INTERACTIONS BETWEEN HOST IMMUNITY AND THE EFFICACY OF CHEMOTHERAPY IN MICE INFECTED WITH *TRYPANOSOMA EVANSI*

by

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Declaration.

I hereby declare that the work presented in this thesis is original and was conducted solely by the author.

I also certify that no part of this thesis has been previously presented in any form to any university for the award of a degree but has been submitted in part in the following scientific paper: Osman, A.S., Jennings, F.W. and Holmes P.H. (1991). The rapid development of drug resistance by *T. evansi* in immunosuppressed mice. *Acta Trop.*, (in press).

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Summary.

Trypanosomiasis caused by the mechanically transmitted organism *Trypanosoma evansi* is the most widely distributed species of the genus *Trypanosoma* and affects a wide range of animal hosts. The control of the disease caused by this organism, surra, relies principally on chemotherapy since there is no effective control strategy against the biting flies which predominantly transmit the disease. The emergence of drug-resistant strains of *T. evansi* in the field is considered to be a major problem which could undermine the efficacy of the small number of trypanocidal drugs currently available. It was reported over 50 years ago that drug-resistance was more likely to develop in immunosuppressed animals than in hosts with an intact immune system. This observation was re-evaluated in the present study using modern trypanocides to treat mice infected with *T. evansi*.

It was found that the efficacy of the trypanocidal drugs currently available for the treatment of trypanosomiasis is substantially reduced in mice immunosuppressed by Co-60 irradiation compared to normal immunocompetent mice. Moreover, continuous passage of clones of *T. evansi* in immunosuppressed mice treated with gradually increasing doses of mel Cy, isometamidium and diminazene was found to rapidly lead to the development of high levels of drug resistance close to the maximum tolerated dose. Furthermore the drug-resistance developed in immunosuppressed mice was a genuine resistance and the clones maintained the same level of resistance when transferred into normal mice. On the other hand it was not possible to produce drug-resistant organisms in immunocompetent mice.

These findings highlight the need for further investigations on the role of the immune response in the development of drug resistance in the field as well the genetic basis of drug resistance in trypanosomiasis.

Chapter 1

GENERAL INTRODUCTION AND REVIEW

Chapter 1

Section 1: General introduction.

Trypanosomes.

Trypanosomes are the causative agents of major diseases of man and domestic animals. In the African continent south of the Sahara, human sleeping sickness occurs in many countries and nagana is a major factor hindering agricultural development in large areas (Peters, 1974), while in South and Central America Chagas disease is of considerable medical importance (Zeledon, 1974). Indeed the World Health Organisation placed trypanosomiasis of man and his domestic animals high on the list of ten major health problems facing mankind (Kershaw, 1970).

The most important group of trypanosomes are cyclically transmitted organisms, particularly in Africa where the tsetse vector is found. Approximately 37% of the continent amounting to 10 million km^2 are infested with tsetse flies (FAO/WHO/OIE, 1982) which are infected with one or more pathogenic trypanosome species (Morrison *et. al.*, 1985). It is thought that 7 million km^2 of this area would otherwise be suitable for livestock and mixed agriculture (Trail *et al.*, 1985). Cattle are the most seriously affected and the disease nagana makes cattle raising either impossible or uneconomical over vast areas of tropical Africa (Desowitz, 1957). Other hosts, including small ruminants are also at risk.

Outside the tsetse belt, the disease surra, caused by *T. evansi* is important in a wide geographical region including North Africa, Asia, Central and South America (Levine, 1973). Camels and horses are particularly susceptible, however other domestic animals are affected as well (Ng and Vaneslow, 1978; Verma and Gautam, 1979; Losos, 1980; Higgins, 1983).

It is believed that trypanosomes were first observed in the seventeenth century, however it was only in the last century that trypanosomes were brought into the limelight as important pathogenic organisms. In 1880, Evans discovered that surra of camels and horses was caused by trypanosomes and described their protozoan nature. The subsequent contribution by Bruce in 1897 that nagana of livestock in the Zululand was caused by trypanosomes transmitted by tsetse flies and that wild game acted as reservoir hosts (Hoare, 1972) focused a great deal of attention on this organism.

Trypanosomes are protozoan parasites which belong to the class Mastigophora, family Trypanosomatidae and genus *Trypanosoma* (Hoare, 1972; Lumsden, 1974; Stephen, 1986). The genus *Trypanosoma* is further sub-divided into two main groups. The stercorarian trypanosomes are generally non-pathogenic and develop in the posterior section of the gut of their insect vectors and as a result their transmission is contaminative. This group consists of a large assemblage of heterogeneous organisms which, apart from *T. cruzi*, an important parasite of man causing Chagas disease in South and Central America, are non-pathogenic and parasitise large numbers of mammalian and non-mammalian hosts. Examples of this group are: *T. lewisi, T. theileri, T. musculi*, and others (Table 1.1). Although stercorarian trypanosomes have none or little veterinary importance their differentiation from pathogenic trypanosomes is important as they can be encountered in surveys and routine diagnostic exercises (Molyneux, 1975).

Table 1.1. Classification of trypanosomes.

GENUS	TRYPANOSOMA						
SECTION	STERCORARIA			SALIVARIA			
SUBGENUS	MEGATRYPANUM	HERBETOSOMA	SCHIZOTRYPANUM	DUTTONONELLA	NANNOMOMAS	PYCNOMONAS	TPYPANOZOON
SPECIES	T.(M) thieleri	T.(H) lewisi	T. (S) cruzi	T. (D) vivax T. (D) uniforme	T. (N) congolense T. (N) simiae	T. (P) suis	T. (T) brucei T. (T) gambiense T. (T) rhodesiense T. (T) evansi T. (T) equiperdum

The salivarian trypanosomes are by far the most significant group of the genus and contain a number of species of tremendous medical and veterinary importance. In the earlier part of this century most species of this group were recognised although confusion surrounded some aspects of the classification of the group (see Stephen, 1986). Hoare (1972) in his monograph subdivided the salivarian group into four subgenera according to their morphological and biological aspects. These are *Duttonella*, which has two species namely *T.(D) vivax* and *T.(D) uniforme*. Second *Nannomonas*, contains *T.(N) congolense* and *T.(N) simiae*. The third subgenus, *Pycomonas*, is represented by only one species *T.(P) suis*. The fourth, *Trypanozoon*, which contains the largest number of species remained a matter of controversy for a long period of time. This subgenus consists of species which are morphologically indistinguishable but differ greatly in biological features.

All species of this subgenus are of significant medical and veterinary importance. The accepted view is to divide this subgenus into five species, namely *T.(T) brucei, T.(T) gambiense, T.(T) rhodesiense, T.(T) evansi* and *T.(T) equiperdum*. The three species, *T. gambiense, T. rhodesiense* and *T. brucei* are parasites cyclically transmitted by species of Glossina and are confined to the African continent south of the Sahara. Moreover *T. gambiense* and *T. rhodesiense* are human parasites causing African sleeping sickness while *T. brucei* is a parasite of animals which together with *T. vivax* and *T. congolense* causes nagana of cattle and other domestic animals in Africa. The remaining two species of the subgenus *T. evansi* and *T. equiperdum* have a wider distribution. It is now believed that these two species evolved from *T. brucei* and adapted themselves in the absence of the tsetse fly.

Transmission of *T. evansi* is principally effected by biting flies and as a result it has gained more wider distribution spreading into many parts of the tropical and subtropical regions where it causes the disease known as surra. The agent of dourine, *T. equiperdum*, on the other hand has a cosmopolitan distribution and is the only pathogenic trypanosome that is normally transmitted directly from one vertebrate host to another of the same species in the absence of a vector. Transmission of dourine takes place through direct contact from the horse to the mare and vice versa during the act of mating. It has also been demonstrated that *T. equiperdum* can be transmitted mechanically by biting flies, however since the organisms are normally localised in the capillaries of the mucous membranes of the urogenital tract, this mode of transmission is considered very rare (Woo, 1977).

The difficulty in subdividing this subgenus is obvious for while they are morphologically similar they greatly differ in biological aspects. Stark evidence of this phenomenon is *T. gambiense* and *T. rhodesiense*, both parasites of man hence their distinction is solely based on nosological features i.e the type of infection they produce (Hoare, 1967). It was later postulated that both *T. gambiense* and *T. rhodesiense* should be regarded a subspecies of *T. brucei*.

Trypanosomes affect a wide range of hosts where they cause different diseases according to the parasite involved as well as the particular strain, the susceptibility of the host and the local epizootiological conditions.

Tsetse-transmitted trypanosomiasis is widespread in Africa affecting a variety of host species. Cattle are highly susceptible and in this host the disease is collectively known as nagana. Trypanosomiasis transmitted by *Glossina* species occurs only in Africa, but groups of the

subgenus *Trypanozoon* that became emancipated from tsetse fly transmission became widespread. *T. evansi* gained access into many countries outside Africa, where it is believed to have evolved from *T. brucei*.

Geographical distribution

Trypanosoma evansi was first recorded in the Punjab in India and this marked the recognition for the first time of the importance of trypanosomes as agents of disease. Evans discovered the presence of motile, spirillum-like organisms in the blood of camels and horses affected with the disease called surra, (surra is a Hindi word meaning rotten), and described their protozoan nature. Later in 1889 Balbiani encountered the same organism and named it *Trypanosoma evansi* (Hoare, 1972).

The distribution of surra and the historical background which led to its spread was reviewed by: Hoare, (1956, 1972), Shaw, (1977) and Stephen, (1986). *T. evansi* has a wide geographical range (Figure 1.1) and occurs in regions with hot and warm-temperate climates extending, in the Old World, in longitude from about 15° W to 125° E. In Africa, where the organism is believed to have originated evolving from *T. brucei*, the disease is found on the fringes of the tsetse belt and occurs in the north along the Atlantic ocean and the Mediterranean littorals affecting Morocco, Algeria, Tunisia, Libya and Egypt. In West Africa it extends across the Sahara Desert and occurs north of the tsetse infested areas such as Senegal, Mali, Chad and parts of Nigeria. In the East the disease spreads southwards down to the Equator in northern Kenya and Somalia. Other areas affected include The Near and Middle Eastern countries, the Trans-Volga

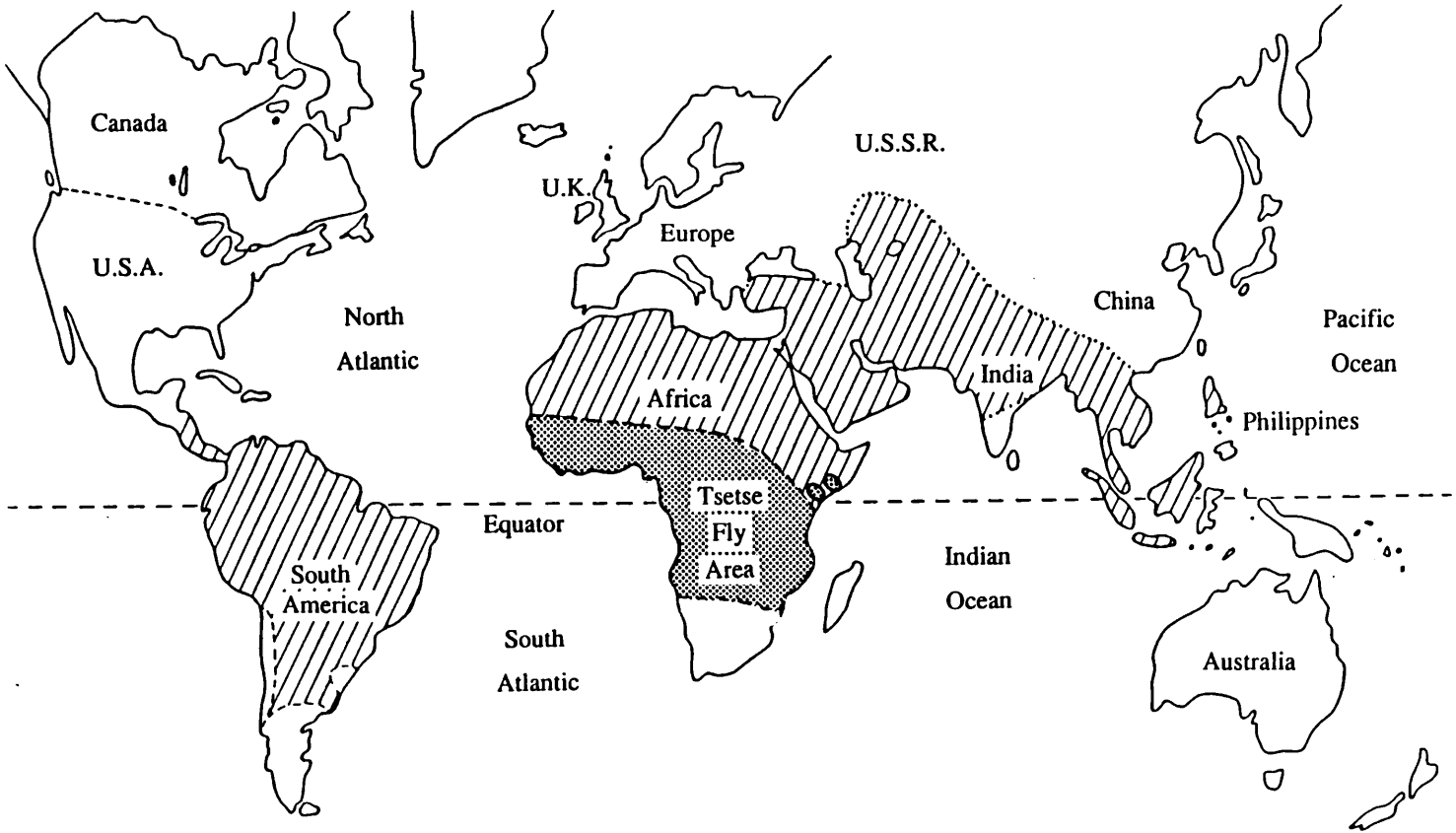

Figure 1.1. Geographical distribution of animal trypanosomiasis
(▩) Tsetse transmitted trypanosomiasis
(▨) Mechanically transmitted trypanosomiasis.

region of the Soviet Union, India, Malay peninsula, Indochina and southern China. Moreover the Indian Ocean islands of Mauritius, Indonesia and also the Philippines are affected.

The first suggestions that *T. evansi* originated from Africa came from Leese (1927) and was supported by others (see review by Hoare, 1972). It has been postulated that camels crossing into tsetse infested regions became infected with *T. brucei* and disseminated to parts of North and West Africa and from there to Asia. In the absence of tsetse flies mechanical inoculators represented by biting flies thereafter ensured transmission and the subsequent spread of the disease in the newly affected areas. This assumption rests on the fact that for centuries the camel was the principal transport animal in North Africa, the Middle East and western Asia and was extensively used for travel, commerce and military campaigns. Although most of Africa's camel populations are found outside the tsetse infested parts significant evidence suggests that tsetse and camels often encroached on each other's territories (Ruttledge, 1928; Godfrey and Killick-Kendrick, 1962; Nash, 1969; Hoare, 1972) supporting the view that *T. evansi* had evolved from *T. brucei*.

One of the main obstacles in relating the origins of *T. evansi* to *T. brucei* was the pleomorphic characteristics of *T. brucei*, however Hoare (1972) provided evidence of occasional pleomorphism in *T. evansi*. Furthermore it has been shown that *T. brucei* when maintained exclusively by syringe passage through animal hosts loses its pleomorphism and develops into monomorphic form incapable of infecting its principal vector the tsetse fly, a fact also encountered in *T. evansi*. Finally the hosts

affected by these two species and the clinical signs produced, as well as the response to chemotherapeutic agents strongly argue in favour of direct development of *T. evansi* from *T. brucei*.

Various names were given to the organism in the Old World such as *T. soudanense*, *T. ninae kohl-yakimov*, *T. marocanum* and others as well as various local names for the disease caused (surra, debab, salaf, su-auru etc.). However thorough statistical analysis of parasite morphology and size revealed these organisms to be variants of *T. evansi* (Hoare, 1972).

In the New World surra is believed to have been introduced by the Spanish conquistadors with their cavalry. Horses shipped from the Barbary Coast of Africa to Spain and later transferred to parts of the New World were claimed to have played a significant role in the spread of the disease. Elsewhere however, the view was expressed that cattle infected with *T. brucei* were perhaps more important in that respect. Horses form an important host of surra and are severely affected by the infection and the stress of a sea voyage in sailing ships would have curtailed still further their chances of surviving the journey and permitting the spread of the infection. *T. brucei* infections of cattle on the other hand are often characterised by mild clinical symptoms despite presence of parasites in the circulation. It is quite conceivable therefore that this parasite could persist in the blood of bovines during such a prolonged journey and upon arrival spread into the horse population through mechanical transmission by vampire bats and biting flies (Stephen, 1986). The West African origin of American surra is gaining support and is further strengthened by the similarity of the isoenzyme patterns of tsetse transmitted stocks of *T. brucei* from West Africa and *T. evansi* stocks from the New World (Gibson *et al.*, 1980).

The three conditions described and the organisms associated with them are mal de caderas (caused by *T. equinum*), murrina (caused by *T. hippicum*) and derrengadera (caused by *T. venezuelensis*). Murrina occurs in Mexico, Central America as well as Venezuela and Colombia while mal de caderas affects the greater part of South America especially Brazil with derrengadera occupying intermediate position (Hoare, 1972). After a closer scrutiny of morphology and other aspects, it was noted that *T. hippicum* was a variant or a serodeme of *T. evansi* and that the recognition of this organism as a separate species could not be justified. In contrast the complete absence of visible kinetoplast in *T. equinum* and the presence of high proportion of dyskinetoplastic forms in *T. venezuelensis* (Hoare and Bennett, 1937) occasioned the classification of these organisms as independent species. However dyskinetoplasty, the main criterion which the differentiation of *T. equinum* and *T. venezuelensis* from *T. evansi* is based, has been reported to be subject to fluctuation in the latter sometimes rising to 100% (Hoare, 1956). Moreover spontaneous transformation of a laboratory strain of *T. evansi* into a dyskinetoplastic form was reported (Hoare and Bennett, 1937) as well as the occurrence of dyskinetoplastic forms under natural conditions or following trypanocidal drug treatment (Hoare, 1954; Killick-Kendrick, 1964). Elsewhere it was shown that a dyskinetoplastic strain, Sudanese akinotoplastic (S.A.K.), isolated from a camel in the Sudan and kept under observation for years remained totally dyskinetoplastic (Hoare, 1959). From these observations it was considered appropriate to assume that the strain of *T. equinum* introduced into the New World was a mutant strain possessing dyskinetoplastic feature and that the subsequent generations continued to display the same

characteristics, thus strengthening the conclusion that *T. evansi* is the only organism responsible for surra and surra-like diseases in all parts of the world (Hoare, 1972).

Morphology.

The morphology of *T. evansi* is typical of the *Trypanozoon* organisms and is similar to the slender and intermediate forms of *T. brucei*. This parasite is almost always monomorphic with a mean length of 24 μm (Hoare, 1956, 1972). However, strains were reported showing pleomorphism with certain percentage of postero-nuclear stumpy forms (Godfrey and Killick-Kendrick, 1962). The slender forms possess a long free flagellum and a blunt posterior end. The kinetoplast, characteristic of the subgenus, is small in size and occupies subterminal or marginal position.

The kinetoplast is the portion of the single mitochondrion that contains the mitochondrial DNA (Simpson, 1972) known as the kinetoplast DNA and consists of a large network of circles held together by extensive catenation into a structural unit (Borst & Hoeijmakers, 1979) containing tens of maxi-circles and thousands of mini-circles (Stuart, 1983). During the multiplication within the tsetse vector the cyclically transmitted trypanosomes possess fully developed mitochondrion and the mitochondrial oxidative phosphorylation seems to be essential, however in the bloodstream stage the mitochondrial biogenesis is repressed and the organisms rely on glycolysis (Borst and Hoeijmakers, 1979).

Apart from *T. evansi* the members of the *Trypanozoon* subgenus have been reported to possess fully developed maxi-circles and the mini-circles to be more heterogeneous, while in *T. evansi* the maxi-circles are totally lacking and the mini-circles are homogeneous (Borst *et al.*, 1987). Thus it is evident that

fundamental differences exist between *T. brucei* and *T. evansi* which are in favour of the specific differentiation between these two species to be retained despite similarities in isoenzyme patterns (Gibson, 1988).

The complete absence of kinetoplast maxi-circles, which contain genes essential for mitochondrial function, therefore is sufficient to explain the inability of *T. evansi* to undergo cyclical transmission (Borst *et al.*, 1987; Gibson, 1988). In dyskinetoplastic forms such as *T. equinum* and S.A.K. Borst *et al.* (1987) were unable to confirm previous reports (Cuthbertson, 1981) that DNA is present as a small core, it is normal organisation having collapsed.

It was postulated that the mean length of *T. evansi* increases from the Morocco to the Philippines and this is supposed to be the underlying cause for the susceptibility of the equine species. In Somalia, Kenya and other African countries horses are refractory to the surra infection (Hoare and Bennet, 1939), while in Indochina horses are the most susceptible host species followed by bovines. *T. evansi* can be considered monomorphic since pleomorphism is quite inconsistent and stumpy forms occur only in small numbers (Woo, 1977).

Transmission

Trypanosomes are organisms with a remarkable potentiality to adapt themselves in an unfavourable environment and this is evident in *T. evansi*. The total loss of dependence on tsetse transmissibility enabled the organism to spread beyond the limits of the tsetse belt. Transmission of *T. evansi* is commonly effected by biting flies mainly *Tabanus* spp. but others such as *Stomoxys*, *Lyperosia* and *Haematopota* have also been implicated (Gatt Rutter, 1967).

The earliest experimental evidence of mechanical transmission by horse-flies (*Tabanidae*) was provided by Rogers (1901) who proved that *T. evansi* can be transmitted by interrupting the blood meal of the flies feeding on infected hosts. However, transmission was found to take place only if the flies were allowed to feed immediately on another animal. Subsequent studies showed that transmission was most efficient when infected flies feed within 15 minutes on a new host and the infectivity was found to disappear after 8 hours.

Most species of the genus *Tabanus* are capable of transmitting the infection particularly the females since the male population is known to feed on plant juices and hence is considered less important in the transmission of surra (Woo, 1977). Gruvel and Balis (1965) listed 11 species of tabanid flies as vectors of camel trypanosomiasis in Chad. In northern Somalia camel surra is associated with members of *Pangonia* spp. Locally known as "dhuug" (Peck, 1936; Derie *et al.*, 1989). However in the south the other more widely distributed tabanid species may be more important.

Investigations on whether *T. evansi* could be transmitted cyclically by arthropods including *Glossina* revealed that vectors of surra act strictly as mechanical inoculators depending on the survival of the organisms in the mouth parts. In an extensive survey Hoare (1940) fed a total 568 *Glossina morsitans* flies on animals infected with *T. evansi* and examined their intestinal contents at intervals from 6 hours to 14 days. It was found that the trypanosomes were disintegrated and completely digested hours after ingestion and 14 days later no traces of the organisms were recognisable in the gut of the insects.

