6 weeks. Cell monolayers used for experiments were those confluent with rounded cells, approximately 4 weeks post-passage.

(b) *Microtus montanus* embryonic fibroblast (MEF) Cells

MEF cells were derived as described by Brun, Jenni, Schoenberger and Schell (1981). MEF cells were maintained in plastic 25cm^2 T-type flasks with MEM medium supplemented with 15% (v/v) foetal bovine serum, 1% (v/v) L-Glutamine (29.2mg ml^{-1} MEM medium) and 40ug ml^{-1} Gentamycin. Passages were conducted every 10-20 days.

For experiments, 24 well Tissue Culture Cluster Plates were seeded with 1x10^5 viable cells per well, 24 hours before addition of trypanosomes. Cell viability was assayed by eosin staining.

2) PREPARATION OF TRYPANOSOMES

(a) *T.congolense* ILNat 3.1

T.congolense ILNat 3.1 was repeatedly passaged every 7 days in sublethally irradiated (650 rads) rats up to passage 15. Bloodstream trypanosomes for in vitro assays were collected just prior to the first parasitaemia peak. Rats were anaesthetised with ether then exsanguinated by cardiac puncture using a 20 guage 1$^1/_2$ inch needle attached to a 20ml syringe containing sterile heparin in Earle's BSS (final concentration approximately 10 i.u. heparin ml^{-1} of blood).

One part of T.congolense-infected rat blood was mixed with nine parts of HEPES (25mM)-buffered RPMI 1640 medium (GIBCO, U.K.) and centrifuged at 160g for 10 minutes to pellet most of the erythrocytes. The supernatant, containing the trypanosomes, was centrifuged at 1500g for

10 minutes and the sediment resuspended in 500ul RPMI 1640 medium. All sediment suspensions were pooled and the number of trypanosomes counted in an improved Neubauer haemocytometer counting chamber.

(b) *T.vivax* IL 2968

T.vivax IL 2968 was isolated from bovine blood as follows: 40ml of RPMI 1640, with the addition of 20mM Hepes, 10mM $NaHCO_3$ and 1% (v/v) L-Glutamine (29.2mg ml^{-1} RPMI 1640), was placed in a Falcon 50ml plastic centrifuge tube. 10ml of bovine blood, collected in Heparin (Novo Industri A/S) (10 i.u. ml^{-1} blood), was added . After centrifugation at 330g for 15 minutes, 45ml was removed leaving behind most erythrocytes and the buffy coat. The supernatant, in 50ml Falcon centrifuge tubes, was centrifuged at 1,300g for 15 minutes. After removal of the supernatant the pellet was resuspended in 1-2ml of PSG, pH 8.0, and pooled with the contents of all other centrifuge tubes. The trypanosome suspension was then placed onto 2cm depth of DEAE-cellulose (DE52, Whatman), newly packed in a small glass funnel with PSG, pH 8.0. Approximately 50ml of eluate was collected and centrifuged at 380g for 10 minutes. The resultant pellet was resuspended in PSG, pH 8.0.

Isolation of T.vivax IL 2968 from rat blood was identical to that described for T.congolense ILNat 3.1.

3) SCREENING OF PLASMA AND SERA FROM CATTLE ADMINISTERED ISOMETAMIDIUM CHLORIDE FOR TRYPANOCIDAL ACTIVITY USING *T.CONGOLENSE* ILNat 3.1 BLOODSTREAM FORMS

(a) 24 Hour Incubation

Cattle sera or plasma for screening were prepared by making 3ml of medium containing 20% (v/v) serum or plasma, 1% (v/v) L-Glutamine (29.2mg ml^{-1} RPMI 1640) and 79% (v/v) RPMI 1640 medium, and filter-sterilised using a 0.2um filter.

Maintenance medium over each bovine endothelial cell monolayer was removed and replaced with 1.000ml of the above described medium. 1.000ml of each medium was placed into each of two wells, taking care not to cause disruption of the confluent monolayer.

For each animal's set of serum or plasma samples, collected after administration of isometamidium chloride, a control sample containing serum or plasma collected on the day of drug administration was also included.

Into each well was added $1.0x10^6$ T.congolense ILNat 3.1 bloodstream forms, in a volume of 20-70ul RPMI 1640 medium.

Immediately following addition of trypanosomes each well was examined for the presence of trypanosomes using a Nikon inverted microscope with a x10 Nikon DLL N.A. 0.30 objective and a x10 Nikon HKW eyepiece. The 24 well plate was then placed in a humidified incubator, gassed with 3-4% CO_2 in air at 37°C, for 24 hours.

After incubation the medium in each well was

vigorously flushed over the cell feeder layer of the same well with a pasteur pipette, so as to cause detachment of all trypanosomes. In so doing some of the cell feeder-layer was also detached. The contents of pairs of wells containing the same serum or plasma sample were pooled and the trypanosome concentration determined using an improved Neubauer haemocytometer chamber. The percentage growth inhibition was calculated by comparing the trypanosome concentration in each post-drug-administration sample with the control sample taken from the same animal.

Determination of trypanosome infectivity: Adult male Balb/c mice were used for infectivity tests. For each trypanosome suspension 5 mice were inoculated i.p. with $1x10^5$ trypanosomes. In some experiments, where lower numbers of trypanosomes were present, $5x10^4$ trypanosomes were inoculated into each mouse. In experiments where insufficient trypanosomes were present to inoculate $5x10^4$ trypanosomes into each of 5 mice, 0.30ml of suspension was inoculated into each mouse. Mouse tail blood was examined twice weekly for parasitaemia for a 30 day period following inoculation.

(b) 24/48/72 hour incubations

This was conducted in a similar manner to that described in the previous section except that 6 wells were filled with the same medium. The contents of individual wells were counted (two at each time point) after intervals of 24, 48 and 72 hours. Infectivity of trypanosomes after

24 hours incubation was determined by i.p. inoculation of $1x10^6$ trypanosomes into each of 5 mice for each serum-trypanosome mixture.

SECTION 1

STUDIES WITH *T.CONGOLENSE*

CHAPTER 1

A STUDY INTO THE FACTORS INFLUENCING THE DURATION OF ISOMETAMIDIUM PROPHYLAXIS IN CATTLE AGAINST EXPERIMENTAL CHALLENGE WITH METACYCLIC FORMS OF *TRYPANOSOMA CONGOLENSE*

INTRODUCTION

The most widely used chemoprophylactic against bovine trypanosomiasis on the African continent is the phenanthridine, isometamidium chloride (Samorin, May and Baker Ltd., Dagenham, U.K.).

The factors which influence the effectiveness of the drug have not been clearly defined; consequently, considerable uncertainty remains as to the most effective use of the drug in the field situation.

In earlier work, Whitelaw, Bell, Holmes, Moloo, Hirumi, Urquhart and Murray (1986) showed that, at a dose of 1mg kg^{-1} bodyweight, isometamidium chloride protected cattle against monthly tsetse challenge with Trypanosoma congolense ILNat 3.1 for at least 5 months. At 6 months post-treatment, two thirds of the cattle (16 of 24) successfully resisted infection, even when subjected to multiple tsetse challenge, or intradermal inoculation of as many as 500,000 in vitro-derived metacyclic trypanosomes. Anti-trypanosomal antibodies were not detected in any animals that successfully resisted challenge.

The work described in this chapter was undertaken to confirm the work of Whitelaw et al. (1986), to test another serodeme of T.congolense and to investigate other factors which might influence the duration of chemoprophylaxis; namely the dose of drug, the level of challenge, and the effect of treatment of an established infection on the subsequent duration of prophylaxis.

MATERIALS AND METHODS

1) CATTLE

The age and breed of cattle were as described in the general materials and methods. For ease of reference, cattle have been allocated individual codes. The relationship between reference codes and animal numbers is shown in table 1, appendix I.

2) METACYCLIC TRYPANOSOMES

Metacyclic trypanosomes of T.congolense ILNat 3.1 and T.congolense IL 2642 were propagated in vitro. Cultures of both populations were initiated by inoculation of rodent-derived bloodstream forms into vitrogen (Vitrogen 100, Collagen corporation, Palo Alto, California)-containing cultures. Primary cultures were maintained at 28°C in HEPES (25mM)-buffered Eagle's MEM with Earle's BSS and 20% (v/v) heat-inactivated foetal bovine serum. After approximately 35 days, transformation through procyclic and epimastigote stages to metacyclic forms had occurred (Hirumi, Hirumi and Moloo, 1982). Coated metacyclic trypanosomes were separated from uncoated procyclic and epimastigote forms by passing the bulk population through a DEAE-cellulose column (Lanham and Godfrey, 1970) suspended in PSG, 0.15M, pH 8.0.

Earlier work had shown infectivity of T.congolense ILNat 3.1 metacyclics for mice to as low as approximately one trypanosome (H.Hirumi, personal communication).

T.congolense IL 2642 metacyclic trypanosomes had not

been produced before. Infectivity of the metacyclics, for mice, was demonstrated down to the level of at least 5 metacyclic forms after both DEAE-cellulose column and plasma separation (see table 2, appendix 1).

3) CHALLENGE OF CATTLE WITH *IN VITRO*-DERIVED METACYCLIC TRYPANOSOMES

Inoculation sites on cattle flanks were prepared as described for the feeding of individual tsetse (see general materials and methods).

Metacyclic trypanosomes cultured in vitro were diluted in culture medium (Hirumi et al., 1982) and inoculated intradermally, in a volume of 0.1ml, using a 25 guage $^5/_8$ inch needle. The production of skin blebs was indicative of intradermal, rather than subcutaneous, inoculation.

4) INFECTIVITY OF CATTLE METACYCLIC INOCULUM

Two suspensions of T.congolense ILNat 3.1 metacyclic trypanosomes were prepared in culture medium (Hirumi et al., 1982) for intradermal inoculation of cattle; tube A contained 10^6 ml^{-1} and tube B contained 10^4 ml^{-1}.

Immediately after inoculation of the metacyclic trypanosomes the remaining populations in each tube were diluted out in ten-fold dilutions to provide in 0.1ml, 10^5, 10^4, 10^3, 10^2, 10^1 and 10^0 trypanosomes for tube A and 10^3, 10^2, 10^1 and 10^0 trypanosomes for tube B. 0.1ml aliquots of each dilution were inoculated into 5 mice each. All metacyclic suspensions were kept on ice. Mice were

monitored twice weekly for 2 months.

5) ANTIBODY ASSAYS

In vitro-derived metacyclic populations of both *T.congolense* ILNat 3.1 and *T.congolense* IL 2642 were used for serological investigations. Two antibody tests were conducted; an infectivity neutralisation test and a trypanolysis test. Both have been described in the general materials and methods.

6) INHIBITION OF *T.CONGOLENSE* ILNat 3.1 METACYCLIC TRYPANOSOME INFECTIVITY BY GAMMA-RADIATION

Metacyclic trypanosomes were suspended in PSG, 0.15M pH8.0, at a concentration of $5x10^5ml^{-1}$, and placed in two $25cm^2$ T-type flasks. Both flasks were placed on ice for the entire experimental period and one of the two flasks was exposed to between 5 and 15 kilorads of gamma-radiation delivered by a caesium source (^{137}Cs). After every dose of 5 kilorads, part of the trypanosome suspension was removed and inoculated i.p. into mice; $1x10^5$ motile trypanosomes per mouse. Similarly, at each time point when irradiated trypanosomes were inoculated into mice, trypanosomes from the flask unexposed to gamma-radiation were also inoculated into mice ($1x10^5$ per mouse) to serve as controls. Mice were monitored twice weekly for one month after inoculation for the presence of parasitaemia.

7) INOCULATION OF CATTLE WITH IRRADIATED *T.CONGOLENSE* ILNat 3.1 METACYCLIC TRYPANOSOMES AND COLLECTION OF SERA

After exposure of *in vitro*-derived metacyclic trypanosomes to 15 kilorads of gamma-radiation, five 0.1ml aliquots of a suspension (in PSG, 0.15M pH8.0) containing $2x10^6$ trypanosomes per ml were inoculated intradermally into each of 6 Boran steers (as described for syringe challenge of cattle administered isometamidium chloride, in section 3)).

Since peak antibody responses to gamma-irradiated *T.brucei* bloodstream forms (at the level of between 10^6 and 10^9) inoculated either subcutaneously or intravenously occurred on days 9 or 11 (Morrison *et al.*, 1982a), serum was collected every second day from the day of inoculation until 16 days following inoculation. All sera collected from cattle were screened for *T.congolense* ILNat 3.1 anti-metacyclic specific antibody responses using infectivity neutralisation and IFA tests.

8) HAEMATOLOGICAL AND PARASITOLOGICAL PARAMETERS

Haematological and parasitological parameters were measured as described in the general materials and methods.

EXPERIMENTAL DESIGN

Twenty-four Boran steers were divided into 6 groups of 4 animals, (groups A-F, table 5). On a monthly basis, from 2 to 7 months after treatment with isometamidium chloride, the cattle were challenged either with infected

Table 5

EXPERIMENTAL DESIGN

GROUP OF CATTLE	DOSE ($mgkg^{-1}$) ISOMETAMIDIUM CHLORIDE	TRYPANOSOMA CONGOLENSE SERODEME	MONTHS AFTER ADMINISTRATION OF ISOMETAMIDIUM CHLORIDE						FORM OF CHALLENGE
			2	3	4	5	6	7	
A	1.0	ILNat 3.1	X	X	X	X	X	X	five infected tsetse
B	1.0	IL 2642	X	X	X	X	X	X	five infected tsetse
C	0.5	ILNat 3.1	X	X	X	X	X	X	five infected tsetse
D*	0.5	ILNat 3.1	X	X	X	X	X	X	five infected tsetse
E	0.5	ILNat 3.1	X	X	X	X	X	X	$5x10^3$ metacyclic trypanosomes
F	0.5	ILNat 3.1	X	X	X	X	X	X	$5x10^5$ metacyclic trypanosomes

*Infected with T.congolense IL 2642 (tsetse infection) 4 weeks before administration of isometamidium chloride.

X = Challenge.

tsetse or *in vitro*-derived metacyclic trypanosomes. Challenges were repeated until the animals became parasitaemic or, for those cattle refractory to challenge, the experiment was terminated after the 7 month challenge.

On day 0 (17.1.85) cattle in groups A and B each received 1.0mg kg^{-1} isometamidium chloride, and those in groups C to F, 0.5mg kg^{-1} (see table 5). With the exception of groups E and F, challenge was by infected tsetse. Groups, A, C and D were challenged with *T.congolense* ILNat 3.1 whilst group B was challenged with *T.congolense* IL 2642. Group C differed from group D in that the latter was infected with *T.congolense* IL 2642 by tsetse 4 weeks prior to drug-treatment. Groups E and F were challenged with *in vitro*-derived metacyclic forms of *T.congolense* ILNat 3.1. At each challenge they received $5x10^3$ and $5x10^5$ organisms, respectively.

For each challenge, 3 control cattle were used to confirm trypanosome infectivity, i.e. one for *T.congolense* ILNat 3.1-infected tsetse, one for *T.congolense* IL 2642-infected tsetse and one for the *in vitro*-derived metacyclic inoculum administered to group E ($5x10^3$).

RESULTS

1) PARASITOLOGICAL DATA

(a)(i) Onset of parasitaemia

Twenty-eight days before administration of isometamidium chloride all 4 animals in group D were fly-infected with T.congolense IL 2642. The presence of trypanosomes in these animals, as detected by the buffy-coat phase-contrast (BC) technique and mouse inoculation, prior to challenge with 5 T.congolense ILNat 3.1-infected tsetse at 2 months post drug-administration, is shown in table 3, appendix I. During the 2 month period following treatment cattle were only detected parasitaemic on the day of treatment or at most one day following treatment. Trypanosomes occurring in cattle on the day following treatment were non-infective for mice.

The development of infection in the cattle following each challenge is shown in table 6.

Following challenge at 2 and 3 months after drug administration none of the drug-administered animals were detected parasitaemic either by the BC technique or by mouse inoculation. In contrast, all control animals were observed parasitaemic by day 16 following each challenge, as detected by the BC technique (see table 4, appendix I). In only one steer (the 2 month challenge control for groups E and F) was parasitaemia detected by mouse inoculation earlier than by the BC technique. Parasitaemia was first

Table 6

NUMBER OF ANIMALS INFECTED FOLLOWING EACH CHALLENGE

GROUP OF CATTLE	DOSE ($mgkg^{-1}$) ISOMETAMIDIUM CHLORIDE	CHALLENGE	FORM OF CHALLENGE	MONTHS AFTER ISOMETAMIDIUM CHLORIDE ADMINISTRATION					
				2	3	4	5	6	7
A	1.0	ILNat 3.1	Tsetse	0	0	0	1	1	0
B	1.0	IL 2642	Tsetse	0	0	0	0	2	0
C	0.5	ILNat 3.1	Tsetse	0	0	1	2	0	0
D	0.5	ILNat 3.1	Tsetse	0	0	0	2	2	-
E	0.5	ILNat 3.1	$5x10^3$ metacyclics	0	0	2	0	2	-
F	0.5	ILNat 3.1	$5x10^5$ metacyclics	0	0	1	2	0	1

detected by the BC technique on day 13 whilst inoculation of day 12 blood into mice revealed the presence of trypanosomes in the blood.

Following all subsequent challenges all challenge controls were detected parasitaemic by at the latest 14 days following challenge, using the BC technique.

At the 4 month challenge, 3 drug-treated animals became parasitaemic; C4, E4 and F3. In each case the onset of parasitaemia was delayed by 2-7 days compared to their respective controls (table 5, appendix I), i.e., days 21, 16, and 21, respectively. Inoculation of day 12 blood from each animal failed to infect mice.

All other drug-administered cattle were refractory to challenge and mice inoculated with their blood were not detected parasitaemic.

Following the 5 month challenge, 8 of the remaining 21 drug-administered animals became parasitaemic (see table 5, appendix I); one in group A (A2), 2 in group C (C1 and C3), 2 in group D (D1 and D4), one in group E (E2) and 2 in group F (F2 and F4). E2 was first detected parasitaemic after only 7 days following the challenge. Since the group E control was first detected parasitaemic on day 14 it is probable that this infection originated from the previous challenge. In only 2 out of the above 8 animals (A2 and D4) was parasitaemia delayed in onset as compared to controls.

Of the 5 drug-administered animals first detected parasitaemic by the BC technique on day 14, only one (C3) was shown to be parasitaemic on day 12 by inoculation of blood into mice.

All mice detected parasitaemic following inoculation of day 24 blood were inoculated with blood from cattle that had been shown to contain trypanosomes by the BC technique.

At the 6 month challenge, 7 of the remaining 13 drug-administered animals became parasitaemic (see table 6, appendix I); one in group A (A4), 2 in group B (B1 and B4), 2 in group D (D2 and D3) and 2 in group E (E1 and E3). In 5 of these animals (A4, B4, D2, E1 and E3) parasitaemia was delayed in onset as compared to controls. Inoculation of blood from all 7 animals failed to reveal the presence of trypanosomes at an earlier day than that detected by the BC technique.

Following the 7 month challenge only one of the remaining 6 drug-treated animals became parasitaemic (F1) (see table 6, appendix I). In a similar manner to animals in the previous month, inoculation of blood from this animal into mice failed to reveal the presence of trypanosomes at an earlier date than that detected by the BC technique.

Throughout the course of the above described experiment all challenge controls became infected with

parasitaemia starting (as detected by the BC technique) on day 12.7 ± 1.0 (S.D.) for group A, C, D controls, day 12.2 ± 1.0 (S.D.) for group B controls and day 14.2 ± 1.1 (S.D.) for group E, F controls.

Parasitaemia onset was delayed in 11 of 19 drug-administered animals susceptible to infection; 2 out of 2 in group A, 1 out of 2 in group B, 1 out of 3 in group C, 2 out of 4 in group D, 3 out of 4 in group E and 2 out of 4 in group F. There appeared not to be a group-associated relationship.

Following onset of parasitaemia in all animals, both controls and those administered isometamidium chloride, the level of parasitaemia rose to a BC score of 4+ or 5+ (Paris et al., 1982) within 7 days of patency and remained at this level until treatment was required.

(ii) Comparison of the sensitivity of mouse inoculation and the BC technique

Nine steers were first detected parasitaemic by the BC technique on day 12 (8 controls and one drug-administered). Blood collected on day 12 from only one of these animals (the drug-administered animal) failed to produce infection in mice.

Inoculation of day 12 blood into mice from the 12 steers first detected parasitaemic by the BC technique on day 14 (6 controls and 6 drug-administered animals) revealed the presence of trypanosomes in the blood of only 4 animals (3 controls and one drug-administered).

In all cattle first detected parasitaemic 16 or more days following challenge, inoculation of day 12 blood into mice failed to reveal the presence of trypanosomes.

Finally, first detection of parasitaemia, using the BC technique, after day 24 occurred in 3 animals; D2 on day 28, B4 on day 29 and D4 on day 30 (all drug-administered animals). Mouse inoculation of day 24 blood from all 3 animals failed to produce infection.

(b) Infectivity of *in vitro*-derived metacyclic trypanosomes used to challenge cattle in groups E and F

Subsequent to the administration of in vitro-derived T.congolense ILNat 3.1 metacyclic trypanosomes to cattle, infectivity titrations were conducted with the same populations in mice. Table 7 shows the resultant data. For each challenge inoculum, except that used for the 3 month challenge, infectivity was demonstrated down to the level of between one and 10 trypanosomes. At the level of 10^3 trypanosomes the inoculum used for the group E three month challenge exhibited zero infectivity for mice. In contrast, the control steer to which $5x10^3$ of the same trypanosome population was administered, developed 5 chancres with diameters at least twice those elicited in cattle fed on by tsetse infected with the same serodeme, thereby indicating that the animal received a larger infective inoculum than that inoculated by tsetse-transmission (Dwinger, 1985a; Akol, Murray, Hirumi, Hirumi and Moloo, 1986). Possibly, in the time taken from

Table 7

T.CONGOLENSE ILNat 3.1 METACYCLIC TRYPANOSOME INFECTIVITY TITRATIONS

Data = No. mice parasitaemic / No. mice inoculated

Inoculum per mouse:	Tube A ($10^6 ml^{-1}$) - Group F						Tube B ($10^4 ml^{-1}$) - Group E + challenge control			
	10^5	10^4	10^3	10^2	10^1	10^0	10^3	10^2	10^1	10^2
2 Months	5/5	5/5	5/5	5/5	5/5	0/5	5/5	5/5	4/5	1/5
3 Months	5/5	3/5	3/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
4 Months	3/5	4/5	5/5	1/5	3/5	1/5	2/4	0/5	1/5	0/5
5 Months	5/5	5/5	4/4	3/3	2/5	3/5	2/4	5/5	3/5	1/5
6 Months	5/5	5/5	5/5	4/5	1/5	1/5	4/5	5/5	2/5	1/5
7 Months	4/4	5/5	5/5	3/5	2/5	0/5	ND	ND	ND	ND

ND = Not done.

inoculating cattle to inoculating mice, the trypanosomes were exposed to undue stress.

2) CLINICAL DATA

(a) Skin thickness and lymph node breadth measurements

A summary of skin thickness changes that occurred in all controls and drug-administered cattle susceptible to challenge is shown in table 8.

(i) Challenge controls

In control animals, skin reactions developed at 100% of sites at which T.congolense ILNat 3.1-infected tsetse had fed, 87% where T.congolense IL 2642-infected tsetse had fed and 84% where in vitro-derived T.congolense ILNat 3.1 metacyclic trypanosomes were inoculated. In association with the pronounced skin thickness changes there occurred, in all cases, a large increase in breadth of the prefemoral lymph node.

Figure 1 shows as an example the changes in skin thickness, lymph node breath, temperature and level of parasitaemia for the 2 month T.congolense ILNat 3.1 tsetse challenge control, during the 30 day period following infection. Skin thickness began to increase on day 9, peaked on day 12 and then returned to its original value by approximately day 20 (skin thickness values are the mean of 5 measurements made on each day). The drainage prefemoral lymph node breadth first increased on day 8 and attained maximal size on day 11. By day 18 the breadth had

Table 8

SKIN THICKNESS AND DRAINAGE LYMPH NODE CHANGES IN ANIMALS SUSCEPTIBLE TO CHALLENGE

GROUP	ANIMAL	CHANGE IN SKIN THICKNESS AT (FIVE) CHALLENGE SITES			% INCREASE IN PREFEMORAL LYMPH NODE BREADTH
		LARGE INCREASE 65+%	MODERATE INCREASE 41-64%	SLIGHT INCREASE 20-40%	
		2 MONTH CHALLENGE (day 61)			
A,C,D control		5	-	-	410
B control		5	-	-	400
E,F control		-	-	1	220
		3 MONTH CHALLENGE (day 91)			
A,C,D control		5	-	-	250
B control		3	1	-	170
E,F control		5	-	-	290
		4 MONTH CHALLENGE (day 119/120)			
C	C4	2	1	1	120+
E	E4	2	3	-	320
F	F3	-	-	1	0
A,C,D control		5	-	-	180
B control		5	-	-	390
E,F control		1	3	1	280
		5 MONTH CHALLENGE (day 151)			
A	A2	-	-	1	30+
C	C1	3	1	-	190
C	C3	4	1	-	240
D	D1	3++	2++	-	230
D	D4	1+	-	4+++	0
E	E2	-	-	3+++	0
F	F2	1	4	-	160+
F	F4	3	2	-	240
A,C,D control		5	-	-	300
B control		4	-	-	270
E,F control		5	-	-	240
		6 MONTH CHALLENGE (day 181)			
A	A4	-	-	2	0
B	B1	-	-	-	0
B	B4	-	2++	-	40+
D	D2	1+	1+	-	80+
D	D3	2	2	1	160
E	E1	3	-	1	200
E	E3	2	2	-	240
A,C,D control		3	2	-	220
B control		3	-	-	240
E,F control		5	-	-	340
		7 MONTH CHALLENGE (day 210/211)			
F	F1	3	1+	-	110+
A,C control		5	-	-	120
B control		5	-	-	240

+Delay in maximal increase compared to control (greater than 2 standard deviations from control mean). The number of +'s indicate the number of measurements where attainment of maximal dimensions were delayed.

FIGURE 1

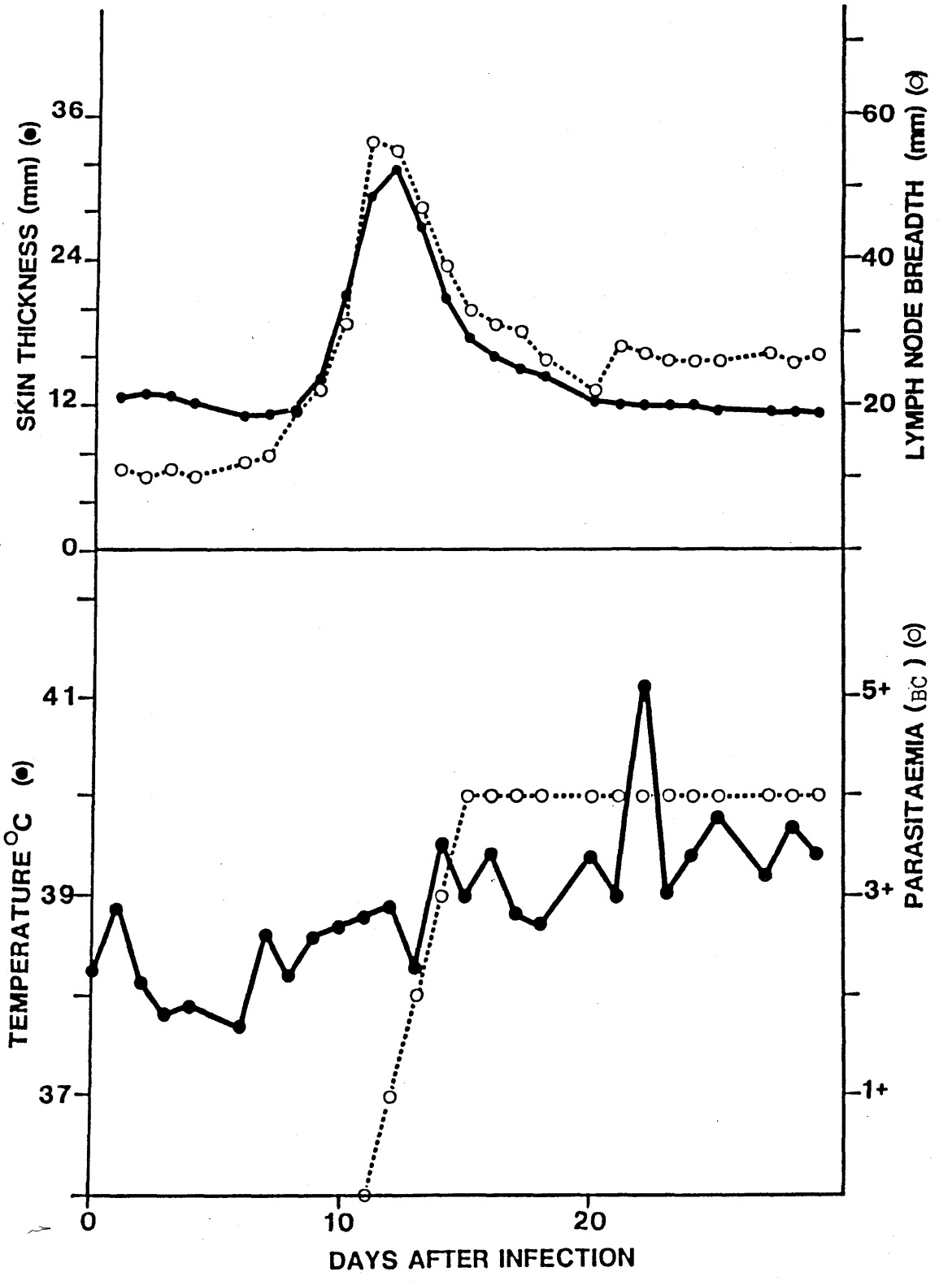

Figure 1: 2 month T.congolense ILNat 3.1 tsetse challenge control. Change in skin thickness at tsetse bite sites and width of draining lymph node; change in temperature and development of parasitaemia.

decreased to approximately half the maximal value. Thereafter, the breadth decreased no further during the observation period, remaining enlarged in size compared to its pre-infection dimension. Parasitaemia was first detected on day 12 and the 4 pyrexic temperature measurements (in excess of 39.4°C) taken during the 30-day observation period were all taken when the animal was parasitaemic.

A similar progression of changes in the same parameters occurred in all other T.congolense ILNat 3.1 tsetse challenge controls.

Changes in the same parameters for the 2 month T.congolense IL 2642 tsetse challenge control are shown graphically in figure 1, appendix I. Both skin thickness and drainage lymph node breadth changes were less pronounced than those described for the 2 month T.congolense ILNat 3.1 tsetse challenge control. The skin thickness began to increase on day 10, peaked on day 12 and decreased to approximately the pre-infection value by day 20. The drainage lymph node breadth first increased on day 9, attained maximal size on day 12 and decreased to approximately half its maximal size by day 20. No further reduction in size occurred during the observation period. Parasitaemia was first detected on day 11 and no pyrexia was detected.

A similar progression in changes of the above described parameters occurred in all other T.congolense IL 2642 challenge controls.

Figure 2, appendix I, depicts graphically the changes in the same 4 parameters for the 2 month T.congolense ILNat 3.1 metacyclic challenge control (challenged with $5x10^3$ in vitro-derived metacyclic trypanosomes). At the level of the skin only one "slight" increase in thickness occurred. In contrast, a large increase in lymph node breadth occurred, first detected on day 9, peaking on day 13 and not decreasing to its pre-infection size by the end of the observation period. Parasitaemia was first detected on day 13 and only one pyrexic temperature measurement was made, during the period of parasitaemia. The reason for the negligible changes at the level of the skin but pronounced changes at the level of the lymph node is unclear. Possibly the metacyclic trypanosomes were inoculated subcutaneously rather than intradermally.

A similar progression of changes in the above described parameters occurred in all other T.congolense ILNat 3.1 metacyclic controls, except that more pronounced skin thickness changes occurred in other animals.

Shown in figure 3, appendix I, are changes in skin thickness, lymph node breadth, PCV and level of parasitaemia for the 3 month T.congolense ILNat 3.1 metacyclic challenge control. Studies in mice on the infectivity of the metacyclic inoculum administered to this animal demonstrated zero infectivity at the level of $5x10^3$ trypanosomes (table 7). It is clear that since pronounced changes in skin thickness and lymph node breadth occurred,

along with the detection of parasitaemia, the inoculum was infective, at least at the level of $5x10^3$ trypanosomes. As previously discussed, since the diameter of chancres produced in this animal were 2 to 3 times the diameter of chancres produced following tsetse-transmission of T.congolense ILNat 3.1, this would indicate that a much larger infective metacyclic inoculum was administered than that inoculated by an infective tsetse fly (Luckins et al., 1981; Akol et al., 1986).

Summaries of data concerning the day of maximal skin thickness and drainage lymph node breadth for control animals are shown in table 9.

(ii) Cattle administered isometamidium chloride

In contrast to control cattle, many cattle administered isometamidium chloride, in addition to exhibiting less pronounced skin and lymph node changes following a successful challenge, exhibited a delay in attainment of maximal skin thickness and lymph node breath.

Data regarding changes in skin thickness and drainage lymph node breadth in animals susceptible to challenge is shown in table 8. Where attainment of skin and lymph node maximal changes were significantly delayed, as compared to controls, this is indicated. The time to development of maximal changes in these two parameters has only been taken as significantly different from controls when data lay outwith two standard deviations from the control mean.

The following text describes the atypical changes at

Table 9

SUMMARIES OF CHALLENGE CONTROL SKIN AND LYMPH NODE DATA

MAXIMAL SKIN THICKNESS (DAY)

GROUP	RANGE	x	S.D.	2(S.D.)	x+2(S.D.)
A, C, D	9-14	11.27	1.31	2.62	13.89
B	10-15	11.35	1.13	2.26	13.61
E, F	10-14	11.54	1.32	2.64	14.18

ATTAINMENT OF MAXIMAL DRAINAGE LYMPH NODE BREADTH (DAY)

GROUP	RANGE	x	S.D.	2(S.D.)	x+2(S.D.)
A, C, D	10-15	12.17	1.94	3.88	16.05
B	11-13	12.17	0.75	1.50	13.67
E, F	11-16	13.00	1.87	3.74	16.74

x = mean

S.D. = standard deviation

the level of the skin and lymph node that occurred in animals both refractory and susceptible to challenge. Where attainment of maximal size was significantly delayed as compared to controls, this is denoted by (+2SD), i.e., the value lay outwith 2 standard deviations from the control mean.

Following the 2 month challenge; although no animal administered isometamidium chloride was detected parasitaemic, D4 developed 4 "slight" skin thickness changes, with maximal thicknesses occurring at all sites on day 25 (+2SD). In the same animal, the drainage lymph node breadth increased by 40% with maximal size measured on day 23 (+2SD).

Subsequent to the 3 month challenge, no animal administered isometamidium chloride became parasitaemic. However, skin and lymph node changes occurred in 3 animals; E2, F3 and F4:

E2 developed one "slight" skin thickness change with maximal thickness occurring on day 8. No lymphadenomegaly was detected.

F3 exhibited 4 "slight" skin thickness changes with maximal sizes attained on day 5 (+2SD). Lymph node breadth increased by 50% and maximal size was attained on day 5 (+2SD).

F4 developed one "slight" skin thickness change with maximal thickness attained on day 6 (+2SD). No

lymphadenomegaly was detected.

Following the 4 month challenge, two drug-administered cattle susceptible to challenge exhibited atypical changes at the level of the skin and/or lymph node; E4 and F3. Figures 4a and 4b, appendix I, show graphically skin, lymph node and parasitaemia data for E4, F3 and the group E,F control.

Two cattle refractory to challenge developed significant changes in skin thickness; E2 and F4:

E2 developed one "slight" increase in skin thickness with attainment of maximal thickness on day 21 (+2SD). No lymphadenomegaly was detected.

F4 developed one "slight" increase in skin thickness; maximal dimension was attained on day 8. No lymphadenomegaly was detected.

Subsequent to the 5 month challenge, atypical changes occurred at the level of the skin and/or lymph node in 5 animals susceptible to challenge:

A2; maximal lymph node breadth was attained on day 19.

D1; skin thickness changes occurred at all 5 sites at which tsetse had fed. Three "large" increases in skin thickness occurred and at 2 of these sites attainment of maximal thickness was delayed; both day 15 (+2SD). "Moderate" increases in thickness occurred at the other 2 sites and attainment of maximal thicknesses at these 2 sites was also delayed; both day 17 (+2SD).

D4; skin thickness changes occurred at all 5 sites at which tsetse had fed. Time to maximal thickness of the one "large" increase was delayed; day 26 (+2SD). Of the 4 "slight" increases, time to maximal thicknesses was delayed in 3; days 15, 16 and 17 (all +2SD). No lymphadenomegaly was detected.

E2; time to maximal skin thickness of all 3 "slight" increases was delayed; days 18, 18 and 21 (all +2SD). No lymphadenomegaly occurred.

F2; maximal lymph node breadth was attained on day 26 (+2SD).

Skin thickness changes were also observed in 3 cattle refractory to challenge:

B1; one "large" increase in skin thickness occurred with maximal thickness attained on day 23 (+2SD).

D2; two "slight" increases in skin thickness occurred.

E3; one "slight" increase in skin thickness occurred with maximal size attained on day 16 (+2SD).

In none of these 3 animals was lymphadenomegaly detected.

Following the 6 month challenge, time to attainment of maximal skin thickness and lymph node breadth was delayed in 2 animals susceptible to challenge:

B4; two "moderate" increases in skin thickness occurred and in both cases attainment of the maximal size was delayed; days 20 and 21 (both +2SD). Maximal lymphadenomegaly occurred on day 20 (+2SD).

D2; skin thickness increases occurred at 2 sites; one "large" and one "moderate". Attainment of maximal sizes was in both cases delayed; day 19 for the large increase and day 26 for the moderate increase (both +2SD). Maximal lymphadenomegaly occurred on day 26 (+2SD).

In only one animal refractory to challenge were changes in skin thickness observed: F1 developed one "large" increase with maximal size attained on day 28 (+2SD) and one "slight" skin increase with maximal size attained on day 11. No lymphadenomegaly was detected.

Following the 7 month challenge, the one animal susceptible to challenge (F1) exhibited a delay in attainment of maximal skin thickness and maximal lymphadenomegaly. Changes in skin thickness occurred at 4 sites and attainment of maximal thickness of the one "moderate" increase was day 26 (+2SD). Maximal lymphadenomegaly occurred on day 18 (+2SD).

Skin thickness changes were also observed in 2 animals refractory to challenge:

B3; one "slight" increase occurred with maximal size attained on day 10.

C2; one "slight" increase occurred with maximal size attained on day 10.

In both animals, lymphadenomegaly did not occur.

In conclusion; of the 19 cattle administered isometamidium chloride and susceptible to challenge, 14

developed less pronounced changes at the level of the skin and/or drainage lymph node as compared to their respective controls. Eight of the 14 animals developed less pronounced changes both at the level of the skin and lymph node. In the other 6 animals, less pronounced changes occurred only at the level of the skin.

In 10 of the 14 animals, first detection of parasitaemia (by the BC technique) was delayed as compared to controls. In contrast, parasitaemia of delayed onset was not observed in any of the 5 cattle that developed skin thickness and lymph node breadth changes comparable to controls.

In 9 of the 19 cattle administered isometamidium chloride and susceptible to challenge, attainment of maximal skin thicknesses and/or lymph node breadth was delayed. In 3 of these animals a delay occurred at both the level of the skin and drainage lymph node, in 3 only at the level of the lymph node and in 3 only at the level of the skin. In 2 of the last 3 animals described, lymphadenomegaly did not occur. In 6 of the 9 animals described above, parasitaemia onset was delayed.

Examination of all the atypical changes that occurred at the level of the skin and/or lymph node in cattle administered isometamidium chloride indicated that there appeared not to be a group-associated relationship.

(b) Temperature measurements

Temperature measurements taken from the 3 challenge

controls during the 30 day observation period following the 2 month challenge are shown graphically in figures 1 and 2, appendix I and figure 1 (in text).

Summaries of temperature measurements taken during the entire experimental period are shown in tables 7, 8 and 9, appendix I. (Pyrexic temperature measurements have been taken as any measurement in excess of 39.4°C.)

A total of 3,515 temperature measurements were taken during the entire experiment of which 452 (12.9%) were made when animals were parasitaemic, as detected by the BC technique. Pyrexia was only detected on 88 occassions. Of these measurements, 76 (86%) were taken when animals were parasitaemic, and 12 (14%) when animals were non-parasitaemic. Thus, only 16.8% (76/452) of temperature measurements taken when animals were parasitaemic were pyrexic. Division of the same data into infections with either T.congolense ILNat 3.1 or T.congolense IL 2642 showed that 13.9% of temperature measurements taken when animals were parasitaemic with T.congolense ILNat 3.1 were pyrexic, whilst 20% were pyrexic when animals were parasitaemic with T.congolense IL 2642.

In all animals, pyrexic temperature measurements occurred intermittently; animals were pyrexic on one day and non-pyrexic the next, rather than persistently pyrexic over a series of consecutive days.

3) HAEMATOLOGICAL DATA

In all controls and drug-administered cattle

susceptible to challenge, the initial decline in PCV always coincided with the first detection of parasitaemia.

Figures 3, 5 and 6, appendix I, show graphically the changes in PCV and level of parasitaemia in the three challenge controls used for the 3 month challenge. The initial PCV decline in all 3 animals coincided with the first detection of parasitaemia.

(In all cattle experiments the criterion used for treatment of infected animals with 7mg kg^{-1} diminazene aceturate was when the PCV fell below 16%.) Without any exception the PCV in all cattle infected with T.congolense ILNat 3.1 and all cattle infected with T.congolense IL 2642 fell below 16% and therefore all infected animals had to be treated. In no animal was self-cure observed.

Earlier workers in the field of chemotherapy have stated that trypanosomes "breaking through" the chemoprophylactic period are less pathogenic than the same challenge administered to cattle that have never received a prophylactic drug (Stephen, 1960, 1962a; Stephen and Gray, 1960). Table 10 has been constructed from the assumption that pathogenicity equates with the time taken for the PCV in experimental animals to decline by 30% following parasitaemia onset. All figures are the number of days taken for the PCV at parasitaemia onset to decline by 30%. For comparative purposes; shown in table 11 are the mean values for the number of days taken for the PCV at parasitaemia onset to decline by 30% in challenge controls.

From the data shown in table 11 there appears to be no

Table 10

TIME (DAYS) FOR PCV AT PARASITAEMIA ONSET TO DECLINE BY 30%

GROUP	CHALLENGE					
	2 MONTHS	3 MONTHS	4 MONTHS	5 MONTHS	6 MONTHS	7 MONTHS
A	-	-	-	34*	30*	-
B	-	-	-	-	25*,31*	-
C	-	-	22(*)	10,19	-	-
D	-	-	-	10,19	12,47*	
E	-	-	23(*)	23(*)	14,23(*)	
F	-	-	18	14,26*	_	28*
CONTROLS						
A, C, D	17	23	14	16	10	13
B	14	13	16	12	16	14
E, F	16	19	21	14	14	NO CONTROL

* Greater than 2 standard deviations from mean control value.

(*) Greater than 1 but less than 2 standard deviations from mean control value.

Table 11

MEAN VALUE (DAYS) FOR PCV AT PARASITAEMIA ONSET TO DECREASE BY 30% IN CHALLENGE CONTROLS

GROUP	*TRYPANOSOMA CONGOLENSE* SERODEME	MEAN(X)	STANDARD DEVIATION (SD)	X+SD	X+2(SD)
A,C,D	ILNAT 3.1	15.5	4.4	19.9	24.3
B	IL 2642	14.2	1.6	15.8	17.4
E,F	ILNat 3.1	16.8	3.1	19.9	23.0
A,C,D,E,F	ILNat 3.1	16.1	3.8	19.9	23.7

significant difference in pathogenicity of infections in the 3 groups of challenge controls; in particular, no significant difference in the pathogenicity of T.congolense ILNat 3.1 and T.congolense IL 2642.

Of the 19 cattle that were administered isometamidium chloride and susceptible to challenge, the time taken for the PCV to decline by 30% following parasitaemia onset was greater than 2 standard deviations from the mean control value in 7 animals, and greater than one but less than 2 standard deviations from the mean control value in 4 other animals.

The 7 animals with 30% PCV decline rates greater than 2 standard deviations from the mean control value were composed of 0 out of 3 cattle susceptible to the 4 month challenge, 2 out of 8 cattle susceptible to the 5 month challenge, 4 out of 7 cattle susceptible to the 6 month challenge and 1 out of 1 susceptible to the 7 month challenge.

4) IMMUNOLOGICAL DATA

(a) Trypanolysis test

Data from trypanolysis tests using T.congolense ILNat 3.1 metacyclic trypanosomes as antigen is shown in tables 12, 13 and 14.

All eleven T.congolense ILNat 3.1 challenge controls developed anti-metacyclic specific trypanolytic antibody following infection; 7 by day 14 and all 11 by day 21. The maximal antibody titres attained during each 28 day

TABLE 12

TRYPANOLYSIS TEST USING T.CONGOLENSE ILNat 3.1 METACYCLIC TRYPANOSOMES

Group	Animal	Day 0	1 month	2 MONTH CHALLENGE					3 MONTH CHALLENGE				
				day 0	day 7	day 14	day 21	day 28	day 0	day 7	day 14	day 21	day 28
A	A1	–	–	–	–	–	–	–	–	–	–	–	–
A	A2												
A	A3												
A	A4												
B	B1	–	–	–	–	–	–	–	–	–	–	–	–
B	B2												
B	B3												
B	B4												
C	C1	–	–	–	–	–	–	–	–	–	–	–	–
C	C2												
C	C3												
C	C4												
D	D1	–	–	–	–	–	–	–	–	–	–	–	–
D	D2												
D	D3												
D	D4												
E	E1	–	–	–	–	–	–	–	–	–	–	–	–
E	E2												
E	E3												
E	E4												
F	F1	–	–	–	–	–	–	–	–	–	1:5	±	1:5
F	F2								–	–	±	±	–
F	F3								–	–	1:5	1:5	1:5
F	F4								–	–	–	–	–
CHALLENGE CONTROLS													
Groups A,C,D				–	–	±	1:5*	1:25	–	–	–	1:125	1:125
Group B				–	–	–	–	–	–	–	–	–	–
Groups E,F				–	–	1:5	1:5	1:25	–	–	–	1:125	1:125

* = Figures indicate maximal dilution at which over 90% trypanosome lysis occured.

± = Trypanosome lysis greater than control but less than 90%, at 1:5 plasma dilution.

TABLE 13

TRYPANOLYSIS TEST USING T.CONGOLENSE ILNat 3.1 METACYCLIC TRYPANOSOMES

		4 MONTH CHALLENGE					5 MONTH CHALLENGE				
Group	Animal	day 0	day 7	day 14	day 21	day 28	day 0	day 7	day 14	day 21	day 28
A	A1	–	–	–	–	–	–	–	–	–	–
A	A2						–	–	–	1:5	1:5
A	A3						–	–	–	–	–
A	A4						–	–	–	–	–
B	B1	–	–	–	–	–	–	–	–	–	–
B	B2						–	–	–	–	–
B	B3						–	–	–	–	–
B	B4						–	–	–	–	–
C	C1	–	–	–	–	–	–	–	1:5	1:25	1:25
C	C2	–	–	–	–	–	–	–	–	–	–
C	C3	–	–	–	–	–	–	–	1:25	1:125	1:125
C	C4	–	–	±	±	1:5 *					
D	D1	–	–	–	–	–	–	–	1:25	1:25	1:25
D	D2						–	–	–	–	–
D	D3						–	–	–	–	–
D	D4						–	–	–	–	±
E	E1	–	–	–	–	–	–	–	–	–	–
E	E2	–	–	–	–	–	1:5	1:5	1:25	1:25	1:125
E	E3	–	–	–	–	–	–	–	–	–	–
E	E4	–	–	–	1:25	1:25					
F	F1	1:5	1:5	1:5	1:5	1:5	±	±	1:5	1:5	1:5
F	F2	±	1:5	1:5	1:5	1:5	1:5	±	1:125	1:125	1:125
F	F3	1:5	1:25	1:5	1:5	1:5					
F	F4	–	±	1:5	1:5	±	–	1:5	1:125	1:125	1:125
CHALLENGE CONTROLS											
Groups A,C,D		–	–	1:5	1:125	1:25	–	–	1:5	1:25	1:5
Group B		–	–	–	–	–	–	–	–	–	–
Groups E,F		–	–	1:5	1:25	1:25	–	–	1:25	1:25	1:125

* = Figures indicate maximum dilution at which over 90% trypanosome lysis occured.

± = Trypanosome lysis greater than control but less than 90%, at 1:5 plasma dilution.

TABLE 14

TRYPANOLYSIS TEST USING T.CONGOLENSE ILNat 3.1 METACYCLIC TRYPANOSOMES

		6 MONTH CHALLENGE					7 MONTH CHALLENGE				
Group	Animal	day 0	day 7	day 14	day 21	day 28	day 0	day 7	day 14	day 21	day 28
A	A1	–	–	–	–	–	–	–	–	–	–
A	A2										
A	A3	–	–	–	–	–	–	–	–	–	–
A	A4	–	–	–	–	–					
B	B1	–	–	–	–	–	Samples not examined				
B	B2	–	–	–	–	–					
B	B3	–	–	–	–	–					
B	B4	–	–	–	–	–					
C	C1										
C	C2	–	–	–	–	–	–	–	–	–	–
C	C3										
C	C4										
D	D1										
D	D2	–	–	–	1:5 ▲	1:25					
D	D3	–	–	–	1:25	1:125					
D	D4										
E	E1	–	–	–	–	1:5					
E	E2										
E	E3	–	–	–	1:5	1:5					
E	E4										
F	F1	1:5	1:5	1:5	1:5	1:5	1:5	1:5	1:5	1:5	1:25
F	F2										
F	F3										
F	F4										
CHALLENGE CONTROLS											
Groups A,C,D		–	–	–	1:25	1:25	–	–	1:25	1:25	1:25
Group B		–	–	–	–	–	Only mice used to assay infectivity of metacyclic inoculum.				
Groups E,F		–	–	1:5	1:5	1:5					

▲ = Figures indicate maximum dilution at which over 90% trypanosome lysis occured.

sampling period were 1:5 for one animal, 1:25 for 6 animals and 1:125 for 4 animals.

Incubation of T.congolense ILNat 3.1 metacyclic trypanosomes in day 28 plasma from T.congolense IL 2642 challenge controls produced no lysis, thereby indicating there to be no metacyclic isotypes in the mVAT repertoires of the two serodemes. Similarly, T.congolense IL 2642 metacyclic trypanosomes were not lysed when incubated in day 28 plasma from T.congolense ILNat 3.1 challenge controls.

During the period when animals in groups A, C, D and E remained refractory to challenge, anti-metacyclic antibodies were not detected. However, antibodies were always detected, without delay in onset, following the development of parasitaemia. Four exceptions to this generalisation were as follows:

A2 was first detected parasitaemic 24 days following the 5 month challenge. Trypanolytic antibody was first detected on day 21 following the 5 month challenge (at a titre of 1:5), 3 days earlier.

E2 was first detected parasitaemic 7 days following the 5 month challenge. Trypanolytic antibody was first detected on day 0 of the same month. Both the presence of antibody on day 0 and the early onset of parasitaemia would suggest that the parasites detected on day 7 originated from the 4 month challenge, rather than the 5 month challenge.

A4 was first detected parasitaemic 24 days following

the 6 month challenge, however antibody was not detected up until and including day 28.

D2 was first detected parasitaemic 28 days following the 6 month challenge. Seven days earlier, on day 21, an antibody titre of 1:5 was detected.

Unlike all the cattle in groups A, C, D and E, all 4 animals in group F (challenged at monthly intervals with $5x10^5$ metacyclic trypanosomes) developed low anti-metacyclic antibody titres 1 to 4 months before being susceptible to challenge:

(F1) Antibody was first detected, at a titre of 1:5, in plasma from F1 fourteen days following the 3 month challenge. F1 did not become infected until following the 7 month challenge.

(F2) In plasma from F2, trypanolytic antibody was first detected 14 days following the 3 month challenge at a titre below 1:5. A titre of 1:5 was detected in plasma collected 7 days following the 4 month challenge and in weekly samples collected thereafter. However, the animal was not susceptible to infection until the 5 month challenge.

(F3) Trypanolytic antibody at a titre of 1:5 was first detected in plasma from F3 fourteen days following the 2 month challenge. F3 did not become infected until following the 4 month challenge.

(F4) Not until following the 4 month challenge was trypanolytic antibody detected in plasma from F4; below a

titre of 1:5 on day 7 and at a titre of 1:5 on day 14. The animal did not succumb to infection until following the 5 month challenge.

Following first detection of antibody in all 4 group F animals, low antibody titres were detected in all subsequent plasma samples until each animal became parasitaemic.

Thus, despite the presence of low anti-metacyclic antibody levels, all group F animals were susceptible to challenge. In no animal did the trypanolytic antibody titre rise above 1:5 before parasitaemia was detected. Once the animals became parasitaemic the antibody titre rose to 1:25 in 2 animals (F1 and F3) and to 1:125 in the other 2 (F2 and F4).

(b) Infectivity neutralisation test

(i) T.congolense ILNat 3.1

The results of infectivity neutralisation tests using T.congolense ILNat 3.1 metacyclic trypanosomes are shown in table 15.

Because of the large number of mice required to conduct neutralisation tests only plasma samples collected on the days of successful challenges were examined for the presence of infectivity neutralising antibody. Initial screening was carried out at a dilution of 1:2. At this dilution, complete neutralisation was produced by plasma from only 4 animals; the 4 animals in group F; F3 had a complete neutralising antibody titre of 1:20 on the day

TABLE 15

INFECTIVITY NEUTRALISATION TEST USING T.CONGOLENSE ILNat 3.1 METACYCLIC TRYPANOSOMES

Data = Number of mice parasitaemic/Number of mice inoculated

				PLASMA DILUTION							
Sample	Animal	Group	Date	1:2	1:5	1:10	1:20	1:40	1:80	1:160	1:320
4 month challenge Day 0▾	C4	C	17.5.85	6/6							
	E2	E	16.5.85	6/6							
	E4	E	16.5.85	5/6							
	F3	F	16.5.85	0/6	0/6	0/6	0/6	2/6	5/6	6/6	5/5
5 month challenge Day 0	A2	A	17.6.85	5/6							
	C1	C	17.6.85	5/6							
	C3	C	17.6.85	4/6							
	D1	D	17.6.85	6/6							
	D4	D	17.6.85	6/6							
	F2	F	17.6.85	0/6	1/6	4/4					
	F4	F	17.6.85	0/6	1/6	5/6					
6 month challenge Day 0	A4	A	17.7.85	6/6							
	B1	B	17.7.85	6/6							
	B4	B	17.7.85	6/6							
	D2	D	17.7.85	6/6							
	D3	D	17.7.85	6/6							
	E1	E	17.7.85	6/6							
	E3	E	17.7.85	6/6							
7 month challenge Day 0	F1	F	16.8.85	0/6	0/6	2/6	6/6	5/6	6/6	4/6	
	A1	A	15.8.85	6/6							
	A3	A	15.8.85	3/6							
	B2	B	15.8.85	5/5							
	B3	B	15.8.85	6/6							
	C2	C	15.8.85	6/6							
5 month challenge Day 28		CONTROL E,F	15.7.85	ND	ND	ND	0/6	0/6	0/6	4/6	

ND = Not done
▾ Day 0 = Day of challenge
Day 28 = 28 days following challenge

of the 4 month challenge, F2 and F4 both had complete neutralising titres of 1:2 on the day of the 5 month challenge and F1 had a complete neutralising titre of 1:5 on the day of the 7 month challenge. In contrast, plasma collected from a 5 month challenge control, 28 days after infection with T.congolense ILNat 3.1, produced complete neutralisation of infectivity at a titre of 1:80.

(ii) T.congolense IL 2642

Data from infectivity neutralisation tests conducted with T.congolense IL 2642 metacyclic trypanosomes is shown in table 16.

All 4 animals in group D were infected with T.congolense IL 2642 by fly-transmission 28 days prior to day 0, when isometamidium chloride was administered; all had high neutralising antibody titres on day 0. Complete neutralisation titres on this day were 1:20 for D1, 1:80 for D2, 1:80 for D3 and 1:40 for D4.

By the end of the cattle experiment only 2 of the 4 animals in group B had become infected; B1 and B4. Both animals became infected following the 6 month challenge. Plasma collected from B4 on the day of the 6 month challenge did not neutralise metacyclic infectivity. However, plasma collected on the same day from B1 produced complete neutralisation of infectivity at a titre of 1:2. Worthy of note are the observations that although B1 was not detected parasitaemic until 14 days following the 6 month challenge, no changes occurred at the level of the

Table 16

INFECTIVITY NEUTRALISATION TEST USING T.CONGOLENSE IL 2642 METACYCLIC TRYPANOSOMES

Data = Number of mice parasitaemic / Number of mice inoculated

				PLASMA DILUTION							
SAMPLE	ANIMAL	GROUP	DATE	1:2	1:5	1:10	1:20	1:40	1:80	1:160	1:320
Day 0	D1	D	17.1.85	ND	0/6	0/6	0/5	4/6	5/5	6/6	6/6
	D2	D	17.1.85	ND	0/6	0/6	0/6	0/5	0/6	4/6	5/6
	D3	D	17.1.85	ND	0/6	0/6	0/6	0/6	0/6	1/6	5/6
	D4	D	17.1.85	ND	0/6	0/6	0/6	0/6	5/6	5/6	6/6
6 month challenge Day 0	B1	B	17.7.85	0/6	5/6	6/6	5/6				
	B4	B	17.7.85	4/4							
7 month challenge Day 0	B2	B	15.8.85	3/6	6/6	6/6	5/5				
	B3	B	15.8.85	3/6	6/6	6/6	6/6				
7 month challenge Day 28	B control		12.9.85	0/6	0/6	0/6	0/5	0/6	1/6	6/6	4/6

ND = Not done

skin or lymph node subsequent to the 6 month challenge. However, following the 5 month challenge one "large" increase (100%) in skin thickness did occur; attainment of maximal thickness was delayed. These observations suggest that the parasitaemia observed in B1 following the 6 month challenge may infact have originated from the 5 month challenge.

Subsequent to the 7 month challenge 2 animals in group A (A1 and A3), 2 animals in group B (B2 and B3), and one animal in group C (C2) remained refractory to challenge. Infectivity neutralising antibody was not detected in plasma collected from any of the 5 animals on the day of the 7 month challenge.

(c) Indirect immunofluorescent antibody (IFA) test

Results from screening plasma samples for antibodies to T.congolense common antigens are shown in table 17. The same set of plasma samples that were used for metacyclic infectivity neutralisation tests were examined.

Plasma collected from all 4 group D animals on day 0 produced strong nuclear staining at a titre of 1:20 or more. All 4 animals were infected with T.congolense IL 2642 twenty-eight days earlier and 3 of them were parasitaemic on day 0. Other workers have described the phenomenon of nuclear staining when conducting IFA tests for antibody to common antigen (Zwart, Perie, Keppler and Goedbloed, 1973; Katende et al., 1987). Nuclear staining

TABLE 17

IFA TEST FOR ANTI-*T.CONGOLENSE* COMMON ANTIGEN ANTIBODIES

SAMPLE	ANIMAL	GROUP	DATE	PLASMA DILUTION 1:20	1:100	1:500	1:2,500
Day 0	D1	D	17.1.85	+N	-		
	D2	D	17.1.85	+N	+N	-	
	D3	D	17.1.85	+N	+N	+N	+N
	D4	D	17.1.85	+N	+N	+N	-
4 month	C4	C	17.5.85	-			
challenge	E2	E	16.5.85	-			
Day 0	E4	E	16.5.85	-			
	F3	F	16.5.85	+N	+N	-	
5 month	A2	A	17.6.85	-			
challenge	C1	C	17.6.85	-			
Day 0	C3	C	17.6.85	-			
	D1	D	17.6.85	(+/-)	-		
	D4	D	17.6.85	(+/-)	-		
	F2	F	17.6.85	-			
	F4	F	17.6.85	+N	-		
6 month	A4	A	17.7.85	-			
challenge	B1	B	17.7.85	-			
Day 0	B4	B	17.7.85	-			
	D2	D	17.7.85	-			
	D3	D	17.7.85	+	+	+	-
	E1	E	17.7.85	+	-		
	E3	E	17.7.85	-			
7 month	A1	A	15.8.85	-			
challenge	A3	A	15.8.85	-			
Day 0	B2	B	15.8.85	-			
	B3	B	15.8.85	-			
	C2	C	15.8.85	-			
	F1	F	16.8.85	+N	-		
6 month	B1	B	14.8.85	+	+	-	
challenge	D3	D	14.8.85	+	+	+	+
Day 28 (positive controls)		A,C,D control	14.8.85	+N	+N	+N	-
		B control	14.8.85	+N	-		
Negative controls	B4		17.1.85	-			
	E,F	control	17.1.85	-			

+ = positive staining.
- = negative staining.
N = nuclear staining only.
(+/-) = staining not as marked as positive samples but more than negative samples.

appears to be more commonly observed when screening for antibody responses to T.congolense common antigen than for similar responses to T.vivax or T.brucei (J.Katende, personal communication). Since, in the work described by Katende et al. (1987), antinuclear antibodies could not be removed by absorption with a lymphocyte lysate containing bovine nuclear material, it was unlikely that the activity was due to antibody directed towards host DNA (Katende et al., 1987). Thus, in the following text the term "antibody titre" includes both whole trypanosome, and nuclear, staining.

Three of the 4 animals in group F (F1, F3 and F4) had an IFA titre of 1:20 on the day of successful challenge. No common antigen antibody response was detected in plasma collected from F2 on the day of successful (5 month) challenge although both trypanolytic and infectivity neutralising anti-metacyclic antibody were detected in the same plasma sample.

Of the 4 animals in group D, all of which had common antibody titres on the day of drug-administration, 3 had a common antibody titre on the day of successful challenge. Since neither trypanolytic nor infectivity neutralising anti-metacyclic antibodies were detected in the latter samples, the anti-common antigen antibody detected in plasma collected on the day of successful challenge probably originated from the animals' earlier infection with T.congolense IL 2642.

Apart from positive controls, the only other sample in

which anti-common antigen antibody was detected was that from E1, on the day of the 6 month challenge. Although an IFA titre of 1:20 was detected in the sample, neither trypanolytic nor infectivity neutralising anti-metacyclic antibody was detected.

5) SCREENING OF BREAKTHROUGH TRYPANOSOME POPULATIONS FOR THEIR *IN VIVO* SENSITIVITY TO ISOMETAMIDIUM

All trypanosome populations arising in drug-administered cattle and controls were screened for their sensitivity to the therapeutic activity of isometamidium in mice.

Since no bovine administered isometamidium chloride became infected following the 2 month or 3 month challenge, drug sensitivity screening was not carried out with trypanosome populations arising in control animals used for these two challenges.

Data from isometamidium sensitivity experiments conducted with trypanosome populations arising as a result of the 4, 5, 6 and 7 month challenges is shown in tables 10, 11, 12 and 13, appendix I, respectively.

Of the infections in mice derived from breakthrough populations occurring in 19 drug-administered steers, all appeared to be sensitive to treatment, both 6 hours post-infection and when parasitaemia attained 10^7 parasites per ml, with isometamidium chloride at dosages of 0.5, 1.0, 2.0 and 4.0mg kg^{-1}. A similar level of sensitivity was expressed by trypanosome populations arising in controls.

Thus, no trypanosome population arising in a drug-administered animal showed any evidence for the expression of an altered level of sensitivity to the therapeutic activity of isometamidium.

The timing of treatment did, however, appear to affect the level of sensitivity expressed by the trypanosome populations, i.e., a slightly lower level of sensitivity appeared to be expressed when infections were treated at peak parasitaemia rather than 6 hours post infection.

6) DETERMINATION OF THE ANTIBODY RESPONSE OF BORAN CATTLE TO INTRADERMAL INOCULATION OF 1.0×10^6 IRRADIATED *T.CONGOLENSE* ILNat 3.1 METACYCLIC TRYPANOSOMES

Since the quantity of gamma-radiation required to completely inhibit infectivity of T.congolense metacyclic trypanosomes had not previously been described, a preliminary experiment was conducted in mice to determine the quantity of gamma-radiation required to completely inhibit the infectivity of in vitro-derived T.congolense ILNat 3.1 metacyclic trypanosomes. Table 14, appendix I, shows the results from this experiment. After exposure to 5 kilorads there was partial inhibition of infectivity, whilst at 10 and 15 kilorads there was complete inhibition of infectivity. A repeat of this experiment produced similar results.

Thus, for the cattle experiment, metacyclic trypanosomes were exposed to 15 kilorads of gamma-radiation before inoculation into cattle.

Sera were collected from each of 6 cattle every second day from the day of inoculation until 16 days following inoculation.

Infectivity neutralisation tests were carried out at a serum dilution of 1:2 using *in vitro*-derived *T.congolense* ILNat 3.1 metacyclic trypanosomes. The infectivity of each metacyclic population for mice, after incubation in serum, is shown in table 15, appendix I. No serum sample produced complete neutralisation. Thus there appeared not to be a significant neutralising antibody response by any of the 6 cattle.

Data from IFA tests is shown in table 16, appendix I. Only in serum collected on day 8 was antibody detected. Day 8 serum from all 6 animals stained between 5% and 48% of the *T.congolense* ILNat 3.1 metacyclic population.

Thus, subsequent to intradermal inoculation of $1x10^6$ irradiated *T.congolense* ILNat 3.1 metacyclic trypanosomes to 6 Boran cattle, only a slight transient indirect immunofluorescent antibody response was detected in each animal (a complete neutralising antibody response was not detected in any animal).

These findings are consistent with those of Morrison *et al*. (1982a) who detected low neutralising activity in sera of cattle intravenously administered 10^6 irradiated *T.brucei* bloodstream forms (antigenically homogenous to the level of 10^4) but no activity if the same inoculum was administered subcutaneously. The same workers conducted immunofluorescence studies on live trypanosomes using

fluorescent conjugates specific for either bovine IgG or bovine IgM. Only in animals administered 10^7 or more trypanosomes was antibody detected. In the work described here a very slight indirect immunofluorescent antibody response was detected on day 8. The fluorescein conjugate used in this work, unlike that described by Morrison et al. (1982a), recognised both bovine IgG and IgM and may therefore, because of antibody isotype polyspecificity, have been more sensitive. This may account for the detection of antibody subsequent to intradermal administration of $1x10^6$ T.congolense metacyclic trypanosomes.

Metacyclic trypanosomes inoculated by feeding tsetse flies are extruded in association with many non-metacyclic antigens (Ellis, Shapiro, ole MoiYoi and Moloo, 1986). That the host may become sensitised to these non-metacyclic antigens has been demonstrated by the occurrence of hypsensitivity reactions in rabbits subsequent to repeated bites by non-infected tsetse (Ellis et al., 1986). By analogy, if the above described experiment had been repeated with metacyclic trypanosomes suspended in serum-containing culture medium (as used to suspend metacyclic trypanosomes for administration to groups E and F) it might be expected that the presence of serum antigens would, by antigenic competition, negate the slight antibody response detected with the indirect immunofluorescence test.

DISCUSSION

In work conducted by Whitelaw et al (1986) a single intramuscular injection of 1.0mg kg^{-1} isometamidium chloride was shown to confer complete protection in cattle for at least 5 months against single or repeated challenge with tsetse-transmitted T.congolense ILNat 3.1. No animal which resisted infection developed skin reactions at the site of inoculation or produced anti-metacyclic trypanosomal antibodies, indicating that prophylaxis was entirely drug-mediated.

In the present study, the duration of complete protection with the same drug dosage against repeated monthly tsetse challenge was 4 months with the serodeme previously investigated (ILNat 3.1; group A) and 5 months with an unrelated T.congolense serodeme (IL 2642; group B) (Table 6). In a similar manner to the work described by Whitelaw et al. (1986), isometamidium protected a significant proportion of cattle beyond 6 months.

Isometamidium chloride is frequently recommended for field administration at 0.5mg kg^{-1} and at this lower dosage (group C), the period of complete prophylaxis was reduced to 3 months. Thereafter, 3 out of 4 cattle were susceptible to challenge during the following 2 months. Analyses of these results indicated a significant ($P<0.06$) dose-dependent relationship for the duration of isometamidium prophylaxis against drug-sensitive strains of T.congolense and showed that within this dose range

(0.5-1.0mg kg^{-1}), 3 to 5 months protection might be expected.

Work from the field in Rhodesia has demonstrated a similar dose-dependent relationship. Using isometamidium chloride at a dosage of 1 or 2mg kg^{-1} in an area of high tsetse challenge (Berenil Index 13), Boyt et al. (1962) indicated that the higher dosage conferred a longer mean period of protection than the lower; the mean period of protection for 4 animals administered 1mg kg^{-1} was 187 days, while of 5 animals administered 2mg kg^{-1}, 3 were still uninfected at 282 days, giving a mean protection period of over 245 days. The period of complete protection for the same animals was 147 days at 1mg kg^{-1} and 173 days at 2mg kg^{-1}. A similar relationship has been described for ethidium bromide, another member of the phenanthridine group of compounds (Leach et al., 1955) to which isometamidium belongs.

Since it has been suggested that the duration of protection afforded by a prophylactic drug is directly related to the intensity of tsetse challenge (Fiennes, 1953; MacOwan, 1955, 1956; Davey, 1957; Whiteside, 1958, 1962a,b), 2 groups of 4 cattle (groups E and F) were administered 0.5mg kg^{-1} and subsequently challenged at monthly intervals with either $5x10^3$ or $5x10^5$ *in vitro*-derived metacyclic trypanosomes. If, as discussed by Whitelaw et al. (1986), it could be assumed that the maximum inoculum of a *T.congolense*-infective tsetse is 100 metacyclic trypanosomes, it would be concluded that the

cattle in groups E and F were exposed each month to the equivalent of 50 and 5,000 infective tsetse respectively. The duration of complete prophylaxis in both groups E and F was found to be the same, i.e., 3 months. This was similar to group C, which was challenged with 5 infective tsetse per month. That the duration of prophylaxis is unaffected by this wide range of challenge conflicts with previous field observations, although is consistent with previous experimental findings (Whitelaw et al., 1986). However, two differences between the experimental conditions described and those in the field may have contributed to this inconsistency. The first was that both populations of T.congolense used in this experiment have been well characterised in terms of their sensitivity to isometamidium at normal therapeutic doses, whereas in the field cattle might encounter strains of trypanosomes less sensitive to the drug. The second was that the challenge was administered on a single day each month, whereas in the field, challenge probably occurs more frequently at a lower intensity. An understanding of the interrelationship between such variables is central to the proper use of prophylactic drugs in the field.

When isometamidium chloride was used to treat an existing infection (group D) the period of prophylaxis against subsequent challenge was the same as in cattle previously uninfected (group C). This observation is of practical importance, since it might have been expected that the duration of prophylaxis would have been shorter

due to drug uptake by the large number of circulating trypanosomes, as suggested by Davey (1957). However, in studies with ^{14}C-ethidium bromide, no difference was observed in either drug distribution or drug excretion between normal and T.congolense-infected calves (Gilbert, Curtis and Newton, 1979).

Results from previous investigations (Whitelaw et al., 1986) and those from 5 of the 6 groups of cattle in the experiment described here (groups A, B, C, D and E), have indicated that prophylaxis was mediated solely by the drug since no antibody was detected in any animal until it became parasitaemic. Furthermore, none of the breakthrough trypanosome populations occurring in the 19 cattle administered isometamidium chloride and susceptible to challenge showed evidence for the expression of a decreased level of sensitivity to isometamidium. An exception to the above conclusion, regarding absence of antibody, was group F in which all 4 animals (which received the largest inoculum of in vitro-derived metacyclic trypanosomes) had low anti-metacyclic antibody titres for 1 to 4 months before becoming parasitaemic, when antibody levels rose five-fold. Thus, it would appear that the repeated administration of $5x10^5$ T.congolense metacyclic parasites was sufficient to induce an antibody response in drug-administered cattle but not at a level that conferred protective immunity.

In none of the 4 animals in group F was trypanolytic antibody detected until following the administration of at

least two inoculae of $5x10^5$ metacyclic trypanosomes. Since no infectivity neutralising antibody and only a very slight indirect immunofluorescent antibody response were detected in cattle after intradermal inoculation of $1x10^6$ irradiated T.congolense ILNat 3.1 metacyclic trypanosome, it would appear that the antibody response occurring in group F animals was an anamnestic, rather than a primary, response.

As to why the trypanolytic antibody titre rose no higher than 1:5 in the group F animals until they became parasitaemic is unclear. Possibly isometamidium exerted an immunosuppressive effect, either by direct action on the mononuclear phagocytic system or by binding to the surface of trypanosomes and rendering them less immunogenic. Alternatively, anti-T.congolense ILNat 3.1 metacyclic antibody may have bound to the metacyclic antigens, and because of the relatively small quantity of antigen administered, masked most, if not all, of the antigen load. In the same manner, Morrison et al. (1982a) found that 10^6 irradiated (non-infective) T.b.brucei gave rise to a detectable antibody response in cattle when inoculated intravenously. The antibody response did not result in complete protection; for this a minimum of 10^7 parasites was required.

It is therefore concluded that acquisition of immunity to metacyclic variable antigen types of T.congolense whilst under chemoprophylactic cover is unlikely to play any significant protective role in animals maintained by a chemoprophylactic regime, as the size of the metacyclic

inoculum required to stimulate a protective immune response is excessively large in relation to what is liable to be encountered in the field.

Dwinger (1985a) has shown that as many as 10^5 irradiated T.congolense ILNat 3.1 in vitro-derived metacyclic trypanosomes did not elicit a skin reaction when inoculated intradermally (at one site). Further work has indicated that the intradermal inoculation of at least 10^7 irradiated metacyclic trypanosomes was required before a change in skin thickness occurred (R.H.Dwinger, personal communication). It must therefore be concluded that only when metacyclic trypanosomes multiply to the level of 10^7 at the site of inoculation that a skin thickness change is elicited.

The absence of skin thickness and lymph node changes in almost all drug-adminstered cattle when refractory to challenge, and the absence of detectable antibody in all animals in groups A, B, C, D and E prior to onset of parasitaemia, would indicate that protection against challenge in groups A-E was mediated solely by drug and that protection occurred at the level of the skin. It must therefore be concluded that isometamidium excerted its trypanocidal activity in these animals at the level of the skin; inhibiting multiplication of the trypanosome inoculum either immediately or before trypanosome numbers required to elicit a skin and antibody response were attained.

Since homidium, another member of the phenanthridine group of compounds, is reported to manifest its

trypanocidal activity *in vivo* after a latent period of 24 hours (Hawking and Sen, 1960), it is possible that trypanosomes exposed to isometamidium are also able to multiply for a limited number of times before the drug's trypanocidal activity is manifested. Thus, a short period of trypanosome multiplication may have occurred after inoculation.