The stable fly (*Stomoxys*) is an inefficient vector of the disease but in this genus both males and the females are involved in the transmission (Hoare, 1970). Other arthropods including mosquitoes and ticks have also been incriminated. Cross and Patel (1921) cited by Gatt Rutter (1967) succeeded in transmitting *T. evansi* from camel to rabbit by means of the soft tick *Ornithodoros* spp., however Leese (1927) dismissed the importance of this method of transmission in the field.

In Central and South America in addition to the biting flies, the vampire bat (*Desmodus rotundus*) is considered an important transmitter of surra (see Hoare, 1965). These haematophagous mammals are widespread from northern Mexico to southern Argentina and may consume from 16 to 50 ml of blood at a feed. Bats contract the disease from infected hosts and suffer from surra sometimes with fatal consequences. However they may survive and transmit the infection to other hosts for a relatively long period of time (Woo, 1977). The transmission of surra by the vampire bat is mechanical in nature since no developmental stages of the parasite in this host have been uncovered. These blood sucking mammals therefore play an important role in the propagation of the disease acting not only as a transmitter but as a host too. Finally dogs and other carnivores may be infected directly by consuming flesh from infected hosts through abrasions of the mucous lining of the alimentary canal.

Clinical features

Clinical features of surra have been reviewed by many authors (Leese, 1927; Losos, 1980; Stephen, 1986 and others). The course of the disease depends on the strain involved, susceptibility of the host and local epizootiological conditions (Hoare, 1970).

In areas where camels are present they are the principal host and the disease occurs throughout the normal range of *Camelus dromedarius* and *Camelus bacterianus*. After an incubation period of about 1 to 3 weeks, parasites invade the blood and can be detected by the routine parasitological techniques. The development of parasitaemia is followed by fever. The acute form of camel surra is characterised by intermittent fever, edamatous swellings, staring coat, progressive anaemia, emaciation and other features such as ataxia and petechial haemorrhages which almost always end in death if treatment is not effected. The acute form of camel surra, although reported in some parts e.g northern Somalia (Derie *et al.*, 1989) does not represent the classical course of *T. evansi* infections. Like other diseases due to trypanosome parasites surra is most commonly a chronic wasting disease. Animals lose condition and become cachectic, pyrexia is associated with successive peaks of parasitaemia and there may be a generalised oedema. The general weakness of the host may lead to secondary complications affecting the respiratory system and other organs. If affected animals are not treated with the appropriate trypanocidal drugs the prognosis is usually unfavourable and death occurs within a few months or years. Occasionally animals may spontaneously recover from the infection (Hoare, 1972).

As well as the camel other hosts of *T. evansi* are horses, donkeys, dogs, cattle, buffaloes, the Asian elephant and others. In these hosts the horse is by far the most susceptible animal and here the infection runs a course of either weeks or months. In any case the outcome is usually fatal with signs of pyrexia, severe anaemia, locomotory disturbance and oedema of the ventral parts of the body. Donkeys and mules usually suffer from chronic forms of surra and may occasionally constitute a potential reservoir host (Bennett, 1933a). Donkeys are readily infected experimentally

with symptoms of intermittent fever, dullness, emaciation, anaemia, persistent nasal discharge and lacrimation (Suryanaryana *et al.*, 1986). The dog is highly susceptible especially imported breeds. Pups usually succumb within a month but in older dogs the disease takes a chronic course and lasts for months. In experimental infection of dogs, deaths occurred between 40-96 days post-infection and the symptoms encountered were recurrent fever, loss of weight and corneal opacity (Shien *et al.*, 1977). Corneal opacity and partial blindness was also encountered in dogs experimentally infected with *T. evansi* (Galhorta *et al.*, 1979).

In cattle and buffaloes in enzootic areas the disease is cryptic characterised by the rare appearance of parasites in the peripheral blood and the possibility that these species act as reservoir hosts has been speculated. None the less the disease occasionally flares up into acute or peracute forms with significant losses (Verma *et al.*, 1973). Symptoms of bovines and buffaloes suffering from surra include fever, anaemia, lacrimation, locomotory disturbance, oedema and abortion. In Thailand Lohr *et al.* (1986) and Lohr *et al.* (1988) investigated outbreaks of abortions which affected herds of buffalo cows and reported that surra is a frequent cause of abortion in buffaloes. Of 40 aborted cases examined 37 were positive by the complement fixation test with 25 cases harbouring *T. evansi* organisms in the circulation (Lohr *et al.*, 1988). However spontaneous recovery from *T. evansi* infections is more common in cattle and buffaloes compared to other hosts.

Surra of sheep and goats is hardly mentioned in the literature although they can be readily infected experimentally (Stephen, 1986). Low parasitaemia was observed in experimental infections of both goats and sheep. The packed cell volume of the sheep was not noticeably changed while in the goats there was slight drop.

Boid *et al.* (1981) failed to diagnose surra in sheep and goats in the Sudan by the thick smear and subinoculation into laboratory rodents, however 54% of the sheep and 58.6% of the goats were found to possess antibodies against *T. evansi*. Similarly serological survey of camels, cattle, goats and sheep in the central regions of Somalia outside the tsetse infested areas revealed 53.8% of the sheep and 45.7% of the goats having antibodies against *T. evansi* (Caille, 1989).

Pathology and pathogenesis.

The information on the pathology of the diseases caused by *T. evansi* in the various species affected seems to be patchy, however there are suggestions that it is comparable to those caused by *T. brucei* (Losos, 1980).

In cattle and buffaloes experimentally infected with a strain of *T. evansi* the gross pathological changes observed were emaciation and cachexia with associated features such as excess of fluid in the pericardial cavity, gelatinisation of the pelvis of the kidneys and base of the heart as well as enlargement of lymph nodes and congestion of the spleen and liver (Verma and Gautam, 1979). Histopathological examination revealed congestion and haemorrhages of the lymph nodes accompanied by loss of the normal architecture of the gland. Moreover the sinuses of the spleen were found to be dilated with an increase in the number of plasma cells. Examination of calves dying during the third week of infection revealed severe congestion and

disorganisation of the spleen with excess deposit of haemosiderin indicative of haemolysis. Elsewhere splenomegaly, swelling of the lymph nodes as well as progressive emaciation and anaemia was reported in both natural and experimental infections of dogs with *T. evansi* (Shien, 1977).

Lymphoadenopathy and splenomegaly are some of the features consistently encountered in infection due to pathogenic trypanosomes (Ormerod, 1970; Fiennes, 1970). During the earlier stages of tsetse transmitted trypanosomiasis of cattle macroscopical changes include gross enlargement of the spleen and the lymph glands throughout the body (Fiennes, 1970). However in the chronic stages the spleen was reported to be atrophic while the lymph nodes remain swollen. The microscopic changes are at first dominated by a lymphoproliferative response showing active germinal centres with subsequent invasion of the lymph nodes and spleen by plasma cells and macrophages (Henson and Noel, 1979). The most serious consequence of the changes in the immunological apparatus is the development of immunosuppression (Goodwin, 1970) which is more severe in laboratory rodents.

Another major factor contributing to the disease process in trypanosomiasis is the development of anaemia (Fiennes, 1970) which is significantly the cause of morbidity and mortality in most animals infected with salivarian trypanosomes (Murray *et al.*, 1974a). Reduction of red blood cell counts and packed cell volume is characteristic in trypanosome infections. Indeed low PCVs in camels is considered to be indicative of *T. evansi* infections in endemic areas (Mahmoud and Gray, 1980).

In camels infected with *T. evansi* the anaemia has been reported to be macrocytic with the appearance of reticulocytes, normoblasts, macrocytes and spherocytes, accompanied by a hyperplastic bone marrow (Jatkar and

Purohit, 1971). Increase of circulating reticulocytes during *T. evansi* infections of rats, horses and camels was also described by Assouku (1975), Ng and Vaneslow (1978) and Raisinghani *et al.*, (1981).

Shien (1977) reported that the anaemia in surra infections of dogs is normocytic and normochromic during the initial stages of the disease and subsequently becomes microcytic and hypochromic.

Other studies involving cattle and buffalo calves and donkeys experimentally infected with *T. evansi* (Singh and Misra, 1986; Suryanarayana *et al.*, 1986) have also reported marked falls in haematocrit value, haemoglobin and the total erythrocyte and leukocyte counts. Moreover a slight increase in osmotic fragility of erythrocytes as well as decreased mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were encountered in both the cattle and buffalo calves (Singh and Misra, 1986).

The causes of the anaemia in animal trypanosomiasis have been suggested to be haemolysis, haemodilution and dyshaemopoiesis acting singly or in concert (Mamo and Holmes, 1975; Suliman and Feldman, 1989). While it is becoming increasingly obvious that the aetiology of the anaemia in trypanosome infection is multifactorial, the major cause is considered to be attributable to red cell damage (Suliman and Feldman, 1989) and subsequent removal by the reticuloendothelial system. In sheep infected with *T. congolense* MacKenzie and Cruickshank (1973) provided evidence of large scale erythrocyte and leukocyte phagocytosis throughout the reticuloendothelial system and emphasised that the phenomenon substantially contributed to the development of the anaemia. Similarly in an experimental study using rats and mice infected with a strain of *T. brucei* Jennings *et al.* (1974) have shown that the anaemia was due to extravascular haemolysis.

Most of the work done to elucidate the mechanisms responsible for the development of the anaemia in trypanosomiasis concentrated on the cyclically transmitted trypanosome species of ruminants and laboratory rodents, and in general the techniques employed to study the pathological features of the other species does not seem to have been extended to *T. evansi* (Luckins, 1988). Richardson and Kendall (1963) singled out dyshaemopoiesis as the main cause behind the development of the anaemia in *T. evansi* infections. However Jatkar and Purohit (1971), in addition to a marked reduction in PCV, found considerable increase of erythrocyte fragility in camels infected with *T. evansi* which could lead to excessive destruction of red blood cells.

Using isotopic tracer techniques erythrokinetic studies in cattle experimentally infected with *T. congolense*, have shown an accelerated rate of loss of red blood cells compared to the uninfected controls (Mamo and Holmes, 1975) as judged from reduced ^{51}Cr-labelled red cell half-lives. Further studies on cattle and rabbits infected with *T. congolense* revealed the development of normochromic and normocytic anaemia and more rapid disappearance of radio-iron from the plasma in the infected animals compared to the controls, indicating that, at least in the early phases, erythropoiesis is not impaired in trypanosome infected animals but on the contrary is generally greatly accelerated (Holmes, 1976).

Various suggestions were put forward explaining the underlying mechanisms behind the destruction of red blood cells in trypanosome infections such as haemolytic factors produced by the trypanosome. Kaukha and Ramasamy (1981) found homogenates of *T. evansi* obtained by freezing and thawing to be haemolytic in both *in vivo* and *in vitro* for rat and mouse erythrocytes. Other factors such as direct trauma to the red cells, immunological mechanisms and disseminated intravascular coagulation among

others have also been implicated (Suliman and Feldman, 1989). A frequent observation in acute trypanosome infections is the rapidity with which the anaemia disappears following trypanocidal drug treatment clearly indicating that the anaemia is associated with the circulating trypanosomes (Murray, 1974; Holmes and Jennings, 1976).

Unlike the congolense-vivax trypanosomes the members of the *Trypanozoon* subgenus are characterised by their ability to invade the host tissues. Artificial infection of the Japanese field vole (*Microtus montebelli*) with *T. evansi* produced interstitial oedema and mononuclear cell infiltration in the subepicardial, myocardium and skeletal muscle (Umeda *et al.*, 1988). The infiltration of plasma cells and lymphocytes as well as the degeneration and atrophy of muscle fibres were reported to be associated with the extra vascular localisation of trypanosomes and subsequent multiplication. Similar lesion were found, in some cases, around the pulmonary arteries, epididymis, pancreas salivary glands adipose tissues around the kidneys and uterus and the choroid plexus of the cerebral ventricles.

In small ruminants experimentally infected with *T. brucei*, lesions were described involving the eyes, heart, reproductive organs and the endocrine system (Ikede and Losos, 1972), and in horses naturally infected with *T. evansi* meningoencephalitis was encountered (Seiler *et al.*, 1981) resembling the lesions in human African trypanosomiasis.

Diagnosis.

Diagnosis of *T. evansi* infections in endemic areas is initially based on clinical symptoms (Woo, 1977). Herdsmen in certain localities particularly in Somalia also diagnose camel surra by the characteristic pungent odour of the urine (Hunter, 1986). However since these features

provide only broad indications specific diagnosis of all trypanosome infections must depend on the demonstration of trypanosomes by light microscopy (Murray *et al.*, 1977).

Direct observations of the blood in either wet films or stained smears are the earliest methods introduced and still remain the techniques most commonly available to the field veterinarian. Together with animal inoculation these methods are termed the Standard Trypanosome Detection Methods (STDM) (Wilson, 1969). In these methods either motile trypanosomes are searched for in a wet film or parasites are looked for in slides stained with Giemsa's stain. The thin smear is less sensitive than the thick smear, but is useful in the identification of the different species of trypanosomes without which diagnosis is only of limited value (Stephen, 1986). The benefit of the thick smear on the other hand lies in mass screening exercises, as more blood is scanned in this method (Fiennes, 1952), to identify cases which could then be examined in greater detail. Since thick films are not fixed but are simultaneously lysed and stained in Giemsa's stain (Killick-Kendrick, 1968), trypanosomes may appear distorted making their recognition somewhat difficult as a result of lysis. However this can be overcome by staining the slide with 0.5% aqueous methylene blue for 1 second prior to the Giemsa's staining (Maclennan, 1957).

Subinoculation of susceptible animals is particularly valuable in cases where trypanosomes are scarce in the peripheral blood and extensive use of this technique has revealed a high incidence of *T. evansi* infections in Nigerian camels (Godfrey and Killick-Kendrick, 1962). Laboratory rodents particularly rats and mice are the most widely used animals (Molyneux, 1975) due to advantages in cost and ease in transportation. Except for *T. vivax* and *T. simiae* which are poorly infective to rodents, animal

inoculation is considered to be as sensitive as some of the concentration techniques particularly for the members of *Trypanozoon* subgenus including *T. evansi* (Kelly and Schillinger, 1983). On one occasion mouse inoculation was reported to have detected 51 positive cases out of 54 camels suspected of suffering from surra (Raisinghani and Lodha 1986).

Other than the STDM, trypanosome concentration techniques are widely used and are thought to be the most sensitive of the parasitological methods available. These include the haematocrit centrifugation technique (Woo, 1970), the dark ground/phase contrast buffy coat method (Murray *et al.*, 1977) and the anion-exchange/ centrifugation technique (Lanham and Godfrey, 1970). The darkground/phase contrast buffy coat method excelled other techniques in the detection of *T. congolense* and *T. vivax* infections of cattle and also provided the extra advantage of allowing species identification and an estimation of the level of parasitaemia (Murray *et al.*, 1977) and at the same time giving a haematocrit reading for anaemia. However with *T. brucei*, mouse subinoculation proved to be the most sensitive followed by the haematocrit technique (Paris *et al.*, 1982). Elsewhere comparison of eight parasitological techniques revealed that the haematocrit centrifugation technique to be the most sensitive method in goats experimentally infected with strains of *T.vivax* and *T. brucei* detecting an average of 78.8% of the infections (Kalu *et al.*, 1986).

Apart from the direct methods which aim to detect the infecting organisms there are other techniques based on host reactions to the infection. Non-specific tests such as the formol-gel and the mercuric chloride test which detect a rise in the globulin levels of the infected hosts have been extensively used in the past to detect *T. evansi* infections (Knowles, 1925; Bennett and Kenny, 1928). However it was found that the results obtained could not be

satisfactorily correlated to active infection and raised IgM levels and hence more recent tests are recommended for epidemiological surveys (Pegram and Scott, 1976; Luckins *et al.* 1979). Currently more sensitive methods are available to detect antibodies produced directly against the infection as well as trypanosome antigen itself.

Serological diagnosis is usually undertaken to supplement parasitological examinations and to provide information on a herd basis since it has been found that antibodies may persist even after elimination of infection by chemotherapy (Losos, 1986). Several serological methods are used e.g. haemagglutination, indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), complement fixation test (CFT) and others for the diagnosis of trypanosomiasis including surra and dourine.

In non-cyclically transmitted trypanosomiasis caused by *T. evansi* and *T. equiperdum* serological methods are very useful and the sensitivity of the CFT enabled the eradication of dourine from many parts of the world (Losos, 1986). The ELISA test was reported to be particularly valuable and detected 96.2% of *T. evansi* infections in buffaloes while the indirect haemagglutination and CFT detected 78% and 82.38% respectively (Shen *et al.*, 1986). The ELISA test also proved to be as sensitive as the IFAT in detecting *T. evansi* infections of rabbits (Luckins *et al.*, 1978). Furthermore the ELISA was found not only suitable to detect host antibodies against the infection but could also be adapted to reveal trypanosomal antigens in the serum. Rae and Luckins (1984) reported that in rabbits infected with *T. evansi* trypanosome antigens could be detected in the serum by

the double antibody sandwich immunoassay four to eight days after infection and that seven days following treatment with suramin the antigens were no longer detectable.

Recently antigen-detection enzyme immunoassays have been described using monoclonal antibodies capable of identifying different species of trypanosomes (Nantulya and Lindqvist, 1989) and this has given promising results.

Elsewhere Raina *et al.* (1985) compared the indirect haemagglutination and the capillary agglutination and found that antibodies could be detected by the IHA a week following experimental infection of buffalo calves with *T. evansi* and three weeks later by the capillary agglutination. Moreover both tests were specific with no cross-reactions with other protozoan infection such as theileriosis, anaplasmosis and babesiosis.

Immunology of trypanosomiasis

The ability of hosts infected with trypanosomiasis to control the initial stages of infection from a given serodeme clearly shows that effective host defence mechanisms are produced. This capability is however circumvented by the phenomenon of antigenic variation and trypanosome-induced immunosuppression (Molyneux and Ashcroft, 1983; Bancroft and Askonas, 1985).

A large body of evidence is now available that the host immune response is mediated by the production of antibodies. According to Stephen (1986) the presence of protective antibodies were demonstrated in trypanosomiasis-infected hosts by Laveran and Mesnil (1912) and was later confirmed by others. Soltys (1957a) reported that infections of rabbits and inoculations of formalised organisms produce neutralising

antibodies to *T. brucei*, and also agglutinating antibodies in experimentally infected or hyperimmunised rabbits (Soltys, 1957b). Similarly experimental studies using normal and congenitally athymic nude mice have clearly demonstrated the production of antibodies against trypanosome infections (Campbell *et al.*, 1978).

Holmes *et al.* (1979) described a technique in which trypanosomes were radiolabelled with [^{75}Se]-methionine with the objective of following the clearance of the organisms from the circulation of the infected hosts and the immune mechanisms involved. In immunised mice the radiolabelled trypanosomes were found to rapidly disappear, while in the controls the organisms remained in circulation. Moreover the liver was found to be the principal site of phagocytosis removing 50% of the trypanosomes (Holmes *et al.*, 1979). Using the same method of trypanosome labelling MacAskill *et al.* (1980) reported that clearance of trypanosomes in immunised mice was accomplished by antibody-mediated hepatic phagocytosis following opsonisation with the participation of complement.

Raised levels of immunoglobulins particularly IgM class are considered a consistent finding in trypanosome infections (Luckins, 1972; Clarkson and Penhale, 1973; Kobayashi and Tizard, 1976). The primary response to the infections was found to initially involve IgM and subsequently augmented by IgG (Urquhart and Holmes, 1987). In experimental infections of camels with *T. evansi* a substantial increase in IgM levels was observed approaching five times the preinfection level (Boid *et al.*, 1980) while IgG levels were found to fluctuate. Significant increase of IgM levels were also encountered in camels naturally infected with surra.

The host immune response is crucial in trypanosomiasis and exerts its influence in various ways. A major aspect of immunity is the supportive role in effective chemotherapy and chemoprophylaxis in trypanosome infections.

However, the introduction of a vaccine is yet to be realised, in spite of intense efforts due mainly to antigenic variation (Gray and Luckins, 1976; Murray and Urquhart, 1977).

Antigenic variation

The trypanosome organisms have the capacity to change their antigenic character and by this means they manage to avoid the host's immune response. Being extracellular parasites in their mammalian host, trypanosomes have devised a mechanism of frustrating the host defence mechanism (Hajduk and Vickerman, 1981) and avoid its lethal consequences.

Two types of antigens have been described in trypanosomes. The common antigens are usually related to organelles and protein substances such as enzymes (Weitz, 1970; Vickerman and Barry, 1982; Losos, 1986) and are considered stable since they can be consistently isolated during an infection of a mammalian host. These antigens are common to various stocks of the same species and even between species. The common antigens have low immunogenicity, but their presence indicates current or earlier contact between host and trypanosomes and can be useful in serodiagnosis (Losos, 1986).

The other type of antigen is variable and changes from one population to the next. According to Gray and Luckins (1976) antigenic variation in trypanosomes was first observed by Franke (1905) who reported that trypanosomes are liable to change immunologically during an infection. Since then, this aspect of the trypanosome organisms has

attracted a great deal of attention to the point that most of the recent immunological work on trypanosomiasis is concerned with it. This characteristic of trypanosomes to change their antigenic properties is widely believed to be the basis for certain features associated with the infection such as the levels of virulence and parasitaemia (Godfrey, 1961; MacNeilage and Herbert 1968; Barry *et al.*, 1979). All salivarian trypanosomes undergo antigenic variation including the non-cyclically transmitted species of *T. evansi* and *T. equiperdum.* In contrast antigenic variation is not considered to have importance in stercorarian trypanosomes (Vickerman and Tetley, 1979). Gill (1971a) reported antigenically distinct trypanosome populations in *T. evansi* infections and currently it is accepted that this organism undergoes extensive antigenic variation (Jones and McKinnell, 1984).

The variable antigen is associated with the surface coat and covers the whole of the trypanosomal body. In *T. brucei*, the variable surface coat lies over the cytoplasmic membrane and measures 12-15 nm as visualised in electromicrograph sections (Vickerman, 1969). Surface labelling techniques have shown that the variable coat consists of a single glycoprotein with an apparent molecular weight of 65000 Daltons (Cross, 1975) and is generally known as "variable surface glycoprotein", (VSG). Less is known about VSG of *T. evansi* however Cross (1977) characterised the VSG of a dyskinetoplastic strain and suggested that it is comparable to the VSG of *T. brucei.*

In cyclical infections multiplication at the site of inoculation takes place with subsequent invasion of the blood stream until a parasitaemic peak is reached. This peak coincides with the appearance of variant specific antibodies followed by decline in the number of organisms as the immune system

expresses its defensive action. However, some organisms with new antigenic properties survive and multiply to form the next variant antigen type (VAT) population (Gray and Luckins, 1976). A succession of parasitaemic peaks followed by relapses is characteristic in trypanosomiasis corresponding to host defence and antigenic variation until death of the infected host or recovery by means of trypanocidal drug treatment or self cure takes effect.