In many of the cattle administered isometamidium chloride and susceptible to challenge there were pronounced differences, as compared to controls, at the level of the skin, lymph node and in time to onset of parasitaemia. There appeared not to be a group-association with regard to these differences. Akol and Murray (1983, 1985) have shown that in cattle immune to homologous rechallenge with cyclically-infected tsetse, skin thickness changes at the site of infected-tsetse bites did not occur. Furthermore, the presence of partial immunity to the intradermally inoculated trypanosome population can result in parasitaemia of delayed onset (R.H.Dwinger, personal communication). In none of the cattle administered isometamidium chloride, except those in group F, could trypanolytic or infectivity neutralising antibody be detected on the day of successful challenge. In the same animals there was also no evidence for alteration in sensitivity of breakthrough trypanosome populations to isometamidium as compared to populations arising in controls. Possibly drug and/or antibody was present, but at levels below the level of detection in the antibody and

drug sensitivity tests; levels sufficient to inhibit growth and infectivity of a large part of the metacyclic inoculum, but not all.

The appearance of trypanosomes in the peripheral blood of cattle at the end of the chemoprophylactic protective period has been described as being often scanty and sporadic (Smith, 1959; Stephen, 1960). These "breakthrough" trypanosomes appear to be less pathogenic than the same parasites in animals that have never received a prophylactic drug (Stephen, 1960, 1962a; Stephen and Gray, 1960). In none of the 19 breakthrough trypanosome populations occurring in drug-administered cattle described in this chapter could the parasitaemia be described as "intermittent and sporadic". All parasitaemias increased to the level of (BC)4+ or (BC)5+ over a period of no more than 7 days (similar to controls) and remained at this level until treatment was required. Although all animals that became infected, both drug-administered and controls, required treatment, the rate of PCV decline in at least 7 of the 19 cattle administered isometamidium chloride indicated the presence of significantly less pathogenic trypanosome populations. The mechanisms involved in alteration of pathogenicity are unknown.

In conclusion, the results from this experiment support previous findings (Whitelaw et al., 1986) and, in addition, indicate that the duration of isometamidium chemoprophylaxis is a function of the dose of drug used and not of the level of metacyclic challenge or the presence of

an existing parasitaemia. They further indicate that antibody responses against challenge by metacyclic forms of T.congolense do not occur in cattle under isometamidium prophylaxis.

CHAPTER 2

STUDIES WITH *T.CONGOLENSE IN VITRO*

INTRODUCTION

The first stage of T.congolense to be successfully cultured in vitro was the procyclic trypomastigote (Cunningham, 1973, 1977; Steiger, Steiger, Trager and Schneider, 1977; Brun, 1982). Gray, Cunningham, Gardiner, Taylor and Luckins (1981) were the first to describe the production of metacyclic forms in vitro. Cultures were initiated by placing infected Glossina proboscides beside a bovine dermal collagen explant. Hirumi et al. (1982) described a similar technique in which the bovine dermal collagen explant was replaced with vitrogen.

Bloodstream forms of T.congolense have proven the most difficult of the life cycle stages to cultivate. By transferring dermal explants from T.congolense-infected skin into culture, the infectivity of trypanosomes was retained for up to 21 days (Gray, Brown, Luckins and Gray, 1979). Five years later Hirumi and Hirumi (1984) described a continuous culture system for T.congolense bloodstream forms. They used a confluent bovine endothelial feeder layer with HEPES-buffered RPMI 1640 medium supplemented with 16% heat inactivated adult goat serum and 4% foetal goat serum. Trypanosomes maintained in this system were shown to possess a surface coat and were infective for mice.

A similar technique to that described by Hirumi and Hirumi (1984) was used in the present studies in conjunction with freshly isolated T.congolense ILNat 3.1 bloodstream forms to screen cattle sera for trypanocidal

activity.

Subsequent to the synthesis of isometamidium chloride only a very few assays have been developed to quantify the levels of drug present in body fluids and tissues. Philips, Sternberg, Cronin, Sodergren and Vidal (1967) described a spectrophotometric technique, utilising the fluorescent properties of isometamidium. Braide and Eghianruwa (1980) and Ali and Hassan (1984) employed the same technique to investigate isometamidium pharmacokinetics in goats and camels, respectively. All three papers reported a drug detection limit of approximately 1 μg ml^{-1} serum and drug was undetectable in serum collected after 24 hours following intravenous inoculation of 1mg kg^{-1} drug. For tissue samples the lower limit of detection was 2.3ug g^{-1} tissue. Appreciable concentrations of isometamidium were still detected in the liver and kidneys 12 weeks after intravenous administration of 1mg kg^{-1} (Braide and Eghianruwa, 1980).

Perschke and Vollner (1985) described an HPLC technique for assaying spiked serum samples; the lower limit of isometamidium detection was 20ng ml^{-1} when 10ml of serum was analysed. Attempts to repeat this work have not been successful and in addition some reservations have been expressed about the experimental protocol employed (W.R. Fish, personal communication).

In the absence of any sensitive technique for detecting isometamidium, it was decided to investigate the

suitability of *in vitro* trypanosome culture techniques for this purpose.

Whitelaw and Murray (1984) described the results of a preliminary study using a biological assay in which bloodstream forms, tsetse-extruded metacyclic trypanosomes and cultured metacyclic trypanosomes of *T.congolense* ILNat 3.1 were incubated for 1 hour in plasma from cattle administered isometamidium chloride, and injected into mice. No anti-trypanosomal activity could be detected in plasma collected beyond 4 weeks after administration of 1mg kg^{-1} isometamidium chloride.

In vitro trypanocidal activity of a compound has been determined by assessing the uptake of radiolabelled precursors (Desjardins, Casero, Willet, Childs and Canfield, 1980) or by examination of trypanosome motility, and infectivity for mice (Williamson and Scott-Finnigan, 1978). The technique used by the latter authors has been utilised in the studies described here.

Using currently employed *in vitro* culture techniques, experiments undertaken in this chapter were conducted to determine whether or not periods of incubation longer than 1 hour proved more sensitive than the technique described by Whitelaw and Murray (1984). In initial studies 24 hour incubation periods were used. Further studies were conducted to ascertain whether or not 48 hours incubation further enhanced the sensitivity of the bioassay.

Because *T.congolense* ILNat 3.1 appeared to express a high level of *in vivo* sensitivity to isometamidium

(personal observation), it was decided to use this population for screening sera from cattle that had been administered drug.

MATERIALS AND METHODS

1) EXPERIMENT 1 - A PRELIMINARY STUDY - *IN VITRO* ANALYSES OF PLASMA SAMPLES COLLECTED FROM CATTLE ADMINISTERED 1mg kg^{-1} ISOMETAMIDIUM CHLORIDE

Plasma samples collected from 7 animals described by Whitelaw et al. (1986) were examined for their effect on freshly isolated T.congolense ILNat 3.1 bloodstream forms with regard to their effect on in vitro growth and rodent infectivity.

The 7 cattle belonged to 4 of the phase 1 experimental groups described by Whitelaw et al. (1986):

		CODE
Group A	: C513	W1
Group C	: C502	W2
"	: C514	W3
Group D	: C504	W4
Group E	: C489	W5
"	: C510	W6
"	: C511	W7

On day 0, isometamidium chloride was administered to all 7 animals at a dosage of 1mg kg^{-1}. Cattle were challenged with 5 tsetse infected with T.congolense ILNat 3.1; groups C, D and E on one occasion at 2, 3 and 4 months, respectively, after drug administration, and group A every month (table 18).

Table 18

CHALLENGE OF BORAN CATTLE WITH TSETSE-TRANSMITTED *T.CONGOLENSE* ILNat 3.1 ONE TO FIVE MONTHS AFTER ADMINISTRATION OF 1mgkg^{-1} ISOMETAMIDIUM CHLORIDE

DRUG STATUS	GROUP	CHALLENGE: MONTHS AFTER DRUG ADMINISTRATION 1	2	3	4	5
Treated	A	x	x	x	x	x
Treated	C	-	x	-	-	-
Treated	D	-	-	x	-	-
Treated	E	-	-	-	x	-

x = challenged

- = not challenged

Subsequent to all challenges, none of the cattle became infected. Thus, all plasma samples collected from cattle up to and including the 5 month challenge were from cattle refractory to challenge.

In phase 2 of the experiment, 6 months after drug-administration, cattle were reallocated into new experimental groups, as outlined in table 19. Only one of the 7 animals (W7) was refractory to the 6 month challenge.

Monthly cattle challenges took place on the following days:

drug administration : day 0
1 month challenge : day 27
2 month challenge : day 58
3 month challenge : day 83
4 month challenge : day 118
5 month challenge : day 148
6 month challenge : day 181

Plasma samples collected on these days were examined. When a particular plasma sample was not available, plasma samples collected a week before and a week after the missing sample were examined. Only in the case of Boran W4 were weekly-collected plasma samples examined.

Table 19

CHALLENGE OF BORAN CATTLE WITH *T.CONGOLENSE* ILNat 3.1 SIX MONTHS AFTER ADMINISTRATION OF ISOMETAMIDIUM CHLORIDE, AND OUTCOME OF THE CHALLENGE

GROUP	CATTLE ADMINISTERED ISOMETAMIDIUM CHLORIDE	UNTREATED CONTROLS	CHALLENGE
1	$W1^s$	A^S	5 tsetse flies on 1 occasion
3	$W3^s$, $W4^s$	B^S	$5x10^2$ metacyclic forms
4	$W2^s$, $W7^R$	C^S	$5x10^3$ metacyclic forms
5	$W6^s$	D^S	$5x10^4$ metacyclic forms
6	$W5^s$	E^S	$5x10^5$ metacyclic forms

^{s} = susceptible to challenge.

^{R} = refractory to challenge.

2) EXPERIMENT 2 -

IN VITRO SCREENING FOR TRYPANOCIDAL ACTIVITY IN SERA FROM CALVES ADMINISTERED 0.25mg kg^{-1} ISOMETAMIDIUM CHLORIDE

(a) PART A

Experiment 2 was conducted to ascertain how closely results from the *in vitro* bioassay correlated with susceptibility of animals to challenge. Thus, more frequently collected samples were examined than in experiment 1, i.e., weekly. So as to reduce the number of samples requiring examination, samples from calves administered 0.25mg kg^{-1} isometamidium chloride were examined.

In this experiment serum rather than plasma was examined with regard to its suitability for screening for trypanocidal activity *in vitro*.

Three Boran calves (X1, X2 and X3), aged 6 months, were administered 0.25mg kg^{-1} isometamidium chloride (1% w/v solution in sterile water) by a deep mid-cervical intramuscular injection. Serum was collected on days 0, 1, 3, 7 and thereafter at weekly intervals. On day 42, 6 weeks after drug administration, the 3 calves along with one control (to which isometamidium chloride had not been administered) were challenged by subcutaneous inoculation of $1x10^5$ *T.congolense* ILNat 3.1 bloodstream forms. Unlike the control animal which was observed parasitaemic within 2 weeks following challenge, all 3 drug-administered animals

were refractory to challenge.

On day 84 the same 3 drug-administered calves, along with a different control calf, were inoculated subcutaneously with $1x10^5$ T.congolense IL 2642 bloodstream forms. All 4 animals were detected parasitaemic within 14 days following challenge.

T.congolense ILNat 3.1 bloodstream forms were incubated in vitro for 24 hours in media containing 20% serum collected on days 0 to 49. Given that pronounced trypanocidal activity was not detected in day 49 samples, sera collected after day 49 were not examined.

(b) PART B

In this part of the experiment, in vitro incubation periods were increased from 24 hours to 48 hours to ascertain whether or not lengthening the incubation period enhanced the sensitivity of the in vitro bioassay. Sera from calves X1 and X2 were examined in such a manner.

(c) PART C

In vitro screening of sera from calves X1, X2 and X3 for trypanocidal effects on *T.vivax* IL 2968 bloodstream forms of bovine and murine origin

Please refer to chapter 4 (in vitro studies with T.vivax) part B.

RESULTS

1) EXPERIMENT 1 -

IN VITRO ANALYSES OF PLASMA SAMPLES COLLECTED FROM CATTLE ADMINISTERED 1mg kg^{-1} ISOMETAMIDIUM CHLORIDE

Results from experiments carried out with plasma collected from 7 of the cattle described by Whitelaw et al. (1986) are shown in tables 1 - 7, appendix II. Wherever experiments were repeated this is indicated by the letter "a" or "b" by the sample number (i.e., 1a denotes sample 1, first test and 1b denotes sample 1, repeat test). All samples with the same test letter were all analysed in the same test. The growth of trypanosomes in each test were expressed as a percentage of the growth of trypanosomes in the control sera used for each test.

Table 20 is a summary of growth inhibition and infectivity data produced by each of the 7 animals' sera.

Day 0 samples from only 3 of the animals (W5, W6 and W7) supported trypanosome growth and rodent infectivity after 24 hours incubation, and were used as control samples for these animals:

(a) W5

Boran W5 was refractory to the 4 month challenge but susceptible to the 6 month challenge.

Since plasma collected on the day of the 6 month

Table 20

24 HOUR IN VITRO INCUBATIONS OF BORAN PLASMA WITH T.CONGOLENSE ILNat 3.1

BLOODSTREAM FORMS; EFFECTS ON GROWTH AND INFECTIVITY

DAY	ANIMAL: W1	W2	W3	W4	W5	W6	W7
0	NT	NT	NT	NT	+	+	+
27-34	NT^r	NT	NT	NT	-	NT	NT
58	NT^r	NT^r	$(NT)^r$	-	-	+/-	NT
83	NT^r	NT	NT	NT^r	NT	-	NT
118	NT^r	NT	NT	NT	NT^r	NT^r	$-^r$
148-153	$+^r$	NT	NT	NT	NT	NT	-
174-181	$+^s$	$+^s$	$+^s$	$+^s$	$+/-^s$	$+^s$	$+/-^r$

NT = No trypanosomes counted.

(NT) = Greater than 95% growth inhibition.

+ = No inhibition of infectivity (i.e., all mice inoculated became parasitaemic).

+/- = Partial inhibition of infectivity (i.e., only some of inoculated mice became parasitaemic).

- = Complete inhibition of infectivity.

r = Refractory to challenge.

s = Susceptible to challenge.

challenge was not available, plasma collected one week before and one week after the 6 month challenge was examined. Trypanocidal activity, as detected by inhibition of growth and infectivity, was detected in samples collected from 1 to 5 months after drug administration (table 1, appendix II). Trypanocidal activity was not detected in plasma collected 1 week before the 6 month challenge.

Unexpectedly, plasma collected 7 days after the 6 month challenge produced 100% growth inhibition. A similar phenomenon was observed with plasma collected from Boran W4; plasma collected on the day of the 6 month challenge did not inhibit trypanosome infectivity whilst that collected 7 days later did (see tables 7a and 7b, appendix II) (W4 was susceptible to the 6 month challenge).

(b) W6

Boran W6 was refractory to the 4 month challenge but susceptible to challenge 2 months later.

Of the plasma samples that were analysed, only that collected on the day of the 6 month challenge did not produce greater than 25% growth inhibition (table 2, appendix II). Trypanocidal activity, as measured by inhibition of growth and/or infectivity, was detected in samples collected on the day of each challenge except the 6 month challenge.

(c) W7

Boran W7 was refractory to both the 4 month challenge and the 6 month challenge.

Plasma collected on the days of the 1, 2 and 3 month challenges produced 100% growth inhibition, i.e., less than $1x10^4$ trypanosomes ml^{-1} remained after 24 hours incubation (table 3, appendix II). Although plasma collected on the days of the 4, 5 and 6 month challenges did not produce appreciable growth inhibition, the presence of trypanocide in these samples was indicated by inhibition of trypanosome infectivity; plasma collected on the days of the 4 and 5 month challenges completely inhibited infectivity whilst that collected on the day of the 6 month challenge partially inhibited infectivity. Since the parasitaemia prepatent period in mice inoculated with the 6 month plasma-trypanosome mixture was 10.7 ± 0.9 (S.D.) days and the parasitaemia prepatent period in mice inoculated the day 0 plasma-trypanosome mixture was 4.0 ± 0 days, this provided further evidence that the 6 month plasma sample exerted an inhibitory effect on trypanosome infectivity.

In contrast to plasma samples collected from W5, W6 and W7, plasma collected on day 0 (day of drug-administration) from W1, W2, W3 and W4 all produced 100% growth inhibition after 24 hours incubation. Because of this anomalous result a later collected sample, that supported growth to the greatest extent, was taken as the control for each animal's samples. In 3 sets of animals'

plasma samples (W1, W2 & W3) this was the 6 month challenge sample. Unfortunately, only a very small quantity of the 6 month challenge sample from W4 was available. Thus, in one set of experiments plasma collected 7 days before the 6 month challenge was used as the control, and in a second set, foetal bovine serum was used.

All 4 animals were susceptible to the 6 month challenge.

(d) W1

All plasma samples from W1, except those collected on the days of the 5 and 6 month challenges, produced pronounced growth inhibition. Plasma samples collected on the days of the 5 and 6 month challenges, in addition to not exerting a growth inhibitory effect, did not inhibit trypanosome infectivity (table 4, appendix II). W1 was refractory to the 1, 2, 3, 4 and 5 month challenges but susceptible to the 6 month challenge.

(e) W2 and W3

Of the 7 plasma samples from W2 that were analysed, all except that collected on the day of the 6 month challenge produced pronounced growth inhibition (table 5, appendix II). The same was true with the samples from W3 (table 6, appendix II). Both W2 and W3 were susceptible to the 6 month challenge.

(f) W4

Data from experiments conducted with plasma from W4 has been presented in two tables since plasma collected 1 week before the 6 month challenge served as a control for one series of experiments (table 7a, appendix II) and foetal bovine serum served as a control for a second series of experiments (table 7b, appendix II).

W4 was refractory to the 3 month challenge but susceptible to the 6 month challenge.

Twenty-one different plasma samples, collected from day 0 to 4 weeks following the 6 month challenge, were examined. In only 3 samples were the trypanosome numbers, remaining after 24 hours incubation, greater than 50% of the number remaining in the control sample; that collected 3 days before the 2 month challenge, 7 days before the 6 month challenge and on the day of the 6 month challenge. Trypanocidal activity, as detected by inhibition of trypanosome infectivity, was only detected in the first of these 3 samples. Of note is the fact that plasma collected 7, 15 and 21 days after the 6 month challenge produced 100% growth inhibition. In contrast, plasma collected 28 days following the 6 month challenge, although exerting pronounced growth inhibition, produced only slight inhibition of trypanosome infectivity.

With reference to the above finding it should be noted that of 12 plasma samples collected from the 7 cattle on days they were refractory to challenge, only one did not

have any pronounced negative effect on growth or rodent infectivity (the plasma sample collected from W1 on the day of the 5 month challenge). Of these 12 samples, 9 exerted pronounced growth inhibition. Of the remaining 3 samples, all of which produced less than 15% growth inhibition, 2 exerted inhibitory effects on trypanosome infectivity for mice. Only with the remaining one sample was no trypanocidal activity detected.

Five of the plasma samples examined were collected on days when cattle were susceptible to challenge; all supported trypanosome growth. In addition, all mice inoculated with each plasma-trypanosome mixture (29/29) became infected.

Since 4 out of the 7 cattle's day 0 plasma samples (taken prior to drug-administration) completely inhibited growth and infectivity of T.congolense ILNat 3.1 bloodstream forms, further studies were conducted only on samples from cattle in which pre-drug-administration samples did not exert inhibitory effects on growth or infectivity.

2) EXPERIMENT 2 -

IN VITRO SCREENING FOR TRYPANOCIDAL ACTIVITY IN SERUM FROM CALVES ADMINISTERED 0.25mg kg^{-1} ISOMETAMIDIUM CHLORIDE

(a) PART A - 24 HOUR INCUBATION

T.congolense ILNat 3.1 bloodstream forms were incubated *in vitro* for 24 hours in media containing 20% serum collected from day 0 to day 49 from 3 calves administered 0.25mg kg^{-1} isometamidium chloride, intramuscularly. (Drug was administered on day 0.) Inhibitory effects on growth and rodent infectivity are shown in table 21.

(i) Growth inhibition

Trypanosome numbers remaining in all post-drug-administration sera from bovine X1 were lower than numbers remaining in the day 0 sample.

With the exception of one sample, the same was true with sera from bovine X2, although trypanosome numbers remaining in days 1, 3 and 35 sera were only very slightly less. In only the day 49 serum sample were there greater trypanosome numbers than in the control sample (i.e., negative growth inhibition); the difference from the control was only slight (3.2%).

Of the 9 post-drug-administration samples from bovine X3 that were examined, trypanosome numbers in 3 were greater than in the control, although in one the difference

Table 21

INFECTIVITY AND PERCENTAGE GROWTH INHIBITION OF MURINE-DERIVED T.CONGOLENSE ILNat 3.1 BLOODSTREAM FORMS EXPOSED FOR 24 HOURS TO SERA FROM CALVES ADMINISTERED 0.25mg kg^{-1} ISOMETAMIDIUM CHLORIDE

		DAY									
TEST	ANIMAL	0	1	3	7	14	21	28	35	42	49
PERCENTAGE	X1	0	46.1	26.0	41.5	62.7	42.4	45.2	15.4	6.2	46.5
GROWTH	X2	0	2.1	0.8	28.4	30.8	33.3	42.6	7.6	22.3	-3.2[¢]
INHIBITION	X3	0	14.5	21.0	-1.8[¢]	5.7	1.8	-13.3[¢]	4.4	12.4	-18.3[¢]
INFECTIVITY	X1	5/5[+]	0/5	0/4	0/5	0/5	1/5*	1/5*	5/5(*)	5/5(*)	5/5
FOR	X2	5/5	5/5	0/4	0/5	0/5	3/5*	4/5	5/5(*)	5/5(*)	5/5
MICE	X3	5/5	0/5	0/4	1/5*	0/5	5/5(*)	5/5	5/5(*)	5/5	5/5

[¢] a negative value indicates that there was a greater concentration of trypanosomes than in the control.

[+] data = no. mice parasitaemic / no. mice inoculated (inoculum = 1 x 10^5 trypanosomes per mouse).

* = parasitaemia delayed in onset.

(*) = no delay in onset of parasitaemia but lower level compared to control on first parasitaemic reading.

was only very slight (less than 5% greater).

(ii) Mouse infectivity

All mice inoculated with trypanosomes incubated in day 0 sera became parasitaemic.

Sera collected on day 1 from X1 and X3 completely inhibited trypanosome infectivity whilst mice inoculated with trypanosomes incubated in day 1 serum-trypanosome mixture from X2 all became parasitaemic. Thereafter, sera collected from all 3 animals up until day 14 completely inhibited trypanosome infectivity.

From day 21 onwards, sera did not completely inhibit infectivity of trypanosomes. All mice inoculated with trypanosomes incubated in sera collected on days 35, 42 and 49 from X1 and X2 became parasitaemic. However, parasitaemia onset was delayed in mice inoculated with trypanosomes incubated in day 35 and day 42 sera from the same two animals.

Sera from X3 differed from other sera examined in that all mice inoculated with trypanosomes incubated in day 21 and day 28 sera became parasitaemic. In addition, the level of parasitaemia in mice inoculated with the day 42 serum-trypanosome mixture, unlike that from X1 and X2, was no lower than controls on the first day parasitaemia was detected.

Although all 3 animals were refractory to challenge on day 42, all mice inoculated with trypanosomes incubated for 24 hours in day 42 sera became parasitaemic. However, as

mentioned above, the occurrence of lower parasitaemia, compared to controls, on the first day parasitaemia was detected in mice inoculated with X1 or X2 day 42 serum-trypanosome mixtures, indicated the presence of trypanocidal activity in these sera.

(b) PART B - LENGTHENING THE INCUBATION PERIOD

In an attempt to increase the sensitivity of the *in vitro* system the incubation period was increased from 24 to 48 hours. The effect on infectivity and growth inhibition using sera from X1 and X2 is shown in table 22.

(i) Calf X1

After 48 hours incubation, all sera collected up to day 35 completely inhibited trypanosome infectivity. Trypanosomes incubated in the day 42 sample infected one out of 5 mice. After 24 hours incubation in the day 42 sample, trypanosomes were infective for 5 out of 5 mice (see table 21).

Of the 9 post-drug-administration sera examined, 8 produced greater growth inhibition when the incubation period was increased from 24 to 48 hours. The mean increase in percentage growth inhibition as a result of doubling the incubation period was 20.4% $\pm$ 16.8% (S.D.).

Thus, after 48 hours incubation, expression of trypanocidal activity appeared to be more pronounced than that occurring after 24 hours incubation.

Table 22

INFECTIVITY AND PERCENTAGE GROWTH INHIBITION OF MURINE-DERIVED T.CONGOLENSE ILNat 3.1 BLOODSTREAM FORMS EXPOSED FOR 48 HOURS TO SERA FROM CALVES X1 and X2, ADMINISTERED 0.25mg kg^{-1} ISOMETAMIDIUM CHLORIDE

		DAY									
TEST	ANIMAL	0	1	3	7	14	21	28	35	42	49
PERCENTAGE GROWTH INHIBITION	X1	0	55.7	62.0	56.0	61.3	55.4	59.2	50.9	57.8	57.1
	X2	0	37.8	ND	47.8	24.4	40.0	47.8	15.6	44.4	7.8
INFECTIVITY FOR MICE	X1	4/5[+]	0/5	0/5	0/5	0/5	0/5	0/5	0/5(*)	1/5	2/4
	X2	3/5	2/5	ND	0/5	0/5	0/5	0/5	0/5	1/5	0/5

[+] data = no. mice parasitaemic / no. mice inoculated (inoculum = 5 x 10^4 trypanosomes per mouse).

(*) = no delay in onset of parasitaemia but lower level compared to control on first parasitaemic reading.

ND = not done.

(ii) Calf X2

After 24 hours incubation, complete inhibition of infectivity occurred in sera collected up until day 14 (table 21). By lengthening the incubation period to 48 hours all sera collected up until day 35 completely inhibited trypanosome infectivity. Trypanosomes incubated in day 42 serum were infective for only 1 out of 5 mice. In a similar manner to the day 42 serum sample from X1, this indicated the presence of trypanocidal activity in the serum and is consistent with resistance of the animal to challenge on the same day. It must be noted, however, that in infectivity experiments conducted with sera from both X1 and X2, mouse inoculae were smaller after 48 hours incubation than after 24 hours incubation. This may have accounted for some of the difference in infectivity occurring between the two incubation periods.

Increasing the incubation period also increased the growth inhibition produced by 8 of the 9 post-drug-administration sera; a 12.7% $\pm$ 12.8% (S.D.) increase in growth inhibition occurred as a result of increasing the incubation period from 24 to 48 hours.

DISCUSSION

The development of *in vitro* culture systems has provided the opportunity to examine the effects of trypanocides on trypanosomes *in vitro*. Borowy, Fink, and Hirumi (1985a) and Borowy, Hirumi, Waithaka and Mkoji (1985b) evaluated the suitability of a *T.b.brucei* continuous cultivation system described by Hirumi, Doyle and Hirumi (1977a,b) for use in screening a series compounds for their trypanocidal activity. Using "fully susceptible" *T.b.brucei* clones, 21 compounds were tested. Minimum effective concentrations of all commonly used trypanocides fell within the range of drug concentrations measured in blood, serum or plasma of drug-administered animals.

Waithaka, Borowy, Gettinby and Hirumi (1985) described an *in vitro* drug screening system for *T.vivax*. The workers used a culture system for *T.vivax* described by ILRAD (1984) along with *in vitro*-derived second generation bloodstream forms (bloodstream forms resulting from cyclical transformation *in vitro*). Second generation bloodstream forms were used since they were said to exhibit more predictable growth patterns *in vitro* than trypanosomes isolated directly from blood. However, no comparative drug sensitivity data was presented concerning the sensitivity of second generation bloodstream forms derived from culture as compared to freshly isolated murine bloodstream forms.

Borowy *et al*. (1985b) described a series of comparative sensitivity studies conducted with their

T.b.brucei system and concluded that the level of drug sensitivity expressed by the trypanosome populations they used bore no relationship to the source from which parasites were obtained.

As well as providing an indication of the trypanocidal activity of compounds the above described system of Borowy et al. (1985b) also provided information on the level of sensitivity of each trypanosome population to the different compounds.

In all the work so far described, trypanosomes were incubated in optimal growth-supportive media spiked with drug. In no case were any of the drugs administered to animals and trypanocidal activity in the animals' sera examined in vitro.

. The work described in this chapter was conducted to examine the suitability of a T.congolense continuous cultivation system described by Hirumi and Hirumi (1984) for screening for trypanocidal activity in sera from cattle administered isometamidium chloride. T.congolense ILNat 3.1 was used for screening purposes since it has been shown to be highly sensitive to the prophylactic and therapeutic activity of isometamidium chloride (Whitelaw et al., 1986; Sones, Njogu and Holmes, 1987). Earlier workers have used second generation bloodstream forms for drug sensitivity screening. However, because of logistical problems in obtaining sufficient numbers of T.congolense second generation bloodstream forms for assays, freshly isolated bloodstream forms of T.congolense ILNat 3.1 were used to

screen plasma and sera for trypanocidal activity.

Of 7 sets of monthly-collected plasma samples from 7 of the animals described by Whitelaw et al. (1986), day 0 samples from 4 animals appeared to exert a trypanocidal action; no trypanosomes remained after 24 hours incubation in media containing 20% plasma. Similarly, sera collected from non-infected goats on a weekly basis can vary widely from one week to the next in their growth-supportive property (N.K. Borowy, personal communication).

Godfrey (1958) showed that feeding animals on a diet containing cod-liver oil markedly depressed T.congolense infections, and that a T.vivax infection maintained in rats with a sheep serum supplement was completely suppressed by cod-liver oil. The toxic activity was shown to be associated with the presence of certain polyunsaturated fatty acids (Godfrey, 1958). It may thus be that individual variation in the dietary intake of the 7 animals described by Whitelaw et al. (1986) could possibly account for the differences in growth supportive properties of day 0 plasma samples. These observations indicate that in the absence of markers for the growth supportive property of any given plasma or serum sample, there can be no true control sample for any plasma or serum sample collected after administration of isometamidium chloride. However, not withstanding this, a summary of results from experiments conducted on plasma from animals described by Whitelaw et al. (1986) showed the following: 100% of samples collected on days when treated animals were

susceptible to challenge supported growth of trypanosomes *in vitro* and had no apparent effect on trypanosome infectivity. In contrast, 75% (9/12) of samples collected on days animals were refractory to challenge produced greater than 90% growth inhibition. In addition, 92% (11/12) of the same samples exerted an inhibitory effect on growth and/or infectivity, i.e., only 8% (1/12) of samples produced a false negative result. Thus, 24 hour *in vitro* incubations of plasma from cattle administered 1mg kg^{-1} isometamidium chloride with *T.congolense* ILNat 3.1 bloodstream forms has shown there to be a close correlation between effects on trypanosome growth and rodent infectivity, and the susceptibility of treated animals to fly-challenge with the same trypanosome population.

The occurrence of trypanocidal effects *in vitro* in the form of loss of infectivity for mice in the absence of gross morphological effects, at the time of inoculation, has been described for quinapyramine and suramin (Hawking and Sen, 1960) and the aromatic diamidine DAPI (Borowy *et al.*, 1985a). Inhibition of *T.congolense* ILNat 3.1 infectivity after *in vitro* incubation appears to be a more reliable and sensitive indicator than growth inhibition for the presence or absence of trypanocidal activity in plasma or sera from an animal administered isometamidium chloride.

Examination of sera from 3 animals administered 0.25mg kg^{-1} isometamidium chloride using *T.congolense* ILNat 3.1 bloodstream forms showed that slight growth inhibitory effects that occurred after 24 hours incubation became more

pronounced after 48 hours incubation. The same was true for inhibition of trypanosome infectivity. Plasma collected on day 42, when animals were refractory to needle challenge with T.congolense ILNat 3.1, failed to inhibit infectivity of the trypanosomes after 24 hours incubation. However, after 48 hours incubation two of the same plasma samples produced almost complete inhibition of trypanosome infectivity. These findings are consistent with those of Borowy et al. (1985a) who, from work conducted with the diamidine DAPI, showed that the minimum exposure time required for expression of anti-trypanosomal activity was dependent on the drug concentration. Thus to produce the same trypanocidal action with a low drug concentation that occurred with a relatively high drug concentration, a longer incubation period was required.

Variation in the innate growth supportive properties of individual animals' serum is a major hindrance to use of the in vitro technique described in this chapter. Future investigations should be directed towards isolation and characterisation of the serum constituent(s) responsible for the trypanocidal activity. Similar work should be conducted to identify trypanosomal growth factors and the quantities of these required for optimal growth of the different species of trypanosome. Only with this knowledge can a rational interpretation be made of trypanocidal activity expressed in vitro by sera from drug-treated animals.

SECTION II

STUDIES WITH *T.VIVAX*

CHAPTER 3

THERAPEUTIC AND PROPHYLACTIC ACTIVITY OF ISOMETAMIDIUM IN BORAN CATTLE AGAINST *TRYPANOSOMA VIVAX* OF WEST AND EAST AFRICAN ORIGIN, TRANSMITTED BY *GLOSSINA MORSITANS CENTRALIS*

INTRODUCTION

Work conducted with T.congolense (chapter 1) has shown that at a dose of 0.5mg kg^{-1} body weight, isometamidium chloride protected cattle against tsetse-transmitted challenge with either of 2 unrelated T.congolense serodemes for at least 3 months (table 6). Furthermore, acquisition of immunity to metacyclic variable antigen types of T.congolense, whilst under chemoprophylactic cover, appeared unlikely to play any significant protective role in animals maintained by an isometamidium prophylactic regime.

The work described in this chapter was conducted to determine the therapeutic and prophylactic activity of isometamidium, in Boran cattle, against four tsetse-transmitted T.vivax populations; one cloned population from Zaria, Nigeria (IL 2968), one cloned population from Kilifi, Kenya (IL 2969), an uncloned population from Galana, Kenya (IL 2982) and an uncloned population from Likoni, Kenya (IL 2986). In addition, work was conducted to ascertain whether or not immunity to metacyclic variable antigen types of T.vivax may be acquired whilst under chemoprophylactic cover.

MATERIALS AND METHODS

1) CATTLE

The age, breed, maintenance and feeding of cattle were as described in the general materials and methods.

All cattle have been allocated reference codes. The relationship between reference codes and animal numbers is shown in table 1, appendix III.

2) ANTIBODY ASSAYS

Trypanolysis and indirect immunofluorescent antibody tests were utilised to examine for the presence of anti-*T.vivax* antibodies in cattle sera. Both tests have been described in the general materials and methods.

3) HAEMATOLOGICAL AND PARASITOLOGICAL PARAMETERS

Haematological and parasitological parameters were measured as described in the general materials and methods.

EXPERIMENT 1

STUDIES USING *T.VIVAX* IL 2968 (ZARIA) AND *T.VIVAX* IL 2969 (KILIFI)

PART A

EXPERIMENTAL DESIGN

1) DETERMINATION OF THE SENSITIVITY OF THE ZARIA *T.VIVAX* AND THE KILIFI *T.VIVAX* TO THE THERAPEUTIC ACTIVITY OF ISOMETAMIDIUM

Two groups of 5 cattle were infected, each with one of the 2 T.vivax populations, using 10 infected Glossina morsitans centralis per animal. Eleven days later, when all cattle had a parasitaemia of approximately $5x10^4$ ml^{-1}, all were treated with 0.5mg kg^{-1} isometamidium chloride. For a period of 200 days following treatment jugular blood was collected from each animal three times weekly and examined for the presence of trypanosomes by the buffy-coat phase-contrast (BC) technique.

2) DETERMINATION OF THE SENSITIVITY OF THE ZARIA *T.VIVAX* AND THE KILIFI *T.VIVAX* TO THE PROPHYLACTIC ACTIVITY OF ISOMETAMIDIUM

To determine the duration of prophylaxis isometamidium conferred against the Zaria T.vivax and the Kilifi T.vivax, 10 Boran steers were administered 0.5mg kg^{-1} isometamidium chloride. The cattle were, thereafter, challenged at monthly intervals using G.m.centralis infected with either T.vivax clone (5 cattle per clone, 10 infective flies per

animal). See table 23.

At each challenge 2 control animals for each group, to which isometamidium chloride had not been administered, were challenged in a similar manner.

In preliminary work, conducted prior to the first challenge, dissection infection rates of almost 100% were obtained in tsetse that had fed on animals infected with either the Zaria T.vivax or the Kilifi T.vivax. When salivary probes from the same fly populations were examined, apparent infection rates of a much lower order were obtained, thereby indicating a poor correlation between results from salivary probes and fly dissection. Thus, for the first challenge (day 32), plastic mesh cages containing 13 flies were placed on each animal that was to be challenged. Thirteen flies were placed into each cage so that each animal would be fed on by at least 10 tsetse. All flies that fed were dissected. The number of infected flies that fed on each animal is shown in table 2, appendix III. Unlike earlier experiments, a low infection rate was obtained in tsetse infected with either of the T.vivax populations. Thus although all animals were fed on by at least 10 tsetse, some were bitten by as few as 4 infected tsetse. Because of this, all animals that had not received 10 infected fly bites were rechallenged 3 days later with additional flies until all had received at least 10 infected fly bites (see table 2, appendix III).

The 4 controls used for the first challenge were challenged by allowing individual tsetse to feed at

Table 23

T.VIVAX CATTLE EXPERIMENT 1, PART A - EXPERIMENTAL DESIGN

GROUP OF CATTLE	DOSAGE OF ISOMETAMIDIUM CHLORIDE ($mgkg^{-1}$)	TRYPANOSOMA VIVAX POPULATION	CHALLENGE: MONTHS AFTER ISOMETAMIDIUM CHLORIDE ADMINISTRATION					FORM OF CHALLENGE
			1	2	3	4	5	
G	0.5	IL 2968*	X	X	X	X	X	ten infected tsetse
H	0.5	IL 2969+	X	X	X	X	X	ten infected tsetse

* Zaria T.vivax (Nigeria)

+ Kilifi T.vivax (Kenya)

separate sites. After feeding, tsetse were dissected and all non-infected tsetse were replaced by others until one infected tsetse had fed at each of the 10 skin sites. This was conducted so as to ascertain the skin reaction associated with tsetse-transmission of both the Zaria and the Kilifi T.vivax.