The number of variant antigen types from a population commonly known as the VAT repertoire, has been suggested to follow a certain orderly pattern with every population after each crisis consisting of organisms with the same VAT, (Gray, 1965). However, recent work using immunofluorescence and trypanolytic tests have shown that even unrelapsed clone populations contain major VATs and also minor heterotype variant antigen types (Vickerman and Barry, 1982). This heterotype population, therefore continues to multiply as the major VAT-expressing population declines as a result of the host immune response.

The number of VATs of a cloned population is yet to be determined but is considered to be inexhaustible (Gray, 1965) and is limited by the survival of the infected host. In *T. equiperdum* Capbern *et al.*, (1977) cited by Vickerman and Barry (1982) have observed more than one hundred VATs in a cloned isolate, while in *T. brucei* a VAT repertoire of more than a thousand have been estimated (Van der Ploeg, *et al.*, 1982).

The interval between successive variant antigen types in an infection varies in relation to the mammalian host and the trypanosome species involved. In laboratory rodents and small ruminants infected with *T. brucei* intervals of 2-4 days have been reported (Gray, 1965) and in rabbits infected with *T. evansi* Gill (1971a) isolated antigenically different populations at weekly intervals. Dar (1972) reported that in infections

of cattle with *T. vivax* the first VATs tended to appear between 18-24 days, while in sheep infected with *T. congolense* variant antigens were collected every week (Wilson and Cunningham, 1972).

The mechanisms behind antigenic variation was attributed to selection and mutation on the part of the trypanosomes in response to the hostile reactions of its host. However this hypothesis is undermined by the appearance of VATs in *in vitro* cultures in the absence of antibodies (Doyle *et al.*, 1979). Recently the concensus seemed to have formed around a genetic basis for antigenic variation (Williams, 1979) with a separate gene for each VSG. There are hundreds of VSG genes in the trypanosome genome of which only one VSG gene is transcribed in each cell (see Aline *et al.*, 1989). Another antigenic switching mechanism has also been described which has been suggested to have the characteristics of gene conversion (Borst, 1986).

Apart from the blood stream VAT repertoire there is also a repertoire of metacyclic VATs (MVATs) in cyclically transmitted trypanosome species. The VSG is reacquired during the metacyclic stage of development in most of the species after it is lost when ingested by the tsetse. However in *T. vivax* the metacyclic stages are apparently devoid of VSG. The metacyclic forms are heterogeneous in their antigenic characteristics and now an MVAT repertoire is recognised (Hajduk and Vickerman, 1981).

The major challenge of trypanosomiasis is antigenic variation. At each crisis the host is presented with a new population expressing different antigenic properties for which it has to produce specific antibodies in order to survive. A second salient feature of the immunology of trypanosomiasis is immunosuppression which predisposes the host to

secondary infections (Goodwin, 1970; Goodwin *et al.*, 1972) and also subverts its efforts to mount effective response to the infection (Sacks and Askonas, 1980).

Immunosuppression

Suppression of host immune response in trypanosome infections to other antigens was first reported by Goodwin (1970), and recent studies have also emphasised a marked depression in parasite-specific immune response (Sacks and Askonas, 1980). The outcome of this effect could be particularly severe in rendering the infected hosts susceptible to secondary infections, and reducing antibody titres following bacterial and viral vaccinations.

Ample evidence is now available confirming the depressed immune response to unrelated antigens in trypanosome infected hosts. Holmes, *et al.*, (1974) demonstrated that cattle infected with *T. congolense* produce less antibodies to polyvalent clostridial vaccination compared to non-infected controls. In goats artificially infected with *T. evansi* Shien (1980) tested their ability to produce antibodies to *Brucella abortus* vaccine by the tube agglutination and also by the complement fixation test. It was found that the production of antibodies was much suppressed compared to uninfected controls. The immunosuppression was reported to develop 25 days after infection and disappeared following suramin treatment with antibody levels coming back almost to the normal values. Recent studies also confirmed significant reduction of antibodies against equine erythrocytes in mice previously infected with *T. evansi* compared to non-infected controls or those infected but treated with suramin (Ye, 1989).

Immunosuppression ensuing from trypanosomiasis has been encountered in a variety of hosts and is considered a major feature of the pathogenesis of the disease. The degree of immunosuppression is generally more pronounced in laboratory rodents compared to larger animals. In mice, vaccinated against louping-ill virus after experimental challenge with *T. brucei* and *T. congolense*, (Whitelaw *et al.*, 1979), antibody response to the vaccine was completely suppressed compared to the uninfected mice or those treated with diminazene aceturate at the time of vaccination. In the same study non-infected vaccinated cattle were more efficient in attaining protective levels of immunity to louping-ill virus in comparison to those previously challenged with *T. brucei, T. congolense* or *T. vivax*. However the antibody response to louping-ill virus was not completely abolished as in the case of the murine host and titres equivalent to 10% of that reached by the uninfected cattle were recorded. Furthermore, groups treated with trypanocidal drugs at the time of vaccination performed better than the untreated ones as far as antibody production was concerned underlining the benefit of chemotherapy during vaccination campaigns in trypanosomiasis endemic areas.

Despite consistent indications confirming the existence of trypanosome mediated immunosuppression, the underlying mechanisms remained the subject of continuing debate and gained significant attention.

In mice infected with a sub-acute strain of *T. brucei* a consistent finding was expansion of the mononuclear phagocytic system including the lymph nodes, spleen, liver and the non-fixed macrophages with the reticuloendothelial system of the liver showing increased activity due to massive plasma cell hyperplasia (Murray *et al.*, 1974b). However although the expanded MPS can possibly play a role in the development of immunosuppression in trypanosome infected hosts as a result of

insufficient localisation of antigenic particulates, it is unlikely to be the only cause and other components of the immunological system are certainly involved.

Elsewhere trypanosome infections were reported to be characterised by the production of large quantities of immunoglobulins particularly of the IgM class part of which have been considered to be non-specific, heterophile antibodies and autoantibodies (Houba *et al.*, 1969; Kobyakawa *et al.*, 1979).

In contrast Musoke *et al.* (1981) claim that experimental evidence for non-specific polyclonal activation comes largely from infected rodents and monkeys and that in cattle the situation is markedly different. All of the IgM and IgG produced during the first two weeks of infection of cattle with *T. brucei* and 85% of that produced during the third week was found to be absorbable with trypanosomes (Musoke *et al.*, 1981).

Other reports blamed antigenic competition as a major cause of trypanosome mediated immunosuppression (Terry, 1976). However complete *in vitro* suppression to sheep red blood cell response was observed when the antigen load in the animal was quite low (Eardley and Jayawardena, 1977). Moreover elimination of the infecting trypanosomes with pentamidine before culturing the infected spleen cells did not abolish the suppressive activity.

According to Murray *et al.*, (1974c) a defect in the thymus-dependent lymphocytes may also play a role in the aetiology of immunosuppression given the far reaching changes occurring in the T-lymphocyte regions of the thymus, spleen and other areas of the lymphoid organ. Moreover cell

mediated immune response, although effective after oxazalone sensitisation in the earlier periods of the infection, is severely impaired (Mansfield and Wallace, 1974) as the infection proceeds.

Alcina and Fresno (1985) reported that spleen cells from *T. brucei* infected BALB/c mice were incapable of responding to the T-cell mitogen Concavalin A with reduced ability to produce detectable amounts of the growth factor required for T-cell proliferation, interleukin 2 (IL. 2) thus linking trypanosome mediated immunosuppression with defective production of IL 2 or the inhibition of its action or both. Similarly, Sileghem *et al.* (1986) incriminated the active inhibition of IL 2 production as the cause in the malfunction of the thymus-dependent lymphocytes in mice infected with *T. brucei*. Lymph node cells (LNC) derived from mice infected with *T. brucei* failed to produce IL 2 after stimulation with Concavalin A, however an exogenous supply of recombinant IL 2 restored proliferative response to the mitogen, with the conclusion that immunosuppression is due to suppressive cells which interfere at the level of IL 2 production.

The roles of other factors such as hypocomplementaemia and enhanced serum protein catabolism were also investigated and may contribute in the development of trypanosome-mediated immunosuppression (Jennings *et al.*, 1973; Nielson *et al.*, 1978).

The striking feature of trypanosome induced immunosuppression is the rapidity with which the effect disappears after trypanocidal drug treatment (Murray *et al.*, 1974c; Roelants *et al.*, 1979), highlighting the close association of this effect with the presence of the trypanosomes rather than a pathological damage developing as a result of infection. This consequently would explain the severity of the immunosuppression in laboratory rodents since these hosts develop high levels of parasitaemia.

Section 2: Chemotherapy and Chemoprophylaxis-review.

Trypanocidal drugs.

The discovery of the protozoan nature of trypanosomes and the appreciation of the multitude of diseases caused by these organisms unleashed tremendous efforts directed towards the control of both human and animal trypanosomiasis. Indeed a widely held view is that modern chemotherapy against infective agents owes its origin to the preoccupation at the turn of the century of disease complex caused by pathogenic trypanosomes (Williamson, 1970; Leach and Roberts, 1981).

Over the years many chemical compounds were developed and tested both in the field and scientific laboratories. Of the host of chemical products that showed a degree of trypanocidal activity only few withstood the test of time and repeated field trials before they had to be abandoned due to shortcomings either in the form delayed toxicity or the development of drug resistant strains (Williamson, 1970). The area where much of the activity took place was in the field of human trypanosomiasis, but inevitably in many instances, compounds active against the human pathogen proved equally effective against animal trypanosomiasis.

Unlike human sleeping sickness animal trypanosomiasis is much less of a straightforward subject and is complicated by the variety of domestic animals affected and the number of trypanosome species involved (Williamson, 1970).

However the watershed in the early endeavours for the search of effective chemotherapy was undoubtedly the synthesis earlier this century of suramin, a product active against both human and animal trypanosomiasis caused by *Trypanozoon* subgenus.

The advantage of this compound in the control of the surra agent was quickly recognised. Knowles (1925) treated camels suffering from surra in the Sudan with "Bayer 205" (suramin) and obtained remarkable success, and in other species Edwards (1926) used "Bayer 205" and concluded that in the equine species it surpassed other trypanocidal agents included in his experiments.

The importance of chemotherapy and chemoprophylaxis lies in the difficulties involved in the eradication of vectors which transmit the various forms of trypanosomiasis either in the cyclical or mechanical forms. This coupled with the constant failure of introducing effective immunoprophylaxis makes chemical control of this important disease the method to be relied on in the foreseeable future. This method itself, however is not devoid of constraints for only a small number of drugs are available in the market (Table 1.2) and these have been bedevilled with development of resistant strains. No new trypanocidal drugs have been introduced into the market for more than 25 years (Holmes and Torr, 1988).

Arsenic

Of the earliest chemical compounds used in the treatment of trypanosomiasis a special place is reserved for the arsenical compounds. Earlier trials against experimental forms of animal trypanosomiasis produced encouraging results and this led to a period of widespread use of a considerable number of arsenical compounds almost bewildering in their chemical structure and configurations (Edwards, 1926).

Table 1.2. Trypanocidal drugs used for the treatment and prophylaxis of animal trypanosomiasis.

Drug	Trade name	Condition of use		Activity on trypanosome		
		Dosage (mg/kg)	Injection	Very active	Less active	Protection
Isometamidium chloride	Samorin Trypamidium	0.25 to 1	IM	*T. vivax* *T. congolense*	*T. brucei*	Prophylactic
Diminazene aceturate	Berenil	3.5	IM	*T. congolense* *T. vivax*	*T. brucei* *T. evansi*	Curative
Homidium bromide Homidium chloride	Ethidium Novidium	1	IM	*T. vivax* *T. congolense*		Curative
Suramin	Naganol Moranyl	10	IV	*T. evansi* *T. brucei*		Prophylactic
Quinapyramine sulphate	Trypacide	3-5	IM	*T. congolense* *T. vivax* *T. brucei* *T. evansi*		Curative
Quinapyramine chloride and sulphate	Trypacide Prosalt	3-5	IM	*T. brucei*		Prophylactic

Adapted from Finelle (1983).

According to Williamson (1970) the earlier efforts to introduce an effective systematic chemotherapy followed along two main lines i.e. synthetic dyes and related compounds on the one hand and arsenicals and antimonials on the other. The first effective trypanocidal end-products of these two lines of development were suramin and tryparsamide. Tryparsamide was extensively used against all forms of human trypanosomiasis over a long period of time until its value was seriously compromised by the emergence of resistant strains (van Hoof, 1947) and severe toxic reactions which could lead to blindness.

Melarsoprol (mel B, Arsobal^{R}) (Friedheim, 1949) was produced later for the treatment of the Gambian forms of sleeping sickness and to date it is the drug of choice because of its ability to cross the blood brain barrier.

Melarsoprol at 3.5 mg/kg was also found to be effective against *T. evansi* infections of camels (Claussen, 1987). However, because melarsoprol is administered by the intravenous route it is rarely used in animal trypanosomiasis.

Other arsenicals such as melarsonyl (mel W; Trimelarsan^{R}) although still available for the treatment of *Dicrofilaria immits* in dogs, appears not to be used in animal trypanosomiasis.

Currently a trivalent arsenical, mel Cy, patented as Cymelarsan^{R} (Raynaud *et al.*, 1989) is being evaluated for use against *T. evansi* infections (Zelleke *et al.*, 1989). Initial trials carried out by Tager-Kagan *et al.* (1989) involving eight dromedaries artificially infected with *T. evansi* and treated with mel Cy at 0.625 mg/kg and 1.25 mg/kg showed that both doses effected complete cure with no relapses during the 60 day post-treatment observation period. However 3 of the treated camels

had a necrotic muscle tissue of 2-3 cm adjacent to the injection site. Significant tissue necrosis was also found in two uninfected cases injected with 3.75 mg/kg of mel Cy.

Antimonials

Potassium antimony tartarate (tartar emetic) and its sodium analogue were among the first chemical substances to show trypanocidal activity. Laboratory tests conducted by Plimmer & Thompson using laboratory rodents infected with *T. evansi* showed promising results (Leach and Roberts, 1981). These and other trials elsewhere led to widespread and prolonged use of this product against many species of pathogenic trypanosomes. However the results obtained with the use of this compound were varied, on some occasions resulting in the death of the treated cases immediately after injection.

Before the introduction of modern and less toxic trypanocides, tartar emetic held sway in the field despite high toxicity often resulting in 6% mortality. This was apparently preferable to the higher losses at times reaching 50% in untreated infections (Wilson, 1958). Despite this obvious handicap tartar emetic had the advantage of not readily giving rise to drug-resistant strains. This and its relatively low cost kept the product in the market for a considerable period of time until as recently as the earlier part of the fifties.

Suramin

Suramin (Bayer 205^{R}; Germanin^{R}; Antrypol^{R}; Moranyl^{R}; Naganol^{R}) (hexa-sodium 3,3-ureylene-*bis* [8-(3-benzamido-*p*-toluido)-1,3,5-naphthalene sulphonate]), a sulphonated naphthalamine synthesised as an offshoot of the dyestuffs was introduced into the

field earlier this century for the treatment of human trypanosomiasis but it proved to be equally effective against experimental infections of *T. equiperdum* (Leach and Roberts, 1981). Trials against natural infections of *T. evansi* of camels, horses and cattle (Knowles, 1925; Edwards, 1926) also met with great success. The advantage of suramin over all other compounds in use at the time of its introduction became clearly obvious and was reported by many workers. Leese (1927) used "Bayer 205" (suramin) against camel surra and obtained superior results to tartar emetic. The following years saw ever increasing doses being administered in the field with a high degree of recovery.

A large dose of 10 g of suramin is recommended and regarded as the standard dose for camels suffering from surra. However Bennett (1933a) compared different doses and found that a dose of 4 g sufficient to eliminate *T. evansi* infections. Gad-El-Mawra and Fayad (1979) also found that a dose of 8 mg/kg of suramin or 2 doses of 4 g administered on two separate occasions with an interval of seven days adequate to effect cure in natural infections of camel surra. In horses a dose range of 10 mg/kg was shown not only to be therapeutic but protected horses for a period of 30 days.

Although some therapeutic activity was reported against *T. simiae* (Stephen, 1966) suramin is known to be ineffective against tsetse-transmitted trypanosomiasis due to the same subgenus (*Nanomonas*) *T. congolense* and also to (*Duttonella*) *T. vivax*.

Another advantage of suramin was its ability to combine with other trypanocides, due to its anionic properties, and form suraminates, a fact which was first noticed in human trypanosomiasis. Simultaneous administration of suramin and pentamidine in sleeping sickness patients

resulted in the elimination of the toxic effects of the later (Williamson, 1957). In animal trypanosomiasis, suraminates were also described (Williamson and Desowitz, 1956) and were extensively applied in the field. Quinapyramine suraminate has prophylactic properties in equine surra (Gill and Malhorta, 1971) and was also found to afford cattle with minimum protection period of five and half months against experimental challenge with *T. vivax*. Moreover the complex was well tolerated even at a high dose of 40.0 mg/kg, while quinapyramine alone at 10.0 mg/kg was observed to cause the death of the treated cases due to toxicity (Desowitz, 1957). Quinapyramine suraminate was also reported to protect pigs exposed to the risk of *T. simiae* trypanosomiasis (Noble, 1958).

Elsewhere Stephen (1958) protected Zebu cattle with ethidium-suramin complex for several months but intense local reaction, swelling and sloughing, ending in loss of prophylaxis was observed. Later Stephen and Williamson (1958) used a lyophilised preparation of suramin-ethidium complex to alleviate the side effects with some success.

Recently a suspension of diminazene and suramin at 10 mg/kg was found to be therapeutic in mares infected with a field strain of *T. evansi* and moreover protected horses for a period of 72 days from infection (Sabanshiev, 1988).

Quinapyramine.

Quinapyramines, (Antrycide^{R}, Trypacide^{R}) is 4-amino-6-(2-amino-6-methylpyramid-4-ylamino)-2-methylquinoline-1,1 dimetho (methyl sulphate or chloride dihydrate) were first introduced into the field as trypanocidal drugs in the early fifties in two forms: The methysulphate and the chloride. Both forms have identical trypanocidal action once they

are in contact with trypanosomes but they have different pharmacological properties due to variation in solubility (Davey, 1950). The methyl sulphate is a readily soluble compound and achieves a maximum concentration in the plasma of 2700 µg/l within 24 hours (Davey, 1950), while the chloride is a sparingly soluble to the extent of 0.12% in water and only reaches a maximum concentration in the plasma of 40 µg/l 24 hours following subcutaneous injection. The combination of the two salts was prepared as Antrycide Prosalt^{R} and became the first true prophylactic drug against cattle trypanosomiasis (Williamson, 1962).

After a subcutaneous injection of Antrycide Prosalt^{R} the soluble methyl sulphate quickly passes into the blood stream to clear current infections while the sparingly soluble chloride forms a depot from where small quantities are gradually released into the bloodstream to afford protection against new infections. The trypanocidal action of quinapyramine is not restricted to the congolense-vivax group of trypanosomes but also extends to the organisms of the *Trypanozoon* subgenus (Curd and Davey, 1950). This property was particularly appreciated in the treatment of surra infections and became a potential alternative in the event of established suramin resistance. In the Sudan quinapyramine was used when relapses occurred in Antrypol^{R} (suramin) treated camels (Leach, 1961).

Quinapyramines were extensively used in the field particularly in tsetse-transmitted trypanosomiasis however because of occurrence of widespread drug resistance the drug was removed from the market in 1974 (Schillinger and Rottcher, 1986). It was later reintroduced exclusively for the treatment of *T. evansi* infections.

The recommended dose for camels, horses and cattle is 7.4 mg/kg consisting of a combination of the two salts (Finelle, 1983). However Gill (1972) found that 5 mg/kg of quinapyramine sufficient to cure ponies infected with *T. evansi.* In buffaloes suffering from surra quinapyramine Prosalt was also reported to clear the infection in 24 hours at 5 mg/kg (Razzaque and Mishra, 1977).

Diminazene

This compound was derived from the quinaldin nucleus Consagin which was found to possess trypanocidal activity. Diminazene (Berenil^{R}), is N-1,3-diamidino-phenyltriazene diaceturate tetrahydrate, a yellow odourless powder moderately soluble in water, and the only diamidine to have been successful in practice after the development of pentamidine which is used against human sleeping sickness (Ruchel, 1975). The action of this drug is rapid on the parasite causing its death in a short time. Fussganger and Bauer (1960) reported that in *in vitro* low concentrations are enough to kill *T. congolense* within hours of exposure.

Shortly after injection, a high blood concentration is produced which tends to disappear in a relatively short time and is completely excreted after 24 hours. While this is the accepted view, evidence came to light suggesting that residual deposits can occur. Lumsden *et al.* (1965) reported that plasma of cattle treated with diminazene aceturate showed significant *in vitro* anti-trypanosomal activity for up to 3 weeks. Moreover mice treated with diminazene aceturate failed to become parasitaemic after repeated challenges with *T. brucei* until the 42nd day post-treatment (Van Hoeve and Cunningham, 1964). Nevertheless diminazene aceturate is considered to be a curative drug with sterilising effect and is less important in

prophylaxis. This consequently is believed to reduce the possibility of trypanosomes developing resistance to the compound. Earlier trials to produce populations of *T. congolense* resistant to diminazene were not successful (Fussganger and Bauer, 1960). However Whiteside (1963) succeeded in producing a strain of *T. congolense* resistant to diminazene aceturate through repeated exposure in a single experimentally infected bovine.

Diminazene aceturate is highly effective against trypanosomes of the *congolense-vivax* species. In addition it is active against babesiosis, an added advantage in the field where the disease is also endemic. The recommended dose for cattle affected with *T. congolense* and *T. vivax* is 3.5 mg/kg bodyweight, while for *T. brucei* infections 5.0 mg/kg is recommended (Milne *et al.*, 1955: Cunningham, 1968). A significant drawback of diminazene was its relative instability in aqueous solutions, however with the addition of antipyrine it was reported that the solution could remain stable for two weeks (Fairclough, 1963). In the treatment of *T. evansi* infections Hiregoudar and Ausahhi (1971) cited by Mahmoud and Gray (1980) succeeded in treating buffaloes suffering from surra with diminazene aceturate at a dose of 8.0 mg/kg. Gill (1973) compared a range of trypanocides against a single strain of *T. evansi* and found diminazene to be more active than other diamidines included in the experiment. Elsewhere Raisinghani and Lodha (1980) reported that diminazene at 1.25 mg/kg and 3.75 mg/kg cured camels infected with *T. evansi* and although two camels died 43 and 45 hours following treatment with 3.75 mg/kg drug toxicity was not suspected. In contrast Leach (1961) found that diminazene at 3.5 mg/kg to be ineffective and increasing the dose to 7.0 mg/kg led to severe toxic reactions. Moreover, Homeida *et al.*, (1981) reported that a high dose of 10 mg/kg diminazene to be extremely toxic to camels with signs of hyperaesthesia, frequent urination

and defecation, tremors, itching, convulsions and frothing at the mouth. Repetition of the same dose proved to be fatal in about a week. In the same study a dose of 40 mg/kg caused death in a matter of hours. Post-mortem findings were dominated by haemorrhages, congestion and oedema of the parenchymatous organs.

However studies carried out by Petrovsky and Khamiev (1977) and quoted by Boid *et al.* (1985) would suggest differences in tolerance to diminazene between the dromedary and the bactrian camel. In the Asian breed infected with *T. ninae kohl-yakimov* (*T. evansi*) 5 mg/kg was effective and cleared the infection without adverse effects. In horses diminazene could be recommended in the event of *T. evansi* strains breaking through prophylaxis with suramin, quinapyramine or quinapyramine suraminate (Gill, 1971c) since no evidence of cross-resistance has been observed.

Phenanthridium compounds

This class of trypanocidal drugs owes its origin to the later works of Ehrlich on the dystuffs based on the acridine nucleus (Ruchel, 1975). Further development and subsequent synthesis of phenanthridine in the 1930s led to the introduction of phenidium chloride (Williamson, 1970). Although phenidium chloride possessed some trypanocidal activity, it was marked by poor solubility in water and low therapeutic index. The more effective and water soluble dimidium bromide was later synthesised and used extensively in East Africa. However, despite these advantages delayed toxicity, photosensitisation and drug resistance which became unavoidable sequelae of this compound was deemed unacceptable and eventually led to its decline.

Further research on the phenanthridine structure led to the introduction of homidium (Watkins and Woolfe, 1952) by substituting a methyl group with ethyl group at the quaternary N atom of dimidium.

Homidium (2,7-diamino-9-phenyl-10-ethylphenanthridinium) bromide or chloride (Ethidium^R and Novidium^R respectively) proved to be more effective and less toxic than its forerunners (Leach and Roberts, 1981). Homidium bromide effected cures at a dose of 0.3 mg/kg in zebu cattle infected with *T. congolense* (Wilde and Robson, 1953) in contrast to dimidium where relapses frequently occurred at 1.0 mg/kg. Another recorded advantage of homidium bromide was its curative and prophylactic activity against both *T. congolense* and *T. vivax* (Wilson and Fairclough, 1953; Ford *et al.*, 1953; Leach *et al.*, 1955). This reported prophylactic properties were later attributed to the stimulation of the host immune system following elimination of the infection by the drug (Gilbert and Newton, 1982).

While Ethidium^R and Novidium^R (a compound equally effective therapeutically as Ethidium, but soluble in cold water) are established trypanocides against infections caused by congolense-vivax trypanosomes, information on its activity against *T. evansi* appears to be scarce (Mahmoud and Gray, 1980). However Srivastava and Ahluwahia (1973) found both homidium bromide (Ethidium^R) and pyrithidium bromide (Prothidium^R) to be ineffective in dogs infected with *T. evansi*.

The recommended therapeutic dose of homidium is 1.0 mg/kg via the intramuscular route as the subcutaneous injection is likely to give rise to severe local reactions (Leach and Roberts, 1981).

Pyrithidium bromide (Prothidium^R) and isometamidium chloride (Samorin^R) were the last of the phenanthridine group to be introduced into the field. Both owe their origin to hybridisation of known trypanocidal

compounds and marked the start of an era of feasible effective chemoprophylaxis in tsetse transmitted trypanosomiasis of cattle. At 1.0 mg/kg isometamidium protected cattle against repeated experimental challenge for a period of 5 months (Whitelaw *et al.*, 1986). Isometamidium is effective against *T. evansi* infections, however because of toxicity to camels (Balis and Richard, 1977) it is rarely used in this species. Avasathi *et al.* (1979) treated fifty six donkeys naturally infected with surra with isometamidium at 0.325 mg/kg and reported that all were cured and no relapses were detected during the 30-day post-treatment observation period.

Both these compounds however have the disadvantage of causing severe local reactions at the injection site. Intramuscular injection of isometamidium is often followed by necrotic reactions of the neck region, a lesion frequently observed in areas where isometamidium is commonly used (Wilson *et al.*, 1976). To counteract this side effect intravenous injection was proposed. A 0.5 mg/kg dose was reported to protect goats and cattle for a period of about 60 and 90 days respectively (Toure, 1973). However intravascular injection of 1.0 mg/kg was found to be extremely toxic with fatal consequences. Nevertheless isometamidium is widely used in the field for curative and prophylactic purposes.

Pyrithidium bromide is capable of protecting cattle at 4.0 mg/kg for a period of four months (Lyttle, 1960). However pyrithidium consistently gave rise to large swellings at the injection site as well as losses in weight of treated animals (Stephen, 1962) and frequent development of resistant strains.

Chemoprophylaxis

The control of trypanosomiasis by chemoprophylactic means has been discussed on many occasions (Maclennan, 1970; Leach and Roberts, 1981). The choice of this line of defence against the consequences of trypanosomiasis seems to warrant careful considerations of the logistical problems involved and in particular the management systems of the target herds. The slow elimination of prophylactic drugs, the mechanism by which they give protection, leads eventually to low concentrations in the tissues giving trypanosomes an opportunity to multiply in the presence of drugs. When this situation arises, it has been suggested that trypanosomes can develop resistance towards the drug involved (Peters, 1974). This fact clearly recommends the repetition of the prophylactic trypanocide to be administered within the period of protection if satisfactory results are to be attained (Finnelle, 1983). In Africa nomadic and semi-nomadic stocks are inaccessible over periods of time, a fact to be taken into consideration in chemoprophylactic operations of herds under extensive management systems.

In tsetse-transmitted trypanosomiasis of cattle significant economic benefits can be obtained with chemoprophylaxis if all the conditions necessary can be satisfactorily fulfilled. A few of the trypanocidal drugs have been shown to possess prophylactic properties namely quinapyramine (Prosalt), pyrithidium and isometamidium. The dose ranges under which these compounds afford protection in relation to tsetse challenge and husbandry practices have been discussed by many authors (Whiteside, 1960; Ford and Blaser, 1971; Lewis and Thompson, 1974). Isometamidium chloride at 1-2 mg/kg can give a protection period of about 3 to 4 months under conditions of heavy trypanosome risk (Kirkby, 1964). Elsewhere protection of Boran cattle with 1 mg/kg of isometamidium was

obtained against a single or multiple challenge with trypanosome-infected tsetse for more than 5 months based on drug cover alone with no signs of priming of the host's immune response (Whitelaw *et al.*, 1986). However, in circumstances where the tsetse challenge is high protection by means of trypanocidal drugs is inadvisable due to the increased possibility of the emergence of resistant populations of trypanosomes. Quinapyramine Prosalt can afford a protection period of two months, however this compound was beset with the development of resistant strains which led to its temporary withdrawl from the market. Likewise pyrithidium consistently gave rise to drug-resistant strains, and severe local reactions which eventually prompted its removal from the market.

In West Africa where trekking of cattle from the countryside to markets in towns is a common practice contact between cattle and tsetse flies is sometimes unavoidable with a consequence of heavy economic losses. In such circumstances the appropriate dose of a preventive drug has been shown to produce tangible results in eliminating the risk (Na'Isa, 1969; Jones-Davies, 1967).

In *T. evansi* infections Gill and Malhorta (1971) reported the possibility of protecting ponies from 6 months to about 23 months with quinapyramine suraminate. Treatment with either suramin or quinapyramine alone is known to afford a protection period of 1 to 2 months depending on the dose administered. Although the importance of the horse has declined in the transport service a significant role is still reserved for this species and where the risk is high chemoprophylaxis is a worthwhile measure.

Chemoprophylaxis against camel surra seems to have attracted less attention. Furthermore, poor tolerance to isometamidium (Balis and Richard, 1977), reduced the significance of this compound in the control of camel

trypanosomiasis. However, since the two drugs available for the treatment of this condition namely suramin and quinapyramine seem to confer a degree of protection, reliance is mainly placed on these for the chemotherapy of *T. evansi* infections of camels. In tsetse-transmitted trypanosomiasis of the camel the high costs incurred in prophylaxis could hardly be justified as this species seldom comes into close contact with heavily infested areas.

The porcine species is highly susceptible to infection with *T. simiae* (Stephen, 1970) which at times leads to the decimation of stocks. However control is only considered where intensive pig keeping is practised. Experimental studies (Stephen and Gray, 1960; Gray, 1961) suggested that quinapyramine suraminate at a high dose of 40 mg/kg could protect stocks at risk.

Drug-resistance

The ability of trypanosomes to become refractory to the action of a trypanocidal compound which they were previously sensitive was observed as early as 1907 by Ehrlich (Gill, 1971b). Recent and more effective trypanocidal drugs are equally liable to give rise to resistant strains. Indeed there is no drug currently in use against which resistant populations have not emerged. Moreover the lack of new trypanocides appearing into the market for more than two decades (Williamson, 1976; Losos, 1986), has exacerbated the situation in the control of this economically important disease.

The development of drug-resistance is attributed to the multiplication of trypanosome organisms in the presence of small amounts of drugs not sufficient to effect cure. Finelle (1983) discussed several ways in which resistant strains could emerge. First the application of insufficient

dose of a trypanocide due to incorrect estimation of body weight or the formation of abscesses or cyst-forming reactions leading to partial rejection or prevention of the diffusion of the drug. Alternatively the erratic use of chemoprophylactic drugs or halting their use altogether while stocks are still at risk could afford the opportunity for trypanosomes to multiply in the presence of drugs and develop resistance.

The mechanism by which drug resistance develops is thought to be one of the following: 1) Altered drug uptake; 2) Altered metabolism; or 3) Inactivation of the drug. Altered drug uptake is believed to be the most likely explanation and could reflect changes at the cell surface and membranes or enzymes responsible for the active transport of drugs or its efflux from the cell (Zakerzewsky, 1973). Hawking (1937) observed that tryparsamide-resistant strains following *in vitro* incubation in nutrient media containing reduced tryparsamide did not remove the drug from the medium while sensitive ones quickly absorbed the drug. The absorption of arsenic and antimony compounds by a sensitive trypanosome is usually quick, however damage to the organism was reported to occur only after a latent period of several hours suggesting that damage is secondary to absorption (Yorke *et al.*, 1931).

The observations that treatment of trypanosome infections often ended in relapse led to attempts aimed at influencing the process and gave the earliest hints of the existence of drug-resistance. Schnitzer and Grunberg (1957) reported that under experimental conditions drug resistance can be induced by repeated or continuous exposure of the trypanosome organisms to subcurative doses of chemotherapeutic agents in laboratory animals or passage in hosts with reduced immune response.

In the field the level of resistance encountered is often around the normal curative doses, in contrast to the levels that can be induced under experimental conditions. However the importance of the field produced drug-resistance is considered to lie in the narrow margin between the curative and the maximum tolerated doses of many trypanocidal drugs (Hawking, 1963), and the possibility that an increase in dosage may lead to manifestations of toxicity symptoms (Stephen, 1986).

Drug-resistance is often expressed in relapse of infection in treated cases, and in situations excluding reinfection and underdosing, drug resistance is usually to blame. However Jennings *et al.* (1977) described another type of relapse related to the interval between treatment and infection in *T. brucei*. In their experiments diminazene aceturate and other trypanocidal drugs led to relapses when treatment was delayed. The relapsed population however was sensitive to the normal doses of the drugs involved if treatment was effected a week after infection. This kind of relapse is due to trypanosomes entering the brain, a tissue not accessible to the action of trypanocidal drugs, before treatment and subsequently emerging into the circulation (Jennings *et al.*, 1979).

Once developed the resistance trait can be stable for a long period of time, even after transmission through tsetse flies (Yorke *et al.*, 1933; Gray and Roberts, 1971) and the loss of resistance described in the field may be caused by the overgrowth of resistant strains by rapidly multiplying sensitive ones. Sones *et al.* (1989) found that superinfection of a drug-resistant strain did not establish infection in goats which had been previously infected with a isometamidium-sensitive strain, while superinfection with the sensitive resulted in temporary remission if the

goats were formerly infected with the resistant. This would imply that resistant strains have a reduced growth potential compared to original sensitive strains.

In view of the fact that many trypanocidal drugs are the result of what is known as "hybrid synthesis" i.e. combination of different chemical substances with trypanocidal activity (Williamson, 1970; Peters, 1974) cross-resistance is frequently observed with compounds of similar structures (Table 1.3). However similarities in chemical structures although important does not account for all the cross-resistance links. Other factors such as anionic properties of the various drugs as well as the receptor sites for the drugs on the trypanosomes have also been proposed to explain the cross-resistance relationships (Williamson, 1962; 1970).

Gill (1971c) tested eleven strains of *T. evansi* breaking through chemoprophylaxis with quinapyramine suraminate and found that the strains had acquired resistance to both quinapyramine and suramin. The resistance to quinapyramine was more pronounced, however they were fully susceptible to diminazene. In the same study it was found that other strains resistant to suramin were sensitive to both diminazene and quinapyramine.

Further studies on the cross-resistance relationships have suggested that strains of *T. evansi* resistant to quinapyramine are also resistant to the drugs of the phenanthridine class but not always to diminazene (Gill, 1971b). Moreover strains made stilbamidine fast were reported to be resistant to diminazene as well as the other diamidines and also to the melaminyl arsenicals.

In bovine trypanosomiasis of Africa Whiteside (1962a) advocated the use of "sanative pairs" i.e. alternation of any of the other trypanocides with diminazene to avoid the development of drug-resistance

Table 1.3. Cross-resistance patterns of the drugs used against trypanosomiasis.

	Response of strain resistant (to drug type)					
Drug	Tryparsamide	Diminazene	Quinapyramine	Homidium	Suramin	Isometamidium
Tryparsamide	++	++	-	-	-	-
Diminazene	-	++	+	-	-	-
Quinapyramine	-	-	++	+	-	+
Homidium	-	-	+	++	-	++
Suramin	-	-	-	-	++	-
Isometamidium	-	-	+	++	-	++

Adapted from Williamson (1970).

+ Cross-resistance to curative dose
++ Cross-resistance to higher dose
- No cross-resistance

and cross-resistance. This was based on the fact that diminazene resistance was rarely encountered in the field and that strains resistant to quinapyramine and phenanthridines were fully susceptible to diminazene. Finnelle (1983) proposed the use of isometamidium in cases were diminazene resistance is suspected and considered these two compounds as an effective sanative pair. However recently there has been reports of field strains of trypanosomes resistant to both diminazene and isometamidium. Ainanshe *et al.* (1989) isolated strains of *T. congolense* expressing high levels of resistance to both diminazene and isometamidium from southern Somalia. The authors described experimental studies where blood samples obtained from cattle infected with trypanosomiasis were inoculated into isolation calves and then treated with standard doses of either diminazene aceturate or isometamidium. When relapses occurred indicative of drug resistance the infection was transferred into groups of calves, goats and mice in order to assess the degree of drug resistance of the isolates. The sensitivity tests in the calves showed both isolates to be resistant to 2.0 mg/kg of isometamidium and 7.0 mg/kg of diminazene.

Immunity and chemotherapy

The value of the host immune response in the achievement of successful control of trypanosomiasis by means of chemical agents has been recognised since the earliest attempts of chemotherapy (Schnitzer *et al.*, 1946). Some of the drugs currently in use against human and animal trypanosomiasis depend on competent host immune response for their trypanocidal activity without which their efficacy is significantly reduced.

Earlier studies on the mode of action of suramin had revealed this naphthalamine compound unlike trivalent arsenicals to possess incomplete *in vitro* trypanocidal activity (Jancso and Jancso, 1934), with the conclusion that suramin had an opsonin-like effect sensitising the trypanosomes to phagocytosis by the reticuloendothelial system (Jancso and Jancso, 1934; Hawking, 1939). Intact host immune response had also been shown to be essential in the efficacy of quinapyramine sulphate in rats experimentally infected with *T. evansi* (Sen *et al.*, 1955).

The recently introduced drug DL-α-difluoromethylornithine (DFMO; Eflornithine^{R}; Ornidyl^{R}), a selective inhibitor of ornithine decarboxylase which is a key enzyme for the synthesis of polyamines (Metcalfe *et al.*, 1978) relies on antibodies against the surface antigen of trypanosomes to be effective. De Gee *et al.*, (1983) have reported that antibody response to surface antigens is important for the rapid elimination of parasites after treatment with DFMO, confirming previous opinions (Bacchi *et al.*, 1980) that DFMO is trypanostatic and not trypanocidal.

In human parasitic infections, praziquantel, the drug of choice currently used in the treatment of schistosomiasis (Harnett, 1988) was found to have its efficacy substantially reduced in immunosuppressed mice (Sabah *et al.*, 1985). Further works demonstrated that the efficacy of praziquantel *in vivo* to depend on humoral immune response and that the effector antibodies act against the surface of the parasite immediately after exposure to the drug (Brindley and Sher, 1987). praziquantel which interacts with the lipid constituents of tegumental membranes causing membrane

destabilisation (Andrews, 1985) is thought to expose antigens on the surface of the schistosome and that the interaction of antibody with these exposed molecules leads to parasite death.

Immunity and chemoprophylaxis

In tsetse infested parts of Africa, Bevan (1928) made the important observation that cattle treated after infection with antimony could be reintroduced into tsetse infested areas and withstand the harmful consequences of trypanosomiasis. Further studies had demonstrated that while such animals harboured small numbers of trypanosome organisms in their blood they nevertheless continued to thrive. From these and subsequent works (Bevan, 1936) the conclusion had emerged that cattle can acquire a state of "tolerance" or immunity to trypanosomiasis comparable to that produced in bovine babesiosis after treatment with trypan blue. Evidence of this form of immunity was further substantiated when infected blood from these animals inoculated into susceptible and untreated cattle developed fully pathogenic trypanosomiasis.

While non-sterile immunity or "tolerance" after chemotherapy was also mentioned by others (Bennet, 1933b), reports on sterile immunity are scanty. However Soltys (1955) suggested that prolonged use of chemoprophylaxis could lead to sterile immunity lasting more than a year. In his experiment cattle bimonthly treated with quinapyramine Prosalt for a period of 28 months resisted both natural and artificial challenge for 18 months and serological studies revealed the development of only low titres of complement fixing antibodies. Furthermore another group treated with the prophylactic quinapyramine Prosalt and later transferred to a tsetse-free location for 10 months equally resisted challenge when

reintroduced into enzootic areas. However Smith (1958) suggested that after successive administration of a prophylactic compound at two-monthly intervals there is sufficient residue to suppress the appearance of circulating organisms for about a year afterwards. As a result evidence of sterile immunity is at best considered inconclusive and its validity still awaits confirmation (Murray and Urquhart, 1977). In marked contrast non-sterile immunity or "tolerance" has been consistently reported and continues to be exploited in commercial situations in endemic areas although details seem to be scarce (Holmes, 1980).

After the earlier work on acquired immunity or "tolerance" (Bevan, 1928, 1936) further evidence came to light lending support to this aspect of trypanosomiasis. Whiteside (1962b) introduced zebu cattle from tsetse free locations into tsetse infested parts of Kenya in the vicinity of lake Victoria. At the start of the experiment the cattle became parasitaemic after every four weeks and were treated with diminazene aceturate a trypanocidal drug with no prophylactic properties. After four successive treatments the interval between treatment and infection lengthened to eight weeks and this was ascribed to the development of immunity.

In a series of experiments in East Africa (Wilson *et al.*, 1975; Wilson *et al.*, 1976) groups of Boran cattle were maintained under different trypanocidal drug regimes in an area of medium tsetse challenge with the aim of assessing the development of immunity in association with treatment. One group was treated with the curative drug diminazene aceturate on the development of clinical disease while two other groups were treated on a group basis either with diminazene or isometamidium when patent parasitaemia was detected in one animal. Development of immunity to trypanosomiasis was assessed on the basis of changes in trypanocidal drug

requirement, ability to maintain normal blood values and response to challenge following withdrawl of drugs. In the group treated with diminazene on clinical grounds the interval between treatments began to lengthen and on withdrawl of drug administration the ability to maintain satisfactory body weights and normal haematological values was not adversely affected in any significant way, while the group treated with diminazene when one animal became parasitaemic did not develop immunity. However from these studies it was concluded that isometamidium administered on group basis was the most suitable for maintaining beef cattle in tsetse infested areas (Wilson *et al.*, 1976). Similar works involving draught oxen in areas of high tsetse challenge provided further evidence that a state of tolerance can be exploited with the strategic use of drugs. However reasonable standards of veterinary supervision and management was found to be essential to achieve successful results in these situations (Bourn and Scott, 1978).

Whitelaw *et al.* (1986) carried out experimental studies with the objective of establishing the duration of drug-induced prophylaxis and the specific antibody responses in animals under chemoprophylactic cover. It was found that Boran cattle treated with isometamidium at 1.0 mg/kg resisted single and multiple challenges by infected tsetse flies for five months. Moreover it was demonstrated that protection was solely associated with the prophylactic activity of isometamidium since there was no priming of the host's immune response. Furthermore single intramuscular injection of isometamidium at 1.0 mg/kg was shown to afford protection against repeated challenge with infected tsetse flies or intradermal inoculation of *in vitro*-derived metacyclic trypanosomes for a period of five months (Peregrine *et al.*, 1988). During the course of the study it became evident that protection was associated with drug cover alone since there were no

signs of skin reaction at the site of inoculation. Similarly a 0.5 mg/kg dose isometamidium chloride was found to protect cattle against trypanosomiasis challenge for a period of 3-5 months.

While the mechanism behind non-sterile immunity or "tolerance" awaits further elucidation the suggestion has been put forward that it is due to a battery of immune responses developed by the host to a range of metacyclic antigens and variant antigen type repertoires in a certain locality, and/or the expansion and activation of the mononuclear phagocytic system (Murray *et al.*, 1983).

Immunity and drug-resistance

In the earlier works on drug-resistance in trypanosomiasis the role of the host was not indisputably obvious (Yorke *et al.*, 1931). However only when Jancso and Jancso (1934) devised a method of excluding the natural defence mechanism of the host did the full impact came to light of the crucial role played by the host immunity in the genesis of drug-resistance. This had been achieved by splenectomy combined with intravenous injection of electro-colloidal copper, allowing for the first time the study of the interaction between trypanosome organisms and trypanocidal agents without interference from the host immune system. The combination of splenectomy and electro-colloidal copper successfully eliminated antibody production and blocked the phagocytic activity of the reticuloendothelial system (Jancso and Jancso, 1934). Later Jancso and Jancso (1935) had produced in immunosuppressed laboratory rodents strains of *T. brucei* highly resistant to Germanin^{R} (suramin) in a very short time by repeatedly treating with subcurative doses. In one strain a degree of resistance was produced in splenectomised and blocked mice of 250 times the normal in only 12 treatments.

Another strain of *T. brucei* was found to have attained a resistance of seven times the normal in immunologically compromised rat after a single treatment, while in contrast production of suramin-fast strains in normal hosts had been shown to be slow and a tedious operation (Jancso and Jancso, 1935; Hawking, 1939).