In all subsequent challenges tsetse were not fed at individual sites.

The exact days following drug-administration on which each of the five challenges took place were as follows:

Drug administration - day 0

1 month challenge - day 32

2 month challenge - day 60

3 month challenge - day 91

4 month challenge - day 119

5 month challenge - day 151

RESULTS

1) DETERMINATION OF THE SENSITIVITY OF THE ZARIA *T.VIVAX* AND THE KILIFI *T.VIVAX* TO THE THERAPEUTIC ACTIVITY OF ISOMETAMIDIUM CHLORIDE AT A DOSAGE OF 0.5mg kg^{-1}

Of the 5 animals infected with the Zaria T.vivax, only one relapsed, 84 days after treatment, whereas relapse was not detected in any of the animals infected with the Kilifi T.vivax during the 200-day post-treatment period.

2) DETERMINATION OF THE SENSITIVITY OF THE ZARIA *T.VIVAX* AND THE KILIFI *T.VIVAX* TO THE PROPHYLACTIC ACTIVITY OF ISOMETAMIDIUM

(a) Parasitological data

The time of appearance of parasites in all experimental animals is shown in table 24. Of the 5 drug-administered animals challenged with the Zaria T.vivax (IL 2968), 2 became infected following challenge at 3-months and one following the fourth monthly challenge. The remaining 2 animals remained aparasitaemic for the 120-day observation period following the fifth challenge. In contrast, of the 5 animals challenged with the Kilifi T.vivax (IL 2969), 3 became parasitaemic following the first challenge and 2 following the second challenge.

All 3 animals susceptible to challenge with the Zaria T.vivax had parasitaemia prepatent periods similar to controls. In contrast, of the 3 group H animals detected

Table 24

DEVELOPMENT OF INFECTION IN CATTLE CHALLENGED WITH *T.VIVAX*-INFECTED TSETSE AFTER ADMINISTRATION OF 0.5mgkg^{-1} ISOMETAMIDIUM CHLORIDE

Animal	Trypanosoma vivax	Challenge: months after isometamidium chloride administration				
		1	2	3	4	5
G1	IL 2968[w]	-	-	-	-	-
G2	"	-	-	-	-	-
G3	"	-	-	11		
G4	"	-	-	-	11	
G5	"	-	-	11		
Control a+	"	10*	11	11	11	11
Control b+	"	10	11	9	11	9
H1	IL 2969[x]	-	7			
H2	"	-	9			
H3	"	12				
H4	"	23				
H5	"	24				
Control a+	"	10	11			
Control b+	"	9	11			

* Numbers correspond to the day following challenge when parasites were first detected.
\- Refractory to challenge.
\+ Different animals used for each challenge.
[w] Zaria T.vivax.
[x] Kilifi T.vivax.

parasitaemic following the first challenge with the Kilifi T.vivax, 2 had extended prepatent periods. The remaining 2 group H animals were both first detected parasitaemic following the second challenge and since one of them (H1) was first detected parasitaemic 7 days following the challenge, earlier than in any control, it is probable that the infection originated from the first challenge.

Twelve and twenty-four days following every challenge blood from cattle challenged with the Zaria T.vivax was inoculated into mice. (Blood from cattle challenged with the Kilifi T.vivax was not inoculated into mice since this population was not infective for mice.) Tables 3 and 4, appendix III, are comparisons of data resultant from examination of blood by the BC technique and mouse inoculation. In no animal did mouse inoculation result in the detection of parasites at an earlier time than their detection by the BC technique. In all cases where inoculation of cattle blood into mice produced mouse infections, trypanosomes were detected in the same blood sample by the BC technique.

In animals susceptible to challenge (both controls and drug-administered) the first peak of parasitaemia always occurred within 7 days following first detection of parasitaemia (see figures 1, 2 and 3, appendix III).

The level of parasitaemia at first peak of parasitaemia was 6+ (greater than 5×10^6 ml^{-1}), as defined

by Paris et al. (1982), in all challenge controls (both IL 2968 and IL 2969). The same level of first peak parasitaemia was attained in all 3 animals administered isometamidium chloride that were susceptible to challenge with the Zaria T.vivax. In contrast, the level of first peak parasitaemia in all 5 drug-administered cattle susceptible to challenge with the Kilifi T.vivax was 5+ (greater than $5x10^5$ ml^{-1} but less than $5x10^6$ ml^{-1}) (figure 3, appendix III).

In contrast to infections with both T.congolense ILNat 3.1 and T.congolense IL 2642 where persistent high levels of parasitaemia followed the first peak of parasitaemia, parasitaemia associated with either the Zaria T.vivax or the Kilifi T.vivax remitted within one to 4 days following the first peak. This finding is consistent with parasitaemia profiles of cattle intravenously infected with T.vivax ILDat 1.3 and T.vivax IL 2133 (Nantulya, Musoke and Moloo, 1986); populations belonging to the same trypanosome lineages as the Zaria T.vivax and the Kilifi T.vivax, respectively.

(b) Clinical data

Following the first challenge, skin thickness at the 10 tsetse bite sites, drainage lymph node breadth and temperature were measured daily in the four challenge controls. Changes in these 3 parameters, along with PCV and parasitaemia, over the 30 day observation period, are shown graphically for the Zaria T.vivax control (1a) and

the Kilifi T.vivax control (1a) in figures 1 and 2, appendix III, respectively. A summary of skin thickness and lymph node breadth measurements from the 4 controls is shown in table 5, appendix III. In contrast to the pronounced increase in skin thickness that occurred following tsetse-transmission of T.congolense ILNat 3.1 and T.congolense IL 2642, tsetse-transmission of the Zaria T.vivax and the Kilifi T.vivax resulted in only very slight changes. Fly-infection of 2 animals with the Zaria T.vivax resulted in a complete absence of skin thickness changes at all 10 sites in control 1b (D331) and only one "slight" (20-40%) increase in control 1a (D330). Exactly the same was observed with 2 animals fly-infected with the Kilifi T.vivax; control 1a (D332) developed only one "slight" increase in skin thickness and control 1b (D334) failed to develop any skin thickness changes . In all 4 animals the percentage increase in drainage prefemoral lymph node breadth ranged from 110% to 140%.

Since only very minor skin thickness changes occurred in all 1 month challenge controls following tsetse-transmission of both the Zaria T.vivax and the Kilifi T.vivax, skin thickness measurements were not made following subsequent challenges. However, because drainage lymphadenomegaly occurred in all 4 controls following the first challenge, drainage lymph node breadth measurements were continued in all experimental animals following all further challenges.

The percentage increase in prefemoral lymph node

breadth in cattle susceptible to the second, third, fourth or fifth challenge is shown in table 6, appendix III. Of the 3 group G animals susceptible to infection, all exhibited lymph node changes that differed by more than 2 standard deviations from the control mean. In one (G4), lymphadenomegaly was not detected.

Lymphadenomegaly in group H controls was less pronounced than that which occurred in group G controls. In both of the group H animals in which lymph node breadth measurements were made following successful challenge (H1 and H2), maximal lymphadenomegaly was less than the H control mean; in H1 it was greater than 2 standard deviations from the control mean.

Temperature measurements were taken from only controls following the first challenge. After all subsequent challenges, temperature measurements were taken from all experimental animals. Tables 7 and 8, appendix III, show a summary of the results. Of 811 temperature measurements taken during the entire course of the experiment, 326 were taken when animals were parasitaemic. Of these 326 measurements, 122 (37%) were in excess of 39.4°C. Subdivision of this last figure into cattle infected with the Zaria T.vivax and those infected with the Kilifi T.vivax showed that 41% of measurements taken when cattle were parasitaemic with the Zaria T.vivax were in excess of 39.4°C, whilst 31% were in excess of 39.4°C when cattle were parasitaemic with the Kilifi T.vivax. Insufficient

data was available to make any valid comparison between drug-administered animals and controls.

(c) Haematological data

In a similar manner to all experimental cattle in the T.congolense experiment, the first decline in PCV in all cattle susceptible to challenge with either the Zaria T.vivax or the Kilifi T.vivax coincided with the onset of parasitaemia. Packed cell volume and parasitaemia data for a group G control, a group H control and 3 group H animals, all susceptible to the first challenge, are shown graphically in figures 1, 2 and 3, appendix III, respectively.

Both the Zaria T.vivax (IL 2968) and the Kilifi T.vivax (IL 2969) are derived from populations which self-cure in cattle 8 to 12 weeks after first detection of parasitaemia (Barry, 1986; Nantulya et al., 1986). Furthermore, parental populations of both T.vivax IL 2968 and T.vivax IL 2969 have also been observed to self-cure in cattle (P.R. Gardiner, personal communication). Thus, unlike T.congolense ILNat 3.1 and T.congolense IL 2642, because of the frequent occurrence of self-cure, no valid comparisons of the pathogenicity of the two T.vivax populations (with regard to rate of PCV decline) could be made.

(d) Immunological data

(i) Indirect immunofluorescent antibody (IFA) test

Data from IFA tests using Zaria T.vivax (IL 2968) antigen and sera from cattle challenged with the Zaria T.vivax is shown in tables 25 and 26. Table 27 shows the results of similar tests using Kilifi T.vivax (IL 2969) antigen and sera from cattle challenged with the Kilifi T.vivax.

In no animal was antibody detected until following the onset of parasitaemia. One exception to this was H5 which although not detected parasitaemic until day 24 following the first challenge, sera collected on day 21 produced strong flagellar staining at a titre of 1:10.

Although challenge controls for groups G and H were all detected parasitaemic by 11 days following challenge, indirect immunofluorescent antibody was detected in day 14 sera from only 2 animals. However, indirect immunofluorescent antibody was detected in day 21 sera from all animals.

Only 2 animals in group G (G1 and G2) were refractory to all 5 challenges. Examination of sera collected from both animals during the entire course of the experiment failed to reveal the presence of indirect immunofluorescent antibody in any sample.

Finally, incubation of day 28 sera from Zaria T.vivax controls with Kilifi T.vivax antigen, and day 28 sera from Kilifi T.vivax controls with Zaria T.vivax antigen (i.e. heterologous antigen) failed to produce any staining,

Table 25

IFA TEST USING *T.VIVAX* IL 2968 ANTIGEN AND SERA FROM GROUP G CATTLE (CHALLENGED WITH *T.VIVAX* IL 2968)

	1 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	-	-	-	-	-
G2	-	-	-	-	-
G3	-	-	-	-	-
G4	-	-	-	-	-
G5	-	-	-	-	-
CONTROLS					
a	-	-	-	1:50	1:250
b	-	-	1:10	1:50	1:50

	2 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	-	-	-	-	-
G2	-	-	-	-	-
G3	-	-	-	-	-
G4	-	-	-	-	-
G5	-	-	-	-	-
CONTROLS					
a	-	-	-	1:250	1:250
b	-	-	-	1:50	1:50

Table 26

IFA TEST USING *T.VIVAX* IL 2968 ANTIGEN AND SERA FROM GROUP G CATTLE (CHALLENGED WITH *T.VIVAX* IL 2968)

	3 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	-	-	-	-	-
G2	-	-	-	-	-
G3	-	-	-	1:250	1:250
G4	-	-	-	-	-
G5	-	-	-	1:50	1:250
CONTROLS					
a	-	-	1:10	1:250	1:250
b	-	-	-	1:250	1:250

	4 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	-	-	-	-	-
G2	-	-	-	-	-
G4	-	-	1:10	1:50	1:50
CONTROLS					
a	-	-	-	1:50	1:250
b	-	-	-	1:50	1:250

	5 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	-	-	-	-	-
G2	-	-	-	-	-
CONTROLS					
a	-	-	-	1:50	1:250
b	-	-	-	1:50	1:250

Table 27

IFA TEST USING *T.VIVAX* IL 2969 ANTIGEN AND SERA FROM GROUP H CATTLE (CHALLENGED WITH *T.VIVAX* IL 2969)

	1 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
H1	-	-	-	-	-
H2	-	-	-	-	-
H3	-	-	-	-	1:10
H4	-	-	-	-	1:50
H5	-	-	-	1:10*	1:10*
CONTROLS					
a	-	-	-	1:50	1:50
b	-	-	-	1:50	1:50

	2 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
H1	-	-	1:50	1:50	1:50
H2	-	-	-	-	1:10
H3	1:10	1:50			
H4	1:50	1:50			
H5	1:10*	1:10*			
CONTROLS					
a	-	-	-	1:250	1:50
b	-	-	-	1:50	1:250

* = strong flagellar staining.

thereby indicating the absence of isoVATs in cattle bloodstream populations occurring within 4 weeks following tsetse-transmission of the Zaria T.vivax and the Kilifi T.vivax.

(ii) Trypanolysis test

Trypanolysis data resulting from analyses on sera from cattle challenged with the Zaria T.vivax (IL 2968) is shown in tables 9a and 9b, appendix III. Data resulting from analyses of sera from cattle challenged with the Kilifi T.vivax (IL 2969) is shown in table 10, appendix III. Background levels of lysis are indicated by the percentage lysis occurring in control samples. In tables 9a and 9b, appendix III, the figures represent the number of trypanosomes lysed out of 100 counted. Since the Kilifi T.vivax did not attain as high a level parasitaemia in cattle as the Zaria T.vivax, the data in table 10, appendix III, is the number of trypanosomes lysed out of 50 counted.

In all instances, greater than 20% lysis only occurred in sera collected subsequent to the onset of parasitaemia in an animal.

Analyses of all sera collected from G1 and G2 (group G animals refractory to all 5 challenges) failed to reveal the presence of trypanolytic antibody in any sample.

(e) Screening of breakthrough trypanosome populations for their sensitivity to isometamidium

Since the Kilifi T.vivax was non-infective for mice,

isometamidium sensitivity studies were only conducted on trypanosome populations arising in cattle challenged with the Zaria T.vivax (i.e., group G animals).

Two drug-administered animals in group G became infected following the third challenge and one following the fourth challenge. Results from drug sensitivity studies conducted in mice on the 3 breakthrough trypanosome populations, as well as on populations occurring in the respective controls, are shown in table 11, appendix III. Trypanosome populations arising in cattle administered isometamidium chloride did not appear to express a decreased level of sensitivity to the therapeutic activity of isometamidium as compared to populations from challenge controls.

In a similar manner to isometamidium sensitivity experiments conducted with T.congolense populations arising in cattle in the T.congolense experiment, treatment of mice when parasitaemia attained 10^7 trypanosomes per ml, as compared to 6 hours post-infection, resulted in an apparent slight decrease in the drug-sensitivity of each trypanosome population.

EXPERIMENT 1

PART B

INTRODUCTION

In the T.congolense experiment described in chapter 1 it was shown that a single intramuscular injection of 0.5mg kg^{-1} isometamidium chloride conferred complete protection to cattle for at least 3 months against repeated monthly challenge with tsetse-transmitted T.congolense ILNat 3.1.

Since the group H cattle (challenged with the Kilifi T.vivax) became infected following the 1 or 2 month challenges, it was decided to rechallenge them with tsetse-transmitted T.congolense ILNat 3.1, three months after administration of isometamidium chloride. If the cattle were refractory to challenge this would indicate that they had not been underdosed with isometamidium chloride, and that the Kilifi T.vivax expressed a much higher level of resistance to the prophylactic activity of isometamidium than T.congolense ILNat 3.1.

EXPERIMENTAL DESIGN

All 5 group H animals were treated with 3.5mg kg^{-1} diminazene aceturate 12 days following the second Kilifi T.vivax challenge. Diminazene aceturate is a curative trypanocide with negligable prophylactic activity (Fairclough, 1963b; Van Hoeve and Cunningham, 1964a,b). Three uninfected cattle to which isometamidium chloride had

not been administered and 2 cattle with primary infections with the same T.vivax clone were also administered diminazene aceturate to serve as controls. A sixth control, non-infected and not administered diminazene aceturate, was also included (table 28). Eighteen days following diminazene aceturate treatment (3 months after administration of isometamidium chloride) all cattle were challenged with tsetse-transmitted T.congolense ILNat 3.1 (5 infective flies per animal).

RESULTS

In contrast to the 6 controls, all of which were detected parasitaemic (using the BC technique) by 17 days post-challenge, none of the 5 cattle previously susceptible to challenge with the Kilifi T.vivax were observed parasitaemic during the 100-day post-challenge observation period (table 28). Furthermore, inoculation of mice with blood taken from cattle 12 and 24 days following challenge with T.congolense ILNat 3.1 failed to reveal the presence of parasitaemia in cattle that was not detected by the BC technique. Mice only became parasitaemic if inoculated with blood detected parasitaemic by the BC technique (see table 12, appendix III).

Finally, following challenge of cattle with T.congolense ILNat 3.1, measurement of the skin thickness at the 5 sites at which tsetse had fed and the drainage lymph node breadth was conducted daily. In contrast to all 6 controls which developed pronounced skin thickness

Table 28

DEVELOPMENT OF *T.CONGOLENSE* ILNat 3.1 INFECTION IN CATTLE PREVIOUSLY SUSCEPTIBLE TO CHALLENGE WITH *T.VIVAX* IL 2969

	Days following Samorin administration								
Animal	0	32	47	60	74	75	93	110	193
H1	Samorin	T.v	-	T.v	+	Berenil	T.c	-	-
H2	Samorin	T.v	-	T.v	+	Berenil	T.c	-	-
H3	Samorin	T.v	+	+	+	Berenil	T.c	-	-
H4	Samorin	T.v	+	+	+	Berenil	T.c	-	-
H5	Samorin	T.v	+	+	+	Berenil	T.c	-	-
Control 1		T.v	+	+	+	Berenil	T.c	+	
2		T.v	+	+	+	Berenil	T.c	+	
3						Berenil	T.c	+	
4						Berenil	T.c	+	
5						Berenil	T.c	+	
6							T.c	+	

Samorin = 0.5mg kg^{-1} isometamidium chloride administered.
Berenil = 3.5mg kg^{-1} diminazene aceturate administered.
T.v = challenge by 10 tsetse infected with T.vivax IL 2969 (Kilifi, Kenya).
T.c = challenge by 5 tsetse infected with T.congolense ILNat 3.1.
+ = parasitaemia.
- = no parasitaemia.

changes and pronounced lymphadenomegaly, 20-40% skin thickness changes occurred in only 2 group H animals and lymphadenomegaly was not detected in any animal (see table 13, appendix III).

EXPERIMENT 2

STUDIES USING *T.VIVAX* IL 2982 (GALANA) AND *T.VIVAX* IL 2986 (LIKONI)

INTRODUCTION

In T.vivax experiment 1, part A, a cloned T.vivax population from Kilifi, Kenya (IL 2969) was shown to be sensitive to the therapeutic activity of isometamidium but resistant to the prophylactic activity of the drug, as defined in the field. In an attempt to ascertain whether or not this finding was restricted to one East African T.vivax population, two other T.vivax populations originating from East Africa were examined in an identical manner to T.vivax experiment 1, part A. Two uncloned populations were used; T.vivax IL 2982 (a derivative of an isolate made in Galana, Kenya) and T.vivax IL 2986 (a derivative of an isolate made in Likoni, Kenya).

EXPERIMENTAL DESIGN

1) DETERMINATION OF THE SENSITIVITY OF THE GALANA *T.VIVAX* AND THE LIKONI *T.VIVAX* TO THE THERAPEUTIC ACTIVITY OF ISOMETAMIDIUM

Two groups of 5 cattle were infected, each with one of the two T.vivax populations, using 10 infected G.m.centralis per animal. Eleven days later, when all cattle had a parasitaemia of approximately 5×10^4 ml^{-1}, all were treated with 0.5mg kg^{-1} isometamidium chloride. For a

200-day period following treatment jugular blood was collected from each animal three times weekly and examined for the presence of trypanosomes using the BC technique.

2) DETERMINATION OF THE SENSITIVITY OF THE GALANA *T.VIVAX* AND THE LIKONI *T.VIVAX* TO THE PROPHYLACTIC ACTIVITY OF ISOMETAMIDIUM

To determine the duration of prophylaxis isometamidium conferred against the Galana T.vivax and the Likoni T.vivax, 0.5mg kg^{-1} isometamidium chloride was administered to 10 Boran steers. Thereafter, cattle were challenged at monthly intervals using G.m.centralis infected with either the Galana or the Likoni T.vivax (5 cattle per population, 10 infected flies per animal) (see table 29). At each challenge one control animal for each group, to which isometamidium chloride had not been administered, was challenged in a similar manner.

Table 29

T.VIVAX CATTLE EXPERIMENT 2 - EXPERIMENTAL DESIGN

GROUP OF CATTLE	DOSAGE OF ISOMETAMIDIUM CHLORIDE ($mgkg^{-1}$)	TRYPANOSOMA VIVAX POPULATION	CHALLENGE: MONTHS AFTER ISOMETAMIDIUM CHLORIDE ADMINISTRATION					FORM OF CHALLENGE
			1	2	3	4	5	
J	0.5	IL 2982*	X	X	X	X	X	ten infected tsetse
K	0.5	IL 2986+	X	X	X	X	X	ten infected tsetse

* Galana T.vivax (Kenya)

\+ Likoni T.vivax (Kenya)

RESULTS

1) DETERMINATION OF THE SENSITIVITY OF THE GALANA *T.VIVAX* AND THE LIKONI *T.VIVAX* TO THE THERAPEUTIC ACTIVITY OF ISOMETAMIDIUM CHLORIDE AT A DOSAGE OF 0.5mg kg^{-1}

Of 5 animals infected with the Galana T.vivax and 5 infected with the Likoni T.vivax, parasitaemia was not detected in any animal on any occassion during the 200-day post-treatment observation period. Thus, infections in all animals appeared to be cured with a dosage of 0.5mg kg^{-1} isometamidium chloride.

2) DETERMINATION OF THE SENSITIVITY OF THE GALANA *T.VIVAX* AND THE LIKONI *T.VIVAX* TO THE PROPHYLACTIC ACTIVITY OF ISOMETAMIDIUM

The time of appearance of trypanosomes in experimental animals, following each challenge, is shown in table 30. All 5 drug-administered animals challenged with the Galana T.vivax became infected following the first challenge; four with parasitaemia prepatent periods identical to the control and one with a parasitaemia prepatent period 5 days longer than the control. The haemorrhagic syndrome, characterised by a rapid and progressive decrease in PCV and death (produced by the parental population of the Galana T.vivax (IL 2982) (P.R. Gardiner, personal communication) was not observed in any group J animal. By approximately 30 days post-infection PCV values in all animals had declined to about 20%. However, after day 30, PCV values in all 6 animals began to gradually increase,

Table 30

DEVELOPMENT OF INFECTION IN CATTLE CHALLENGED WITH *T.VIVAX*-INFECTED TSETSE AFTER ADMINISTRATION OF 0.5mgkg^{-1} ISOMETAMIDIUM CHLORIDE

ANIMAL	TRYPANOSOMA VIVAX	CHALLENGE: MONTHS AFTER ISOMETAMIDIUM CHLORIDE ADMINISTRATION				
		1	2	3	4	5
J1	IL 2982[y]	9*				
J2	"	9				
J3	"	9				
J4	"	14				
J5	"	9				
CONTROL	"	9				
K1	IL 2986[z]	-	12			
K2	"	-	12			
K3	"	-	12			
K4	"	-	12			
K5	"	-	14			
CONTROL+	"	11	12			

* Numbers correspond to the day following challenge when parasites were first detected.
\- Refractory to challenge.
\+ Different animals used for each challenge.
[y] Galana T.vivax.
[z] Likoni T.vivax.

indicating that all were self-curing.

Of the 5 animals administered isometamidium chloride and challenged with the Likoni T.vivax, all were refractory to the first challenge. However, all became infected following the second challenge; 4 with parasitaemia prepatent periods identical to the control and one 2 days longer than the control.

DISCUSSION

With apparently "sensitive" trypanosome populations of both T.congolense and T.vivax, 0.5mg kg^{-1} isometamidium chloride can be expected to confer complete protection for at least 12 weeks (Fairclough, 1963a; Chapter 1). The early occurrence of breakthrough infections following drug administration has generally been taken as evidence of resistance to isometamidium (Jones-Davis and Folkers, 1966a; Na'Isa, 1967; Bourn and Scott, 1978; Pinder and Authie, 1984).

Following treatment of infections with 0.5mg kg^{-1} isometamidium chloride, no relapses occurred in 5 cattle infected with the Kilifi T.vivax (IL 2969) and in only one out of 5 infected with the Zaria T.vivax (IL 2968). Thus, neither clone appeared to show evidence for the expression of marked resistance to isometamidium's therapeutic activity. However, prophylactic administration of 0.5mg kg^{-1} isometamidium chloride afforded cattle less than one month's complete protection against challenge with the Kilifi T.vivax. Hence, the Kilifi T.vivax appears to be resistant to the prophylactic activity of the drug, although sensitive to the therapeutic activity of the drug. It would also appear that since the 5 cattle, susceptible to Kilifi T.vivax challenge, were still refractory to challenge with T.congolense ILNat 3.1 three months after administration of isometamidium chloride, the susceptibility was not due to absence of drug activity in the animals. Further evidence for this conclusion comes

from studies *in vitro* (see chapter 4).

These findings provide corroborative evidence for the expression of a low level of sensitivity to the prophylactic activity of isometamidium by the Kilifi *T.vivax*, in contrast to the high level of sensitivity expressed by both *T.congolense* populations investigated in the cattle experiment described in chapter 1.

In a similar manner to the Kilifi *T.vivax*, the Galana *T.vivax* (IL 2982) has also been shown to be sensitive to the therapeutic activity of isometamidium but resistant to the prophylactic activity of the drug.

The Likoni *T.vivax* (IL 2986) has also been shown to be sensitive to the therapeutic activity of isometamidium. However, the level of resistance expressed against the prophylactic activity of isometamidium was less than that expressed by the Kilifi *T.vivax* and the Galana *T.vivax*, but greater than that expressed by *T.congolense* ILNat 3.1 and the Zaria *T.vivax*.

In a similar manner to studies conducted with *T.congolense*, antibody was not detected in any animal refractory to challenge with the Zaria *T.vivax* or the Kilifi *T.vivax*, thereby indicating that prophylaxis was entirely mediated by the action of drug. Furthermore, rodent-infective breakthrough populations occurring in drug-administered cattle did not appear to express a decreased level of sensitivity to the therapeutic activity of isometamidium in mice, when compared to trypanosome populations from controls.

In a similar manner to the Zaria T.vivax and the Kilifi T.vivax, tsetse-transmission of a T.vivax stock from Likoni, Kenya, to a cow, failed to produce a detectable skin reaction (Dwinger, Grootenhuis, Murray, Moloo and Gettinby, 1986a). The absence of skin reactions may be associated with rapid migration (within a few days) of parasites away from the skin (Emery et al., 1980b). In so doing, localised trypanosome multiplication to numbers required to elicit a skin reaction would not, presumably, occur.

Temperature measurements made during the T.congolense cattle experiment showed that of measurements made when cattle were parasitaemic, 13.9% were in excess of 39.4°C when parasitaemic with T.congolense ILNat 3.1, and 20.6% were in excess of 39.4°C when parasitaemic with T.congolense IL 2642. In contrast, of the temperature measurements made when animals were parasitaemic with T.vivax, 41% were in excess of 39.4°C when infected with the Zaria T.vivax and 31% in excess of 39.4°C when infected with the Kilifi T.vivax. Losos and Ikede (1972) stated that both T.congolense and T.vivax reside only within the blood circulation. However, the presence of T.vivax within extravascular sites, such as the myocardium of cattle (Van den Ingh and De Neijs-Bakker, 1979), and within the eye (Hornby, 1952; Ilemobade and Schilhorn van Veen, 1974; D.D. Whitelaw, personal communication) suggests that the parasite may invade certain tissues. The release of inflammatory mediators in association with tissue invasion

may account for the more frequent occurrence of pyrexia in animals infected with T.vivax.

CHAPTER 4

IN VITRO EXAMINATION OF SERA FROM *T.VIVAX* EXPERIMENT 1 CATTLE, AND STUDIES WITH *T.VIVAX IN VITRO*

INTRODUCTION

In chapter 3, the susceptibility of all 5 group H cattle to challenge with the Kilifi T.vivax (IL 2969) one and 2 months after administration of 0.5mg kg^{-1} isometamidium chloride (T.vivax experiment 1, part A) suggested that the population was resistant to the prophylactic activity of the drug.

In an attempt to ascertain whether or not trypanocidal activity, associated with the administration of isometamidium chloride, could be demonstrated in vitro in the sera of group H infected cattle, serum samples from all 5 animals were incubated in vitro with T.congolense ILNat 3.1 bloodstream forms to examine for (a) their growth inhibitory effect after 24, 48 and 72 hours incubation, and (b) their effect on mouse infectivity after 24 hours incubation.

Maintenance of T.vivax in vitro has proven more difficult than T.congolense. Bloodstream forms cultured within tsetse tissues were shown to be infective for sheep after 39 days culture (Trager, 1959) and transformation to epimastigote forms using similar culture conditions has also been described (Trager, 1975). Brun and Moloo (1982) maintained animal infective forms of T.vivax in culture for 3 months using a Microtus montanus fibroblast-like (MEF) feeder-layer using MEM medium with Earle's salts, supplemented with 20% heat-inactivated goat serum. ILRAD (1984) has developed a culture system using bovine

fibroblasts in MEM, and Matrix Green Gel A beads, for complete cyclical development of T.vivax.

Using similar culture conditions to that described by Brun and Moloo (1982), Waithaka et al. (1985) described a technique for screening the activity of drugs on T.vivax second generation bloodstream forms (bloodstream forms produced after cyclical transformation in vitro). A similar technique has been used in this chapter for screening for trypanocidal activity in serum from cattle administered isometamidium chloride, except that freshly isolated bovine and murine bloodstream forms were used.

MATERIALS AND METHODS

SCREENING OF SERA FROM CALVES ADMINISTERED 0.25mg kg^{-1} ISOMETAMIDIUM CHLORIDE WITH BOVINE AND RODENT-DERIVED ZARIA *T.VIVAX* (IL 2968) BLOODSTREAM FORMS

24 Hour Incubation

These experiments were set up in a similar manner to that described in section 3(a) of the *in vitro* general materials and methods, except that MEF cell monolayers were used and the medium into which trypanosomes were added consisted of 20% (v/v) serum, 1% (v/v) L-Glutamine (29.2mg ml^{-1} MEM medium) and 79% (v/v) MEM medium.

All other techniques utilised in this chapter have been described in the general materials and methods section.

RESULTS

1) PART A -

SCREENING OF SERA FROM CATTLE IN *T.VIVAX* EXPERIMENT 1

Three group H animals (H3, H4 and H5) became parasitaemic following the first challenge. Four serum samples from each animal were examined in vitro for their trypanocidal activity; those collected 0, 32, 39 and 46 days after treatment. Day 0 sera were used as controls for other sera from the same animal. Growth inhibition produced by each sample is shown in table 31. All 9 post-drug-administration sera inhibited trypanosome growth after 48 and 72 hours incubation and only one of the 9 sera (H4, day 39) failed to do so after 24 hours incubation. All 9 sera produced greater growth inhibition when the incubation period was increased from 24 to 48 hours. Incubation for 72 hours further accentuated growth inhibition in all but one serum sample; H3, day 39.

The number of trypanosomes remaining after each period of incubation is shown graphically for H4 in figure 2. In a similar manner to sera from H3 and H5, trypanosome numbers remaining in all sera after 48 hours incubation were lower than the initial well inoculum. In addition, numbers remaining after 72 hours incubation were always lower than after 48 hours. Since the described loss of trypanosome viability also occurred in day 0 sera it would indicate that a "die out" occurred in all trypanosome populations. A similar pattern in loss of trypanosome viability occurred in sera collected on days 0, 60, 67 and

Table 31

TRYPANOSOME NUMBERS AND GROWTH INHIBITION OF T.CONGOLENSE ILNat 3.1 BLOODSTREAM FORMS AFTER 24, 48 AND 72 HOUR INCUBATIONS IN SERA FROM GROUP H CATTLE

		H3						H4						H5					
	INCUBATION:	24 HOURS		48 HOURS		72 HOURS		24 HOURS		48 HOURS		72 HOURS		24 HOURS		48 HOURS		72 HOURS	
SAMPLE	DAY	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
1	0	129.8*	18.9	55.0	5.9	10.1	4.1	128	11.6	69.8	13.6	22.5	5.1	79.8	10.2	47.5	7.7	5.5	2.3
2	32	67.9	8.4	20.5	6.8	2.4	1.1	117	18.3	49.3	6.3	4.8	1.0	55.3	8.7	12.4	4.0	0.7	1.2
3	39	86.0	13.4	24.0	9.4	4.8	2.2	152	11.6	55.5	6.8	6.8	2.1	53.5	10.3	11.3	3.1	0.8	0.8
4	46	94.0	22.9	35.3	5.8	3.4	1.4	91.3	8.3	23.0	8.5	3.3	1.9	40.3	6.3	8.8	5.1	0.2	0.4
PERCENTAGE GROWTH INHIBITION																			
1	0	0		0		0		0		0		0		0		0		0	
2	32	47.7		62.7		76.5		8.4		29.4		78.9		30.7		73.9		86.4	
3	39	33.7		56.4		53.1		-18.8		20.4		70.0		32.9		76.3		84.0	
4	46	27.6		35.9		66.6		28.7		67.0		85.6		49.5		81.2		95.5	

* Figures = Number of trypanosomes x 10^4 ml $^{-1}$

FIGURE 2

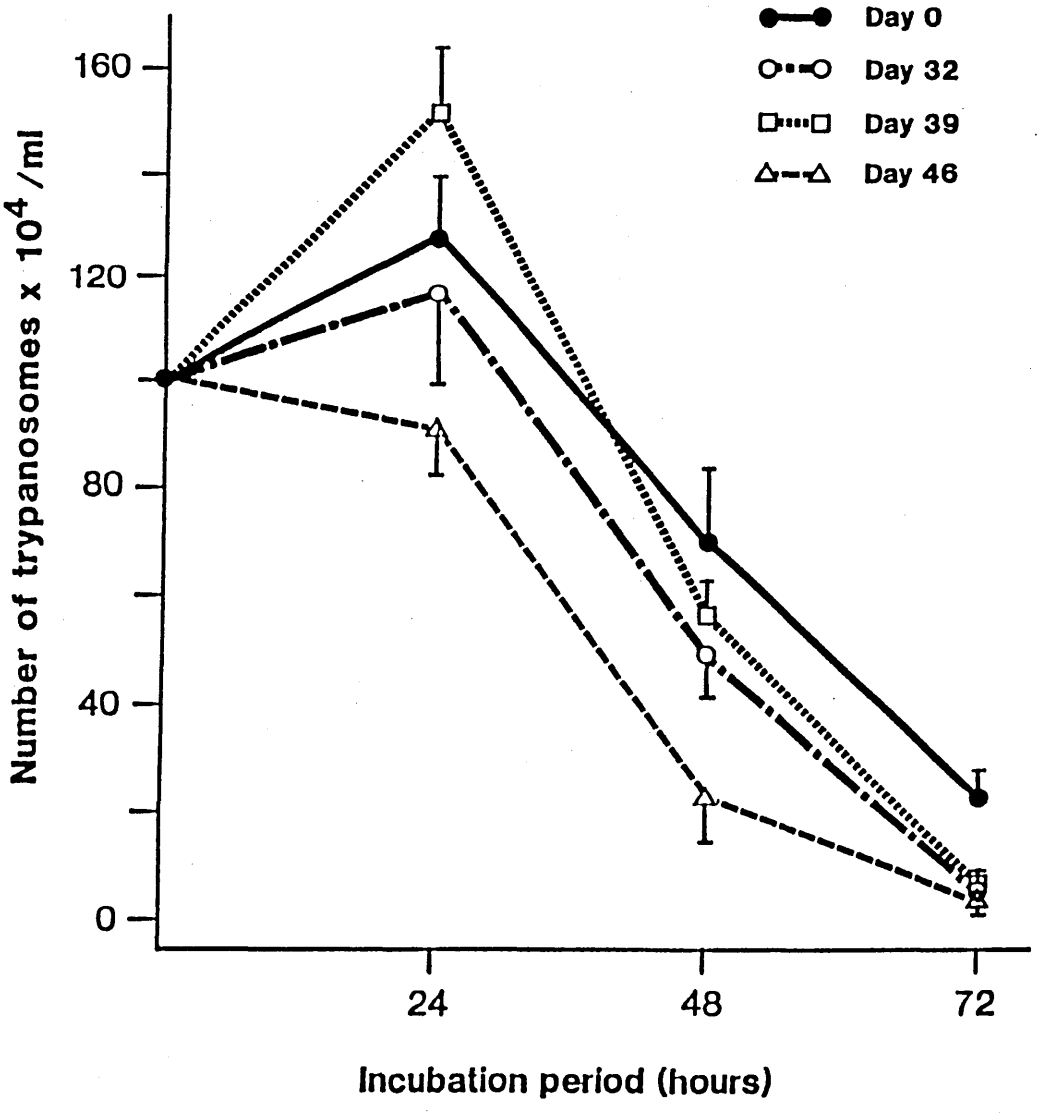

Figure 2: In vitro incubation of sera from bovine H4 with T.congolense ILNat 3.1 bloodstream forms; trypanosome numbers remaining after 24, 48 and 72 hours incubation.

74 from H1 and H2; group H animals first detected parasitaemic following the second (day 60) challenge (see table 1, appendix IV).

Although trypanosome numbers remaining in day 0 sera from all 5 group H animals decreased with time, in all cases the number of trypanosomes remaining in day 0 sera after 72 hours incubation was always greater than numbers remaining in post-drug-administration sera. However, differences in the growth inhibition produced after 72 hours incubation in post-drug-administration sera as opposed to pre-drug-administration sera were not pronounced because of the inherent statistical inaccuracy of the neubauer chamber counting method at low trypanosome numbers (Dacie and Lewis, 1970). Even so, on a growth inhibition basis, there were indications for the presence of trypanocidal activity in all post-drug-administration sera collected from all group H animals at a time when they were infected with the Kilifi T.vivax.

Further evidence for the presence of trypanocide in the same post-drug-administration sera was derived from mouse infectivity studies conducted with trypanosome populations (T.congolense ILNat 3.1) after 24 hours incubation in the sera (see table 32). All mice inoculated with trypanosomes incubated in day 0 sera from all group H animals became infected. In contrast, when inoculated with trypanosomes incubated in day 32, day 39 or day 46 sera from H3, H4 and H5, no mouse became parasitaemic. An exception to this generalisation was the day 32 serum

Table 32

MURINE INFECTIVITY OF T.CONGOLENSE ILNat 3.1 AFTER 24 HOURS INCUBATION IN SERA FROM CATTLE CHALLENGED WITH EITHER T.VIVAX IL 2968 OR T.VIVAX IL 2969

	DAY FOLLOWING ADMINISTRATION OF 0.5mgkg^{-1} ISOMETAMIDIUM CHLORIDE											
ANIMAL	0	7	14	21	28	32	39	46	60	67	74	91
G1	5/5*											0/5^{R}
G2	5/5											0/5^{R}
G3	5/5											5/5^{S}
G5	5/5											5/5^{S}
H1	8/8					0/5^{R}	0/5	0/5	0/4^{S}	0/5	1/5	
H2	5/5								0/5^{S}	0/5	1/5	
H3	10/10	0/4	0/5	0/5	0/5	0/10^{S}	0/10	0/10				
H4	5/5					0/5^{S}	0/4	0/4				
H5	10/10					5/10^{S}	0/9	0/10				

*Data = Number of mice parasitaemic / Number of mice inoculated.

R = Refractory to challenge.

s = Susceptible to challenge.

G = Monthly tsetse-challenge with T.vivax IL 2968.

H = Monthly tsetse-challenge with T.vivax IL 2969.

sample from H5 which partially inhibited trypanosome infectivity (5/10 mice became infected). H3, H4 and H5 were all susceptible to challenge with the Kilifi T.vivax on day 32.

In a similar manner, sera collected from H1 and H2 on days 60, 67 and 74, inhibited trypanosome infectivity. H1 and H2 were first observed parasitaemic 7 and 9 days, respectively, following challenge with the Kilifi T.vivax on day 60.

Thus, all sera collected from all 5 group H animals when infected with the Kilifi T.vivax (IL 2969) inhibited infectivity of T.congolense ILNat 3.1 for mice. The presence of trypanocidal activity in all these sera was therefore confirmed.

Only day 0 and day 91 sera from group G animals were analysed in vitro for their effect on T.congolense ILNat 3.1 infectivity (table 32). Day 0 samples from all 4 animals examined failed to inhibit trypanosome infectivity. Day 91 sera from G1 and G2 inhibited infectivity whilst day 91 sera from G3 and G5 had no apparent effect on infectivity. G1 and G2 were refractory to challenge on day 91 with the Zaria T.vivax whilst G3 and G5 were susceptible to challenge on the same day with the same population.

2) PART B -

IN VITRO SCREENING OF SERA FROM CALVES X1, X2 AND X3 FOR TRYPANOCIDAL EFFECTS ON ZARIA *T.VIVAX* (IL 2968) BLOODSTREAM FORMS OF BOVINE AND MURINE ORIGIN

(See chapter 2 for T.congolense in vitro experiments conducted with sera from calves X1, X2 and X3.)

(a) Bovine-derived *T.vivax* IL 2968

Using bovine-derived T.vivax IL 2968 bloodstream forms, sera collected on day 0 through to day 49 from X1, X2 and X3 were screened for their effect on trypanosome infectivity and growth after 24 hours incubation. The results from these experiments are shown in table 33.

(i) Growth Inhibition

Nine post-drug-administration sera from each animal were examined. In many samples, trypanosomes grew better than in control sera; 8 from X1, 5 from X2 and 6 from X3. In addition, growth inhibition produced by sera from each animal varied widely from one sample to the next (see table 33 and figure 3); to a much greater extent than did T.congolense ILNat 3.1 bloodstream forms after the same incubation period in the same sera (see table 21).

(ii) Mouse infectivity

All mice inoculated with T.congolense ILNat 3.1 bloodstream forms incubated for 24 hours in day 0 sera,

Table 33

INFECTIVITY AND PERCENTAGE GROWTH INHIBITION OF BOVINE-DERIVED T.VIVAX IL 2968 BLOODSTREAM FORMS EXPOSED FOR 24 HOURS TO SERA FROM CALVES ADMINISTERED 0.25mgkg^{-1} ISOMETAMIDIUM CHLORIDE

	DAY									
ANIMAL	0	1	3	7	14	21	28	35	42	49
X1[a]	0	-21.4	-42.1	-1.3	-10.7	-6.3	2.5	-15.1	-69.2	-20.1
X2[a]	0	-78.4	-168	-68.6	10.2	38.1	-58.9	4.2	-102	3.4
X3[a]	0	0.8	36.5	-50.8	-33.3	-16.7	-50.8	-29.4	-67.5	22.2
X1[b]	0/5	0/5	0/5	0/5	0/5	3/5	1/5	2/5	5/5	5/5
X2[b]	0/5	5/5	0/5	1/5	0/4	1/5	1/5	0/5	5/5	0/5
X3[b]	3/5	0/5	0/5+	0/5	0/5	1/5	5/5	5/5	5/5	1/5+

a = % growth inhibition.

b = infectivity for mice;

data = no. mice parasitaemic / no. mice inoculated (inoculum = 1 x 10^5 trypanosomes per mouse).

+ inoculum per mouse = 5 x 10^4 trypanosomes.

FIGURE 3

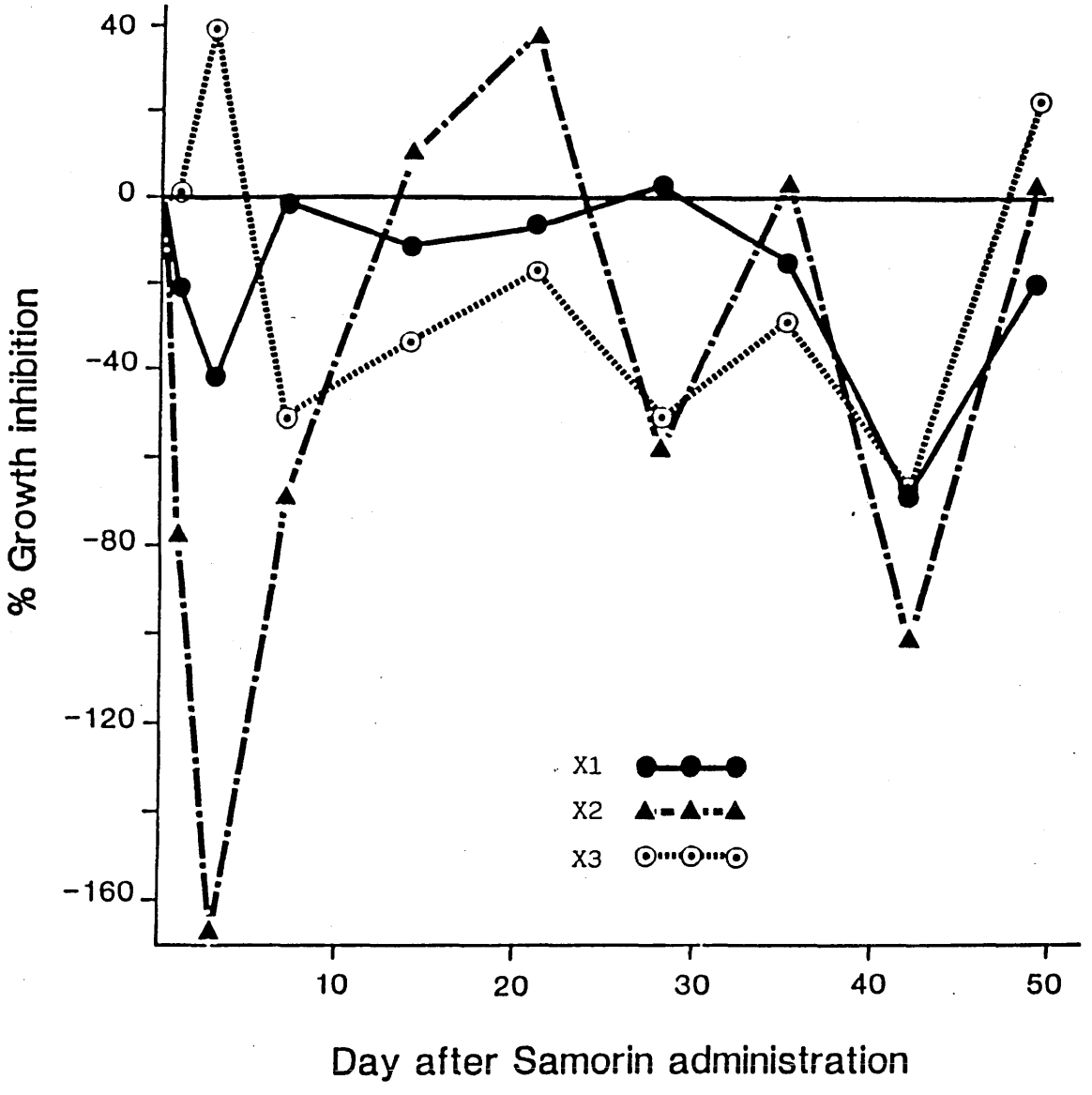

Figure 3: 24 hour in vitro incubation of bovine-derived T.vivax IL 2968 in 20% serum from Boran cattle administered 0.25mgkg^{-1} isometamidium chloride; percentage growth inhibition.

from each calf, became infected (see table 21). In contrast, day 0 sera from X1 and X2 completely inhibited infectivity of T.vivax IL 2968. Furthermore, the mouse infectivity of trypanosomes incubated in all 3 animals' sets of sera appeared highly inconsistent, e.g., day 35 and day 49 sera from X2 completely inhibited trypanosome infectivity whilst that collected on day 42 from the same animal had no apparent effect on infectivity.

(b) Murine-derived *T.vivax* IL 2968

Since all sets of data, in table 33, regarding growth inhibition and mouse infectivity appeared highly variable from one animals' sample to the next, sera from X3 was analysed again, in an identical manner, using murine-derived T.vivax IL 2968 bloodstream forms. (Murine-derived T.vivax IL 2968 appears to exhibit a higher level of infectivity for rodents than the same trypanosome population derived from cattle (personal observation)). Table 2, appendix IV, shows the resultant data.

Growth inhibition followed an approximately similar pattern to that previously described for bovine-derived bloodstream forms of T.vivax IL 2968.

Serum collected on days 0, 28, 35, 42 and 49 had no apparent effect on trypanosome infectivity whilst that collected on days 1, 3, 7 and 14 completely inhibited infectivity. Rodent infectivity data thus followed a more logical and consistent progression from week to week when murine-derived rather than bovine-derived trypanosomes were

used. The infectivity of murine-derived T.vivax IL 2968 after exposure for 24 hours to sera from calf X3 was almost identical to the infectivity of T.congolense ILNat 3.1 after 24 hours incubation in the same sera (compare table 21 with table 2, appendix IV).

DISCUSSION

Studies have been conducted *in vitro* with sera from cattle in the first *T.vivax* experiment to ascertain whether or not trypanocidal activity, associated with the administration of isometamidium chloride, could be detected.

All 5 animals in group H were susceptible to challenge with the Kilifi *T.vivax* either 32 or 60 days following administration of 0.5mg kg^{-1} isometamidium chloride, and all sera collected from these animals from day 32 to day 74 (when infected with the Kilifi *T.vivax*) inhibited the infectivity of *T.congolense* ILNat 3.1 after 24 hours incubation *in vitro*. The same sera, when compared to pre-drug-administration sera, also exerted growth inhibitory effects on *T.congolense* ILNat 3.1 which, although not pronounced, became greater as the incubation period was extended from 48 to 72 hours.

Studies with sera from 4 group G cattle showed a complete correlation between *in vitro* inhibition of *T.congolense* ILNat 3.1 infectivity and susceptibility of the 4 animals to challenge with *T.vivax* IL 2968 on day 91; (a) day 91 sera from G1 and G2 completely inhibited trypanosome infectivity and both were refractory to challenge on day 91, (b) day 91 sera from G3 and G5 did not inhibit trypanosome infectivity and both were susceptible to challenge on day 91.

In contrast to *T.congolense*, *T.vivax* appears to be

much more sensitive to the growth supportive properties of individual sera (R.T.Nelson, personal communication). This may account for the great range in growth inhibition of both bovine and murine-derived Zaria T.vivax (IL 2968) bloodstream forms when incubated in sets of weekly collected sera from 3 calves administered 0.25mg kg^{-1} isometamidium chloride. Inhibitory effects on infectivity for mice appeared almost identical to murine-derived T.congolense ILNat 3.1 bloodstream forms when murine-derived Zaria T.vivax bloodstream forms were used. This therefore indicated that an approximately similar level of sensitivity was expressed by the two trypanosome populations to the prophylactic activity of isometamidium. When bovine-derived Zaria T.vivax was incubated in the same sera the effect on infectivity was highly variable and inconsistent. This would appear to indicate that T.vivax populations freshly isolated from cattle are unsuitable for direct in vitro drug-sensitivity studies.

Borowy et al. (1985b) suggested that the mammalian feeder-layer system they described for T.b.brucei might be suitable for the determination of the level of trypanocide sensitivity expressed by field isolates. However, in addition to the difficulties encountered in adapting field isolates to continuous culture in vitro (Hill and Hirumi, 1983) there is a lack of information on the effect adaption to culture has on the level of drug sensitivity expressed by a trypanosome population. However, Brown, Ross, Holmes, Luckins and Taylor (in press) have shown, in a small

experiment, that adaption of three T.congolense populations to in vitro culture did not appear to alter their sensitivity to isometamidium.

In vitro drug sensitivity screening on trypanosomes freshly isolated from a parasitaemic animal could circumvent the above described problems and prove considerably cheaper. However, of the 3 trypanosome species pathogenic for cattle only T.vivax commonly attains levels of parasitaemia in cattle sufficiently high so as to make trypanosome separation procedures reasonably manageable for trypanocide sensitivity-screening purposes. Using a bovine-derived rodent-infective T.vivax population from Zaria, Nigeria (IL 2968), inhibitory effects on growth and infectivity, associated with the presence of trypanocide in serum, have been shown to be masked by the growth supportive properties of the serum per se. Since the Zaria T.vivax used in these experiments appears to be much less sensitive to the growth supportive properties of individual sera than the 3 T.vivax populations from Kenya (IL 2969 (Kilifi), IL 2982 (Galana) and IL 2986 (Likoni) (R.T.Nelson, personal communication), it would appear that drug sensitivity screening on T.vivax populations freshly isolated from cattle is not a useful scientific exercise. An alternative, indirect, approach to determine the level of resistance expressed by a trypanosome population in an infected animal, would be to analyse the serum in which trypanosomes were growing. Examination of sera from cattle with regard to its effect on infectivity of an

isometamidium "sensitive" T.congolense population (ILNat 3.1) has been shown to be a reliable indicator for the presence or absence of trypanocidal activity in sera from cattle administered isometamidium chloride.

SECTION III

DRUG SENSITIVITY STUDIES

CHAPTER 5

DETERMINATION OF THE *IN VIVO* AND *IN VITRO* ISOMETAMIDIUM-SENSITIVITY OF TRYPANOSOME POPULATIONS USED IN THE CATTLE EXPERIMENTS

INTRODUCTION

In the past, determination of the drug sensitivity of rodent-infective trypanosome populations has been conducted by infecting groups of mice and then, at a later date, treating different groups of mice with different dosages of drug (Browning and Gulbransen, 1935; Murgatroyd, Yorke and Corson, 1937; Goble, 1950; Hawking, 1963a; Mwambu et al., 1969; Gill, 1971; Arowolo and Ikede, 1977; Pinder and Authie, 1984; Nyeko, Golder and Otieno, 1985b). The level of resistance expressed by a population can be expressed in a number of different ways: The minimum effective dose (MED_x) refers to the dosage of drug that clears trypanosomes from the blood of a certain percentage (x) of mice, and the curative dose (CD_x) refers to the dosage of drug that cures a certain percentage (x) of infected mice; i.e., the CD_{50} value denotes the dose which cures 50% of infected animals. Similarly, the IC_{50} (50% inhibitory concentration) refers to the concentration of drug required to inhibit in vitro growth by 50% (Borowy et al., 1985b).

In this chapter CD_{50} and IC_{50} values have been derived for rodent-infective trypanosome populations described in this thesis. For comparative purposes an isometamidium-"resistant" T.congolense from Burkina Faso, T.congolense IL 2856 (derived from isolate Banankeladaga 83/CRTA/67 described by Pinder and Authie, 1984 (see figure 7, appendix V)), has also been included.

MATERIALS AND METHODS

1) ISOMETAMIDIUM CHLORIDE CD_{50} DETERMINATION

Isometamidium chloride CD_{50} values were determined at both 6 hours post infection and when parasitaemia attained 10^7 trypanosomes per ml. For the 6 hour CD_{50} determination, mice were inoculated intraperitoneally with $1x10^6$ rodent-derived bloodstream forms at time 0 and treated 6 hours later: Doubling dilutions of isometamidium chloride in 0.2ml, down to a concentration equivalent to a dosage of 0.244ug kg^{-1}, were administered intraperitoneally to groups of 6 mice each. Mice were monitored twice weekly for 30 days following treatment.

2) DETERMINATION OF THE ISOMETAMIDIUM CHLORIDE IC_{50} FOR *T.CONGOLENSE* ILNat 3.1

IC_{50} values were determined in flat-bottomed 24-well culture plates, each test well containing a confluent bovine aorta endothelial cell monolayer. Maintenance medium was removed from each well and replaced with 1.000ml of medium containing 20% (v/v) foetal bovine serum, 1% (v/v) L-Glutamine (29.2mg ml^{-1} RPMI 1640) and 79% (v/v) RPMI 1640.

In each experiment seven different concentrations of isometamidium chloride were used, along with control medium where no drug was added. Drug was prepared in sterile distilled water in a concentrated (100X) form (100ug ml^{-1}, 30ug ml^{-1}, 10ug ml^{-1}, 3ug ml^{-1}, 1ug ml^{-1}, 0.3ug ml^{-1} and 0.1ug ml^{-1}) in order to maintain the osmolarity of the

medium. 10ul aliquots of isometamidium chloride solutions were added to wells; two wells per concentration. (When the ratio of added drug volume to the total volume was 1:100, the osmolarity did not alter by more than 0.8% and had no influence on T.b.brucei cultures (Borowy et al., 1985a,b).)

Trypanosomes were isolated as described in part 2 of the in vitro general materials and methods and $5x10^5$ (suspended in the test medium) added to each well. Before adding drug and trypanosomes to each well an identical volume to that to be added (10ul drug + volume of trypanosome suspension containing $5x10^5$ trypanosomes) was removed from each well so that a volume of 1.000ml was contained in each well, thereby resulting in test isometamidium chloride concentrations of 1ug ml^{-1}, 0.3ug ml^{-1}, 100ng ml^{-1}, 30ng ml^{-1}, 10ng ml^{-1}, 3ng ml^{-1} and 1ng ml^{-1}. Immediately following addition of trypanosomes, all wells were checked for the presence of trypanosomes using an inverted microscope.

After 24 hours incubation the number of trypanosomes present was determined by counting the contents of individual wells.

The growth inhibition produced was calculated by comparing the mean number of trypanosomes in each drug concentration with the number of trypanosomes in the control sample.

Each experiment was repeated at least twice.

3) DETERMINATION OF THE ISOMETAMIDIUM CHLORIDE IC_{50} FOR *T.VIVAX* IL 2968

The isometamidium chloride IC_{50} value for T.vivax IL 2968 was determined in an identical manner to that described for T.congolense ILNat 3.1 except that the medium into which drug and trypanosomes were added was composed of 10% (v/v) goat serum, 10% (v/v) foetal bovine serum, 1% (v/v) L-Glutamine (29.2mg ml^{-1} MEM), 1% (v/v) non-essential amino acids (GIBCO, U.K.) and 79% (v/v) MEM medium.

RESULTS

1) MURINE CD_{50} VALUES

CD_{50} values were determined for all trypanosome populations using a minimum logit chi-square analysis (Hewlett and Plackett, 1979) and are shown, along with 95% confidence intervals, in table 34.

Both T.congolense ILNat 3.1 and T.congolense IL 2642 appeared to express approximately the same level of sensitivity to the therapeutic activity of isometamidium. In contrast, the Zaria T.vivax (IL 2968) expressed a level of sensitivity a factor of 8 times lower than both T.congolense ILNat 3.1 and T.congolense IL 2642 when treated 6 hours post-infection, and 16 times lower when treated at a parasitaemia of 10^7 trypanosomes per ml. However, a comparison of the CD_{50} value of T.congolense IL 2856 (putatively drug-resistant) with other trypanosome CD_{50} values indicated that T.congolense ILNat 3.1, T.congolense IL 2642 and T.vivax IL 2968 all express a relatively high level of sensitivity to the therapeutic activity of isometamidium.

2) *IN VITRO* IC_{50} VALUES

The sensitivity of T.congolense ILNat 3.1 and the Zaria T.vivax (IL 2968) to the therapeutic activity of isometamidium in vitro has been determined after 24 hours incubation in medium containing 20% foetal bovine serum. The sensitivity has been expressed as the drug concentration required to inhibit parasite growth by 50%

Table 34

ISOMETAMIDIUM CHLORIDE CD_{50} VALUES, AS DETERMINED IN MICE

TRYPANOSOME POPULATION	TIME OF TREATMENT	CD_{50} ($mgkg^{-1}$)	95% CONFIDENCE INTERVAL
T.congolense ILNat 3.1	6 hours*	0.0066	0.0055 - 0.0079
T.congolense IL 2642	6 hours	0.0072	0.0056 - 0.0093
T.vivax IL 2968	6 hours	0.0583	0.0522 - 0.0652
T.congolense ILNat 3.1	10^7 ml^{-1}+	0.0177	0.0125 - 0.0251
T.congolense IL 2642	10^7 ml^{-1}	0.0210	0.0153 - 0.0287
T.vivax IL 2968	10^7 ml^{-1}	0.3021	0.2033 - 0.4489
T.congolense IL 2856	10^7 ml^{-1}	6.537	3.864 - 11.06

* Mice treated 6 hours after intraperitoneal inoculation of $1x10^6$ trypanosomes.
+ Mice treated when parasitaemia attained approximately 10^7 ml^{-1}.

(IC_{50}). Experiments were repeated at least twice. Before determination of the IC_{50} value by the same minimum logit chi-square analysis as used for CD_{50} values, consistency between experiments was determined using an analysis of variance technique with a 3-factor design (G. Gettinby, unpublished data). Any inconsistent data was not included in the chi-square analysis. IC_{50} values are expressed as ug ml^{-1} and are shown in table 35.

Table 35

ISOMETAMIDIUM CHLORIDE IC_{50} VALUES, AS DETERMINED *IN VITRO*

TRYPANOSOME POPULATION	IC_{50} (ug ml-1)	95% CONFIDENCE INTERVAL
T.congolense ILNat 3.1	0.1109	0.1037 - 0.1186
T.vivax IL 2968	0.0053	0.0041 - 0.0067

DISCUSSION

Comparison of the 6 hour and 10^7 ml^{-1} CD_{50} values for each trypanosome population showed there to be consistently a higher CD_{50} value when treated at 10^7 ml^{-1} rather than 6 hours post-infection. Davey (1957), with little experimental evidence, suggested that another chemoprophylactic, antrycide prosalt, became bound to trypanosomes and was excreted along with the trypanosomes. He thereby suggested that drug would be more rapidly excreted in infected animals. However, Gilbert *et al*. (1979) showed that the levels of radioactivity in blood and tissue fluids, associated with the administration of [^{14}C]-ethidium bromide, followed a similar course in non-infected and *T.congolense*-infected calves. Although the pharmacokinetics of ethidium bromide were apparently unaltered in *T.congolense*-infected calves it may be that in mice the pharmacokinetics in non-infected animals do differ from infected animals since parasitaemia in murine *T.congolense* infections may attain anything up to 100 times the maximum level attained in cattle. The occurrence of more rapid pharmacokinetics in infected mice would explain the occurrence of higher CD_{50} values when mice were treated at a parasitaemia of 10^7 ml^{-1} as compared to 6 hours post-infection.

Determination of *in vitro* IC_{50} values has previously only been conducted with culture-adapted bloodstream forms of *T.b.brucei* (Borrowy *et al*., 1985a, b) and second generation bloodstream forms of *T.vivax* (Waithaka *et al*.,

1985). The only isometamidium chloride IC_{50} value described in the literature is for T.vivax IL 1392, a derivative of the same isolate from which T.vivax IL 2968 was derived; Zaria Y486 (Waithaka et al., 1985). The IC_{50} value given by Waithaka et al. (1985) for T.vivax IL 1392 was 0.545ug ml^{-1}. No confidence interval was indicated. Thus, there is a great difference in the level of sensitivity expressed by T.vivax IL 1392 and T.vivax 2968 (IC_{50} = 0.0053ug ml^{-1}). Both populations are directly related. However, since both are clones, this may account for the different levels of sensitivity expressed by the two populations.

Finally, the isometamidium chloride IC_{50} value for T.congolense ILNat 3.1 was shown to be much greater than that for T.vivax IL 2968. A comparison with the mouse isometamidium chloride CD_{50} values for the same trypanosome populations shows the reverse. This difference in the therapeutic activity of isometamidium in vivo and in vitro may be associated with different active forms of the drug: Hutchings (1979), without presenting any experimental evidence, stated that Samorin was a mixture of 3 substances; isometamidium, a purple isomer and a *bis*-compound, and was not 100% isometamidium. Since Wragg et al. (1958) indicated that the p-amidino and m-amidino isomers of isometamidium differed in their in vivo therapeutic activity against a T.congolense population, it may be that some of Samorin's constituents exert the drug's in vivo activity, and others the in vitro activity.

GENERAL DISCUSSION

Isometamidium chloride has been marketed for 25 years. However, it is only very recently that an extensive survey has been carried out on the cost benefit to the farmer using a regular isometamidium-administration programme. Under an efficient animal health programme, cattle maintained under isometamidium prophylaxis on the Mkwaja ranch, Tanzania (a region where cattle cannot survive without the use of chemotherapy), were 80% as productive as Boran cattle in a tsetse-free area of Kenya (ILCA, 1985).

At a dosage of 1mg kg^{-1}, isometamidium chloride has been shown to confer cattle prophylaxis against trypanosomiasis from 2 to 22 weeks (Kirkby, 1964; Lwembandisa, 1970; Lewis and Thomson, 1974; Omwero-Wafula and Mayende, 1979; Pinder and Authie, 1984; Whitelaw et al., 1986). In a similar manner, quinapyramine in its prophylactic formulation (pro-salt) has been shown to confer cattle 1 to 4 months' protection (Davey, 1950; Unsworth and Chandler, 1952; Fiennes, 1953; Robson and Wilde, 1954). Recognising the wide variation in apparent prophylaxis that quinapyramine pro-salt conferred to cattle, workers suggested that the duration of protection afforded by quinapyramine pro-salt was directly related to the intensity of tsetse challenge (Fiennes, 1953; MacOwan, 1955, 1956; Davey, 1957; Whiteside, 1958, 1962a). Whiteside (1958) suggested that a combination of "trypanosome challenge" and "accessory factors" determined the apparent prophylactic period. The term "trypanosome challenge" encompassed both the intensity of infection and

the trypanosome characteristics. Included in "intensity of infection" were the fly species, the number of flies, the fly feeding habits, the fly infection rate and mechanical transmission. Thus, "intensity of infection" was almost synonymous with the number of infective testse bites per unit time.

"Characteristics of the trypanosome" included the trypanosome species, their pathogenicity, their drug susceptibility and their liability to become drug-fast.

Finally, "accessory factors" were divided into two groups: those concerned with the innate resistance of cattle to trypanosomiasis (breed, sex, place of origin, previous history) and those modifying the innate resistance (cattle condition, pregnancy, lactation, intercurrent disease, condition of grazing and climatic condition).

The demonstration from field studies that the prophylactic period of quinapyramine pro-salt appeared to be inversely related to the tsetse density (MacOwan, 1955, 1956; Davey, 1957; Whiteside, 1958, 1962a) indicated to these workers that the essential factors determining the period of prophylaxis may be the density of infected flies and the transmission rate of those flies (Whiteside, 1962a). Relating the natural incidence of trypanosomiasis (expressed as the mean time in weeks between individual treatments with diminazene aceturate) to the period of prophylaxis conferred by prothidium, in 22 field trials in Kenya, Whiteside (1962a,b) showed that the higher the incidence of trypanosomiasis in an area the shorter the

period of prophylaxis conferred by a chemoprophylactic in the same area.

The work described in this thesis has been directed towards determining the importance of a number of the factors discussed by Whiteside (1958) in determining the duration of prophylaxis conferred to cattle by administration of isometamidium chloride; namely, dosage of drug, infection at the time of treatment, trypanosome species, trypanosome species serodeme and the level of metacyclic challenge.

Using an experimental protocol identical to that described in this thesis, Whitelaw et al. (1986) showed that isometamidium chloride at a dosage of 1mg kg^{-1} completely protected cattle against monthly tsetse challenge with T.congolense ILNat 3.1 for 5 months. Work described in this thesis using the same drug dosage has shown that in cattle challenged in a similar manner, complete prophylaxis was conferred for 4 months against T.congolense ILNat 3.1 and for 5 months against T.congolense IL 2642. At a dosage of 0.5mg kg^{-1} the period of complete prophylaxis against T.congolense ILNat 3.1 was reduced to 3 months. Analyses of the prophylaxis results indicated a significant ($P<0.06$) dose-dependent relationship, in that a higher dosage of drug afforded a longer period of prophylaxis. This finding is consistent with that of Boyt et al. (1962) who showed that isometamidium conferred complete prophylaxis for 147 days at a dosage of 1mg kg^{-1} and for 173 days at a dosage of 2mg

kg^{-1}.

In Burkina Faso, the occurrence of T.congolense infections in cattle as little as 2 weeks after administration of 1mg kg^{-1} isometamidium chloride suggested that the trypanosome populations were highly resistant to the prophylactic activity of isometamidium. The same populations expressed a high level of resistance to the therapeutic activity of isometamidium in mice (Pinder and Authie, 1984). Since 1mg kg^{-1} isometamidium chloride conferred complete prophylaxis for 4 months against T.congolense ILNat 3.1 and for 5 months against T.congolense IL2642 it would suggest that both populations are highly sensitive to the prophylactic activity of isometamidium.

Both T.congolense ILNat 3.1 and T.congolense IL 2642 also appeared to be highly sensitive to isometamidium's therapeutic activity in mice; whilst T.congolense ILNat 3.1 and T.congolense IL 2642 had (10^7 parasites ml^{-1}) CD_{50} values of 0.0177mg kg^{-1} and 0.0210mg kg^{-1}, respectively, that for T.congolense IL 2856 was 6.537mg kg^{-1}, 340-fold greater. T.congolense IL 2856 is a derivative of isolate Banankeladaga/83/CRTA/67 (see figure 7, appendix V), an isometamidium-resistant isolate described by Pinder and Authie (1984).

As noted previously, Whiteside in a personal communication to D.G. Davey (1957) suggested that the level of trypanosome challenge determined the duration of prophylaxis conferred to cattle by quinapyramine pro-salt

(Davey, 1957). Davey himself supported this theory (Davey, 1957).

To ascertain whether this theory was true for isometamidium, 3 groups of 4 cattle each were administered 0.5mg kg^{-1} isometamidium chloride and subsequently challenged at monthly intervals with metacyclic forms of T.congolense ILNat 3.1; either 5 infective tsetse (approximately 500 metacyclic trypanosomes (Whitelaw et al., 1986)) or one of two different doses of in vitro-derived metacyclic trypanosomes, $5x10^3$ or $5x10^5$. Complete protection lasting 3 months was afforded to all 3 groups. That the level of metacyclic challenge had no effect on the duration of prophylaxis conflicts with previous field observations, although is consistent with previous experimental findings (Whitelaw et al., 1986). Although earlier workers had suggested (on the basis of field observations) an inverse relationship between fly density and the duration of quinapyramine prophylaxis, the findings presented in this thesis have shown that the level of metacyclic challenge appears not to influence the prophylactic period. There are several possible reasons for this difference: Firstly, both populations of T.congolense used in the cattle experiment have been well characterised in terms of their sensitivity to isometamidium at normal therapeutic doses, whereas in the field cattle might encounter trypanosome populations less sensitive to the drug. Secondly, the challenge was administered on a single day each month, whereas in the

field, challenge probably occurs more frequently at a lower intensity.

If there is an inverse relationship between the tsetse density and the duration of prophylaxis in the field, one might hypothesise that a fly-associated factor influences the prophylactic period. Following repeated bites of uninfected tsetse, both immediate and delayed hypersensitivity reactions have been observed in humans and animals in response to salivary components (Gordon and Crewe, 1948; Ellis et al., 1986). Since metamidium and isometamidium act as depot prophylactics, being stored at the site of injection (Smith and Brown, 1960; Hill and McFadzean, 1963), and isometamidium appears to reside as deposits within the cytoplasm of macrophages at the injection site (Bell, 1987), one might propose that the release of systemically acting inflammatory mediators in response to such hypersensitivity reactions may cause a more rapid efflux of drug-containing-macrophages from the injection site, thereby shortening the prophylactic period.

Infection, associated with clinical signs of trypanosomiasis, at the time of drug administration has been suggested as a factor that may shorten the prophylactic period (Davey, 1957). To provide information on this factor, two groups of 4 cattle were administered 0.5mg kg^{-1} isometamidium chloride and then challenged at monthly intervals with T.congolense ILNat 3.1-infected tsetse. One group was fly-infected with T.congolense IL 2642 four weeks before drug administration. Complete

protection was afforded the group previously infected with T.congolense IL 2642 for 4 months whilst 3 months complete protection was afforded the other group. Thus, it appears that heterologous infection at the time of drug administration had no shortening effect on the prophylactic period. Furthermore, since T.congolense ILNat 3.1 and T.congolense IL 2642 are cloned populations and belong to different serodemes (Dwinger, 1985b) it can be concluded that immunity did not contribute to the period of prophylaxis conferred to the group originally infected with T.congolense IL 2642.

Jennings et al. (1977a,b, 1979) demonstrated that the brain, inaccessible to most trypanocides, may act as a source of relapse infections following treatment. Thus, infections occurring in animals maintained on a chemoprophylactic regime, may in reality be the result of a relapse from a drug-privileged site rather than from a new infection. This phenomenon cannot have occurred in any of the experiments described within this thesis since no animals, except the 4 group D animals, were infected with trypanosomiasis prior to administration of isometamidium chloride. The four animals in group D were infected with T.congolense IL 2642 at the time of drug administration and were then challenged with T.congolense ILNat 3.1. Infections that subsequently developed in all 4 animals were shown serologically to be T.congolense ILNat 3.1.

Whiteside (1962a) suggested that antrycide pro-salt's prophylactic period consisted of two parts; one due to the

drug and independent of the trypanosome challenge, and the other, added to it, due to an immune response by the host, which may be antigenically primed during part or all of the protective period. The experiments described here, both with T.congolense and T.vivax, have shown that antibody was not detected in any animal under isometamidium prophylaxis until it became parasitaemic. The only exception to this finding was one group of 4 cattle, (group F) administered 0.5mg kg^{-1} isometamidium chloride and challenged at monthly intervals with $5x10^5$ in vitro-derived T.congolense ILNat 3.1 metacyclic trypanosomes. Low anti-metacyclic antibody titres were detected in all 4 animals one to 4 months before they became parasitaemic, when antibody titres rose five-fold. Thus, acquisition of immunity whilst under chemoprophylactic cover appears highly unlikely in animals maintained by an isometamidium prophylactic regime, as the size of metacyclic inoculum required to stimulate a protective immune response was excessively large in relation to what is likely to be encountered in the field.

Although immunity appears not to be acquired whilst under chemoprophylactic cover it may be acquired under therapeutic regimes and contribute to the chemoprophylactic period. Wilson et al. (1975b, 1976) described a field based experiment in which partial immunity, as determined by a lengthening of the intertreatment period, appeared to develop after 2 years in a group of cattle treated on an individual basis with diminazene aceturate when animals developed clinical disease or the PCV had gone below 20%.

Immunity did not appear to develop in a group of cattle treated on a group basis with diminazene aceturate when one animal in the group was detected parasitaemic. Thus, development of immunity in cattle was greatest in animals in which infections were well established before treatment. Similarly, cattle treated with diminazene aceturate 5 days after cyclical infection with T.congolense were completely susceptible to homologous challenge, whilst cattle treated 15 days following infection were completely immune to homologous challenge (Akol and Murray, 1985). Cattle treated on days 10-12 exhibited a range of susceptibility to homologous challenge.

In addition to the development of immunity following treatment being dependent on the timing of treatment following infection, the presence of concomitant heterologous serodemes may interfere with the development of immunity (Luckins, 1986). Induction of immunity to a particular T.congolense serodeme by a regime of infection and treatment with homidium chloride appeared to be adversely affected if animals were concurrently infected with a heterologous T.congolense serodeme. Immunity was impaired either when infection was conducted simultaneously with both serodemes (when establishment of both infections appeared to proceed normally) or when infection with the second serodeme was conducted at a later date. In the latter case this may have been due to interference in establishment of the second infection (Morrison, Wells, Moloo, Paris and Murray, 1982b; Luckins and Gray, 1983;

Dwinger, Luckins, Murray, Rae and Moloo, 1986b). All experiments on interference have been conducted with cloned populations belonging to two different serodemes. In the field the situation is probably more complex since animals are constantly exposed to mixed infections with different serodemes and different trypanosome species (Luckins, 1986).