Further studies have recognised defective antibody responses as the major factor behind the the genesis of drug-resistance in immunologically compromised hosts (Schnitzer *et al.*, 1946). In a strain of *T. equiperdum* a high level of resistance to p-rosaniline was produced in splenectomised rats using the short passage technique. The short passage, i.e. transfer of infection to a clean host hours after treatment, in combination with splenectomy avoided any contact between antibodies and trypanosome organisms and led to a rapid development of p-rosaniline resistance. In contrast, the development of drug-resistance to p-rosaniline in splenectomised mice was counteracted by passive immunisation clearly indicating that the rapid development of the drug-fastness was due to the elimination of the role played by the host antibodies (Schnitzer *et al.*, 1946).

Reduced host immune response was also found to select drug-resistant populations of trypanosomes in experimental infections of mice after treatment with melarsoprol (Frommel, 1988). In this report two groups of mice immunosuppressed either with cyclophosphamide treatment or exposure to irradiation and a further group with an intact immune system were treated with different doses of melarsoprol after a syringe challenge with a strain of *T. rhodesiense*. Initially all groups were treated with 20 µg/kg of melarsoprol and this had been followed by relapses in all groups. However in the immunocompetent group the relapses were significantly delayed. Increasing the dose to 50 µg/kg achieved complete cure in the normal

mice while in the immunologically compromised groups almost all had relapsed and the trend was not reversed even when the dose was increased to 80 μg/kg. Relapsed populations inoculated into immunocompetent and irradiated mice resisted treatment at 50 μg/kg, a dose which in normal mice results in the elimination of infection indicating that the strain had acquired resistance (Frommel, 1988).

Selection of drug-resistant bacterial organisms in human patients with impaired immune responses has also been described (Follath, *et al.*, 1987) and is considered relatively frequent with serious clinical consequences.

The experiments described in the next chapter were designed to re-evaluate the role of immunosuppression in the possible development of drug resistance by clones of *T. evansi* to currently available drugs.

Chapter 2

THE DEVELOPMENT OF DRUG RESISTANCE BY CLONES OF *T. EVANSI* IN IMMUNOSUPPRESSED MICE.

Chapter 2

The development of drug resistance by clones of *T. evansi* in immunosuppressed mice.

Introduction.

Despite recent advances in tsetse control methods, particularly the development of olfactory attractants (Vale *et al.*, 1985) which tremendously increase the efficacy of traps and targets used for tsetse control schemes, chemotherapy remains the most widely adopted control strategy against all forms of trypanosomiasis. Unfortunately chemotherapeutic control suffers from the drawback of relying on a small number of drugs (Newton, 1974) which have been in use for many years. Although a new arsenical compound, mel Cy, has recently been developed (Raynaud *et al.*, 1989) specifically for the treatment of surra infections, no new trypanocides against the other African animal trypanosomiasis are known to be in the process of development and the situation has remained unchanged for more than 30 years. In Africa the three most widely used drugs are homidium bromide, diminazene aceturate and isometamidium chloride. Homidium and diminazene are used solely for curative purposes while isometamidium has both therapeutic and prophylactic properties (Leach and Roberts, 1981). In addition suramin, quinapyramine and the recently introduced mel Cy are available for the treatment of *T. evansi* infections (Schillinger and Rottcher, 1986; Zweygarth and Kaminsky, 1990).

Although trypanocidal drugs can be very effective, the continuous use of a small number of established compounds could undermine their value and lead to a widespread drug resistance. Drug resistance is believed to develop when trypanosomes are exposed to levels of drugs not sufficient to result in their elimination; a condition which can arise in many circumstances in the field (Finnelle, 1983). Reduced host immune response was also considered to assist the development of resistant strains by Schnitzer and Grunberg (1957).

It was reported over 50 years ago that splenectomy and blockage of the host reticuloendothelial system could lead to the development of suramin resistance (Jancso and Jancso, 1934, 1935). The aim of the experiments described in this chapter was to establish whether immunosuppression by means of ^{60}Co-irradiation would lead to the development of drug-resistance by *T. evansi* using the currently available trypanocidal drugs and if the resistance produced would remain stable in normal hosts.

Materials and methods

Mice

The mice used in all experiments were a strain of Swiss white mice CD-1 purchased from a commercial source (Charles River, England), of both sexes and weighing about 30 grams. The mice were kept in plastic /metal cages (North Kent Plastics, England), bedded with wood shavings at a room temperature of 21°C and 50% relative humidity.

The animals were fed with pelleted concentrates (Special Diet Services, England) and fresh drinking water was provided *ad libitum.*

Trypanosomes.

Three clones of *T. evansi* were used in these experiments. Two clones of *T. evansi*, GRVPS 13/3 and GRVPS 18/2, cloned from GVR 74 were derived from TREU 1444 which was originally isolated from a camel naturally infected with surra in the Sudan and was suramin resistant. The other clone, *T. evansi* GRVPS 19/5 a derivative of TREU 1412, was also originally isolated from a camel in the Sudan and was suramin sensitive.

All stocks were kept as cryopreserved stabilates in liquid nitrogen.

Preparation of stabilates.

Infected mice, at the first peak of parasitaemia, were bled by cardiac puncture while under terminal anaesthesia (Trilene^{R}, ICI, England), using a heparinised syringe. Blood obtained was measured and glycerol added dropwise until a final overall concentration of 12.5% was attained. This

blood/glycerol mixture was then drawn up into capillary tubes and one end sealed with Cristaseal^{R} (Hawksley & Sons Ltd, England). All capillary tubes were placed in screw top plastic tubes, which had holes made to allow ingress of liquid nitrogen and were then suspended overnight in the vapour phase in the liquid nitrogen container. Later they were fully immersed in liquid nitrogen for storage.

Infection of mice.

The mice were infected with inocula prepared either directly from the frozen stabilates or from fresh infected blood. In both cases, the inocula were diluted with a solution of phosphate buffered saline glucose (pH 8.0) containing 10% foetal calf serum. Optimum concentration of 1 x 10^5 organisms per ml was obtained and 0.2ml of this suspension was injected intraperitoneally.

Measurement of parasitaemia.

Blood was checked for circulating parasites from the third day post-infection and every second day from there onwards. A drop of blood obtained by means of tail snip, was microscopically examined at x400 magnification. The level of the parasitaemia was assessed by the matching technique of Herbert and Lumsden (1976). Twenty fields were examined before a sample was regarded as negative.

Immunosuppression of mice.

The mice were immunosuppressed by sublethal whole body irradiation (6.5 grays) from a cobalt-60 source 24 hours prior to infection.

Trypanocidal drugs.

The trypanocidal drugs used were mel Cy (Cymelarsan^{R}) and suramin (Moranyl^{R}) (Rhone Merieux, Toulouse, France), isometamidium chloride (Samorin^{R}) and quinapyramine sulphate (Trypacide^{R}) (RMB, Daghenham,

England) and diminazene aceturate (Berenil^{R}, Hoechst AG, Frankfurt, Germany). Dilutions of the various drugs were made so that the appropriate dose rates (mg/kg) could be administered at a rate of 0.05 ml per 5g bodyweight.

Determination of packed cell volume.

The PCV was determined by the method of haematocrit centrifugation technique (Woo, 1970) using battery operated minicentrifuge (Compur M 1100; Compur-Electronic, Germany). Mouse blood (9 µl) obtained by tail snip was drawn up into heparinised capillary tubes and centrifuged in the minicentrifuge. The PCV values expressed as percentages were read directly on the minicentrifuge disc.

Body Weights.

The mice were weighed by electronic balance (Oertling, England) before and after infection.

Results

Section 1 A: Studies with mel Cy in normal and immunosuppressed mice

Experiment 1: The sensitivity of *T. evansi* GRVPS 13/3 and *T. evansi* GVR 74/1 to mel Cy in normal and immunosuppressed mice.

Experimental Design.

It is necessary to determine the sensitivity of the original stabilates to mel Cy so that a realistic subcurative dose of mel Cy can be given when "drug pressure" is being applied. A test was carried out in normal and immunosuppressed mice with the objective of determining the sensitivity of *T. evansi* GRVPS 13/3 and *T. evansi* GVR 74/1 to mel Cy. To this effect 25 immunocompetent mice were divided into 5 groups of 5 mice each and infected with an inocula prepared from a cryopreserved stabilate of *T. evansi* GRVPS 13/3. A further 6 groups of 3 normal mice for each group were infected with *T. evansi* GVR 74/1, (GVR 74/1 is a working stabilate of GVR 74), the original stabilate from which GRVPS 13/3 had been cloned. A range of doses of mel Cy were injected in all mice excluding the controls after the first peak of parasitaemia. The doses administered were 0.125, 0.25, 0.5, 0.75 and 1.0 mg/kg. Monitoring resumed for detection of possible relapses of infection and continued for a period of 60 days post treatment.

The sensitivity of both GRVPS 13/3 and GVR 74/1 was also tested in immunosuppressed mice. Groups of three mice immunosuppressed by whole body irradiation were infected with either of the stabilates and treated following development of parasitaemia. The doses administered were similar to those used in the normal mice.

Results.

For the immunocompetent groups infected with *T. evansi* GRVPS 13/3 the first parasites appeared in the circulation on the fifth day post-infection and continued to increase progressively. Treatment was effected on the ninth day after infection and coincided with the period of high parasitaemia. Initially all the doses injected cleared the trypanosomes from the circulation, however on the tenth day post-treatment relapses occurred in the groups treated at 0.125 and 0.25 mg/kg (Table 2.1.1). More relapses followed until four weeks post treatment 3 out of the group treated at 0.125 mg/kg and all those injected at 0.25 mg/kg had relapsed (Table 2.1.1). No breakthroughs were observed in the other groups treated at 0.5 and 1.0 mg/kg mel Cy during the experimental period. The control group, infected on the same day as the treated groups remained parasitaemic and the animals in that group subsequently died as a result of the infection (Fig. 2.1).

On the other hand in the groups infected with *T. evansi* GVR 74/1 relapse of infection was detected in all the three mice treated at 0.125 mg/kg, two of those treated at 0.25 mg/kg and one mouse of the group treated at 0.5 mg/kg mel Cy whilst treatment at 0.75 and 1.0 mg/kg effected complete cure (Table 2.1.1).

In marked contrast all the doses failed to eliminate the infections in the immunosuppressed mice. There was no reduction of the parasitaemia in the groups irrespective of whether they were infected with GRVPS 13/3 or with GVR 74/1 despite treatment at 1.0 mg/kg (Table 2.1.2). These results highlight the importance of the host immune response in the chemotherapy of trypanosomiasis.

Experiment 2: The development of mel Cy resistance by *T. evansi* in immunosuppressed mice.

Experimental Design.

The objective of this experiment was to attempt to increase the resistance of *T. evansi* GRVPS 13/3 to mel Cy in immunosuppressed mice. Groups of three mice were immunosuppressed by irradiation and subsequently infected. At the first peak of parasitaemia the mice were treated with a subcurative dose of mel Cy (0.5 mg/kg) determined in Experiment 1. Following relapse the trypanosomes were transferred to three new irradiated mice. At the first peak of parasitaemia one of the mice was treated at the same dose from which the infecting organisms had relapsed whilst the other two mice were treated at slightly higher doses. This process was repeated to determine whether increases in drug resistance could be generated and the level of drug resistance which could be obtained

Results.

The successive stages enacted to increase mel Cy resistance are shown in Fig. 2.2. Initially a group of three immunosuppressed mice were infected with GRVPS 13/3 and treated at 0.5 mg/kg on the third day post-infection

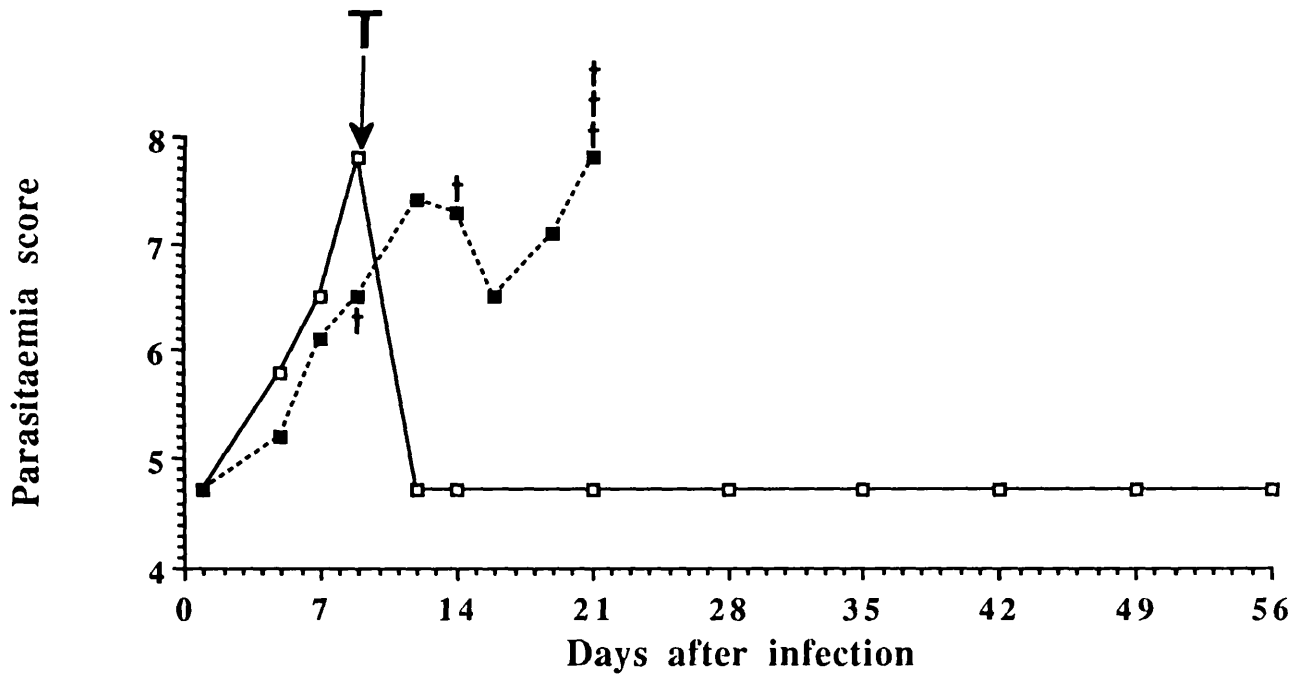

Figure 2.1. Prasitaemia profiles of normal mice infected with *T. evansi* GRVPS 13/3 and treated with 1.0 mg/kg mel Cy (□—□) or untreated (■-■). Death (†). (T) day of treatment.

Table 2.1.1. The sensitivity of *T. evansi* GRPVS 13/3 and *T. evansi* GVR 74/1 to mel Cy in normal mice.

Group	Mel Cy Dose (mg/kg)	GRVPS 13/3 Relapsed/ Treated	GRVPS 13/3 Days to Relapse	GVR 74/1 Relapsed/ Treated	GVR 74/1 Days to Relapse
A	0.125	3/5	9; 7; 28	3/3	7; 7; 7
B	0.25	5/5	10; 14; 15; 21; 21	2/3	23; 23
C	0.5	0/5	-	1/3	23
D	0.75	-	-	0/3	-
E	1.0	0/5	-	0/2	-

Table 2.1.2. The sensitivity of *T. evansi* GRVPS 13/3 and *T. evansi* GVR 74/1 to mel Cy in immunosuppressed mice.

Group	Mel Cy Dose (mg/kg)	GRVPS 13/3 Relapsed/ Treated	GRVPS 13/3 Days to Relapse	GVR 74/1 Relapsed/ Treated	GVR 74/1 Days to Relapse
A	0.125	*/3	*	*/3	*
B	0.25	*/3	*	*/3	*
C	0.5	*/3	*	*/3	*
D	0.75	-		*/3	*
E	1.0	*/3	*	*/3	*

* All remained positive

following onset of parasitaemia. The relapse population was then transferred into a fresh group of three irradiated mice where one mouse was treated at 0.5 mg/kg and the other two at 0.75 mg/kg on the seventh day post-infection. Relapses occurred at 0.75 mg/kg on the second day post-treatment and the breakthrough from these mice was inoculated into a further group of immunologically compromised mice. This time one mouse was treated at 0.75 mg/kg and the other 2 at a slightly higher dose (1.0 mg/kg) on the sixth day post-infection. Treatment at 1.0 mg/kg did not affect the level of parasitaemia, and on the second day after treatment the trypanosomes were transferred into a fresh group of irradiated mice. This process was repeated several times. At each step 3 irradiated mice were subinoculated whereby one mouse was treated with the dose from which the infecting organisms had relapsed and the others with slightly higher doses. At each step an increase in drug resistance was detected. In 13 passages within a period of about 5 months it was possible to markedly enhance the resistance of *T. evansi* GRVPS 13/3 to mel Cy in irradiated mice. However there is significant evidence to suggest that the process could have been speeded up by shortening the steps. At 0.5 mg/kg there was a temporary disappearance of parasites, however in the other steps the parasitaemia continued to increase despite treatment at a higher dose. During the process it was found that the infection from a mouse treated at 5.0 mg/kg and transferred into a fresh group of mice was equally resistant to 20.0 mg/kg. Finally the trypanosomes breaking through 40 mg/kg mel Cy were passaged once in irradiated mice to a obtain a working stabilate designated *T. evansi* GRVPS 13/3R19. The findings of this experiment confirmed previous reports (Hawking, 1939) that reduced host immune response leads to the rapid development of drug resistance by trypanosome organisms.

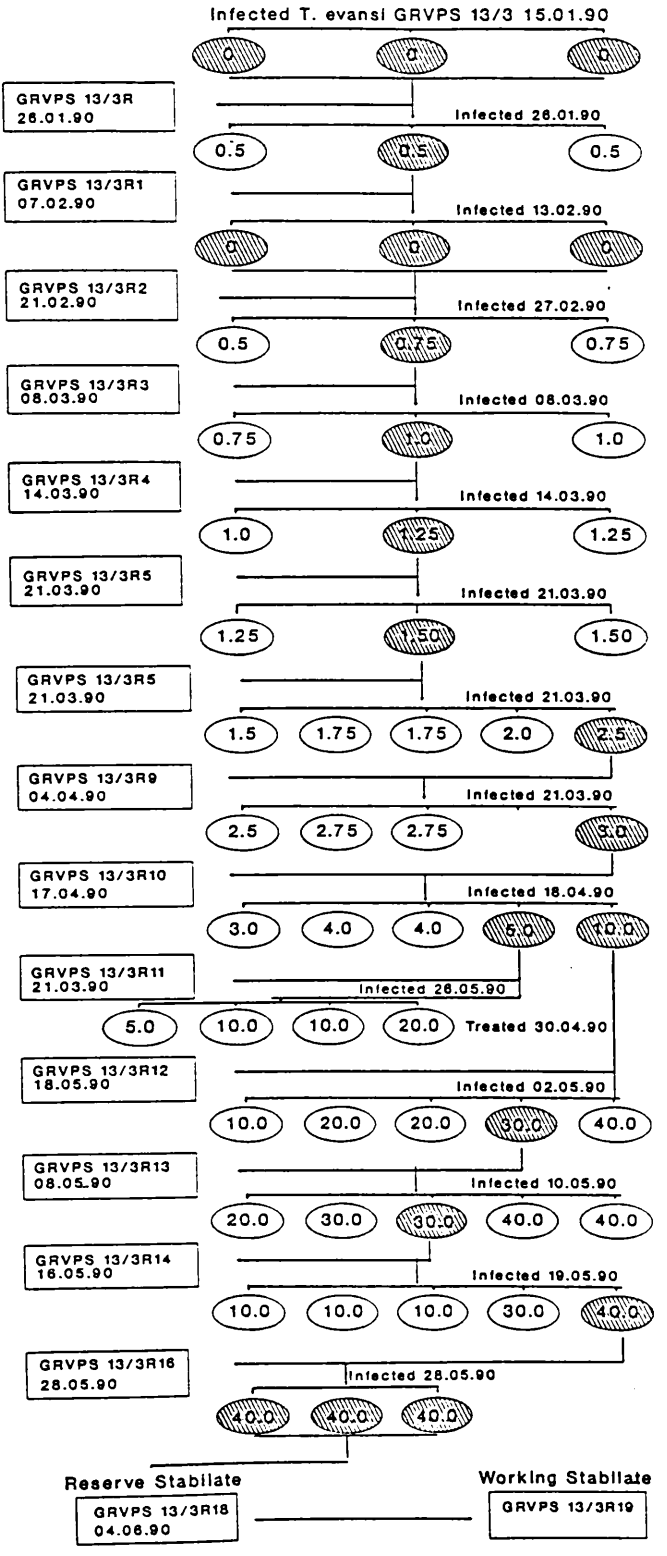

Figure 2.2. The development of mel Cy resistance by *T. evansi* GRVPS 13/3 in immunosuppressed mice.

Experiment 3: A comparison of mel Cy sensitivity of resistant (GRVPS 13/3R19) and parent (GRVPS 13/3) clones in normal mice.

Experimental Design.

The experiment described here was intended to establish whether mel Cy resistance developed in immunosuppressed mice by *T. evansi* GRVPS 13/3R19 would retain this level of resistance in immunocompetent hosts. The parent clone *T. evansi* GRVPS 13/3 was included for comparison. For this purpose 36 normal mice were divided into groups of 3 mice each and infected either with the mel Cy-resistant or with the parent clone. Altogether 12 groups were involved in which 6 groups were infected with either parent or mel Cy-resistant clone. Treatment was made following the onset of parasitaemia. The doses administered to the groups infected with the parent clone were 0.5, 1.5, 2.0, 3.0 and 5.0 mg/kg while for the resistant the doses were 5.0, 10.0, 30.0 and 40.0 mg/kg. Tail blood was regularly examined for relapses and any deaths recorded.

Results.

All groups were treated on the third day post-infection following development of parasitaemia. At 0.5 mg/kg relapse of infection occurred in one mouse out of the three mice treated in the group infected with parent clone. All the other doses effected complete cure and no breakthroughs occurred during the course of the experiment (Table 2.2). On the other hand none of the groups infected with the resistant clone were cured and the trypanosomes continued to multiply in the circulation despite treatment. At 40.0 mg/kg one

of the mice treated died hours after treatment due to toxicity since 40 mg/kg is close to the maximum tolerated dose in mice. The rest continued to be parasitaemic until they died from the infection within 2 weeks.

These results confirm that mel Cy resistance developed in irradiated mice is a true resistance and persists in normal hosts.

Experiment 4: The sensitivity of the mel Cy-resistant clone GRVPS 13/3R19 to other trypanocides in normal mice..

Experimental Design.

The response of the trypanosomes made highly resistant to mel Cy to some of the other trypanocidal drugs currently available was investigated in mice in order to study the cross-resistance pattern. The drugs tested were isometamidium, diminazene aceturate, pentamidine, quinapyramine and suramin. For each drug, 12 groups of 3 mice each were used. Six groups were infected with the mel Cy-resistant clone (*T. evansi* GRVPS 13/3R19) and the other 6 groups with the parent clone (*T. evansi* GRVPS 13/3). A series of doses were selected for each compound and injected intraperitoneally. Regular checks for relapses were subsequently made.

Results.