Chemoprophylaxis in neonatal animals may be enhanced by an immunoprophylactic contribution. Whitelaw and Urquhart (1985) showed that immunity to T.brucei could be conferred to mice when allowed to suckle, from birth, a mother either infected or whose infection was cured before parturition with a trypanocide. Immunity lasted until approximately 25 days of age and was transmitted via the mother's colostrum. Administration of diminazene aceturate to normal newbown mice conferred protection against T.brucei challenge for approximately 25 days. In a similar group of drug-administered mice, also receiving colostral antibody, combined immunochemoprophylaxis lasted 40-50 days.

In contrast to mice, little protection appeared to be afforded neonatal goats by colostral transfer of antibody from their dams. Studies on East African goats showed that whilst 3 out of 5 kids fed colostrum from immunised dams resisted homologous T.brucei challenge at 12 days of age, none out of 3 were immune at 28 days of age (Whitelaw and Jordt, 1985).

In studies with T.vivax (described in this thesis)

isometamidium chloride at a dosage of 0.5mg kg^{-1} has been shown to confer complete prophylaxis for 2 months against T.vivax IL 2968, a derivative of an isolate made in Zaria, Nigeria, and for 1 month against T.vivax IL 2986, a derivative of an isolate made in Likoni, Kenya. The same dosage of isometamidium chloride conferred less than 1 month's prophylaxis against T.vivax IL 2969 and T.vivax IL 2982, derivatives of isolates made in Kilifi and Galana, Kenya, respectively. Likoni, Kilifi and Galana are situated within 100km of each other in the Kenyan coastal province.

Thus, experiments have been conducted with two East African T.congolense populations (ILNat 3.1 and IL 2642) and three East African T.vivax populations (Kilifi (IL 2969), Galana (IL 2982) and Likoni (IL 2986)). In a similar manner to the two T.congolense populations, parental populations of the Kilifi T.vivax and the Galana T.vivax have been shown to belong to different serodemes (Dwinger, 1985b; Nantulya et al., 1986). In contrast, parental populations of the Kilifi T.vivax and the Likoni T.vivax may belong to the same serodeme (Nantulya et al., 1986). All 3 T.vivax populations expressed a much greater level of resistance to the prophylactic activity of isometamidium, as compared to both T.congolense populations. If the 3 T.vivax populations and the two T.congolense populations are representative of the respective trypanosome species in East Africa as a whole then one would hypothesise that T.vivax populations in East

Africa express a greater level of resistance to isometamidium's prophylactic activity than East African T.congolense populations. This being so, it would be expected that T.vivax infections in East Africa would occur more frequently in cattle maintained by an isometamidium prophylaxis regime than in cattle maintained in the same area without the use of isometamidium. Some evidence to support this hypothesis was provided from the findings of Njogu, Dolan, Sayer, Wilson and Alushula (1985): They described a series of experiments conducted on the Galana ranch, Kenya, and compared the percentage T.vivax infections occurring in two groups of cattle from 1982 to 1984; one group was maintained on a regular isometamidium prophylaxis regime and the other (a sentinel group) was maintained in the same area on an individual basis with diminazene aceturate, i.e., a sentinel animal was only treated with diminazene aceturate when detected parasitaemic. In each of the three years a greater percentage of T.vivax infections occurred in the group maintained with isometamidium as compared to the sentinel group. In 1985 and 1986 almost 100% of infections occurring in the isometamidium prophylaxis group were associated with T.vivax (R.B. Dolan, personal communication).

Reports of resistance to isometamidium by trypanosomes in East Africa are lacking. Rottcher and Schillinger (1985) reported the occurrence of multiple drug resistance associated with a haemorrhagic type T.vivax from the Tana

river district of Kenya. Experiments were conducted with blood from a donor cow that had been infected with 11 isolates. The resultant trypanosome "cocktail" was shown to be resistant to the therapeutic activity of isometamidium chloride, quinapyramine sulphate, diminazene aceturate and homidium chloride at dosages of 2mg kg^{-1}, 5mg kg^{-1}, 3.5mg kg^{-1} and 2mg kg^{-1}, respectively. Commenting on this work, Njogu and Heath (1986) stated that the association of a drug-resistant strain of T.vivax with a haemorrhagic syndrome could not be confirmed. Furthermore, although the Kenyan Trypanosomiasis Research Institute had confirmed the resistance of the cocktail to isometamidium, they had failed to establish from which cattle group the resistant strain originated. Only 7 of the original isolates were available and all were sensitive to isometamidium chloride at .1mg kg^{-1}. Thus the report of Rottcher and Schillinger (1985) was not confirmed. Herds of cattle are still being maintained on the Galana ranch using an 8-weekly isometamidium prophylaxis regime with no apparent evidence of resistance (R.B. Dolan, personal communication).

Following on from the work described by Rottcher and Schillinger (1985), Schonefeld and Rottcher (1987) reported on drug resistance studies conducted with 7 T.vivax isolates from Kenya and Somalia. All 7 isolates were resistant to the recommended therapeutic dosages of isometamidium chloride, homidium chloride and quinapyramine sulphate. Sensitivity to isometamidium's prophylactic

activity was investigated with only one isolate; that from Bamburi, Kenya (situated on the coast, approximately 50 km south of Kilifi). Cattle administered 1mg kg^{-1} isometamidium chloride were fly-challenged with the Bamburi T.vivax either 10 or 20 days following drug administration. All cattle were detected parasitaemic within 7-10 days following each challenge.

Drug-resistant trypanosomes are a potential major threat to cattle productivity in Africa. However, this threat may be reduced, firstly by the finding that some cattle infected with populations of T.vivax, T.congolense or T.brucei, all of East African origin, may self-cure (Wellde, Hockmeyer, Kovatch, Bhogal and Diggs, 1981; Nantulya et al., 1984,1986), and secondly, trypanosome populations arising in cattle maintained by a chemoprophylactic regime in the field may express decreased pathogenicity (Stephen, 1962a). In the T.congolense experiment described in chapter 1, greater than 50% of T.congolense infections arising in cattle administered isometamidium chloride expressed decreased pathogenicity as compared to the same populations in controls.

The ability of a trypanosome population to acquire an increased level of drug resistance has been suggested as a reason for shortening of antrycide pro-salt's prophylactic period (Fiennes, 1953; Whiteside, 1958). However, this did not appear to occur in any of the experiments described in this thesis. Extensive isometamidium sensitivity screening was conducted in mice on all T.congolense ILNat 3.1,

T.congolense IL 2642 and Zaria T.vivax (IL 2968) populations arising in cattle administered isometamidium chloride. In no case (22 infections) did breakthrough populations appear to express a greater level of resistance to isometamidium as compared to those in controls.

In addition to isometamidium exerting trypanocidal activity within the host, it may also exert trypanocidal activity within the tsetse fly. Using wild caught Glossina pallidipes Hawking (1963b) showed that infections with T.congolense, T.vivax or T.brucei were eliminated when the flies were allowed to feed through membranes on blood containing 0.1mg ml^{-1} isometamidium chloride. Using a similar feeding technique and the same drug concentration, both immature and mature T.vivax infections were eliminated from G.p.palpalis (Agu, 1985). Furthermore, when used at a lower concentration of 0.1ug ml^{-1}, isometamidium chloride has also been shown to decrease the number of mature T.b.brucei infections in G.m.centralis when fed to flies throughout the whole period of trypanosome development (Jefferies and Jenni, 1987). Since trypanocidal activity was evident at the level of the salivary glands and not in the midgut, the authors suggested that isometamidium interacted with kinetoplast DNA preventing expression of genes important for trypanosome transformation.

All the above described studies have examined the effect of feeding infected flies on blood spiked with native drug. Since in vivo activity of Samorin may be associated with constituents that do not effect the drug's

in vitro activity (see chapter 5 discussion) there is a danger in extrapolating *in vitro* findings to the *in vivo* situation. In an attempt to examine the situation *in vivo*, Agu (1984) fed *T.vivax*-infected *G.p.palpalis* on sheep, 24 hours after intramuscular administration of 1mg kg^{-1} isometamidium chloride. Both immature and mature *T.vivax* infections were eliminated from the flies. In contrast, when *T.congolense*-infected *G.m.morsitans* were fed on drug-administered rabbits, no alteration was observed in midgut or proboscis infections, or transmissibility of parasites (Nyeko, Golder and Otieno, 1985a). These authors also showed that infections in mice, resulting from feeding of the above described tsetse, expressed a decreased level of sensitivity to isometamidium.

There is thus little experimental evidence to indicate that trypanosome transmissibility of tsetse in the field is influenced by the feeding of tsetse on drug-administered animals.

Techniques for the determination of isometamidium levels in body fluids and tissues are lacking. Ali and Hassan (1984), using an insensitive spectrophotometric technique described by Philips *et al*. (1967), were unable to detect isometamidium in camel plasma 48 hours after intravenous inoculation of either 0.5mg kg^{-1} or 1.0mg kg^{-1} isometamidium chloride. Using the same technique to examine slaughterhouse material, Shetty (1986) showed that highest levels of drug were detected in liver, then kidney, skeletal muscle, heart ventricle and spleen; decreasing in

that order (the time interval between drug administration and slaughter was unknown). A similar tissue distribution has been reported in dogs, monkeys and goats (Philips et al., 1967; Braide and Eghianruwa, 1980). The level of sensitivity of the technique described by Braide and Eghianruwa (1980) was down to approximately 1.0ug ml^{-1} of serum and 2.3ug g^{-1} of tissue. That described by Shetty (1986) was 2.1ug g^{-1} of wet tissue. Because of the insensitivity of these techniques the actual duration of pharmacologically active drug within body fluids and tissues may infact be much longer than that currently reported.

Perschke and Vollner (1985) described a high performance liquid chromatography technique for the measurement of isometamidium in bovine plasma or serum. Using spiked samples their lower limit of detection was 20ng ml^{-1}. This technique was not, however, applied to the examination of sera from drug-administered animals.

Other workers have failed to reproduce the results of Perschke and Vollner (1985) (D.D. Whitelaw, personal communication).

Using a T.congolense in vitro culture system described by Hirumi and Hirumi (1984), a qualitative technique for the detection of trypanocidal activity in plasma or sera from drug-administered cattle has been developed. Trypanocidal activity was detected in cattle plasma collected as long as 6 months after administration of 1mg kg^{-1} isometamidium chloride; levels that correlated with

susceptibility of cattle to challenge. The same technique was used to confirm the high level of resistance expressed by the Kilifi T.vivax (IL 2969) to the prophylactic activity of isometamidium: Trypanocidal activity was detected in serum taken from cattle 1 and 2 months after administration of 0.5mg kg^{-1} isometamidium chloride, at a time when they were susceptible to challenge with the Kilifi T.vivax.

There are, however, problems with the in vitro technique. In particular, the detection of trypanocidal activity in serum from some animals prior to drug-administration and, therefore, the absence of a true serum control for any animal.

In the past, putative isometamidium-resistant trypanosome populations have been investigated by a strategy of infection and treatment (Jones-Davies and Folkers, 1966a; Na'Isa, 1967; Gray and Roberts, 1971; Lewis and Thomson, 1974; Gitatha, 1979; Kupper and Wolters, 1983; Rottcher and Schillinger, 1985). Studies described in this thesis on T.vivax populations originating from Kilifi (IL 2969), Galana (IL 2982) and Likoni (IL 2986), in Kenya, have indicated them to be sensitive to the therapeutic activity of isometamidium but resistant to the prophylactic activity of the drug, as defined in the field situation. Differences between prophylactic and therapeutic activity have also been reported for the 2,7-di-m-amidinophenyldiazoamino derivative of homidium (Berg, Brown, Hill and Wragg, 1961). Although the compound

was much less active therapeutically than metamidium against a T.congolense population, it was no less active prophylactically. These findings might suggest that the form(s) of isometamidium chloride (Samorin) exerting the drug's therapeutic activity may be different from the form(s) exerting the prophylactic activity. Possibly, the therapeutic activity is associated with native drug whilst drug metabolite(s) exert(s) the drug's prophylactic activity. Alternatively, different constituents of Samorin may be responsible for the different activities (Wragg et al., 1958; Hutchings, 1979).

Since isometamidium is principally used for conferring prophylaxis against bovine trypanosomiasis, it is suggested that future in vivo studies on putative isometamidium-resistant trypanosomes should ideally be conducted using a prophylactic rather than a therapeutic protocol. In vitro studies on putative drug-resistant isolates should be conducted in a similar manner; instead of adding native isometamidium chloride to cultures (Waithaka et al., 1985), it is suggested that sensitivity screening of trypanosome isolates should be conducted by incubating trypanosomes in sera collected from cattle after administration of isometamidium chloride. Incubation of trypanosome isolates with native drug should only be conducted with isolates collected from areas where isometamidium chloride is used as a therapeutic.

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Table 1, appendix I

T.CONGOLENSE EXPERIMENT - CATTLE CODES

ANIMAL CODE	GROUP	NUMBER	ANIMAL CODE	GROUP	NUMBER
A1	A	C862	D1	D	C890
A2	A	C882	D2	D	C892
A3	A	C933	D3	D	C893
A4	A	C954	D4	D	C894
B1	B	C879	E1	E	C871
B2	B	C888	E2	E	C885
B3	B	C936	E3	E	C891
B4	B	C953	E4	E	C952
C1	C	C870	F1	F	C860
C2	C	C875	F2	F	C861
C3	C	C886	F3	F	C872
C4	C	C932	F4	F	C889

CONTROLS

CHALLENGE MONTH	GROUPS A,C,D	GROUP B	GROUPS E,F
2	C868	C934	C874
3	C887	C730	C869
4	C935	C731	C938
5	C597	C535	C420
6	C865	C734	C937
7	C730	C887	-

Table 2, appendix I

INFECTIVITY TITRATIONS OF *T.CONGOLENSE* IL 2642

METACYCLIC TRYPANOSOMES FOR MICE

INOCULUM	DEAE-COLUMN SEPARATION		PLASMA SEPARATION	
	No. Parasitaemic /No. inoculated	Parasitaemia onset (days)	No. Parasitaemic /No. inoculated	Parasitaemia onset (days)
$5x10^4$	4/4	4,5	4/4	3
$5x10^3$	4/4	3-5	4/4	3
$5x10^2$	4/4	5-7	4/4	4-6
$5x10^1$	4/4	5-9	4/4	5,6
$5x10^0$	3*/4	6-9	4/4	7,8

*Negative mouse monitored for 40 days.

Table 3, appendix I

DETECTION OF TRYPANOSOMES IN GROUP D ANIMALS PRIOR TO THE 2 MONTH CHALLENGE

DATE:	17.1.85		18.1.85		25.1.85		15.2.85	
DAY:	0*		1		8		29	
ANIMAL NO.	B.C.	M.I.	B.C.	M.I.	B.C.	M.I.	B.C.	M.I.
D1	3+	5/5	-	0/5	-	0/5	-	0/5
D2	2+	5/5	-	0/5	-	0/5	-	0/5
D3	-	5/5	3+	0/5	-	0/5	-	0/5
D4	3+	5/5	2+	0/5	-	0/5	-	0/5

DATE:	22.2.85		1.3.85		15.3.85	
DAY:	36		43		57	
ANIMAL NO.	B.C.	M.I.	B.C.	M.I.	B.C.	M.I.
D1	-	0/5	-	0/5	-	0/5
D2	-	0/5	-	0/5	-	0/5
D3	-	0/5	-	0/5	-	0/5
D4	-	0/5	-	0/5	-	0/5

B.C. = Buffy-coat phase-contrast technique
M.I. = Mouse inoculation: Data = No. parasitaemic/No. inoculated
* = All four animals observed consistently parasitaemic for at least 17 days prior to day 0.

Table 4,appendix I

DETECTION OF TRYPANOSOMES BY THE BUFFY-COAT PHASE-CONTRAST (BC) TECHNIQUE AND MOUSE INOCULATION (M.I.) FOLLOWING CHALLENGE AT 2 AND 3 MONTHS

	2 MONTH CHALLENGE			3 MONTH CHALLENGE		
		M.I.			M.I.	
ANIMAL NO.	BC/DFOP	DAY 12	DAY 24	BC/DFOP	DAY 12	DAY 24
A1	-	0*	0	-	0	0
A2	-	0	0	-	0	0
A3	-	0	0	-	0	0
A4	-	0	0	-	0	0
B1	-	0	0	-	0	0
B2	-	0	0	-	0	0
B3	-	0	0	-	0	0
B4	-	0	0	-	0	0
C1	-	0	0	-	0	0
C2	-	0	0	-	0	0
C3	-	0	0	-	0	0
C4	-	0	0	-	0	0
D1	-	0	0	-	0	0
D2	-	0	0	-	0	0
D3	-	0	0	-	0	0
D4	-	0	0	-	0	0
E1	-	0	0	-	0	0
E2	-	0	0	-	0	0
E3	-	0	0	-	0	0
E4	-	0	0	-	0	0
F1	-	0	0	-	0	0
F2	-	0	0	-	0	0
F3	-	0	0	-	0	0
F4	-	0	0	-	0	0
A,C,D CONTROL	12	2	2	12	1	2
B CONTROL	11	2	2	12	2	2
E,F CONTROL	13	1	2	16	0	2

BC/DFOP = Day first observed parasitaemic using BC technique.

* = Number of mice parasitaemic (2 inoculated).

Table 5, appendix I

DETECTION OF TRYPANOSOMES BY THE BUFFY-COAT PHASE-CONTRAST (BC) TECHNIQUE AND MOUSE INOCULATION (M.I.) FOLLOWING CHALLENGE AT 4 AND 5 MONTHS

	4 MONTH CHALLENGE			5 MONTH CHALLENGE		
		M.I.			M.I.	
ANIMAL NO.	BC/DFOP	DAY 12	DAY 24	BC/DFOP	DAY 12	DAY 24
A1	-	0*	0	-	0	0
A2	-	0	0	24	0	2
A3	-	0	0	-	0	0
A4	-	0	0	-	0+	0
B1	-	0	0	-	0	0
B2	-	0	0	-	0	0
B3	-	0	0	-	0+	0
B4	-	0	0	-	0+	0
C1	-	0	0	14	0	2
C2	-	0	0	-	0	0
C3	-	0	0	14	1	2
C4	21	0	2			
D1	-	0	0	14	0	2
D2	-	0	0	-	0	0
D3	-	0	0	-	0	0
D4	-	0	0	30	0	0
E1	-	0	0	-	0	0
E2	-	0	0	7	2	1
E3	-	0	0	-	0	0
E4	16	0	2			
F1	-	0	0	-	0	0
F2	-	0	0	14	0	2
F3	21	0	2			
F4	-	0	0	14	0	2
A,C,D CONTROL	14	0	2	12	1	2
B CONTROL	12	2	2	12	1	2
E,F CONTROL	14	0	2	14	2	2

BC/DFOP = Day first observed parasitaemic using BC technique.

+ = Inoculation on day 15.

* = Number of mice parasitaemic (2 inoculated).

Table 6, appendix I

DETECTION OF TRYPANOSOMES BY THE BUFFY-COAT PHASE-CONTRAST (BC) TECHNIQUE AND MOUSE INOCULATION (M.I.) FOLLOWING CHALLENGE AT 6 AND 7 MONTHS

	6 MONTH CHALLENGE			7 MONTH CHALLENGE		
		M.I.			M.I.	
ANIMAL NO.	BC/DFOP	DAY 12	DAY 24	BC/DFOP	DAY 12	DAY 24
A1	-	0*	0	-	0	0
A3	-	0	0	-	0	0
A4	24	0	0			
B1	14	0	2			
B2	-	0	0	-	0	0
B3	-	0	0	-	0	0
B4	29	0	0			
C2	-	0	0	-	0	0
D2	28	0	0			
D3	12	0	2			
E1	21	0	2			
E3	19	0	2			
F1	-	0	0	24	0	2
A,C,D CONTROL	14	1	1	12	1	2
B CONTROL	12	2	2	14	2	2
E,F CONTROL	14	0	2			

BC/DFOP = Day first observed parasitaemic using BC technique.

* = Number of mice parasitaemic (2 inoculated).

Table 7, appendix I

TEMPERATURE MEASUREMENTS OVER 39.4°C

	2 MONTH CHALLENGE (27*)				3 MONTH CHALLENGE (26*)			
ANIMAL NO.	DFOP	NTMP	NPM	NPM NTMP %	DFOP	NTMP	NPM	NPM NTMP %
A1	-	-	0	-	-	-	0	-
A2	-	-	0	-	-	-	0	-
A3	-	-	0	-	-	-	0	-
A4	-	-	0	-	-	-	0	-
B1	-	-	0	-	-	-	0	-
B2	-	-	0	-	-	-	0	-
B3	-	-	0	-	-	-	0	-
B4	-	-	0	-	-	-	0	-
C1	-	-	0	-	-	-	0	-
C2	-	-	(1)	-	-	-	0	-
C3	-	-	(1)	-	-	-	0	-
C4	-	-	(1)	-	-	-	0	-
D1	-	-	0	-	-	-	0	-
D2	-	-	0	-	-	-	0	-
D3	-	-	0	-	-	-	0	-
D4	-	-	0	-	-	-	0	-
E1	-	-	0	-	-	-	0	-
E2	-	-	0	-	-	-	0	-
E3	-	-	0	-	-	-	0	-
E4	-	-	0	-	-	-	0	-
F1	-	-	0	-	-	-	0	-
F2	-	-	0	-	-	-	0	-
F3	-	-	0	-	-	-	0	-
F4	-	-	(1)	-	-	-	0	-
CONTROLS								
A,C,D	12	16	4	25	12	15	5	33
B	11	17	0	0	12	15	3	20
E,F	13	15	1	7	16	11	0	0

DFOP = Day first observed parasitaemic.
NTMP = Number of temperature measurements when parasitaemic.
NPM = Number of pyrexic temperature measurements.
( ) = Not parasitaemic.
* = Number of measurements.

Table 8, appendix I

TEMPERATURE MEASUREMENTS OVER 39.4°C

	4 MONTH CHALLENGE (28*)				5 MONTH CHALLENGE (28*)			
ANIMAL NO.	DFOP	NTMP	NPM	NPM NTMP %	DFOP	NTMP	NPM	NPM NTMP %
A1	-	-	0	-	-	-	0	-
A2	-	-	0	-	24	5	1	20
A3	-	-	(1)	-	-	-	0	-
A4	-	-	0	-	-	-	(1)	-
B1	-	-	0	-	-	-	0	-
B2	-	-	0	-	-	-	0	-
B3	-	-	0	-	-	-	0	-
B4	-	-	0	-	-	-	0	-
C1	-	-	0	-	14	14	0	-
C2	-	-	0	-	-	-	0	-
C3	-	-	0	-	14	14	1	7
C4	21	8	1	13				
D1	-	-	0	-	14	14	(1)3	21
D2	-	-	0	-	-	-	0	-
D3	-	-	0	-	-	-	0	-
D4	-	-	(1)	-	30	0	0	0
E1	-	-	0	-	-	-	0	-
E2	-	-	0	-	7	21	0	0
E3	-	-	0	-	-	-	0	-
E4	16	14	2	14				
F1	-	-	0	-	-	-	0	-
F2	-	-	0	-	14	14	3	21
F3	21	9	0	0				
F4	-	-	0	-	14	14	2	14
CONTROLS								
A,C,D	14	15	4	27	12	16	2	13
B	12	17	4	24	12	16	9	56
E,F	14	16	(1)2	13	14	14	4	29

DFOP = Day first observed parasitaemic.
NTMP = Number of temperature measurements when parasitaemic.
NPM = Number of pyrexic temperature measurements.
( ) = Not parasitaemic.
* = Number of measurements.

Table 9, appendix I

TEMPERATURE MEASUREMENTS OVER 39.4°C

	6 MONTH CHALLENGE (27*)				7 MONTH CHALLENGE (28*)			
ANIMAL NO.	DFOP	NTMP	NPM	NPM NTMP %	DFOP	NTMP	NPM	NPM NTMP %
A1	-	-	0	-	-	-	0	-
A3	-	-	(2)	-	-	-	0	-
A4	24	6	0	0				
B1	14	14	1	7				
B2	-	-	0	-	-	-	0	-
B3	-	-	0	-	-	-	0	-
B4	29	2	0	0				
C2	-	-	0	-	-	-	0	-
D2	28	3	0	0				
D3	12	16	4	25				
E1	21	9	0	0				
E3	19	10	3	30				
F1	-	-	0	-	24	6	0	-
CONTROLS								
A,C,D	14	14	1	7	12	17	5	29
B	12	16	3	19	14	15	(1)7	47
E,F	14	14	1	7				

DFOP = Day first observed parasitaemic.
NTMP = Number of temperature measurements when parasitaemic.
NPM = Number of pyrexic temperature measurements.
( ) = Not parasitaemic.
* = Number of measurements.

Table 10, appendix I

STUDIES IN MICE ON THE THERAPEUTIC ACTIVITY OF ISOMETAMIDIUM ON TRYPANOSOME POPULATIONS ISOLATED FROM CATTLE SUSCEPTIBLE TO THE 4 MONTH CHALLENGE

Data = No. mice parasitaemic/No. mice treated

TREATMENT	ANIMAL:	*(A,C,D)	*(E,F)	C4	E2	E4	F3
6 hours							
0.5mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	1/6
1.0mgkg^{-1}		0/5	0/6	0/6	0/6	0/6	0/6
2.0mgkg^{-1}		0/5	0/6	0/5	0/6	0/6	0/6
4.0mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	0/6
10^7 tryps.ml^{-1}							
0.5mgkg^{-1}		0/6	0/4	0/6	1/6	1/6	0/6
1.0mgkg^{-1}		0/5	0/6	0/6	0/6	0/6	0/6
2.0mgkg^{-1}		0/6	0/6	1/6	0/6	0/6	0/5
4.0mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	0/6
None							
Control		6/6	6/6	6/6	6/6	6/6	6/6

* Control

Table 11, appendix I

STUDIES IN MICE ON THE THERAPEUTIC ACTIVITY OF ISOMETAMIDIUM ON TRYPANOSOME POPULATIONS ISOLATED FROM CATTLE SUSCEPTIBLE TO THE 5 MONTH CHALLENGE

Data = No. mice parasitaemic/No. mice treated

TREATMENT	ANIMAL:	*(A,C,D)	*(B)	*(E,F)	A2	C1	C3	D1	D4	F2	F4
6 hours											
0.5mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/5
1.0mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/5
2.0mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6	0/5
4.0mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/5
10^7 tryps.ml^{-1}											
0.5mgkg^{-1}		1/6	0/6	0/6	0/6	1/6	0/6	0/6	1/6	0/6	0/6
1.0mgkg^{-1}		0/6	1/6	1/6	0/6	2/6	0/6	0/6	0/6	1/6	0/6
2.0mgkg^{-1}		1/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6
4.0mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
None											
Control		6/6	6/6	6/6	5/5	4/4	5/5	6/6	5/5	6/6	6/6

*Control animal

Table 12, appendix I

STUDIES IN MICE ON THE THERAPEUTIC ACTIVITY OF ISOMETAMIDIUM ON TRYPANOSOME POPULATIONS ISOLATED FROM CATTLE SUSCEPTIBLE TO THE 6 MONTH CHALLENGE

Data = No. mice parasitaemic/No. mice treated

TREATMENT	ANIMAL:	*(A,C,D)	*(B)	*(E,F)	A4	B1	B4	D2	D3	E1	E3
6 hours											
0.5mgkg^{-1}		0/5	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
1.0mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
2.0mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
4.0mgkg^{-1}		0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
10^7 tryps.ml^{-1}											
0.5mgkg^{-1}		0/6	0/6	0/6	2/6	0/6	0/6	0/6	0/6	0/6	1/6
1.0mgkg^{-1}		1/6	0/6	1/6	1/6	1/6	0/5	1/6	0/6	0/6	0/6
2.0mgkg^{-1}		0/6	0/6	0/6	0/6	2/6	0/5	0/6	1/6	0/6	1/6
4.0mgkg^{-1}		0/6	0/6	0/6	0/6	2/6	0/5	0/6	0/5	1/6	0/6
None											
Control		6/6	6/6	6/6	5/5	6/6	4/6	6/6	5/5	6/6	6/6

*Control animal

TABLE 13, appendix I

STUDIES IN MICE ON THE THERAPEUTIC ACTIVITY OF ISOMETAMIDIUM ON TRYPANOSOME POPULATIONS ISOLATED FROM CATTLE SUSCEPTIBLE TO THE 7 MONTH CHALLENGE

Data = No. mice parasitaemic/No. mice treated

TREATMENT	ANIMAL: *(A,C)	F1
6 hours		
0.5mgkg^{-1}	0/6	0/6
1.0mgkg^{-1}	0/6	0/6
2.0mgkg^{-1}	0/6	0/6
4.0mgkg^{-1}	0/6	0/6
10^7 tryps.ml^{-1}		
0.5mgkg^{-1}	0/6	1/6
1.0mgkg^{-1}	0/5	0/6
2.0mgkg^{-1}	0/6	0/5
4.0mgkg^{-1}	0/5	1/4
None		
Control	6/6	6/6

* Control

Table 14, appendix I

MOUSE INFECTIVITY OF *IN VITRO*-DERIVED *T.CONGOLENSE* ILNat 3.1 METACYCLIC TRYPANOSOMES SUBSEQUENT TO GAMMA-IRRADIATION

(Mouse inoculum = $1x10^5$ trypanosomes)

Data = No. mice parasitaemic/No. mice inoculated

GAMMA-RADIATION (KILORADS)	MOUSE GROUPS	
	IRRADIATED	CONTROL
0	-	6/6
5	3/6	6/6
10	0/6	6/6
15	0/6	6/6

Table 15, appendix I

INFECTIVITY NEUTRALISATION TEST USING *T.CONGOLENSE* ILNat 3.1 METACYCLIC TRYPANOSOMES

Figures = No. mice parasitaemic/No. mice inoculated.

All sera tested at a dilution of 1:2.

ANIMAL:	D127	D128	D129	D133	D134	D135
DAY						
0*	6/6	6/6	6/6	5/6	6/6	6/6
2	6/6	6/6	6/6	6/6	5/6	5/6
4	5/6	6/6	6/6	6/6	6/6	6/6
6	6/6	6/6	6/6	5/6	6/6	5/6
8	5/5	6/6	5/6	6/6	5/6	3/6
10	6/6	6/6	4/6	5/6	5/6	6/6
12	6/6	5/6	5/6	6/6	6/6	5/6
14	6/6	6/6	2/6	6/6	6/6	5/6
16	6/6	6/6	4/6	4/4	2/6	4/6

*Day 0 - cattle administered $1x10^6$ gamma-irradiated T.congolense ILNat 3.1 metacyclic trypanosomes, intradermally.

Positive controls included (data not shown).

Table 16, appendix I

IFA TEST USING FORMALDEHYDE FIXED *T.CONGOLENSE* ILNat 3.1 METACYCLIC TRYPANOSOMES

Figures = % population strongly fluorescent.

All sera tested at a dilution of 1:10.

ANIMAL:	D127	D128	D129	D133	D134	D135
DAY						
0*	0	0	0	0	0	0
2	0	0	0	0	0	0
4	0	0	0	0	0	0
6	0	0	0	0	0	0
8	5	30	48	26	36	44
10	0	0	0	0	0	0
12	0	0	0	0	0	0
14	0	0	0	0	0	0
16	0	0	0	0	0	0

*Day 0 - cattle administered $1x10^6$ gamma-irradiated T.congolense ILNat 3.1 metacyclic trypanosomes, intradermally.

Positive sera included (data not shown).

Appendix I,

FIGURE 1

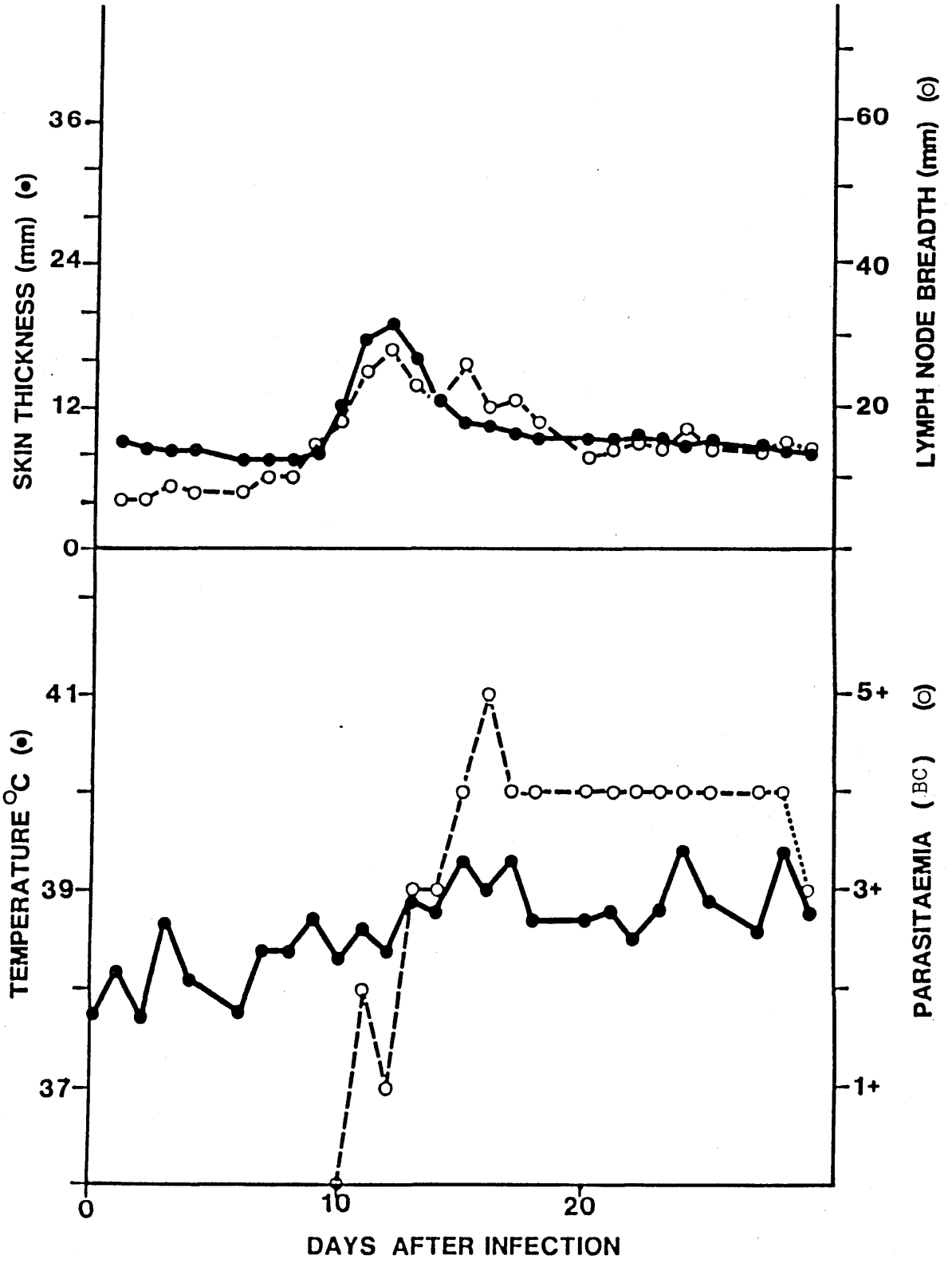

Figure 1: 2 month T.congolense IL 2642 tsetse challenge control. Change in skin thickness at tsetse bite sites and width of draining lymph node; change in temperature and development of parasitaemia.

Appendix I,

FIGURE 2

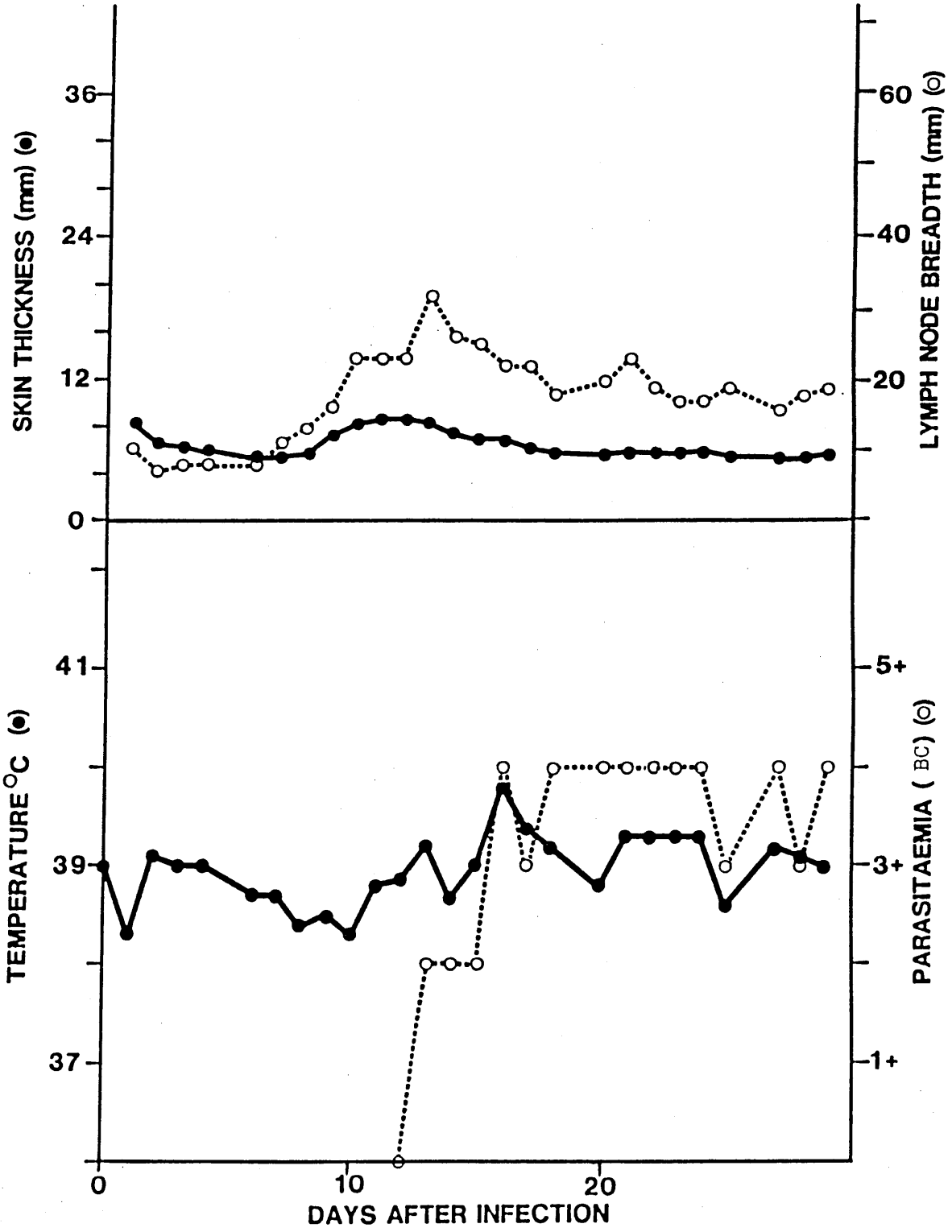

Figure 2: 2 month T.congolense ILNat 3.1 metacyclic ($5x10^3$) challenge control. Change in skin thickness at inoculation sites and width of draining lymph node; change in temperature and development of parasitaemia.

Appendix I,
FIGURE 3

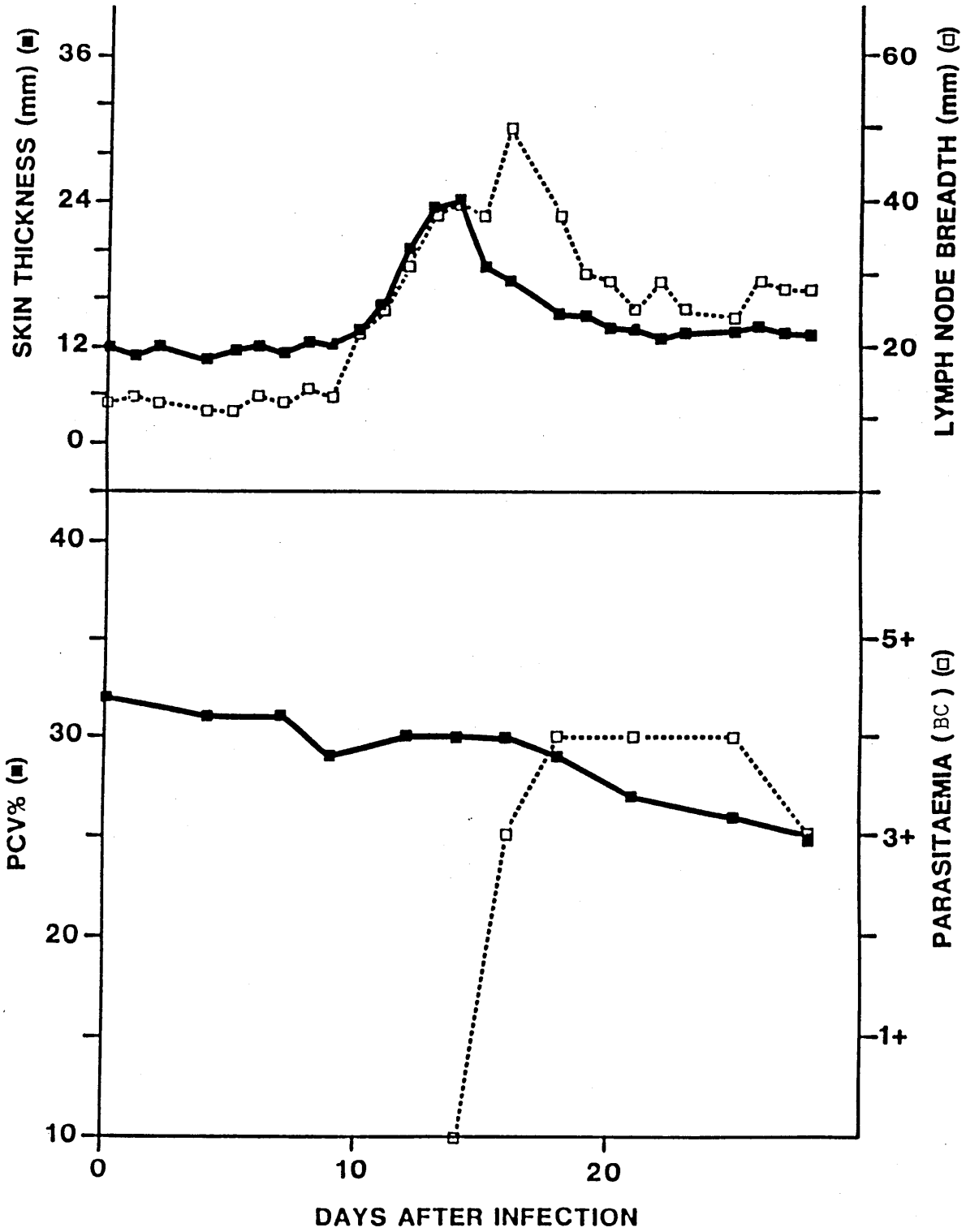

Figure 3: 3 month T.congolense ILNat 3.1 metacyclic ($5x10^3$) challenge control. Change in skin thickness at inoculation sites and width of draining lymph node; change in packed red cell volume (PCV) and development of parasitaemia.

Appendix I,

FIGURE 4a

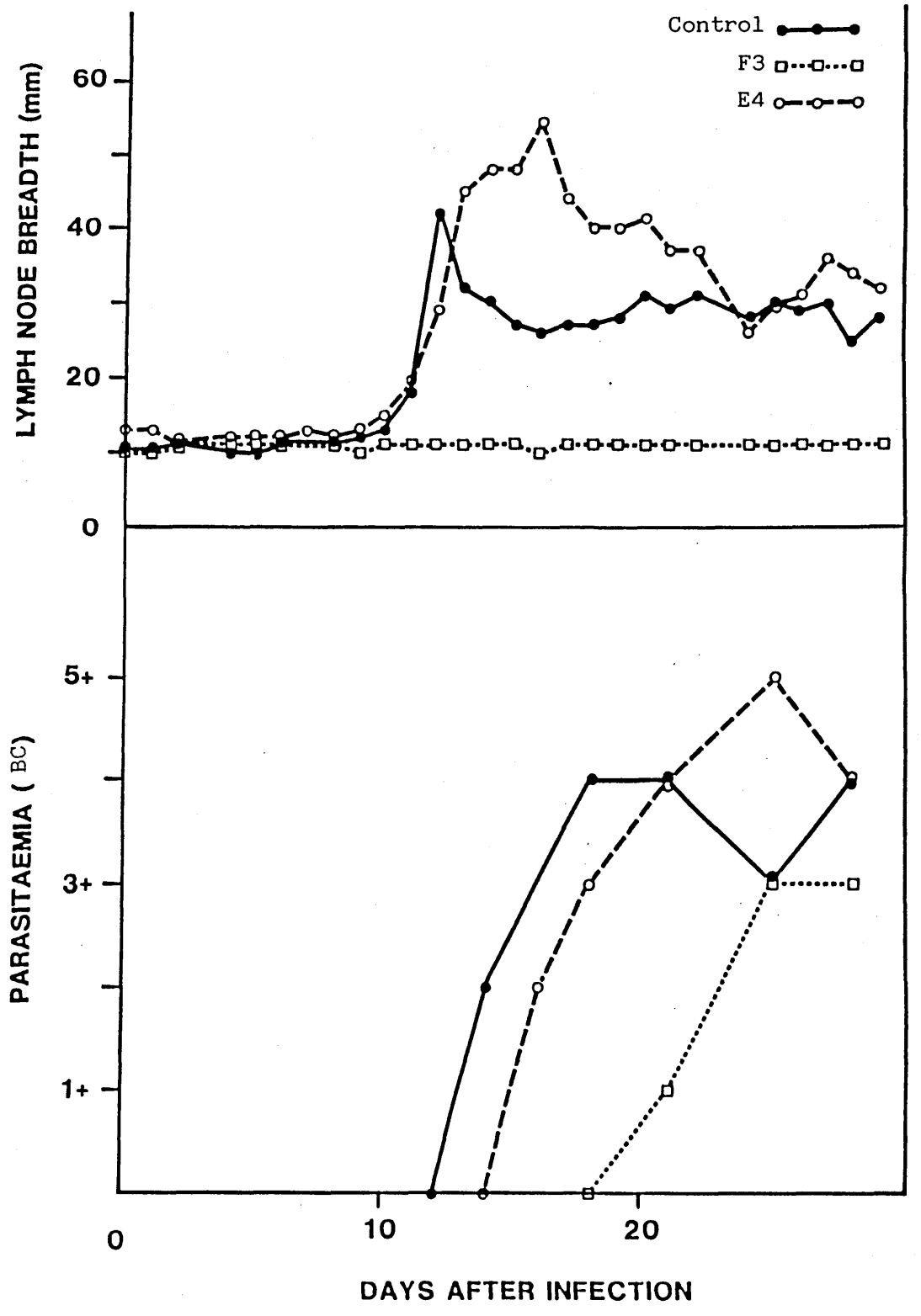

Figure 4a: 4 month challenge. Change in width of the draining lymph node; and development of parasitaemia in E4, F3 and the T.congolense ILNat 3.1 metacyclic ($5x10^3$) challenge control.

Appendix I,

FIGURE 4b

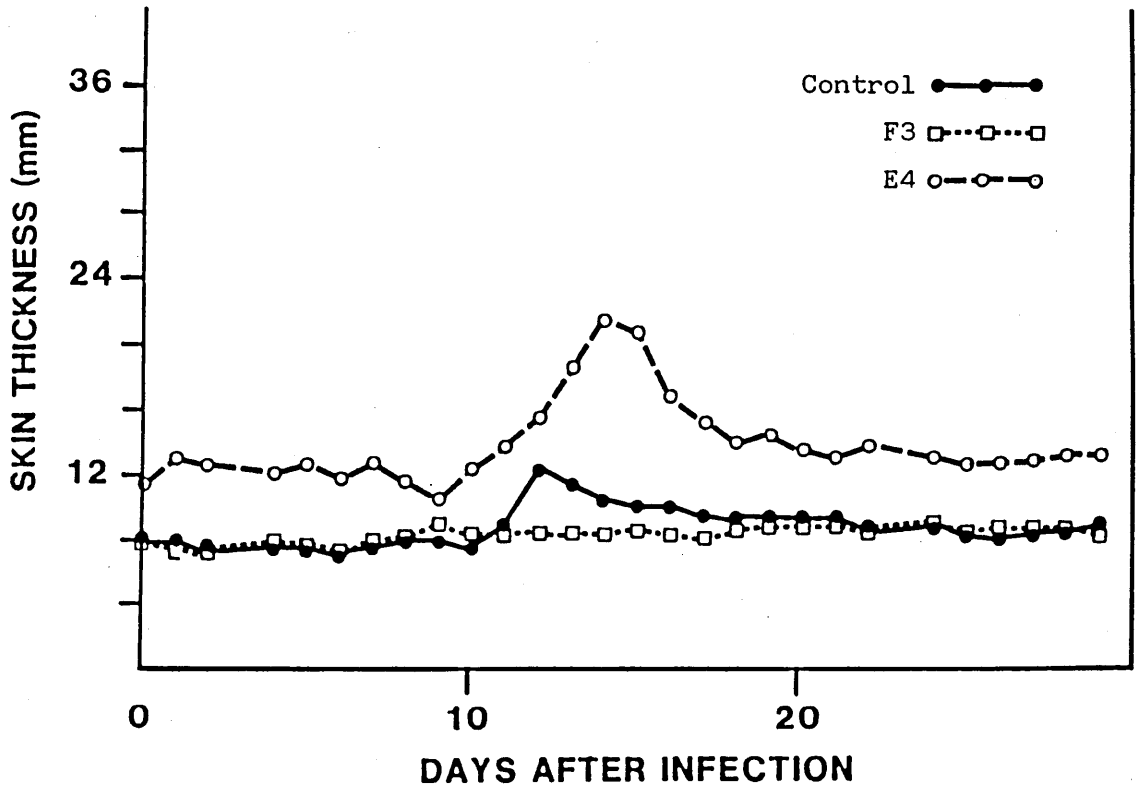

Figure 4b: 4 month challenge. Change in skin thickness at inoculation sites in E4, F3 and the T.congolense ILNat 3.1 metacyclic ($5x10^3$) challenge control.

Appendix I,

FIGURE 5

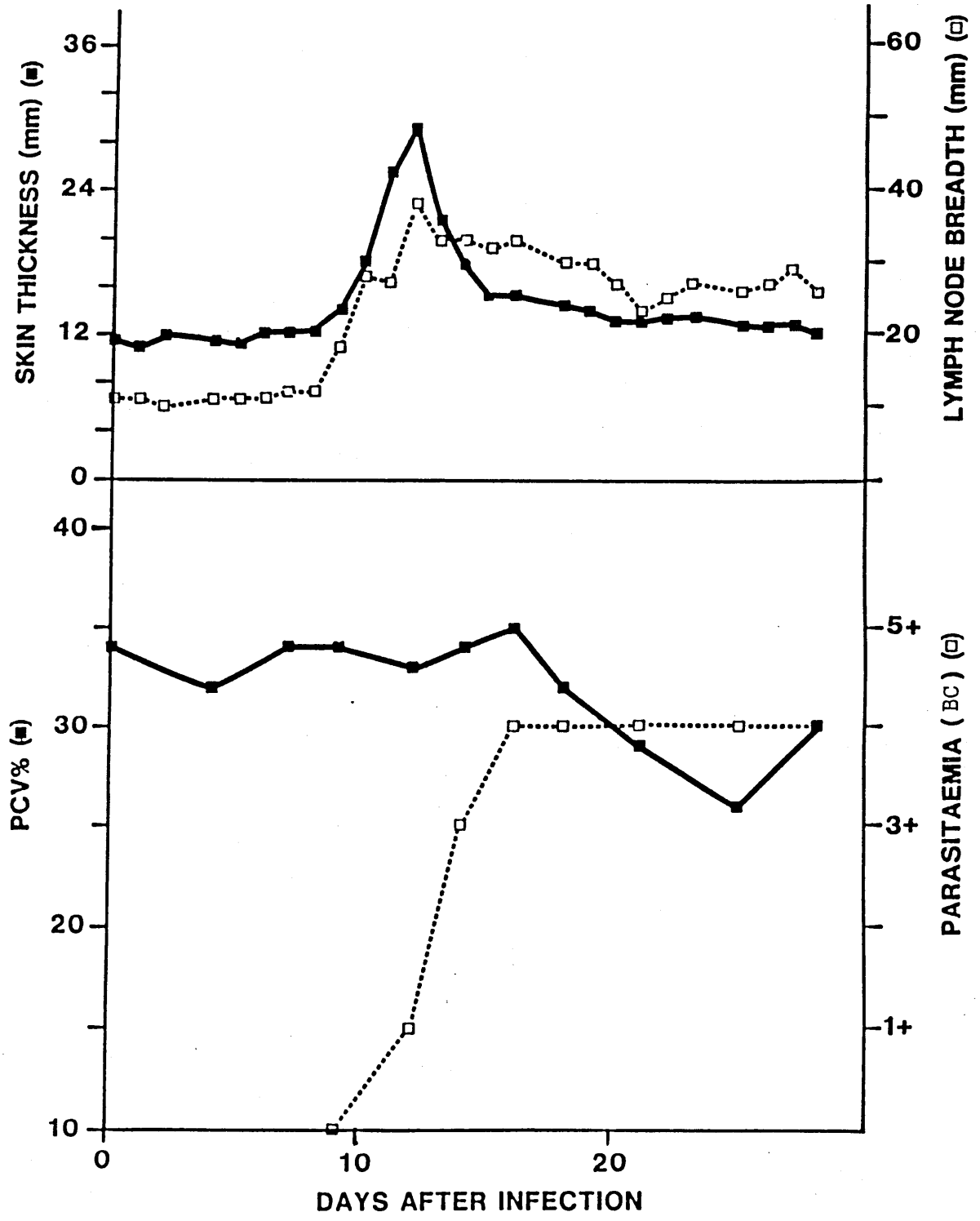

Figure 5: 3 month T.congolense ILNat 3.1 tsetse challenge control. Change in skin thickness at tsetse bite sites and width of draining lymph node; change in packed red cell volume (PCV) and development of parasitaemia.

Appendix I,

FIGURE 6

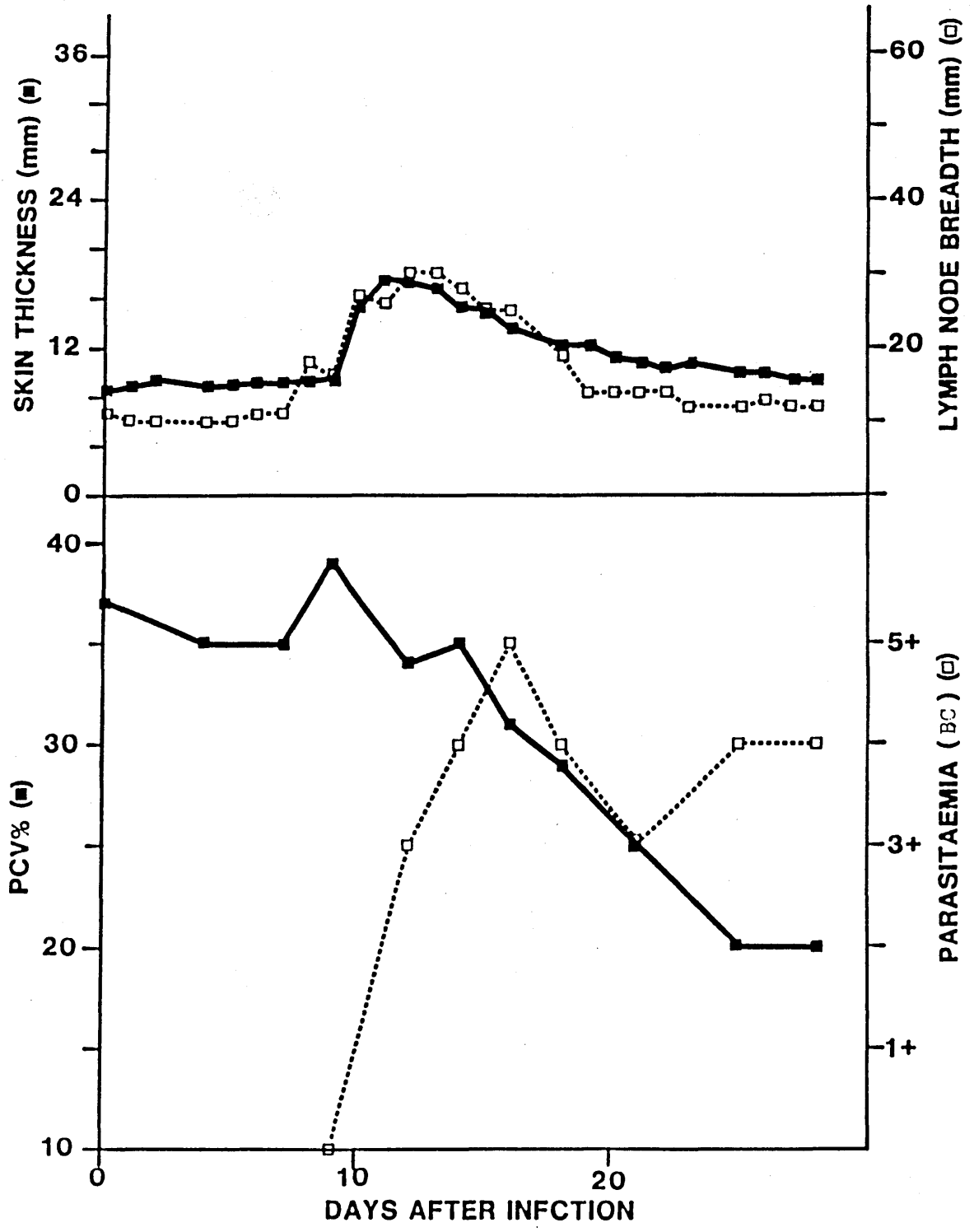

Figure 6: 3 month T.congolense IL 2642 tsetse challenge control. Change in skin thickness at tsetse bite sites and width of draining lymph node; change in packed red cell volume (PCV) and development of parasitaemia.

APPENDIX II

Table 1, appendix II

BORAN W5 - 24 HOUR *IN VITRO* INCUBATION OF PLASMA WITH *T.congolense* ILNat 3.1 BLOODSTREAM FORMS

(susceptible to challenge on day 181)

SAMPLE	DAY	% GROWTH INHIBITION	MOUSE INOCULUM	INFECTIVITY FOR MICE*
1a	0	0	$1.0x10^5$	5/5
1b	0	0	$1.0x10^5$	5/5
2a	34	57.1	$7.8x10^4$	0/5
2b	34	-3.5	$1.0x10^5$	0/5
3a	58	100	ND	
3b	58	95.8	$3.5x10^3$	0/5
4a	83	100	ND	
4b	83	100	ND	
5a	118^R	100	ND	
5b	118^R	100	ND	
6a	153	100	ND	
6b	153	100	ND	
7b	174	-17.2	$1.0x10^5$	4/5
8a	188	100	NTC	0/5
8b	188	100	NTC	

* Data = No. mice parasitaemic / No. mice inoculated.
a = Experiment 1.
b = Experiment 2.
ND = Not done.
NTC = No trypanosomes counted.
^{R} = Refractory to challenge.

Table 2, appendix II

BORAN W6 - 24 HOUR *IN VITRO* INCUBATION OF PLASMA WITH *T.congolense* ILNat 3.1 BLOODSTREAM FORMS

SAMPLE	DAY	% GROWTH INHIBITION	MOUSE INOCULUM	INFECTIVITY FOR MICE*
1a	0	0	$1.0x10^5$	5/5
1b	0	0	$1.0x10^5$	5/5
2a	27	100	ND	
2b	27	100	ND	
3a	58	67.1	$5.8x10^4$	4/5
3b	58	25.5	$1.0x10^5$	1/5
4a	83	99.3	$1.3x10^3$	0/5
4b	83	94.7	$1.9x10^4$	0/5
5a	118^R	100	ND	
5b	118^R	100	ND	
6b	148	100	NTC	0/5
7a	181^s	6.7	$1.0x10^5$	5/5
7b	181^s	-0.8	$1.0x10^5$	5/5

* Data = No. mice parasitaemic / No. mice inoculated.
a = Experiment 1.
b = Experiment 2.
ND = Not done.
NTC = No trypanosomes counted.
R = Refractory to challenge.
s = Susceptible to challenge.

Table 3, appendix II

BORAN W7 - 24 HOUR *IN VITRO* INCUBATION OF PLASMA WITH *T.congolense* ILNat 3.1 BLOODSTREAM FORMS

SAMPLE	DAY	% GROWTH INHIBITION	MOUSE INOCULUM	INFECTIVITY FOR MICE*
1a	0	0	$1.0x10^5$	5/5
2a	27	100	ND	
3a	58	100	ND	
4a	83	100	ND	
5a	118[R]	-7.5	$1.0x10^5$	0/5
6a	148	9.3	$1.0x10^5$	0/5
7a	181[R]	8.6	$1.0x10^5$	3/5

* Data = No. mice parasitaemic / No. mice inoculated.
a = Experiment 1.
ND = Not done.
NTC = No trypanosomes counted.
[R] = Refractory to challenge.

Table 4, appendix II

BORAN W1 - 24 HOUR *IN VITRO* INCUBATION OF PLASMA WITH *T.congolense* ILNat 3.1 BLOODSTREAM FORMS

(day 181 plasma sample = control)

SAMPLE	DAY	% GROWTH INHIBITION	MOUSE INOCULUM	INFECTIVITY FOR MICE*
1a	0	100	ND	
2a	27^{R}	100	ND	
3a	58^{R}	93.6	ND	
4a	83^{R}	100	ND	
5a	118^{R}	100	ND	
6a	148^{R}	12.5	$1.0x10^{5}$	5/5
7a	181^{s}	0	$1.0x10^{5}$	5/5

* Data = No. mice parasitaemic / No. mice inoculated.
a = Experiment 1 (only one experiment conducted).
ND = Not done.
^{R} = Refractory to challenge.
^{s} = Susceptible to challenge.

Table 5, appendix II

BORAN W2 - 24 HOUR *IN VITRO* INCUBATION OF PLASMA WITH *T.congolense* ILNat 3.1 BLOODSTREAM FORMS

SAMPLE	DAY	% GROWTH INHIBITION	MOUSE INOCULUM	INFECTIVITY FOR MICE*
1a	0	100	ND	
2a	27	100	ND	
3a	58^{R}	100	ND	
4a	83	100	ND	
5a	118	100	ND	
6a	148	100	ND	
7a	181^{s}	0	$6.7x10^{4}$	5/5

* Data = No. mice parasitaemic / No. mice inoculated.
a = Experiment 1 (only one experiment conducted).
ND = Not done.
R = Refractory to challenge.
s = Susceptible to challenge.

Table 6, appendix II

BORAN W3 - 24 HOUR *IN VITRO* INCUBATION OF PLASMA WITH *T.congolense* ILNat 3.1 BLOODSTREAM FORMS

(day 181 plasma sample = control)

SAMPLE	DAY	% GROWTH INHIBITION	MOUSE INOCULUM	INFECTIVITY FOR MICE*
1a	0	100	ND	
2a	27	100	ND	
3a	58^{R}	93.6	ND	
4a	83	100	ND	
5a	118	100	ND	
6a	148	100	ND	
7a	181^{s}	0	1.0×10^{5}	4/4

* Data = No. mice parasitaemic / No. mice inoculated.
a = Experiment 1 (only one experiment conducted).
ND = Not done.
R = Refractory to challenge.
s = Susceptible to challenge.

Table 7a, appendix II

BORAN W4 - 24 HOUR *IN VITRO* INCUBATION OF PLASMA WITH *T.congolense* ILNat 3.1 BLOODSTREAM FORMS

(day 174 sample = control)

SAMPLE	DAY	% GROWTH INHIBITION	MOUSE INOCULUM	INFECTIVITY FOR MICE*
1a	0	100	NTC	0/5
1b	0	100	NTC	0/5
2a	34	100	ND	
2b	34	100	ND	
3a	58	96.9	$6.9x10^3$	0/5
3b	58	99.2	$3.1x10^3$	0/5
4a	83R	100	ND	
4b	83R	100	ND	
5a	118	100	ND	
5b	118	100	ND	
6a	153	100	ND	
7b	157	100	ND	
8a	174	0	$1.0x10^5$	5/5
8b	174	0	$1.0x10^5$	5/5
9a	188	100	ND	0/5
9b	188	100	ND	

* Data = No. mice parasitaemic / No. mice inoculated.
a = Experiment 1.
b = Experiment 2.
ND = Not done.
NTC = No trypanosomes counted.
R = Refractory to challenge.

Table 7b, appendix II

BORAN W4 - 24 HOUR *IN VITRO* INCUBATION OF PLASMA WITH *T.congolense* ILNat 3.1 BLOODSTREAM FORMS

(foetal bovine serum (FBS) = control)

SAMPLE	DAY	% GROWTH INHIBITION	MOUSE INOCULUM	INFECTIVITY FOR MICE*
1a	FBS	0	$1.0x10^5$	5/5
1b	FBS	0	$1.0x10^5$	5/5
2a	41	100	ND	
3a	48	100	ND	
4a	55	3.4	$1.0x10^5$	0/5
5a	58	66.5	$1.0x10^5$	0/5
6a	62	100	ND	
7a	70	100	ND	
8a	76	100	ND	
9b	153	100	ND	
10b	160	100	ND	
11b	167	100	ND	
12b	181s	23.0	$1.0x10^5$	5/5
13b	196	100	ND	
14b	202	100	ND	
15b	209	73.3	$1.0x10^5$	4/5

* Data = No. mice parasitaemic / No. mice inoculated.
a = Experiment 1.
b = Experiment 2.
ND = Not done.
s = Susceptible to challenge.

APPENDIX III

Table 1, appendix III

T.VIVAX EXPERIMENTS - CATTLE CODES

EXPERIMENT 1			EXPERIMENT 2		
ANIMAL CODE	GROUP	NUMBER	ANIMAL CODE	GROUP	NUMBER
G1	G	D335	J1	J	D316
G2	G	D339	J2	J	D317
G3	G	D343	J3	J	D318
G4	G	D359	J4	J	D319
G5	G	D363	J5	J	D374
H1	H	D338	K1	K	D321
H2	H	D340	K2	K	D329
H3	H	D347	K3	K	D365
H4	H	D350	K4	K	D366
H5	H	D355	K5	K	D370

CONTROLS

MONTH	CODE	GROUP G	GROUP H	GROUP J	GROUP K
1	1a	D330	D332	D536	D561
1	1b	D331	D334	-	-
2	2a	D336	D348	-	D562
2	2b	D341	D349	-	-
3	3a	D354	-	-	-
3	3b	D356	-	-	-
4	4a	D337	-	-	-
4	4b	D357	-	-	-
5	5a	D21	-	-	-
5	5b	D51	-	-	-

Table 2, appendix III

1 MONTH CHALLENGE - TSETSE DATA

T.VIVAX	ANIMAL	DAY	No.+ve BITES	DAY	No.+ve BITES	TOTAL +ve BITES
IL 2968	G1	32	6	35	5	11
"	G2	"	7	"	3	10
"	G3	"	4	"	7	11
"	G4	"	4	"	7	11
"	G5	"	5	"	8	13
IL 2969	H1	32	10	35	-	10
"	H2	"	7	"	3	10
"	H3	"	8	"	2	10
"	H4	"	11	"	-	11
"	H5	"	13	"	-	13
		C O N T R O L S				
IL 2968	a	32	10	-	-	10
"	b	"	10	-	-	10
IL 2969	a	32	10	-	-	10
"	b	"	10	-	-	10

Table 3, appendix III

DETECTION OF TRYPANOSOMES BY THE BUFFY-COAT PHASE-CONTRAST (BC) TECHNIQUE AND MOUSE INOCULATION (MI) FOLLOWING CHALLENGE 1 AND 2 MONTHS AFTER ADMINISTRATION OF ISOMETAMIDIUM CHLORIDE

		1 MONTH CHALLENGE			2 MONTH CHALLENGE		
			MI			MI	
Group	Animal	BC/DFOP	d12	d24	BC/DFOP	d12	d24
G	G1	-	0*	0	-	0	0
G	G2	-	0	0	-	0	0
G	G3	-	0	0	-	0	0
G	G4	-	0	0	-	0	0
G	G5	-	0	0	-	0	0
H	H1	-	ND	ND	7	ND	ND
H	H2	-	ND	ND	9	ND	ND
H	H3	12	ND	ND			
H	H4	23	ND	ND			
H	H5	24	ND	ND			
G CONTROL a		10	2	3	11	3	3
G CONTROL b		10	3	3	11	3	3
H CONTROL a		10	ND	ND	11	ND	ND
H CONTROL b		9	ND	ND	11	ND	ND

BC/DFOP = Day first observed parasitaemic using BC technique.
* = Number of mice parasitaemic (3 mice inoculated).
ND = Not done; non-infective for mice.

Table 4, appendix III

DETECTION OF TRYPANOSOMES BY THE BUFFY-COAT PHASE-CONTRAST (BC) TECHNIQUE AND MOUSE INOCULATION (MI) FOLLOWING CHALLENGE 3, 4 AND 5 MONTHS AFTER ADMINISTRATION OF ISOMETAMIDIUM CHLORIDE

		3 MONTH CHALLENGE			4 MONTH CHALLENGE		
			MI			MI	
Group	Animal	BC/DFOP	d12	d24	BC/DFOP	d12	d24
G	G1	-	0*	0	-	0	0
G	G2	-	0	0	-	0	0
G	G3	11	2	3			
G	G4	-	0	0	11	3	3
G	G5	11	3	3			
G CONTROL a		11	3	3	11	3	3
G CONTROL b		9	3	3	11	3	3

		5 MONTH CHALLENGE		
			MI	
Group	Animal	BC/DFOP	d12	d24
G	G1	-	0	0
G	G2	-	0	0
G CONTROL a		11	3	3
G CONTROL b		9	3	3

BC/DFOP = Day first observed parasitaemic using BC technique.
* = Number of mice parasitaemic (3 mice inoculated).

Table 5, appendix III

SKIN THICKNESS AND DRAINAGE LYMPH NODE BREADTH CHANGES IN 1 MONTH CHALLENGE CONTROLS

Group	Animal	Change in skin thickness at (ten) challenge sites			% increase in prefemoral lymph node breadth	1st day detected parasitaemic
		Large increase 65+%	Moderate increase 41-64%	Slight increase 20-40%		
G*	D330	-	-	1	110	10
G	D331	-	-	-	140	10
H+	D332	-	-	1	120	10
H	D334	-	-	-	130	9

* = Infected with T.vivax IL 2968 (Zaria, Nigeria).

+ = Infected with T.vivax IL 2969 (Kilifi, Kenya).

Table 6,appendix III

DRAINAGE PREFEMORAL LYMPH NODE BREADTH CHANGE IN ANIMALS SUSCEPTIBLE TO CHALLENGE

GROUP	ANIMAL	% INCREASE IN PREFEMORAL LYMPH NODE BREADTH
	2 MONTH CHALLENGE (DAY 60)	
H	H1	40
H	H2	50
G control	a	240
G control	b	180
H control	a	60
H control	b	130
	3 MONTH CHALLENGE (DAY 91)	
G	G3	150
G	G5	270
G control	a	210
G control	b	220
	4 MONTH CHALLENGE (DAY 119)	
G	G4	0
G control	a	180
G control	b	200
	5 MONTH CHALLENGE (DAY 151)	
G control	a	180
G control	b	210

G control mean = 203% ± 22% (S.D.).
H control mean = 110% ± 34% (S.D.).

Table 7, appendix III

TEMPERATURE MEASUREMENTS OVER 39.4°C FOLLOWING THE FIRST AND SECOND CHALLENGE

		1 MONTH CHALLENGE (25*)				2 MONTH CHALLENGE (27*)			
GROUP	ANIMAL	DFOP	NTMP	NPM	NPM NTMP %	DFOP	NTMP	NPM	NPM NTMP %
G	G1	-	ND	ND	-	-	-	0	-
G	G2	-	ND	ND	-	-	-	0	-
G	G3	-	ND	ND	-	-	-	0	-
G	G4	-	ND	ND	-	-	-	0	-
G	G5	-	ND	ND	-	-	-	0	-
H	H1	-	ND	ND	-	7	21	3	14
H	H2	-	ND	ND	-	9	19	2	11
H	H3	12	ND	ND	-				
H	H4	23	ND	ND	-				
H	H5	24	ND	ND	-				
CONTROLS									
G	a	10	16	9	56	11	17	5	29
G	b	10	16	(1)9	56	11	17	5	29
H	a	10	16	4	25	11	17	8	47
H	b	9	17	(1)2	12	11	17	(1)14	82

G = T.vivax IL 2968 challenge challenge; H = T.vivax IL 2969 challenge.
DFOP = Day first observed parasitaemic.
NTMP = Number of temperature measurements made when parasitaemic.
NPM = Number of pyrexic temperature measurements.
( ) = Non parasitaemic.
* = Number of measurements.
ND = Not done.

Table 8, appendix III

TEMPERATURE MEASUREMENTS OVER 39.4°C FOLLOWING THE THIRD, FOURTH AND FIFTH CHALLENGE

		3 MONTH CHALLENGE (25*)				4 MONTH CHALLENGE (27*)				5 MONTH CHALLENGE (26*)			
GROUP	ANIMAL	DFOP	NTMP	NPM	NPM NTMP %	DFOP	NTMP	NPM	NPM NTMP %	DFOP	NTMP	NPM	NPM NTMP %
G	G1	-	-	0	-	-	-	0	-	-	-	0	-
G	G2	-	-	0	-	-	-	0	-	-	-	0	-
G	G3	11	16	6	38								
G	G4	-	-	0	-	11	18	3	17				
G	G5	11	16	3	19								
CONTROLS													
G	a	11	16	6	38	11	18	11	61	11	16	(1)8	50
G	b	9	17	10	59	11	18	7	39	9	18	7	39

G = T.vivax IL 2968 challenge.
DFOP = Day first observed parasitaemic.
NTMP = Number of temperature measurements made when parasitaemic.
NPM = Number of pyrexic temperature measurements.
( ) = Non parasitaemic.
* = Number of measurements.