It is of interest that GRVPS 13/3 was originally derived from a suramin-resistant stock. Therefore attempts were made to establish whether the mel Cy-resistant clone had retained the original suramin resistance. Various doses of suramin were selected and injected intraperitoneally in groups of mice infected either with the resistant or the parent clone. There was a slight decrease in the intensity of the parasitaemia in those infected with

Table 2.2. A comparison of mel Cy sensitivity of the parent clone (GRVPS 13/3) and the resistant clone (GRVPS 13/3R19) in normal mice.

Group	Mel Cy Dose (mg/kg)	GRVPS 13/3 Relapsed/ Treated	GRVPS 13/3 Days to Relapse	GRVPS 13/3R19 Relapsed/ Treated	GRVPS 13/3R19 Days to Relapse
A	0.5	1/3	11	-	-
B	1.0	0/3	-	-	-
C	1.5	0/3	-	-	-
D	2.0	0/3	-	-	-
E	3.0	0/3	-	-	-
F	10.0	-	-	*/3	-
G	20.0	-	-	*/3	-
H	30.0	-	-	*/3	-
I	40.0	-	-	*/2 (1)	-

* All remained Positive

(1) One mouse died because of toxicity

the mel Cy-resistant clone irrespective of the doses administered, although there was no case in which the parasitaemia completely disappeared. However, after the initial decrease the parasitaemia began to gain in intensity and subsequently all the groups developed a fulminating parasitaemia. On the other hand in those infected with the parent clone the parasitaemia continued to rise despite treatment. It is evident that these clones had remained highly resistant to suramin with a dose of 200.0 mg/kg not effecting cure (Table 2.3.1). Thus GRVPS 13/3R19 had not only gained mel Cy resistance but had also maintained its original suramin resistance.

However there was a marked difference in sensitivity between the mel Cy-resistant and the parent clones to diminazene aceturate and pentamidine. The mel Cy-resistant was found to be also resistant to diminazene. There was a relapse at 40.0 mg/kg while treatment at 20.0 mg/kg caused only temporary disappearance of the parasites from the circulation with a subsequent relapse of all treated cases. In contrast a dose of 10.0 mg/kg diminazene was sufficient to result in permanent cure of those infected with the parent clone (Table 2.3.2). Similarly the mel Cy-resistant clone displayed a substantial degree of resistance to pentamidine with a dose of 100.0 mg/kg not effecting cure compared against the successful elimination of the infections ensuing from the parent clone by treatment with 20.0 mg/kg (Table 2.3.3).

These results provide further evidence of the cross-resistance links between arsenicals and diamidines (Rollo and Williamson, 1951; Zweygarth and Kaminsky, 1990).

On the other hand both the mel Cy-resistant and the parent clones exhibited unusually high levels of resistance to isometamidium chloride. However in the groups infected with the mel Cy-resistant clone only one mouse relapsed following treatment at 10.0 mg/kg while in those infected with parent clone all the three mice injected with 10.0 mg/kg had relapsed (Table 2.3.4).

Finally the sensitivity of the mel Cy-resistant and the parent clone to quinapyramine sulphate was tested in normal mice. No significant variation in sensitivity to quinapyramine could be discerned between the clones (Table 2.3.5). Despite relapses which occurred in the group infected with the resistant clone after treatment with 2.0 mg/kg quinapyramine, both were sensitive to 3.0 mg/kg.

The clone which developed mel Cy-resistance, at the same time as retaining its original sensitivity to isometamidium and quinapyramine, had retained its original resistance to suramin and had acquired a cross-resistance to both diminazene aceturate and pentamidine.

Table 2.3.1. A comparison of suramin sensitivity of the resistant clone (GRVPS 13/3R19) and the parent clone (GRVPS 13/3) in normal mice.

Group	Suramin Dose (mg/kg)	GRVPS 13/3		GRVPS 13/3R19	
		Relapsed/ Treated	Days to Relapse	Relapsed/ Treated	Days to Relapse
A	10..0	*/3	-	*/3	-
B	20.0	*/3	-	*/3	-
C	40.0	*/3	-	*/3	-
D	80.0	*/3	-	*/3	-
E	160.0	*/3	-	*/3	-
F	200.0	*/3	-	*/3	-

* All remained positive

Table 2.3.2. A comparison of diminazene sensitivity of the resistant clone (GRVPS 13/3R19) and the parent clone (GRVPS 13/3) in normal mice.

Group	Diminazene Dose (mg/kg)	GRPVS 13/3 Treated/ Relapse	GRPVS 13/3 Days to Relapse	GRVPS 13/3R19 Treated/ Relapse	GRVPS 13/3R19 Days to Relapse
A	2.5	3/3	16;, 20, 28	*/3	-
B	5.0	3/3	43; 20; 28	*/3	-
C	10.0	0/3	-	*/3	-
D	20.0	0/3	-	3/3	6; 15; 20
E	40.0	0/3	-	1/3	20
F	80.0	0/2(1)	-	0/3	-

(1) One mouse died because of toxicity

* All remained positive

Table 2.3.3. A comparison of pentamidine sensitivity of the resistant clone (GRVPS 13/3R19) and the parent clone (GRVPS 13/3) in normal mice.

Group	Pentamidine Dose (mg/kg)	GRVPS 13/3 Relapsed/ Treated	GRVPS 13/3 Days to Relapse	GRVPS 13/3R19 Relapsed/ Treated	GRVPS 13/3R19 Days to Relapse
A	2.5	*/3	-	*/3	-
B	5.0	*/3	-	*/3	-
C	10.0	3/3	14; 4; 14	*/3	-
D	20.0	0/3	-	*/3	-
E	40.0	0/3	-	*/3	-
F	100.0	-	-	3/3	9; 14; 23

* All remained positive

Table 2.3.4 A comparison of isometamidium sensitivity of the resistant clone (GRVPS 13/3R19) and the parent clone (GRPVS 13/3) in normal mice.

Group	Isometamidium Dose (mg/kg)	GRVPS 13/3 Relapsed/ Treated	GRVPS 13/3 Days to Relapse	GVR 13/3R19 Relapsed/ Treated	GVR 13/3R19 Days to Relapse
A	0.5	3/3	*; *; 8	3/3	*; 2; 2
B	1.0	3/3	8; 8; 12	3/3	4; 8; 2
C	2.0	3/3	18; 8; 30	3/3	6; 6; 16
D	4.0	3/3	18; 18; 32	2/3	7; 22
E	5.0	3/3	18; 8; 32	3/3	7; 7;23
F	10.0	3/3	18; 32; 18	1/3	37

*Remained positive

Table 2.3.5 A comparison of quinapyramine sensitivity of the resistant clone (GRVPS 13/3R19) and the parent clone (GRVPS 13/3) in normal mice

Group	Quinapyramine Dose (mg/kg)	GRVPS 13/3 Relapsed/ Treated	GRVPS 13/3 Days to Relapse	GRVPS 13/3R19 Relapsed/ Treated	GRVPS 13/3R19 Days to Relapse
A	0.05	3/3	8; 24; 10	3/3	10; 24; 36
B	0.5	3/3	15; 24; 24	3/3	19; 36; 36
C	1.0	3/3	53; 45; 31	2/3	39; 52
D	2.0	0/3	-	2/3	44; 44
E	4.0	0/3	-	0/3	-
F	5.0	0/3	-	0/3	

Section 1 B: Studies with mel Cy and diminazene in normal mice

Experiment 5: Attempts to develop mel Cy and diminazene resistance in normal mice.

Experimental Design.

Attempts were made to increase the resistance of *T. evansi* GRVPS 13/3 to mel Cy and *T. evansi* GRVPS 19/5 to diminazene in normal mice. The protocol adopted was similar to that using irradiated mice. Infected mice were treated with subcurative doses of the drugs. Relapses were subsequently transferred into a fresh group of normal mice and treated.

Results.

In both cases it was not possible to achieve resistance exceeding 0.5 mg/kg. At 0.5 mg/kg mel Cy the trypanosomes relapsed, but the parasitaemia was rather low and was therefore expanded in irradiated mice. However infection ensuing from this relapse later disappeared following treatment at 0.75 mg/kg mel Cy.

Similarly a relapse from 0.5 mg/kg diminazene failed to establish infection in a fresh group of normal mice on two separate occasions.

From these observations the view was taken that the development of mel Cy and diminazene resistance in normal mice would be difficult and time consuming.

Section 1 C: Studies on the pathogenecity and antigenic relationship of drug resistant and drug sensitive clones of *T. evansi* in normal mice.

Experiment 6: To examine the pathogenicity of drug-resistant and drug sensitive clones of *T. evansi* in normal mice.

Experimental Design.

The objective of this experiment was to investigate whether drug-resistant trypanosomes are less pathogenic than normal sensitive ones. Twelve mice were divided into 2 groups of 6 mice each. One group was infected with the *T. evansi* GRVPS 13/3R19, made highly resistant to mel Cy while the remaining group was infected with the parent clone *T. evansi* GRVPS 13/3. A further group of six mice which served as uninfected controls was also included. The packed cell volume (PCV) and the body weights of all groups was determined prior to infection and three times a week afterwards. The parasitaemia of the infected groups was also monitored on a daily basis.

Results.

The parasitaemia profile, PCV and body weight changes of the groups of mice are shown in Figs 2.3.1, 2.3.2 and 2.3.3. In both infected groups parasitaemia was first detected on the third day post-infection and increased progressively from then onwards. The highest level of parasitaemia in the group infected with the parent clone was recorded on the seventh day post-infection, while in the mice infected with the resistant clone peak parasitaemia developed on the ninth day post-infection. On the eighth day post-infection one of the mice infected with the sensitive clone

died and further two mice in this group died twelve and thirteen days following infection. However in the remaining three mice a marked decrease of parasitaemia was observed although it started to rise again until on the sixteenth day post-infection when they all died as result of intense parasitaemia. In the group infected with the resistant trypanosomes all the mice remained highly parasitaemic until death from fulminating parasitaemia. One mouse of this group died on the tenth day after infection, three mice died by twelve days and in the remaining two one mouse died on the fourteenth day post-infection while the last one died on the sixteenth day following infection. The PCVs in both infected groups were significantly lower than the controls. While the mice infected with the resistant clone showed steady decline of PCV until death, there was a sharp drop which coincided with the peak parasitaemia in the group infected with parent clone. However the PCV recovered in the three mice which controlled the initial rise of parasitaemia.

It is apparent from Fig. 2.3.3 that the group infected with the parent clone was more seriously affected by weight loss compared to the others. Nevertheless the results from the initial stage of the experiment show a drop in the body weights of the infected groups while the controls slightly gained weight. However the subsequent body weight increases of the infected groups appears to be related with the smaller number of mice sampled as a result of deaths from the infection.

The results presented show that pathogenecity was not significantly changed following the development of drug resistance. Both clones caused fulminating parasitaemias and eventually led to the death of all the infected animals.

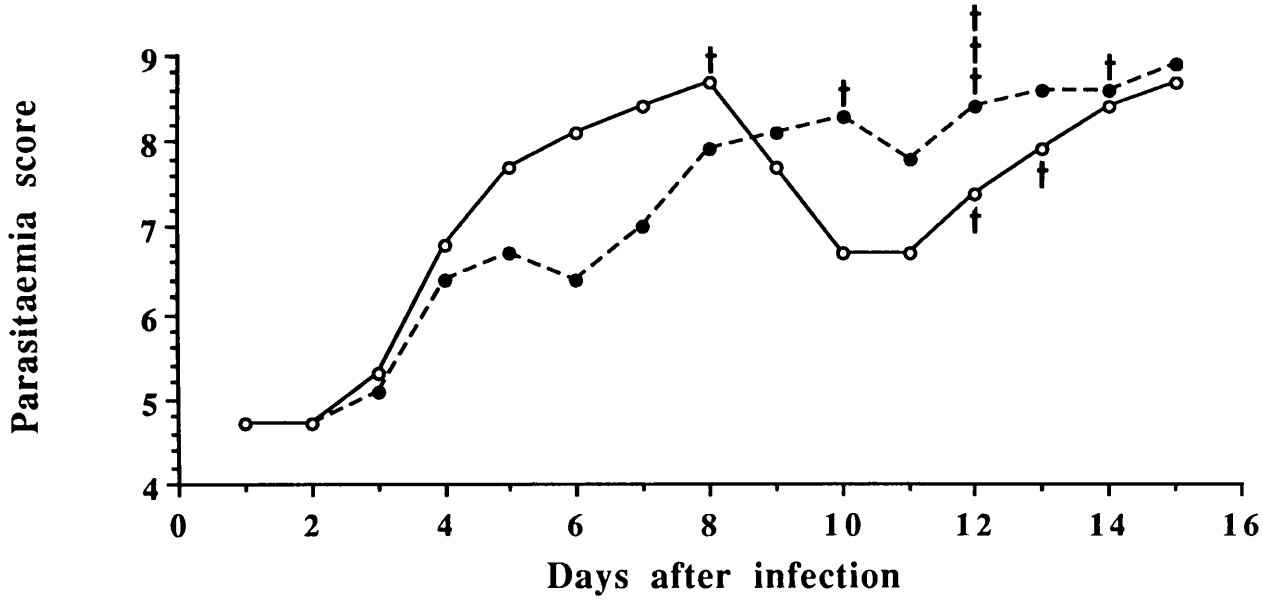

Figure 2.3.1. Parasitaemia profile of the mice infected with the mel Cy-resistant clone GRVPS 13/3R19 (●-●) or with the parent clone GRVPS 13/3 (○—○). Death (†).

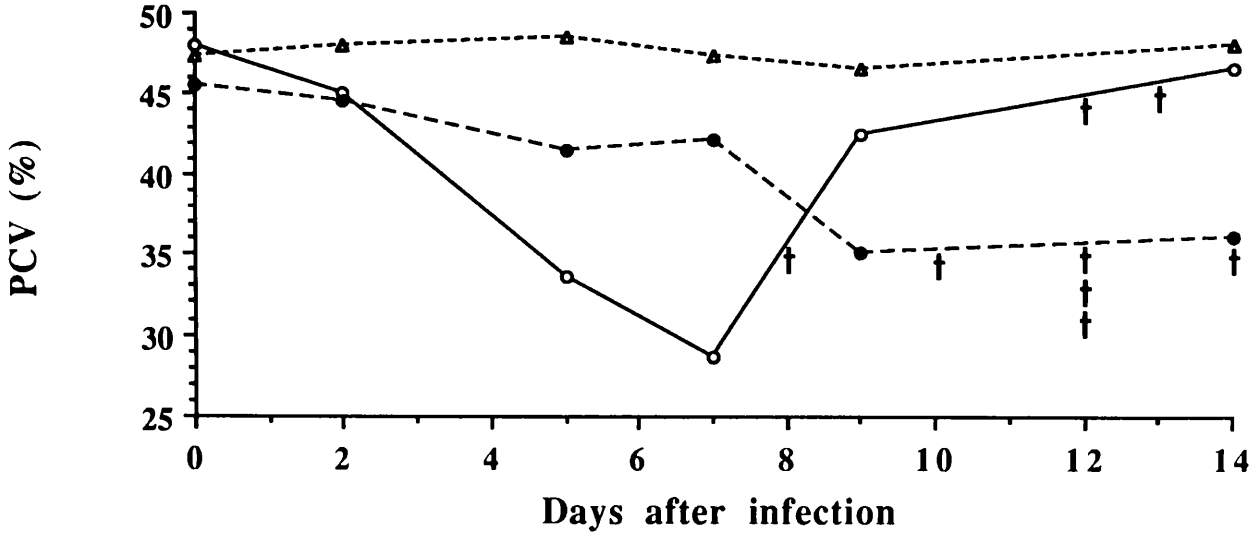

Figure 2.3.2 PCV changes of the mice infected with the mel Cy-resistant clone GRVPS 13/3R19 (●-●) and the parent clone GRVPS 13/3 (○—○) compared with the uninfected controls (△- -△). Death (†).

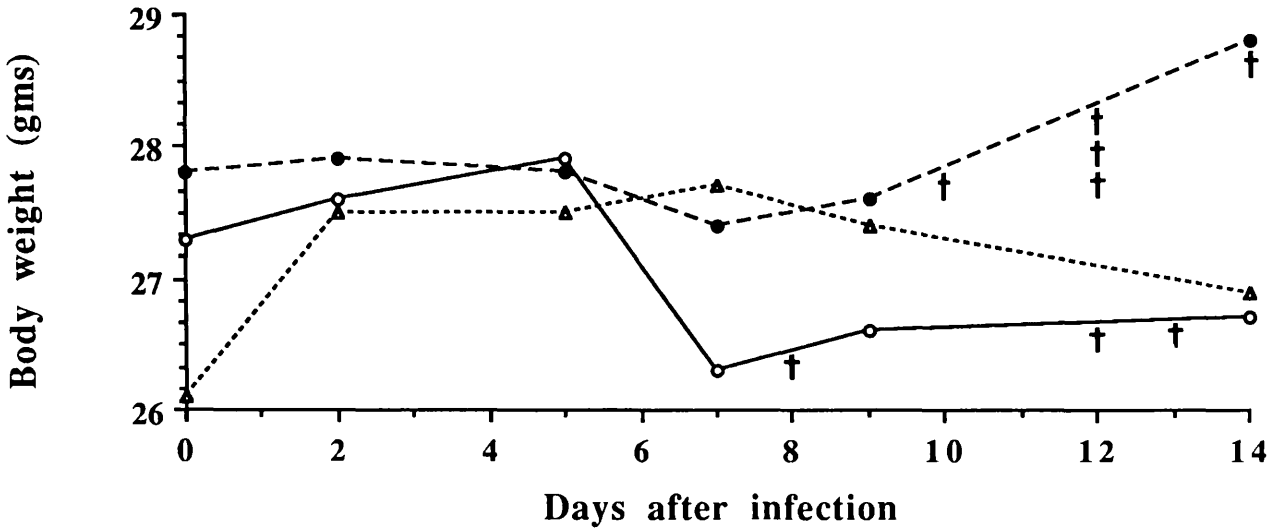

Figure 2.3.3. Body weight changes of the mice infected with the mel Cy-resistant clone GRVPS 13/3R19 (●--●) and the parent clone GRVPS 13/3 (○—○) compared with the uninfected controls (△--△). Death (†).

Experiment 7: To examine the antigenic relationship of *T. evansi* GRVPS 13/3R19 with its parent clone (GRVPS 13/3) in normal mice.

Experimental Design.

The aim of this experiment was to establish whether the clone of *T. evansi* which developed mel Cy resistance acquired different antigenic characteristics from its parent clone during the process of attaining drug resistance. A group of three normal mice was infected with the parent clone GRVPS 13/3 and treated with a dose of 10.0 mg/kg mel Cy on the sixth day post-infection following the development of high levels of parasitaemia. Previous studies had shown this clone to be sensitive to 1.0 mg/kg mel Cy (see Expt. 1). Eight days after treatment the mice were infected with the resistant clone GRVPS 13/3R19. Tail blood was regularly examined during the following three weeks after the second infection. A further five groups of mice were also included in the experiment to serve as controls.

Results.

Table 2.4 summarises the results of the experiment. After treatment at 10.0 mg/kg the mice infected with the parent clone became aparasitaemic and continued to be free of infection while the untreated controls developed a fulminating parasitaemia. The mice were challenged with the mel Cy resistant clone eight days after treatment and monitored for development of parasitaemia for three more weeks. All three mice reinfected with the drug-fast trypanosomes resisted establishment of infection during the whole period of observation. In contrast a group of mice infected with the mel Cy-resistant on the same day developed patent infection and subsequently

died as result of intense parasitaemia. Moreover a group of three mice treated with 10.0 mg/kg mel Cy before infection and challenged eight days later with the mel Cy-resistant clone developed patent infection providing evidence that the failure of the establishment of the second infection in the first group was not caused by drug residue. Similarly a group of mice infected with the resistant population and treated with mel Cy at 10.0 mg/kg continued to be parasitaemic.

The results show that the mel Cy-resistant clone maintained a similar antigenic relationship with the parent clone despite frequent passage in immunosuppressed mice during the process of developing mel Cy resistance.

Table 2.4. The antigenic relationship between *T. evansi* GRVPS 13/3R19 and GRVPS 13/3 the parent clone in normal mice.

Group	1st infection (Day 0)	Parasitaemia	Treatment mel Cy (mg\kg) (Day 6)	Response to treatment	2nd infection (Day 14)	Prasitaemia
A	GRVPS 13/3	Positive	10	Cure	GRVPS 13/3R19	Negative
B	GRVPS 13/3	Positive	10	Cure	-	Negative
C	GRVPS 13/3	Positive	-	Dead		
D	-	-	10	-	GRVPS 13/3R19	Positive
E	GRVPS 13/3R19	Positive	10	Dead		
F	GRVPS 13/3R19	Positive	-	Dead		

Section 2: Studies with isometamidium chloride in normal and immunosuppressed mice

Experiment 8: The sensitivity of *T. evansi* GRVPS 18/2 to isometamidium, mel Cy and diminazene in normal mice.

Experimental Design.

The drug susceptibility of a clone of *T. evansi* (*T. evansi* GRVPS 18/2), isolated from the same stabilate as GRVPS 13/3 and known to be suramin resistant (Abebe *et al.*, 1983) was investigated in normal mice. The drugs tested were isometamidium, diminazene and mel Cy. Infected mice were treated with different doses of either of the drugs and monitored for relapses.

Results.

The results are presented in Table 2.5. This clone was found to be relatively sensitive to all trypanocidal drugs tested. Treatment at 0.5 mg/kg isometamidium resulted in the relapse of the treated cases. Two of the relapses occurred five days after treatment while the remaining mouse relapsed on day nineteen post-treatment. However at 1.0 mg/kg isometamidium the infection was cleared and no relapses were detected during the period of the experiment. Treatment at 0.5 mg/kg diminazene was also found not to effect cure in all the injected mice. One mouse of the group treated at 0.5 mg/kg remained positive while the other two mice were cured. However at 1.0 mg/kg all three treated cases were cured with no relapses during the course of the experiment. On the other hand a dose of 0.5 mg/kg mel Cy was found adequate

to attain a state of aparasitaemia during the post-treatment observation period. Thus although this clone was known to be suramin resistant it was not resistant to the other trypanocides tested.

Experiment 9: Development of isometamidium resistance by *T. evansi* in immunosuppressed mice.

Experimental Design.

The aim of this experiment was to increase the resistance of the clone (*T. evansi* GRVPS 18/2) to isometamidium chloride in immunosuppressed mice. The protocol adopted was similar to that used in the development of mel Cy-resistance and described in experiment 2.

Results.

Details of the steps taken to increase the drug resistance of the clone are shown in Fig. 2.4. At the beginning of this experiment a group of 3 mice immunosuppressed by whole body irradiation were infected with *T. evansi* GRVPS 18/2 and treated with isometamidium. One mouse was given a dose of 0.5 mg/kg while the other 2 were treated at 1.0 mg/kg. In contrast to the normal mice, treatment at 1.0 mg/kg isometamidium did not lead to the disappearance of trypanosomes from the circulation in irradiated mice, suggesting a decrease of the trypanocidal efficacy of this compound associated with the abolition of the host immune response. Subsequent subinoculations and treatments with gradually increasing doses of isometamidium consistently led to relapses until the clone attained a marked degree of resistance close to the maximum tolerated dose in the murine host. Trypanosome populations breaking through at 20.0 mg/kg were passaged once

Table 2.5. Drug sensitivity of *T. evansi* GRVPS18/2 to isometamidium, mel Cy and diminazene.