Table 9a, appendix III

TRYPANOLYSIS TEST USING *T.VIVAX* IL 2968 ANTIGEN AND SERA FROM GROUP G CATTLE (CHALLENGED WITH *T.VIVAX* IL 2968)

Figures = Percentage trypanosomes lysed (100 counted)

	1 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	1	1	0	4	3
G2	1	1	0	8	12
G3	0	3	1	2	2
G4	3	3	1	3	1
G5	0	3	3	4	9
CONTROLS					
a	2	0	4	73	100
b	0	1	4	59	93

Serum controls = 7, 12

	2 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	3	1	3	6	2
G2	12	1	2	3	9
G3	2	0	1	0	2
G4	1	2	0	1	9
G5	9	1	0	7	17
CONTROLS					
a	6	0	4	85	100
b	13	0	3	82	95

Serum controls = 7, 12

Table 9b, appendix III

TRYPANOLYSIS TEST USING *T.VIVAX* IL 2968 ANTIGEN AND SERA FROM GROUP G CATTLE (CHALLENGED WITH *T.VIVAX* IL 2968)

Figures = Percentage trypanosomes lysed (100 counted)

	3 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	0	4	0	0	0
G2	0	0	2	0	2
G3	0	0	2	68	82
G4	0	0	3	0	1
G5	0	2	5	83	92
CONTROLS					
a	3	2	6	84	85
b	2	0	3	87	93
	Serum controls = 5, 3				

	4 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	3	3	3	2	3
G2	1	1	6	3	4
G4	2	4	3	68	84
CONTROLS					
a	1	0	3	76	81
b	1	1	5	74	98
	Serum controls = 7, 1				

	5 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
G1	0	0	1	0	3
G2	0	0	0	1	0
CONTROLS					
a	1	1	3	72	84
b	2	1	1	56	75
	Serum controls = 1, 2				

Table 10, appendix III

TRYPANOLYSIS TEST USING *T.VIVAX* IL 2969 ANTIGEN AND SERA FROM GROUP H CATTLE (CHALLENGED WITH *T.VIVAX* IL 2969)

Figures = Number trypanosomes lysed/50 counted

	1 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
H1	1	0	0	1	0
H2	0	0	0	6	0
H3	1	0	0	46	8
H4	1	2	1	4	1
H5	1	1	2	1	2
CONTROLS					
a	0	0	1	50	50
b	0	0	1	50	50
	Serum controls = 2, 1				

	2 MONTH CHALLENGE				
ANIMAL	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
H1	0	0	25	48	46
H2	0	0	2	41	38
H3	8	37	ND	ND	ND
H4	1	34	ND	ND	ND
H5	2	32	ND	ND	ND
CONTROLS					
a	0	6	0	46	40
b	1	0	2	45	48
	Serum controls = 2, 1				

ND = Not done.

Table 11, appendix III

ISOMETAMIDIUM SENSITIVITY STUDIES ON BREAKTHROUGH TRYPANOSOME POPULATIONS OCCURRING IN GROUP G CATTLE

ANIMAL:	3 MONTH CHALLENGE			4 MONTH CHALLENGE	
TREATMENT	G3	G5	(a)	G4	(a)
6 hours					
0.5mgkg^{-1}	0/6*	0/6	3/6	0/6	0/6
1.0mgkg^{-1}	0/5	0/6	0/6	0/6	0/6
2.0mgkg^{-1}	1/6	0/6	0/6	0/6	0/6
4.0mgkg^{-1}	0/6	0/6	0/3	0/6	0/3
10^7 tryps.ml^{-1}					
0.5mgkg^{-1}	4/6	5/6	2/6	2/6	2/6
1.0mgkg^{-1}	0/4	2/6	3/6	1/5	1/6
2.0mgkg^{-1}	0/5	0/6	0/6	0/6	0/6
4.0mgkg^{-1}	0/6	0/6	0/6	1/6	0/6
None	6/6	6/6	6/6	6/6	6/6

( ) = Control animal.
* = Number of mice parasitaemic / Number of mice inoculated.

Table 12, appendix III

DETECTION OF PARASITAEMIA IN GROUP H ANIMALS FOLLOWING 3 MONTH CHALLENGE WITH *T.CONGOLENSE* ILNat 3.1

Animal/Status	1st Day detected parasitaemic by BC technique	Mouse inoculation Day 12	Day 24
H1*	-	0/3+	0/3
H2*	-	0/3	0/3
H3*	-	0/3	0/3
H4*	-	0/3	0/3
H5*	-	0/3	0/3
Controls			
1 - Group H 1 month control*	12	3/3	3/3
2 - Group H 1 month control*	12	3/3	3/3
3 - Berenil only*	12	3/3	3/3
4 - Berenil only*	12	3/3	2/3
5 - Berenil only*	17	0/2	3/3
6 - Non-infected + no Berenil	12	2/3	3/3

* = 3.5mg kg^{-1} diminazene aceturate administered 18 days prior to 3 month (day 93) challenge.
+ Figures = Number mice parasitaemic / Number mice inoculated.

Table 13, appendix III

SKIN THICKNESS AND DRAINAGE LYMPH NODE BREADTH CHANGES IN GROUP H ANIMALS FOLLOWING 3 MONTH CHALLENGE WITH *T.CONGOLENSE* ILNat 3.1

Animal/Status	Change in skin thickness at (five) challenge sites			% Increase in prefemoral lymph node breadth
	Large 65+%	Moderate 41-64%	Slight 20-40%	
H1*	-	-	-	-
H2*	-	-	2	-
H3*	-	-	3	-
H4*	-	-	-	-
H5*	-	-	-	-
Controls				
1 - Group H 1 month control*	5	-	-	210
2 - Group H 1 month control*	2	3	-	210
3 - Berenil only*	1	4	-	340
4 - Berenil only*	5	-	-	220
5 - Berenil only*	5	-	-	320
6 - Non-infected + no Berenil	5	-	-	240

* = 3.5mg kg^{-1} diminazene aceturate administered 18 days prior to 3 month (day 93) challenge.

Appendix III,

FIGURE 1

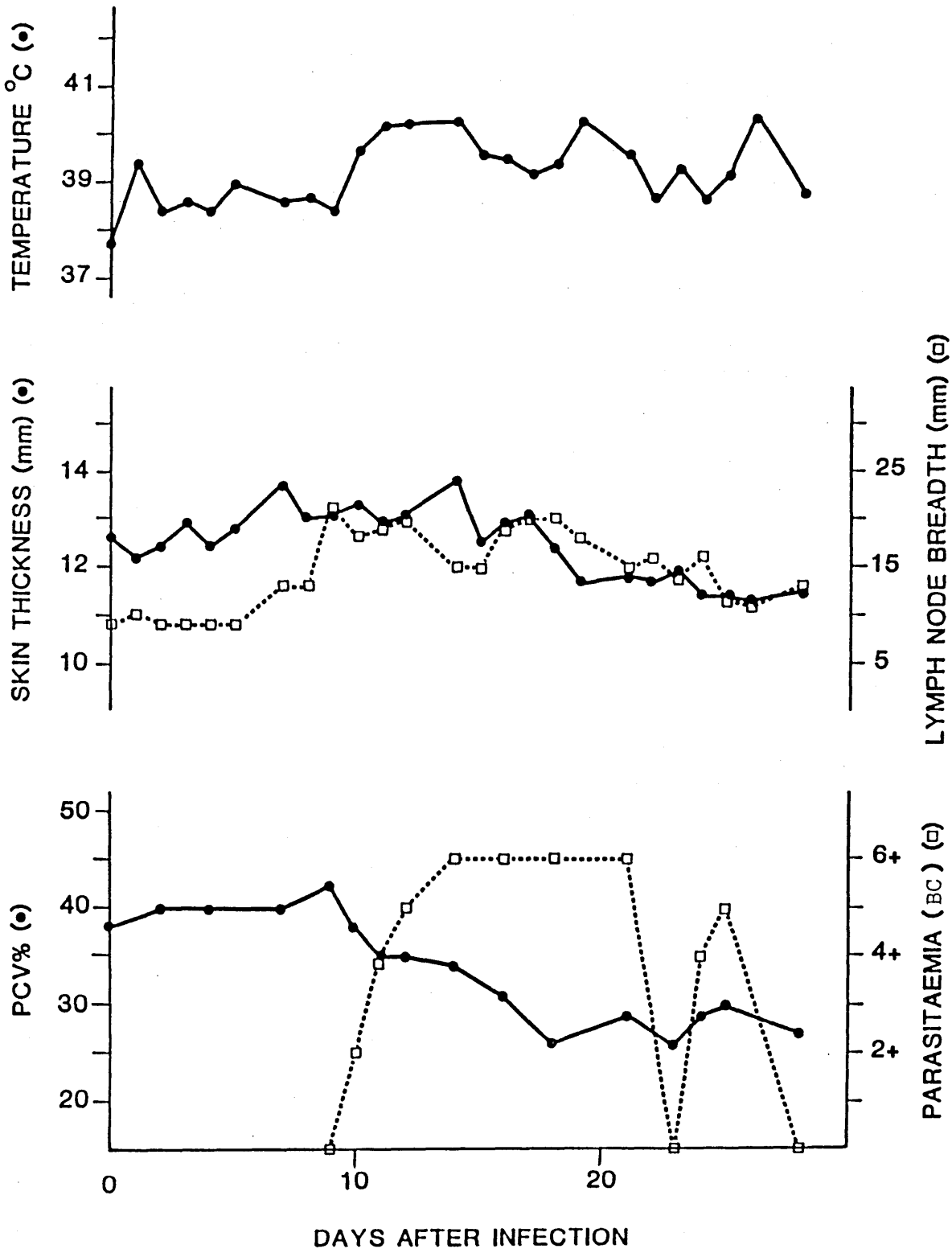

Figure 1: Group G (T.vivax IL 2968) 1 month challenge control (1a). Change in temperature; change in skin thickness at tsetse bite sites and width of draining lymph node; change in packed red cell volume (PCV) and development of parasitaemia.

Appendix III,
FIGURE 2

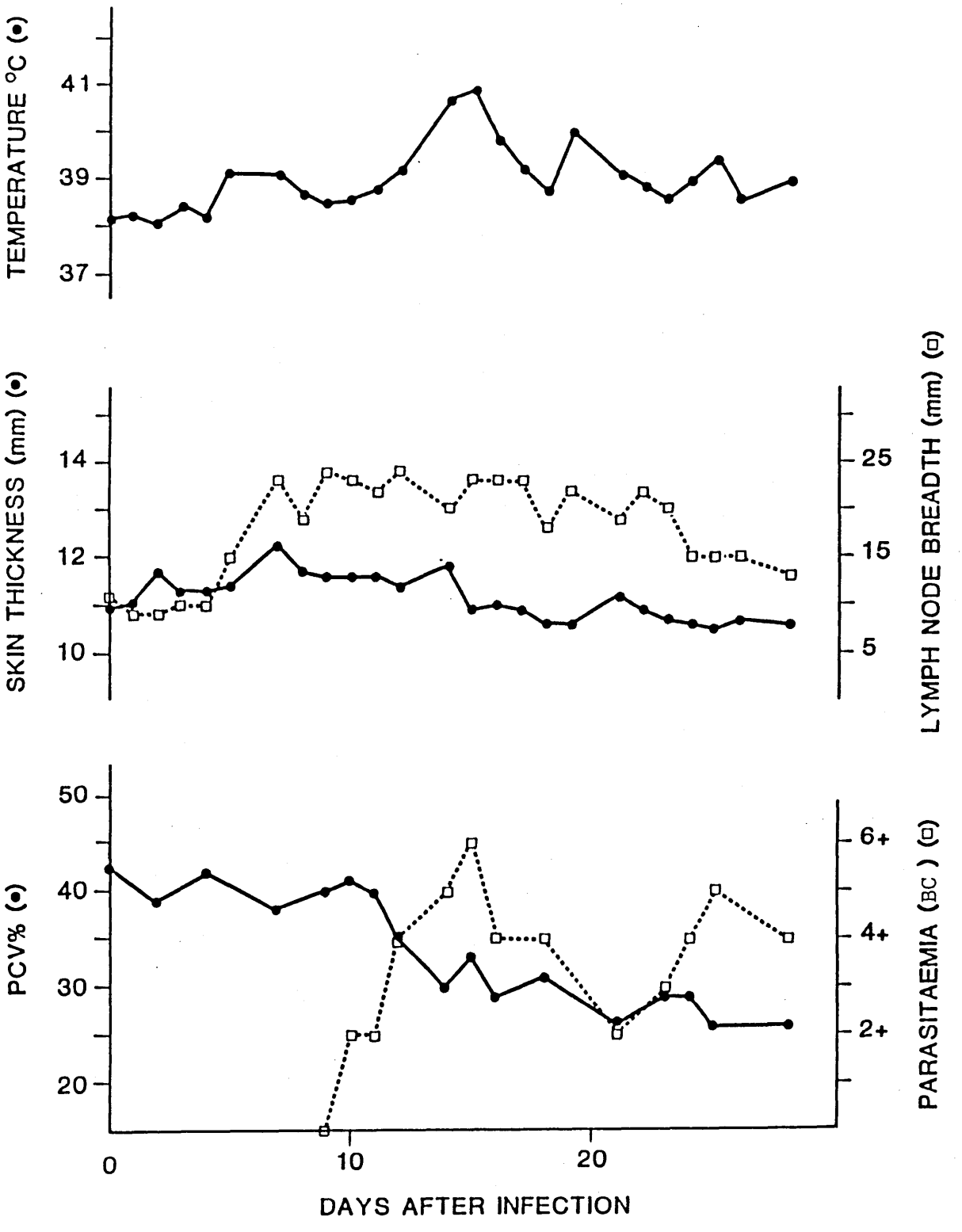

Figure 2: Group H (T.vivax IL 2969) 1 month challenge control (1a). Change in temperature; change in skin thickness at tsetse bite sites and width of draining lymph node; change in packed red cell volume (PCV) and development of parasitaemia.

Appendix III,

FIGURE 3

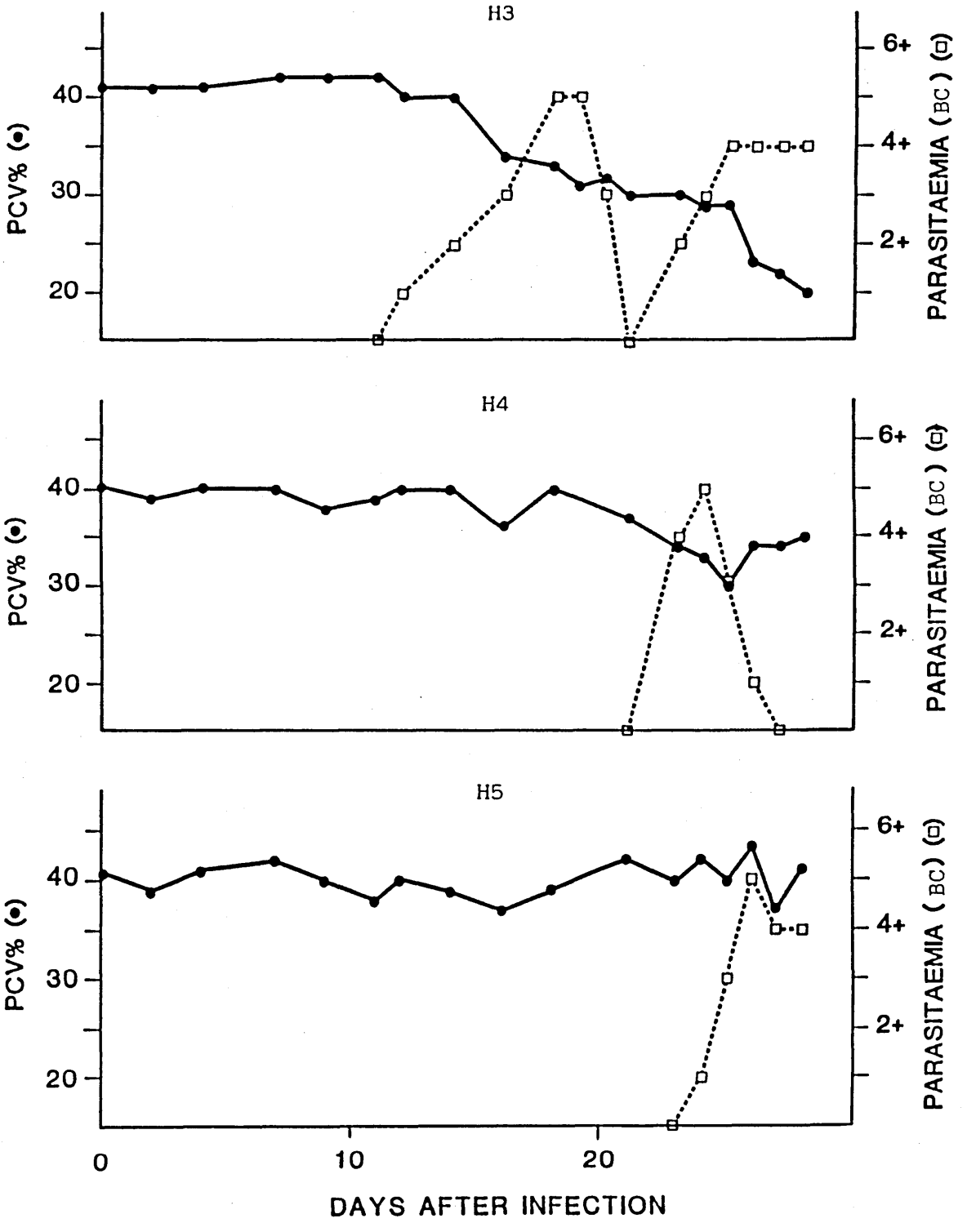

Figure 3: 1 month T.vivax IL 2969 tsetse challenge. Change in packed red cell volume (PCV) and development of parasitaemia in H3, H4 and H5.

APPENDIX IV

Table 1, appendix IV

IN VITRO INCUBATION OF GROUP H CATTLE SERA WITH T.CONGOLENSE ILNat 3.1 BLOODSTREAM FORMS

		H1						H2					
	INCUBATION:	24 HOURS		48 HOURS		72 HOURS		24 HOURS		48 HOURS		72 HOURS	
SAMPLE	DAY	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
1	0	104.6*	9.9	31.6	6.2	6.6	4.5	86.5	9.6	28.5	6.6	2.4	1.1
2	60	40.4	9.5	13.6	2.3	1.5	1.2	66.1	10.0	25.0	5.5	0.5	0.8
3	67	37.9	7.5	12.0	2.3	0.5	0.8	61.5	6.6	21.6	4.3	1.1	1.1
4	74	96.5	15.4	35.3	6.0	4.3	2.2	105.6	10.8	33.1	5.0	1.3+	1.0
PERCENTAGE GROWTH INHIBITION													
1	0	0		0		0		0		0		0	
2	60	61.4		56.9		82.9		23.6		12.3		79.0	
3	67	63.8		62.1		94.3		28.9		24.2		52.5	
4	74	7.7		−11.4		51.4		−22.1		−16.1		47.5	

* Figures = Number of trypanosomes x 10^4 ml $^{-1}$

+ only one well counted

Table 2, appendix IV

INFECTIVITY AND PERCENTAGE GROWTH INHIBITION OF MURINE-DERIVED T.VIVAX IL 2968 BLOODSTREAM FORMS EXPOSED FOR 24 HOURS TO SERA FROM CALF X3 , ADMINISTERED 0.25mgkg^{-1} ISOMETAMIDIUM CHLORIDE ON DAY 0

	DAY									
	0	1	3	7	14	21	28	35	42	49
Percentage growth inhibition	0	15.3	24.3	-21.5	-70.0	ND	-55.4	-67.8	-67.8	-97.7
Mouse infectivity*	5/5	0/5	0/5	0/5	0/5	ND	5/5	5/5	5/5	5/5

* Data = no. mice parasitaemic / no. mice inoculated (inoculum = 5 x 10^4 trypanosomes per mouse).

ND = not done.

APPENDIX V

Appendix V,

FIGURE 1

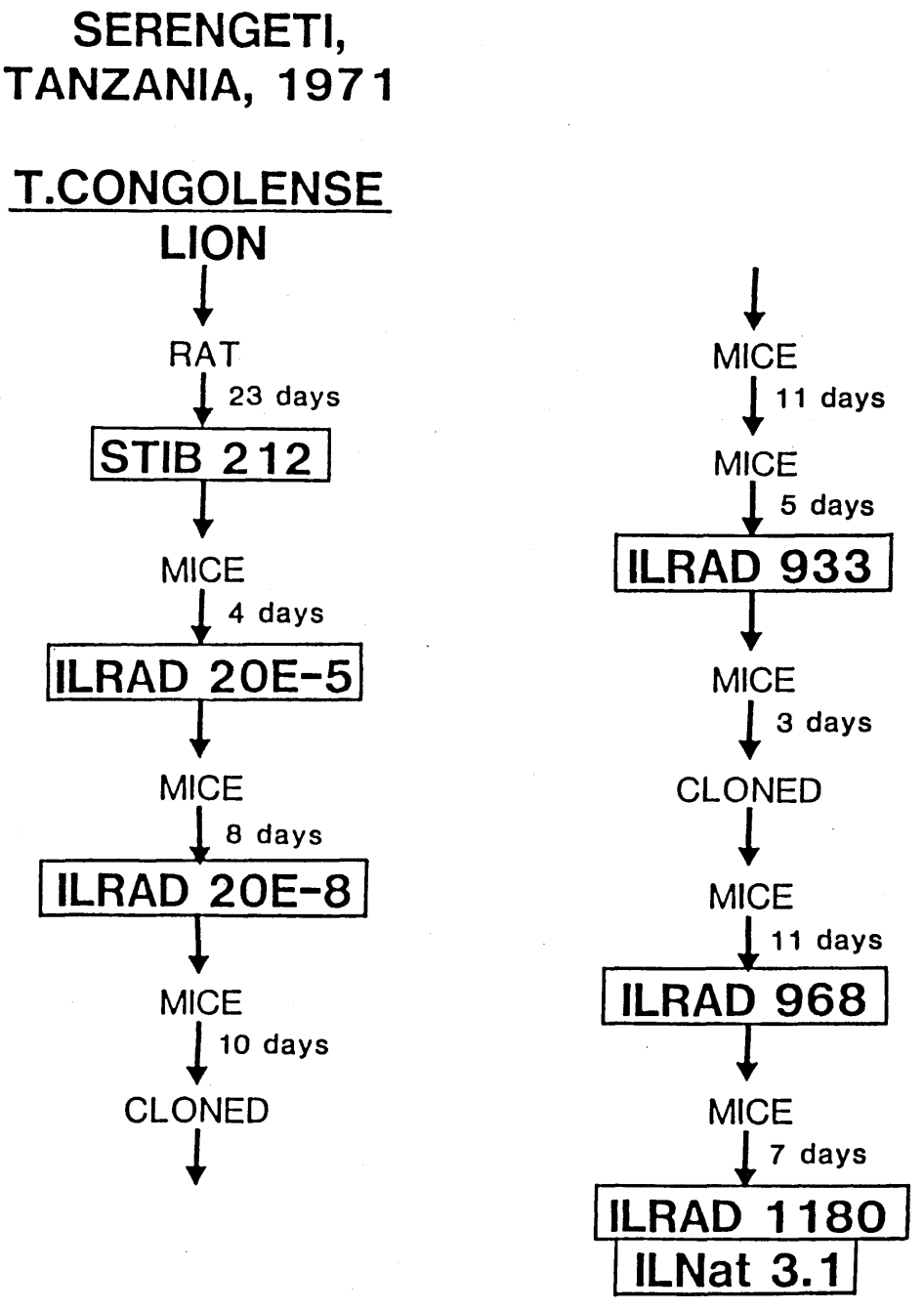

Figure 1: T.congolense ILNat 3.1

Appendix V,
FIGURE 2

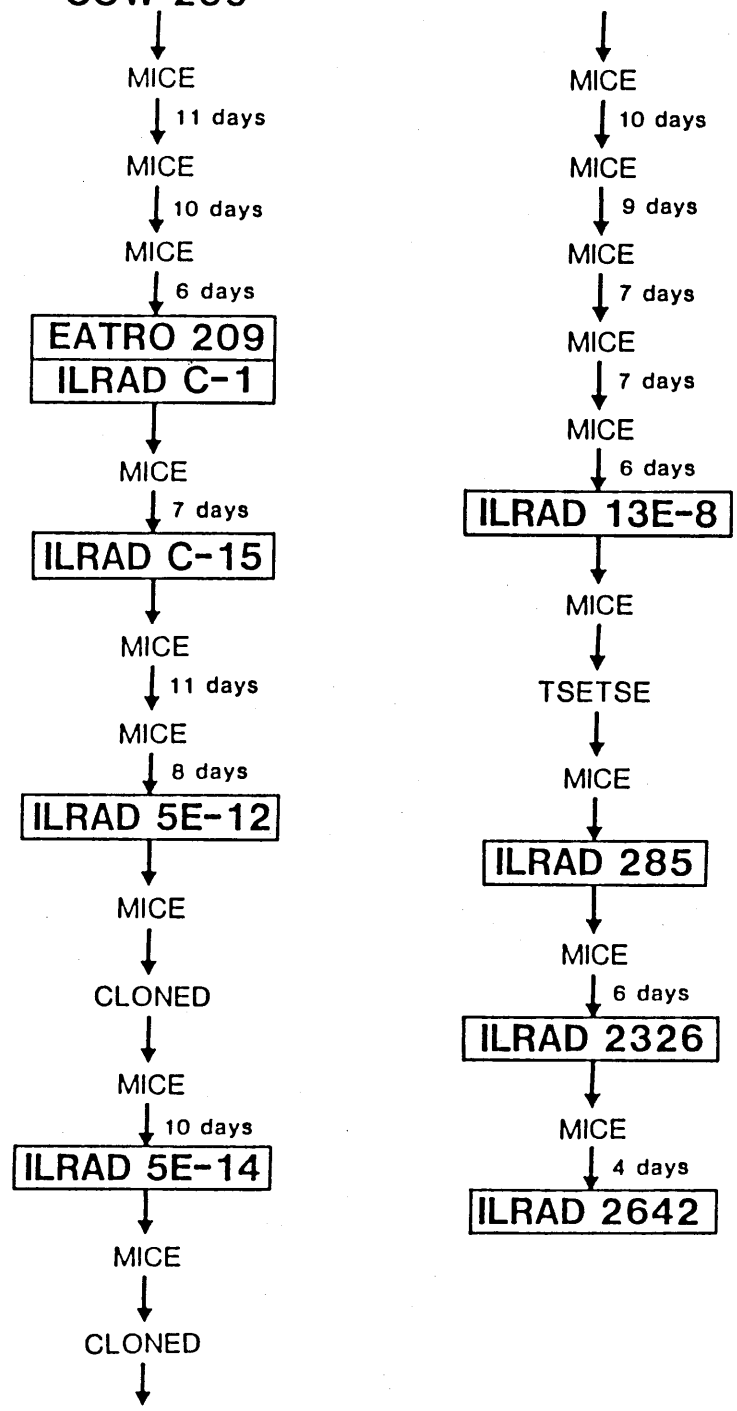

Figure 2: T.congolense IL 2642

Appendix V,
FIGURE 3

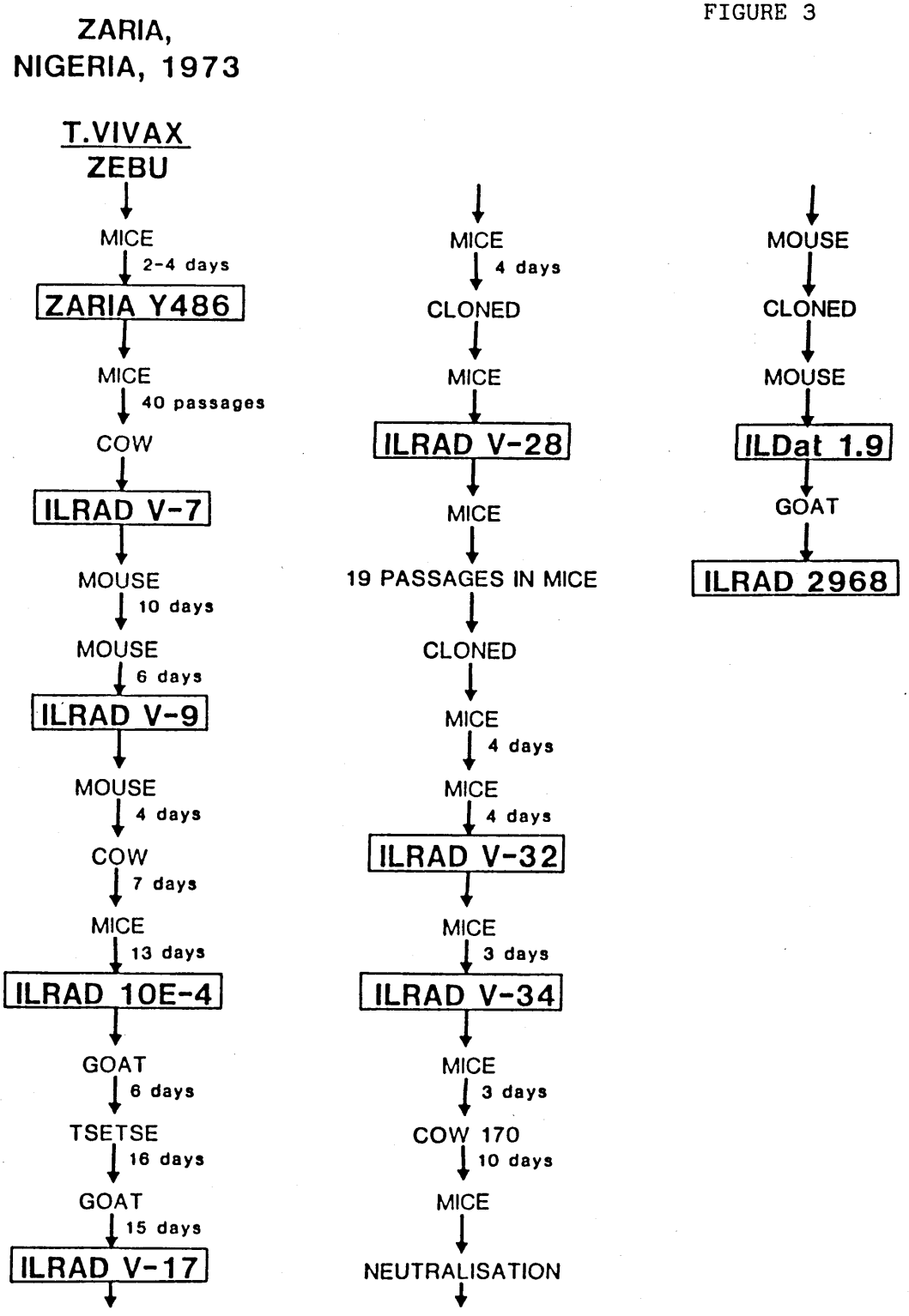

Figure 3: T.vivax IL 2968

Appendix V,
FIGURE 4

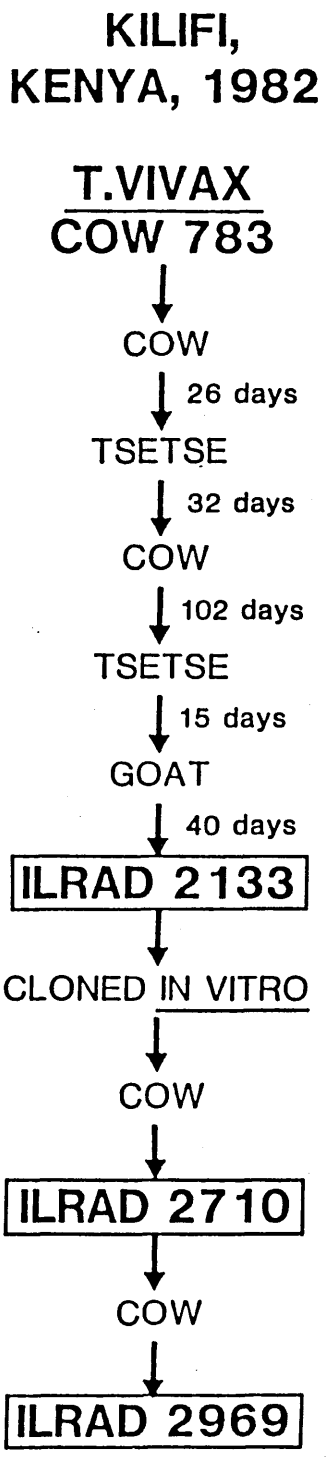

Figure 4: T.vivax IL 2969

Appendix V,
FIGURE 5

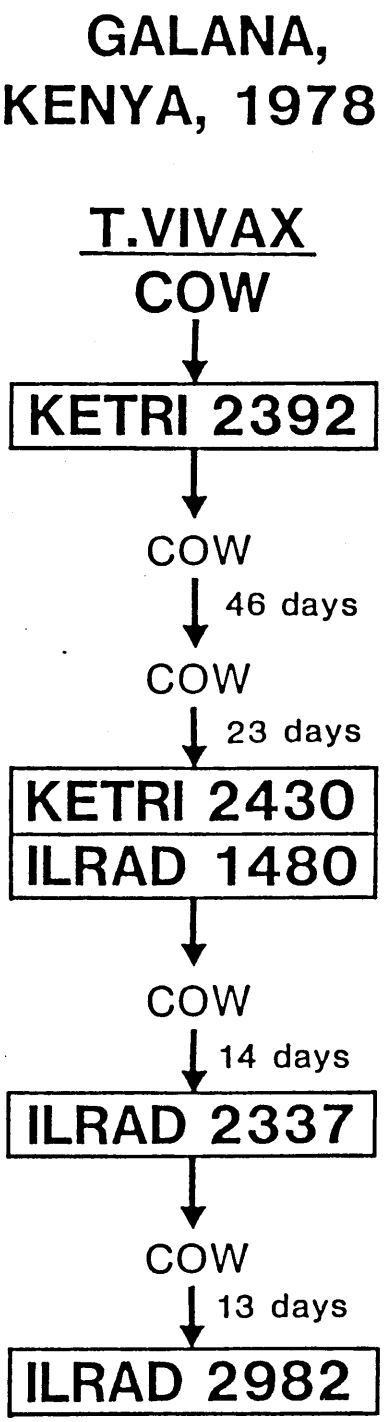

Figure 5: T.vivax IL 2982

Appendix V,
FIGURE 6

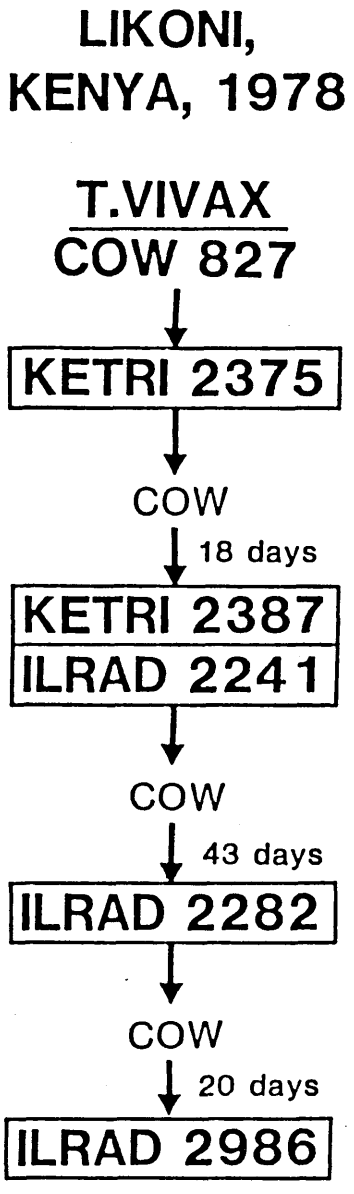

Figure 6: T.vivax IL 2986

Appendix V,
FIGURE 7

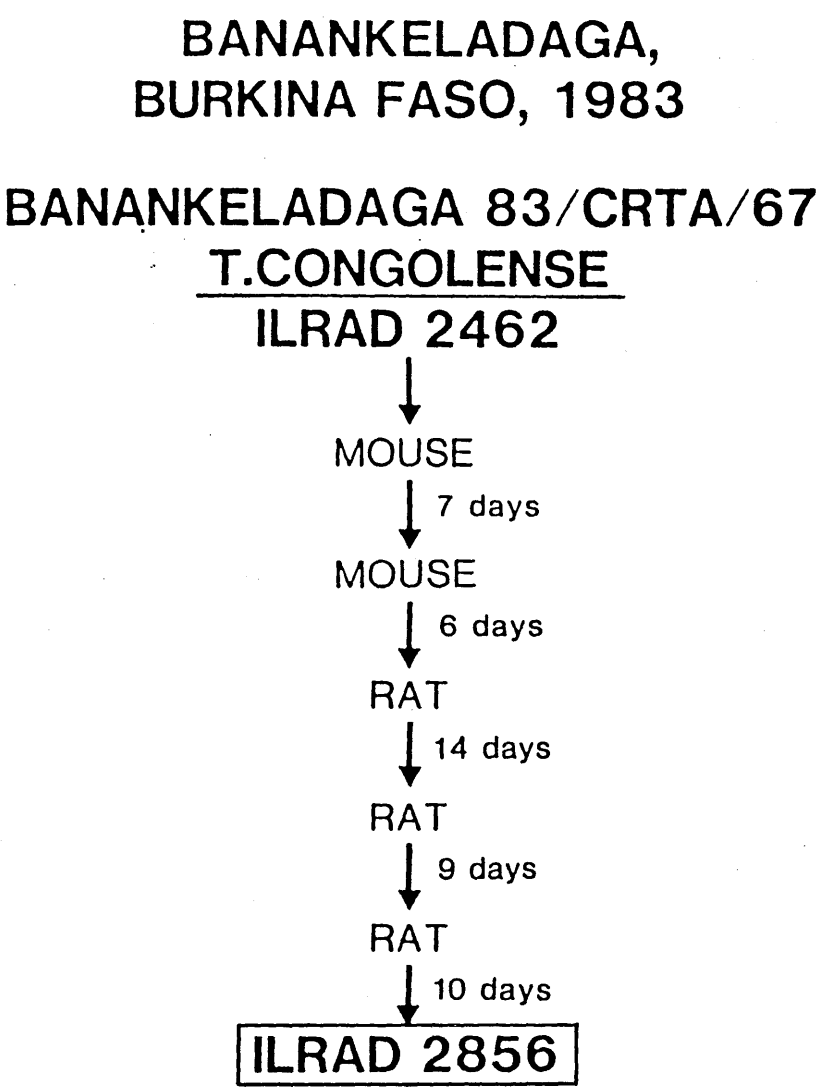

Figure 7: T.congolense IL 2856