Trypanocide Tested	Dose (mg/kg)	GRVPS 18/2 *T. evansi* Relapsed/ Treated	Days to Relapse
Isometamidium	0.5	3/3	5; 19; 5
	1.0	0/3	-
	7.0	0/3	-
Mel Cy	0.25	3/3	19; 19; 19
	0.5	0/3	-
	5.0	0/3	-
Diminazene	0.5	1/3	*
	1.0	0/3	-
	5.0	0/3	-

* Remained positive

in irradiated mice without drug treatment to obtain a working stabilate *T. evansi* GRVPS 18/2R5. Thus within a period of three months and eleven passages in immunologically compromised hosts it was possible to significantly increase the resistance of this clone to isometamidium chloride.

Experiment 10: A comparison of isometamidium sensitivity of the resistant (GRVPS 18/2R5) and the parent (GRVPS 18/2) clones in normal mice..

Experimental Design.

In view of the high degree of isometamidium resistance developed in irradiated mice, the sensitivity of this drug-resistant clone in normal mice was investigated and compared against the parent clone. Groups of mice were either infected with the parent clone (*T. evansi* GRVPS 18/2) or with the resistant clone (*T. evansi* GRVPS 18/2R5) and treated with a range of doses of isometamidium chloride following the onset of parasitaemia. For each clone six groups of mice were used. The doses chosen for the groups infected with the parent clone were 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 mg/kg. Those infected with the resistant, on the other hand, were treated at 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 mg/kg isometamidium chloride. All groups were monitored for breakthroughs during the following eight weeks and any relapsed mouse was removed from the experiment.

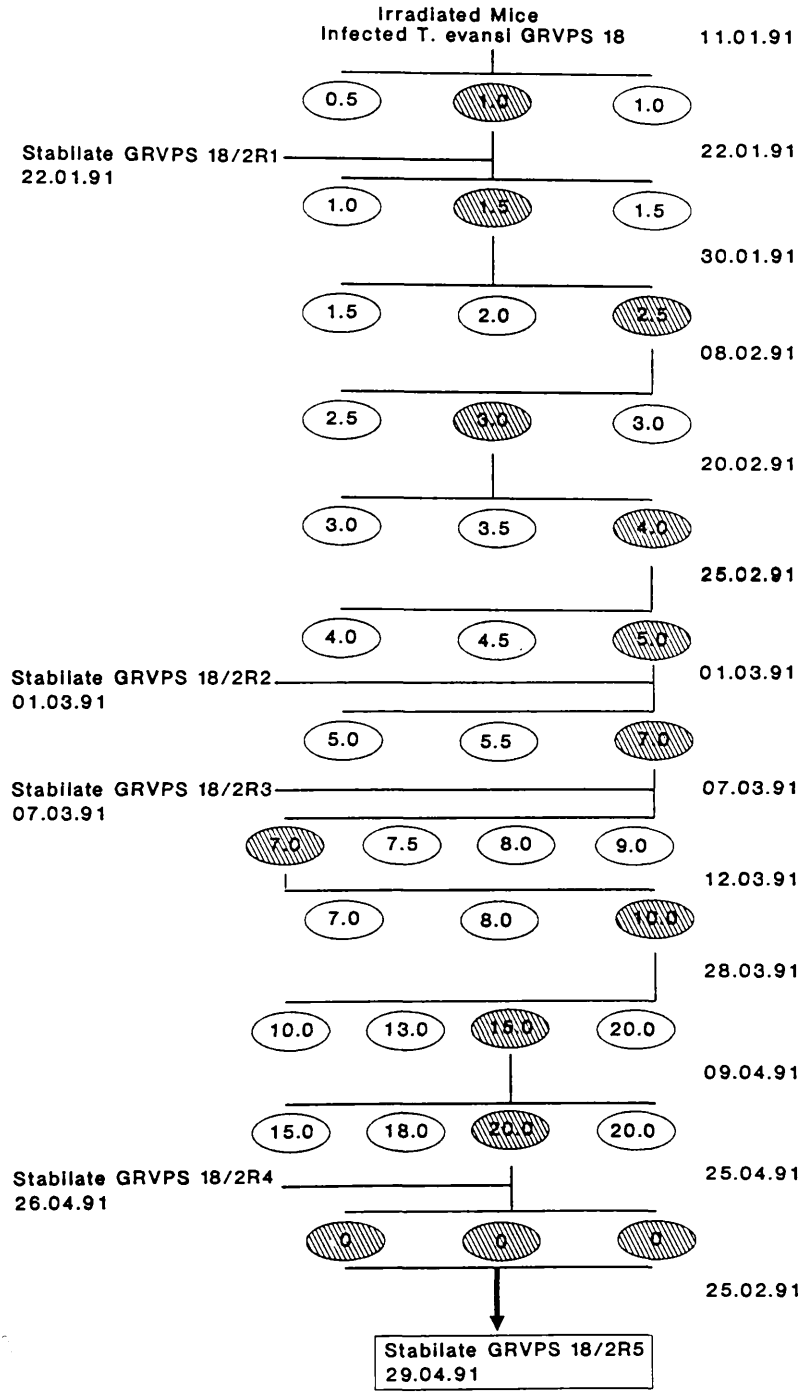

Figure 2.4. The development of isometamidium resistance by *T. evansi* GRVPS 18/2 in immunosuppressed mice.

Results.

Parasitaemia in all cases developed during the first week of infection at which time the mice were treated. In the groups infected with the parent clone relapses occurred in those treated with 0.25 and 0.5 mg/kg (Table 2.6), however at 1.0 mg/kg complete cure was achieved. In contrast relapses were detected in all the groups infected with the resistant clone (Table 2.6). At 20.0 mg/kg the three mice infected had all relapsed. Time to relapse was 10 and 21 days post-treatment. From these observations the conclusion can be drawn that isometamidium resistance developed in irradiated mice was retained when the clone was tested for drug sensitivity in normal immunocompetent mice.

Table 2.6. A comparison of isometamidium sensitivity of the parent clone (GRVPS 18/2) and the resistant clone (GRVPS 18/2R5) in normal mice.

Group	Isometamidium Dose (mg/kg)	GRVPS 18.2 Relapsed/ Treated	GRVPS 18.2 Days to Relapse	GRVPS 18/2R5 Relapsed/ Treated	GRVPS 18/2R5 Day to Relapse
A	0.25	*/3	*; *; *;	-	-
B	0.5	3/3	*; *; 8	-	-
C	1.0	0/3	-	3/3	*; *; *
D	2.5	0/3	-	3/3	8; 8; 8
E	5.0	0/3	-	3/3	8; 8; 11
F	10.0	0/3	-	3/3	19; 11; 8
G	15.0	-	-	2/3	10; 10
H	20.0	-	-	3/3	21; 10; 21

*Remained positive

Section 3: Studies with diminazene aceturate in normal and immunosuppressed mice.

Experiment 11: The development of diminazene resistance by *T. evansi* in immunosuppressed mice.

Experimental design.

A clone of *T. evansi* (*T. evansi* GRVPS 19/5), isolated from GVR 72, was subjected to continuous passage in irradiated mice treated with gradually increasing doses of diminazene aceturate in order to enhance its resistance to the drug. This clone was sensitive to suramin (Abebe *et al.*, 1983) and was not known to be resistant to any of the other trypanocidal drugs. The procedure followed was similar to that described in experiment 2 and involved infecting irradiated mice with the clone and treating with gradually increasing doses of diminazene aceturate.

Results.

Fig. 2.5 outlines the sequence of steps undertaken. The entire process of increasing the resistance of the clone to the highest possible level of diminazene took a period of twelve weeks and eleven passages in immunosuppressed mice. At the beginning of the experiment 3 irradiated mice were infected with *T. evansi* GRVPS 19/5 where one mouse was treated with 0.5 mg/kg while the remaining two were treated with 1.0 mg/kg of diminazene. Only a modest reduction of parasitaemia was recorded following treatment at 1.0 mg/kg, hence the infection was transferred into a fresh group of four immunosuppressed mice. In this group treatment was effected on the

fifth day post-infection. The doses administered were 1.0 (1 mouse), 1.5 (2 mice) and 2.0 (1 mouse) mg/kg. In all the animals the level of the parasitaemia remained high and the infection was then transferred into a new group and treated at a higher dose. This process continued until the trypanosomes developed a high level of diminazene resistance. The populations breaking through 80.0 mg/kg were stabilated and stored as a reserve stabilate. Subsequently a working stabilate designated *T. evansi* GRVPS 19/5BR6 was prepared in irradiated mice.

Experiment 12: A comparison of diminazene sensitivity of the resistant clone (GRVPS 19/5BR6) and the parent (GRVPS 19/5) clone in normal mice.

Experimental Design.

With the success encountered in increasing the drug-resistance of clone of *T. evansi* to diminazene in immunosuppressed mice further attempts were made to establish the stability of this resistance in immunocompetent hosts. Previous experiments have confirmed that mel Cy and isometamidium resistance produced in irradiated mice persist in normal mice. This experiment was intended to determine the sensitivity of the clone subjected to continuous passage in irradiated mice and treated with increasing doses of diminazene aceturate and compare these results with those of the parent clone. To this effect 36 normal mice were divided into groups where 6 groups of 3 mice each were infected with the parent clone (*T. evansi* GRVPS 19/5). Further 6 groups were inoculated with the clone rendered diminazene resistant (GRVPS 19/5BR6). Treatment was made when examination of wet blood films revealed significant numbers of trypanosomes in the circulation. The doses

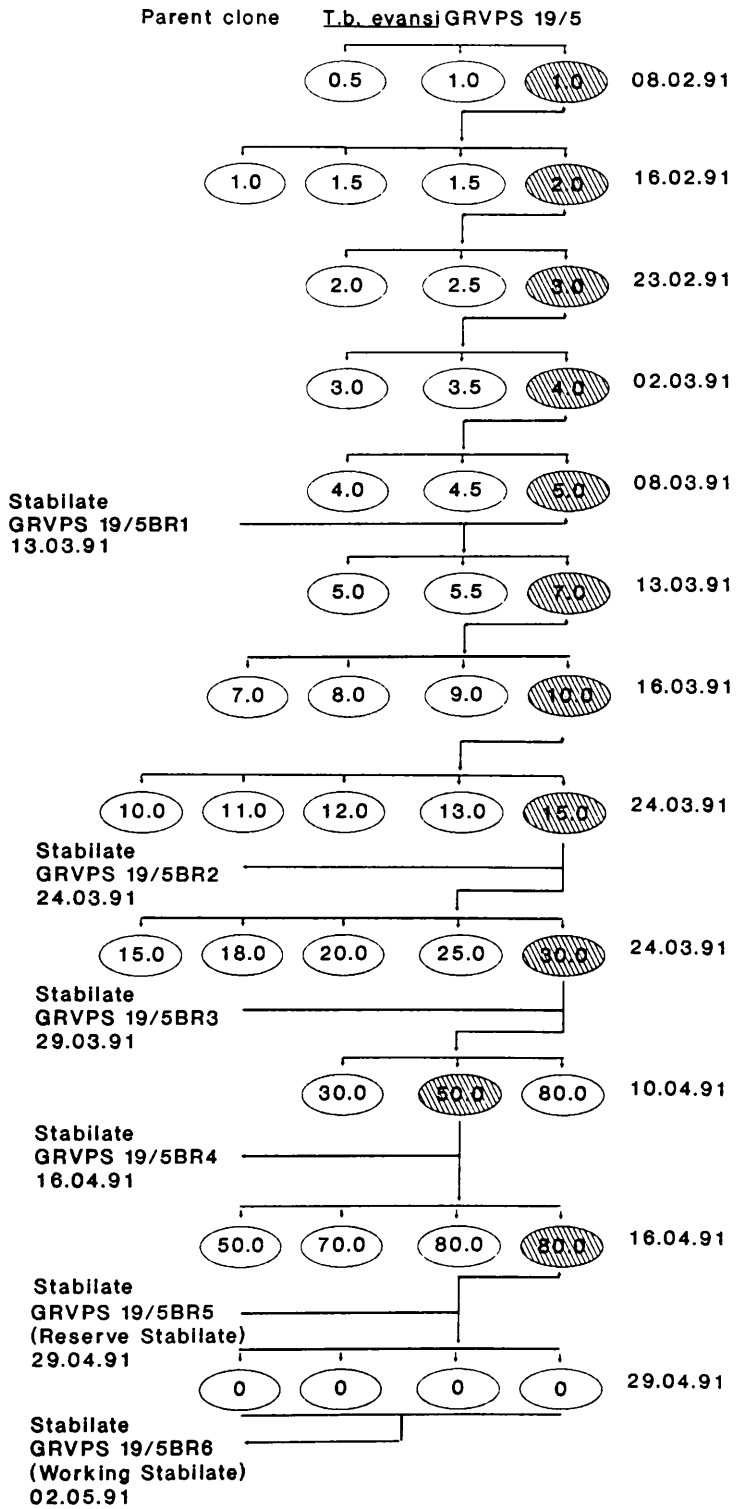

Figure 2.5. The development of diminazene resistance by *T. evansi* GRVPS 19/5 in immunosuppressed mice.

administered were 0.5, 1.0, 2.5, 5.0, 10.0, and 20.0 mg/kg diminazene aceturate for the parent clone and 2.5, 5.0, 10.0, 20.0, 40.0 and 80.0 mg/kg for the resistant clone.

Results.

Parasitaemia was quicker to develop in the groups infected with the parent clone compared to those infected with the resistant. The two clones showed a marked difference in their sensitivity to diminazene (Table 2.7). In the groups infected with the parent clone, *T. evansi* GRVPS 19/5 a dose of 1.0 mg/kg cured all cases. On the other hand no cures were achieved at 80.0 mg/kg in those infected with the resistant clone. This dose is close to the maximum tolerated dose in mice. Indeed one mouse died immediately after treatment because of toxicity.

The results provide further evidence that the drug resistance developed by *T. evansi* clones in immunosuppressed mice is a true resistance and is maintained in normal hosts.

Table 2.7. A comparison of diminazene sensitivity of the parent clone (GRVPS 19/5) and the resistant clone (GRVPS 19/5BR6) in normal mice.

Group	Diminazene Dose (mg/kg)	GRPVS 19/5 Relapsed/ Treated	GRPVS 19/5 Days to Relapse	GRVPS 19/5BR6 Relapsed/ Treated	GRVPS 19/5BR6 Days to Relapse
A	0.5	3/3	29; 23; 15	-	-
B	1.0	0/3	-	3/3	*; *; 2
C	2.5	0/3	-	3/3	*; *; 2
D	5.0	0/3	-	3/3	*; *; 2
E	10.0	0/3	-	3/3	*; 2; 4
F	20.0	0/3	-	3/3	*; 2; 4
G	40.0	-	-	3/3	10; 24; 24
H	80.0	-	-	2/3(1)	24; 40

* Remained positive

(1) One mouse died because of toxicity

Discussion

The results presented here indicate that immunosuppression of the host considerably reduces the efficacy of the trypanocidal drugs currently available for the treatment of trypanosomiasis and can lead to the rapid development of high levels of drug resistance.

In the course of this study clones of *Trypanosoma evansi* resistant to many of the trypanocidal drugs currently in use against animal trypanosomiasis was rapidly achieved. Initial tests on the sensitivity of the clones GRVPS 13/3 and GRVPS 18/2 as well as GVR 74/1, the stock from which the two clones were derived and known to be suramin resistant as well as GRVPS 19/5 known to be suramin sensitive revealed that the clones were relatively sensitive to the other trypanocides. However following rapid passage in immunosuppressed hosts under increasing drug pressure clones of *T. evansi* were obtained which were highly resistant to each specific trypanocidal drug.

In all the three clones tested, significant differences were detected concerning efficacy of the treatment depending on the immunological status of the mice. The doses of trypanocidal drugs which achieved cure in normal mice were found incapable of clearing the infection if the hosts were previously immunocompromised.

Attempts to produce drug resistance in normal immunocompetent mice on the other hand failed to lead to the development of drug resistance.

In the present studies ionising radiation from Co-60 source was used to achieve immunosuppression. Although ionising radiations cause substantial damages to all living cells it has been reported that lymphocytes are more

sensitive compared to other cells (Anderson and Warner, 1976). Following exposure to sublethal doses of radiation it was found that the more sensitive lymphocytes such as B-cells and some T-cell subpopulations die quickly and this type of cell death is known as the interphase death. The more radio-resistant lymphocytes on the other hand were reported to survive the initial effects of radiation, however death of these cells occurs when they begin to divide, the mitotic death, as a result of damages sustained by the cell chromosomes (Anderson and Warner, 1976). The sublethal dose used in the experiments was sufficient to impair the immune system for approximately one week followed by slow recovery of normal immune function.

When the irradiated mice were infected with *T. evansi* and the infection is treated with trypanocidal drugs the efficacy of the drugs was markedly impaired, indicating the desirability of having fully functional immune system for effective chemotherapy. The curative dose of mel Cy for both GRVPS 13/3 and GVR 74/1 was below 1.0 mg/kg in normal mice, however treatment at that dose was found to be insufficient to effect cure in the immunosuppressed mice. This finding is in agreement with the previous observations that the efficacy of melarsoprol is significantly lowered in mice immunosuppressed either by whole body irradiation or cyclophosphamide treatment (Frommel, 1988).

Having successfully developed both a mel Cy-resistant and isometamidium-resistant clones from an original suramin-resistant clone, and a diminazene-resistant clone from an initially suramin-sensitive clone of *T. evansi* respectively, further investigations on the nature of the resistance and particularly whether the resistance would be stable in normal immunocompetent hosts were made. In all the clones the high levels of drug resistance developed in immunosuppressed hosts was found to be stable and

persisted when the clones were subsequently tested in normal immunocompetent mice. The results highlight in a dramatic manner the important role that the host immune system plays in the efficacy of trypanocidal drugs.

In the clone which gained mel Cy resistance it was observed that the mice which survived treatment at 40 mg/kg (maximum tolerated dose) the level of parasitaemia was not affected by the drug and continued to increase progressively thereafter. Indeed no difference could be discerned between them and the controls which received no treatment. On the other hand in the clones which attained isometamidium and diminazene resistance most of the doses administered temporarily cleared the infection, however they all subsequently relapsed usually within two to three weeks. This difference in response could be attributed to the degree of parasitaemia at the time of drug administration. In both isometamidium and diminazene resistant clones, parasitaemia was relatively slow to develop and reached only moderate levels.

Nevertheless the results unequivocally demonstrate that resistance generated in immunosuppressed mice is stable and subsequently continues to be expressed consistently irrespective of the immune status of the host.

The role of the host defence mechanism in the chemotherapy of trypanosomiasis was described by Ehrlich at the turn of this century who argued that the combined action between drugs and the host immune response was a prerequisite for the achievement of cure (Williamson, 1970; Doenhoff *et. al.* 1991). Furthermore this interdependence may have special bearing on the treatment of trypanosomiasis particularly *T. evansi.* It has been suggested that in the treatment of surra the two trypanocidal drugs currently

used, namely suramin and quinapyramine, both depend on the participation of the host immune response to be effective (Jancso and Jancso, 1935; Sen *et al.*, 1955).

Studies showing that suppression of the host immune response can lead to the development of drug resistance in trypanosomiasis were first reported in the 1930s. Jancso and Jancso (1934) devised a method which would exclude the immune response by splenectomy and inoculation of ellectro-colloidal copper. In their experiment it was found that in rodents immunocompromised in this manner chicken erythrocytes inoculated intravenously remained in the circulation for 24 to 36 hours while in intact animals the red blood cells disappeared within 2 to 3 hours. It was later reported that suramin-resistant strains of *T. brucei* could be produced in mice and rats immunosuppressed by splenectomy and blockage of the reticuloendothelial system (Jancso and Jancso, 1935). Further attempts made to establish the specific components of the immune response involved in the rapid development of resistance demonstrated the crucial role of antibodies in effective chemotherapy. Schnitzer *et al.* (1946) reported that splenectomy alone led to the development of p-rosalinine resistance by *T. equiperdum* while on the other hand the development of the resistance could be counteracted by passive transfer of immune serum.

The mechanism behind the rapid development of resistance in immunosuppressed hosts is not clear and merits further investigation. Jancso and Jancso (1935) concluded that trypanosomes have the capacity to adapt themselves to trypanocidal drugs and by excluding the host immune response "free play" is given to the process of adaptation of the parasites. Recent studies on chloroquine-resistance by *Plasmodium falciparum* have shown that resistant organisms rid themselves of chloroquine 40-50

fold faster than do sensitive parasites and that the rapid-efflux phenotype is linked to a single genetic locus on *P. falciparum* chromosome 7 (Wellems, 1991). Therefore it may be possible that small number of trypanosome organisms which develop resistance as a result of mutational changes have the opportunity to multiply thus passing the genes responsible for the resistance to their progenies. This opportunity would not be available in immunocompetent hosts since immune response would have ensured opsonisation and subsequent elimination of individuals which had survived drug treatment. Diesing *et al.* (1986) reported that addition of variable antigen type populations of *T. evansi* to immune serum led to specific opsonisation of trypanosomes resulting in an intense metabolic activation and chemiluminescence response of phagocytic cells.

Whether variation in drug sensitivity is associated with variable antigen types also requires further elucidation. However as part of these experiments normal immunocompetent mice infected with *T. evansi* GRVPS 13/3 and challenged with the mel Cy-resistant *T. evansi* GRVPS 13/3R19 a week later after the primary infection was eliminated by mel Cy treatment failed to become parasitaemic during the three week observation period. The failure of the challenge to establish infection could not be attributed to drug residue, since a control group treated with mel Cy and infected with the mel Cy-resistant clone developed a patent infection. Moreover it has been noted that mel Cy is rapidly excreted and the plasma trypanocidal activity was found to be less than 48 hours (Baltz *et al.*, 1989). According to Jones and McKinnell (1985) passage in immunosuppressed rodents does not interfere with antigenic variation in *T. evansi*, however the rapidly multiplying VATs were found to predominate over the others in these circumstances.

It has been reported that trypanosomes which attain drug resistance or break through chemoprophylaxis tend to multiply rather slowly and display diminished pathogenicity (Tobie and Von Brand, 1953; Sones *et al.*, 1989). Cantrell (1956) argued that trypanosomes which develop drug-resistance are less well adapted to survival in the absence of the drug compared to their unmodified parents. Furthermore Silayo and Marandu (1989) reported that a strain of *T. congolense* which developed diminazene resistance through repeated exposure exhibited reduced pathogenecity with the infected mice surviving upto 120 days while the survival time following infection with two sensitive strains was 20 to 60 days. However in the present studies no variation in pathogenicity was detected between the clone rendered highly resistant to mel Cy and the parent clone. In both cases survival time did not exceed 16 days post infection.

It is also noteworthy that the clone of *T. evansi* which developed a high level of resistance to mel Cy was found to be also resistant to diminazene and pentamidine. This finding agrees with earlier reports which suggested cross-resistance links between arsenicals and diamidines (Williamson, 1970; Zweygarth and Kaminsky, 1990).

A major area where the cross-resistance between diamidines and arsenicals coupled with the reduced efficacy of trypanocidal drugs in immunosuppressed hosts could be important is in the control of human trypanosomiasis in Africa. Both pentamidine, a diamidine, and melarsoprol which is a melaminyl arsenical are used against this condition (Williamson, 1970).

Whereas it is relatively easy to produce diminazene aceturate resistance using immunocompromised animals (this thesis) it is reputedly very difficult, either to find or produce under field conditions. In endemic areas of human

trypanosomiasis the possibility exists that animals may be chronically affected with *T. rhodesiense* or *T. gambiense*. Repeated dosing with diminazene aceturate will not eliminate these CNS-trypanosomes and it is unknown what the effects of repeated dosing will have on the CNS-trypanosomes. If these are rendered diminazene-resistant, not only will they be resistant to diminazene but also to the two main drugs used in human medicine. They will be resistant to pentamidine, another diamidine and also they will be cross-resistant to melarsoprol, virtually the only compound capable of curing late-stage sleeping sickness. These resistant trypanosomes may spread and infect the human population.

There is also the possibility that patients with diseases which are known to impair the immune system, namely malaria, which is endemic in the same regions as trypanosomiasis, and especially those patients with AIDS may form a milieu for the generation of drug-resistant strains. Although, to our knowledge no AIDS patient has yet been reported to have been also infected with trypanosomes such patients may be routinely treated with trypanocidal drugs such as pentamidine, suramin and DFMO to control concomitant infections associated with AIDS. If trypanosomes were also present the generation of drug-resistance could occur.

The emergence of drug resistance in immunocompromised hosts could also have serious implications in animal trypanosomiasis since there are other protozoan infections which induce various degrees of immunosuppression in animals (Phillips and Wakelin, 1976) and which commonly occur in trypanosome endemic areas. There is a large body of evidence, supported with experimental confirmations, that such protozoan infections as babesiosis and toxoplasmosis are capable of inducing immunosuppression of the infected hosts (Callow and Stewart, 1978; Phillips and Wakelin, 1976;

Strickland *et. al.*, 1972). It has been reported that calves experimentally infected with *Babesia bovis* develop significantly less resistance to the one-host tick *Boophilus microplus* compared to non-infected controls (Callow and Stewart, 1978), as judged from the number of engorged females developed. Elsewhere *Babesia microti* has been shown to temporarily depress the immune response of mice to the nematode *Trichuris muris* thus delaying the expulsion of the adult worms from the intestine (Phillips and Wakelin, 1976).

Because of the difficulties and expenses involved in the introduction of new trypanocides further work on the causes and extent of immunosuppression in the field as well as the possible impact it might have on the development of trypanocide resistance would be warranted.

The relative ease by which drug-resistant lines of parent cloned stabilates can be produced means that various tests such as isoenzyme characterisation as well as chromosome karyotyping and investigations on kinetoplast DNA (kDNA) minicircles can be carried out, comparing each drug-resistant stabilate not only with its parent clone but also with other lines produced against unrelated trypanocides. Studies on these clones together with intermediate clones may give some indication if changes in drug resistance are a gradual process or a single event.

It should be possible to produce from a single parent clone a series of clones resistant to each of the trypanocidal drugs as well as clones with various combinations of multi-drug resistance. Analysis of these may give a clearer insight into the mechanisms of drug resistance per se as well as cross-resistance and possibly lead to the development of new tests for drug resistance and improved strategies for combating drug resistance.

Such studies not only provide information of considerable scientific interest but may also assist in the future control of trypanosomiasis.

REFERENCES

REFERENCES

Abebe, G., Jones, T.W. and Boid, R. (1983). Suramin sensitivity of stocks of *Trypanosoma evansi* isolated in the Sudan. *Trop. Anim. Hlth. Prod.*, **15**, 151-152.

Ainanshe, O., Jennings, F.W. and Holmes, P.H. (1989). Isolation of drug-resistant strains of *Trypanosoma congolense* in Somalia. International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). 20th meeting. Mombasa, Kenya. Pub. No. 115.

Alcina, A. and Fresno, M. (1985). Suppressor factor of T-cell activation and decreased interleukin 2 activity in experimental African trypanosomiasis. *Infec. Immun.*, **50**, 382-387.

Aline, R.F., Myler, P.J., and Stuart, K.D. (1989). *Trypanosoma brucei*: frequent loss of a telomeric variant surface glycoprotein gene. *Exp. Parasitol.*, **68**, 8-16.

Anderson, R.E. and Warner, N.L. (1976). Ionising radiation and the immune response. *Adv. Immunol.*, **24**, 215-235.

Andrews, P. (1985). Praziquantel: mechanism of anti-schistosomal activity. *Pharmacol. Ther.*, **29**, 129-156.

Assouku, R.K.G. (1975). Immunological studies of the mechanism of anaemia in experimental *Trypanosoma evansi* infections in rats. *Inter. J. Parasitol.*, **5**, 137-145.

Avsatthi, B.L., Mannari, M.N. and Shukla, D.C. (1979). Curative effect of Samorin against *Trypanosoma evansi* infection in donkeys. *Indian J. Parasitol.*, **3**, 165-166.

Bacchi, C.J., Nathan, H.C., Hunter, S.H., McCann, P.P. and Sjoerdsma, A. (1980). Polyamine metabolism: a potential target in trypanosomes. *Science*, **210**, 332-334.

Balis, J. and Richard, D. (1977). Trypanocide action of isometamidium chloride hydrochlorate on *Trypanosoma evansi* and an attempt to control trypanosomiasis in dromedary. *Rev. Elev. Med. Vet. Pays Trop.*, **30**, 369-372.

Baltz, T., Oukessou, M., Benlamlih, S., Laurentie, M.P. and Toutain, P.L. (1989). Plasma arsenic concentrations vs trypanocidal activity for Cymelarsan^{R}, Camels results. International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). 20 th meeting. Mombasa, Kenya. Pub. No. 115

Bancroft, G.J. and Askonas, B.A. (1985). Immunology of African trypanosomiasis in laboratory rodents. In: Immunology and Pathogenesis of Trypanosomiasis. Ed. I. Tizard. pp. 75-101. CRC Press, Inc., Boca Raton, Florida.

Barry, J.D., Le Ray, D. and Herbert, W.J. (1979). Infectivity and virulence of *Trypanosoma* (*Trypanozoon*) *brucei* for mice. IV. Dissociation of virulence and variable antigen type in relation to pleomorphism. *J. Comp. Path.*, **89**, 465-470.

Bennett, S.C.J. (1933a). The control of camel trypanosomiasis. *J. Comp. Path.*, **46**, 67-77.

Bennett, S.C.J. (1933b). The control of camel trypanosomiasis. Immunity following recovery. *J. Comp. Path. Ther.*, **46**, 174-185.

Bennett, S.C.J. and Kenny, P.A.C. (1928). Mercuric chloride test as diagnostic agent for trypanosomaisis of camels. *J. Comp. Path. Ther.*, **41**, 341-358.

Bevan, L.E.W. (1928). A method of inoculating cattle against trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.*, **22**, 2147-2156.

Bevan, L.E.W. (1936). Notes on immunity in trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.*, **30**, 199-206.

Boid, R., Jones, T.W. and Luckins, A.G. (1985). The camel in health and disease. 3: protozoological diseases of camels. *Brit. Vet. J.*, **141**, 87-104.

Boid, R., El Amin, E.A., Mahmoud, M.M. and Luckins, A.G. (1981). *Trypanosoma evansi* infections and antibodies in goats, sheep, and camels in the Sudan. *Trop. Anim. Hlth. Prod.*, **13**, 141-146.

Boid, R., Luckins, A.G., Rae, P.F., Gray, A.R., Mahmoud, M.M. and Malik, K.H. (1980). Serum immunoglobulin levels and electrophoretic patterns of serum proteins in camels infected with *Trypanosoma evansi*. *Vet. Parasitol.*, **6**, 333-345.

Borst, P. (1986). Gene rearrangements controlling gene expression. *Biochem. Intern.*, **12**, 567-591.

Borst, P. and Hoeijmakers, H.J. (1979). Review: kinetoplast DNA. *Plasmid.*, **2**, 20-40.

Borst, P., Fase-Fowler, F. and Gibson, W. (1987). Kinetoplast DNA of *T. evansi*. *Mol. Biochem. Parasitol.*, **23**, 31-38.

Bourn, D. and Scott, M. (1978). The successful use of work oxen in agricultural development of tsetse infested land in Ethiopia. *Trop. Anim. Hlth. Prod.*, **10**, 191-203.

Brindlay, P.J. and Sher, A. (1987). The chemotherapeutic efficacy of praziquantel against *Schistosoma mansoni* is dependent on host antibody response. *J. Immunol.*, **139**, 215-220.

Caille, J.Y. (1989). Serological survey of the prevalence and seasonal incidence of haemoprotozoa in livestock in Somalia. *Protozool. Abst.*, **13**, [48].

Callow, L.L. and Stewart, N.P. (1978). Immunosuppression by *Babesia bovis* against its tick vector *Boophilus microplus*. *Nature.*, **272**, 818-819.

Campbell, G.H., Esser, K.M. and Phillips, S.M. (1978). *Trypanosoma rhodesiense* infection in congenitally athymic (nude) mice. *Infec. Immun.*, **20**, 714-720.

Cantrell, W. (1956). Behaviour of mixtures of oxophenarsine-resistant and unmodified strains of *Trypanosoma equiperdum* in the rat. *Exp. Parasitol.*, **5**, 178-190.

Clarkson, M.J. and Penhale, W.J. (1973). Serum protein changes in trypanosomiasis in cattle. *Trans. R. Soc. Trop. Med. Hyg.*, **67**, 273.

Claussen, P.H. (1987). IgG and IgM antibodies during experimental *Trypanosoma evansi* infections in camels. *Protozool. Abst.*, **11**, [1716].

Cross, G.A.M. (1975). Identification, purification and properties of variant-specific glycoprotein antigens constituting the surface coat of *Trypanosoma brucei*. *Parasitol.*, **71**, 393-417.

Cross, G.A.M. (1977). Antigenic variation in trypanosomes. *Am. J. Trop. Med. Hyg.*, **26**, 240-244.

Cunningham, M.P. (1968). Vaccination of cattle against trypanosomes by infection and treatment. In: Isotopes and Radiation in Parasitology., pp. 89-91. International Atomic Energy Agency. Vienna.

Cuthbertson, R.S. (1981). Kinetoplast DNA in *Trypanosoma equinum*. *J. Protozool.*, **28**, 182-188.

Curd, F.H.S. and Davey, D.G. (1950). "Antrycide" a new trypanocidal drug. *Brit. J. Pharmacol.*, **5**, 25-32.

Dar, F.K. (1972). Antigenic variation of *Trypanosoma vivax* in cattle infected with strains from wild caught tsetse flies. *Trop. Anim. Hlth. Prod.*, **4**, 237-244.

Davey, D.G. (1950). Experiments with "Antrycide" in the Sudan and East Africa. *Trans. R. Soc. Trop. Med. Hyg.*, **43**, 583-616.

De Gee, A.L.W., McCann, P.P. and Mansfield, J.M. (1983). Role of antibody in the elimination of trypanosomes after DL- x -difluoromethylornithine chemotherapy. *J. Parasitol.*, **69**, 818-822.

Derie, M.F., Wallbanks, K.R., Aden, A.A., Bornstein, S and Ibrahim, M.D. (1989). Camel trypanosomiasis and its vectors in Somalia. *Vet. Parasitol.*, **32**, 285-291.

Desowitz, R.S. (1957). Suramin complexes. Part II: prophylactic activity against *Trypanosoma vivax* in cattle. *Ann. Trop. Med. Parasitol.*, **51**, 457-464.

Diesing, L., Ahmed, J.S. and Schein, E. (1986). Application of chemi- and bio-luminescence in the studies of immunological reactions against protozoa. *Vet. Parasitol.*, **20**, 229-235.

Doenhoff, M.J., Modha, J., Lambertucci, J.R. and McLaren D.J. (1991). The immune dependence of chemotherapy. *Parasitol. Today.*, **7**, 16-18.

Doyle, J.J., Hirumi, H. and De Gee, A.L.W. (1979). Mechanisms of antigenic variation in salivarian trypanosomes. In: Pathogenicity of Trypanosomes. Eds. G. Losos and A. Chouinard., pp. 44-45. IDRC Ottawa, Canada.

Eardley, D.D. and Jayawardena, A.N. (1977). Suppressor cells in mice infected with *Trypanosoma brucei*. *J. Immunol.*, **119**, 1029-1033.

Edwards, J.T. (1926). The chemotherapy of surra (*Typanosoma evansi* infections) of horses and cattle in India. *Tran. R. Soc Trop Med. Hyg.*, **20**, 10-71.

FAO-WHO-OIE. (1982). Animal Health Yearbook 1981. FAO, Animal production and health Series No. 18, FAO Rome.

Fairclough, R. (1963). Observations on the use of Berenil against trypanosomiasis of cattle in Kenya. *Vet. Rec.*, **75**, 1107-1112.

Fiennes, R.N.T.-W. (1952). Trypanosome infections of cattle - diagnosis of blood infections of cattle when parasites are rare. *Vet. Rec.*, **46**, 733.

Fiennes, R.N.T.-W. (1970). Pathogenesis and pathology of animal trypanosomiasis. In: The African Trypanosomiases. Ed. H.W. Mulligan., pp. 729-773. Allen and Unwin, London.

Finelle, P. (1983). African animal trypanosomiasis. Part II. Chemoprophylaxis and the raising of trypanotolerant livestock. In: African Animal Trypanosomiasis. *World Anim. Rev.*, **37**, 8-11.

Follath, F., Costa, E., Thommen, A., Frei, R., Burdeska, A. and Meyer, J. (1987). Clinical consequences of development of resistance to third generation cephalosporins. *Eur. J. Clin. Microbiol.*, **6**, 446-450.

Ford, J. and Blaser, E. (1971). Some aspects of cattle raising under prophylactic treatment against trypanosomiasis on the Mkwaya Ranch, Tanzania. *Acta Trop.*, **28**, 69-79.

Ford, E.J.H., Karib, A.A. and Wilmhurst, E.C. (1953). Studies on ethidium bromide. II. The treatment of *Trypanosoma congolense* infections in cattle. *Vet. Rec.*, **65**, 589-590.

Freeman, J., Hudson, K.M., Longstaffe, J.A. and Terry, R.C. (1973). Immunodepression in trypanosome infections. *Parasitol.*, **67**, xxii.

Freidheim, E.A.H. (1949). Mel B in the treatment of human trypanosomiasis. *Am. J. Trop. Med.*, **29**, 173-180.

Frommel, T.O. (1988). *Trypanosoma brucei rhodesiense*: effect of immunosuppression on the efficacy of melarsoprol treatment of infected mice. *Exp. Parasitol.*, **67**, 364-366.

Fussganger, R. and Bauer, F. (1960). Investigations on Berenil resistance in trypanosomes. *Vet. Rec.*, **72**, 1118-1121.

Gad-El-Mawla, B.I, and Fayed, A.A. (1979). The efficacy of suramin in the treatment of trypanosomiasis in Egyptian camels under desert conditions. *J. Egyptian Vet. Med. Assoc.*, **32**, 89-94.

Galhorta, A.P., Malik, P.D., Gautam, O.P. and Banerjee, D.P. (1979). Clinico-pathological changes and therapeutic trials in experimental trypanosomiasis in dogs. *Harayana Agric. Univer. J. Res.*, **9**, 69-74.

Gatt Rutter, T.E. (1967). Diseases of camels. *Vet. Bull.*, **37**, 611-618.

Gibson, W.C. (1988). Nomenclature of trypanosomes. *Trans. R. Soc. Trop. Med. Hyg.*, **82**, 175-176.

Gibson, W.C., Marshal, T.F. de C. and Godfrey, D.G. (1980). Numerical analysis of enzyme polymorphism - a new approach to the epidemiology and taxonomy of trypanosomes from the subgenus *Trypanozoon*. *Adv. Parasitol.*, **18**, 175-239.

Gilbert, R.J. and Newton, B.A. (1982). Ethidium bromide: pharmacokinetics and efficacy against trypanosome infections in rabbits and calves. *Parasitol.*, **85**, 127-148.

Gill, B.S. (1971a). Studies on surra. III. Agglutinogens and precipitinogens of the serological variants of *Trypanosoma evansi*. *Indian J. Anim. Sci.*, **41**, 618-623.

Gill, B.S. (1971b). Resistance of *Trypanosoma evansi* to quinapyramine, suramin, stilbamidine and tryparsamide and analysis of cross-resistance. *Trans. R. Soc. Trop. Med. Hyg.*, **65**, 352-357.

Gill, B.S. (1971c). Drug-resistance in *Trypanosoma evansi*. *Trop. Anim. Hlth. Prod.*, **3**, 195-198.

Gill, B.S. (1972). Studies on surra. VIII. Therapeutic activity of oxophenarsine hydrochloride, Mel W, pentamidine, quinapyramine suraminate and terephthalnilide compounds in equine surra. *Indian J. Anim. Sci.*, **42**, 278-280.

Gill, B.S. (1973). Studies on surra. IX. Chemotherapeutic susceptibility of *T. evansi*. *Indian J. Anim. Sci.*, **43**, 226-229.

Gill, B.S. and Malhorta, M.N. (1971). Chemoprophylaxis of *T. evansi* infections in ponies. *Trop. Anim. Hlth. Prod.*, **3**, 199-202.

Godfrey, D.G. (1961). Types of *Trypanosoma congolense*. II. Differences in the courses of infection. *Ann. Trop. Med. Parasitol.*, **55**, 154-166.

Godfrey, D.J. and Killick-Kendrick, R.R. (1962). *Trypanosoma evansi* of camels in Nigeria: a high incidence demonstrated by inoculation of blood into rats. *Ann. Trop. Med. Parasitol.*, **56**, 14-19.

Goodwin, L.G. (1970). The pathology of African trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.* **64**, 797-817.

Goodwin, L.G., Green, D.G., Guy, M.W. and Voller, A. (1972). Immunosuppression during trypanosomiasis. *Brit. J. Exp. Path.*, **53**, 40-43.

Gray, A.R. (1961). Suramin complexes. VIII. A further trial of the prophylactic activity of Antrycide-suramin complex and Antrycide chloride against *Trypanosoma simiae* in pigs. *Ann. Trop. Med. Parasitol.*, **55**, 432-439.

Gray, A.R. (1965). Antigenic variation in a strain of *Trypanosoma brucei* transmitted by *Glossina morsitan* and *G. palpalis*. *J. Gen. Microbiol.*, **44**, 195-214.

Gray, A.R. and Luckins, A.G. (1976). Antigenic variation in salivarian trypanosomes. In: Biology of the Kinetoplastida. (Eds) W.H.R. Lumsden and D.A. Evans., pp. 493-542. Academic Press, London.

Gray, A.R. and Roberts, C.J. (1971). The stability of resistance to diminazene aceturate and quinapyramine sulphate in a strain of *Trypanosoma vivax* during cyclical transmission through antelope. *Parasitol.*, **63**, 163-168.

Gruvel, J. and Balis, J. (1965). La trypanosomiase a *Trypanosoma evansi* chez le dromedaire au Tchad et ses principaux vecteurs. *Rev. Elev. Vet. Pays. Trop.*, **18**, 435-439.

Hajduk, S.L. and Vickerman, K. (1981). Antigenic variation in cyclically-transmitted *Trypanosoma brucei*. Variable antigen type composition of the first parasitaemia in mice bitten by trypanosome-infected *Glossina morsitans*. *Parasitol.*, **83**, 609-621.

Harnett, W. (1988). The antihelmintic action of praziquantel. *Parasitol. Today*, **4**, 144-146.

Hawking, F. (1937). Studies on chemotherapeutic action. I. The absorption of arsenical compounds and tartar emetic by normal and resistant trypanosomes and its relation to drug-resistance. *J. Pharmacol.*, **59**, 123-156.

Hawking, F. (1939). Contribution on the mode of action of Germanin (Bayer 205). *Ann. Trop. Med. Parasitol.*, **33**, 13-19.

Hawking, F. (1963). Drug-resistance of *Trypanosoma congolense* and other trypanosomes to quinapyramine, phenanthridines, Berenil and other compounds in mice. *Ann. Trop. Med. Parasitol.*, **57**, 262-282.

Henson, J.B. and Noel, J.C. (1979). Immunology and pathogenesis of African animal trypanosomiasis. *Adv. Vet. Sci. Comp. Med.* **23**, 161-182.

Herbert, W.J. and Lumsden, W.H.R. (1976). *T. brucei*: a rapid "matching" technique for estimating the host's parasitaemia. *Exp. Parasitol.* **40**, 427-431.

Higgins, A.J. (1983). Observations on the diseases of the Arabian camel and their control - a review. *Vet. Bull.*, **53**, 1089-1098.

Hoare, C.A. (1940). Studies on the behaviour of *Trypanosoma evansi* in tsetse flies, with special refrence to its phylogeny. *Parasitol.*, **32**, 105-121.

Hoare, C.A. (1954). The loss of the kinetoplast in trypanosomes, with special refrence to *Trypanosoma evansi*. *J. Protozool.*, **1**, 28-33.

Hoare, C.A. 1956). Morphological and taxonomic studies on mammalian trypanosomes VIII. Revision of *Trypanosoma evansi*. *Parasitol.*, **46**, 130-172.

Hoare, C.A. (1959). Present condition of a mutant akinetoplastic strain of *Trypanosoma evansi*. *J. Protozool.*, **6** (Suppl.) 26.

Hoare, C.A. (1965). Vampire bats as vectors and hosts of equine and bovine trypanosomes. *Acta Trop.*, **22**, 204-216.

Hoare, C.A. (1967). Evolutionary trends in mammalian trypanosomes. *Adv. Parasitol.*, **5**, 41-91.

Hoare, C.A. (1970). The mammalian trypanosomes of Africa. Systematic description of mammalian trypanosomes of Africa. In: The African trypanosomiases. Ed. H.W. Mulligan.,pp. 3-66. George Allen & Unwin, London.

Hoare, C.A. (1972). The trypanosomes of mammals. A zoological monograph. Blackwell Scientific Publications (Oxford).

Hoare, C.A. and Bennett, S.C.J. (1937). Morphological and taxonomic studies on mammalian trypanosomes. III. Spontaneous occurrence of strains of *Trypanosoma evansi* devoid of the kinetonucleus. *Parasitol.*, **29**, 43-56.

Hoare C.A. and Bennett, S.C.J. (1939). Morphological and taxonomic studies on mammalian trypanosomes. VI. Further observations on the absence of the kinetoplast in *Trypanosoma evansi*. *Parasitol.*, **30**, 529-542.

Holmes, P.H. (1976). Use of radioisotopic tracer techniques in the study of the pathogenesis of trypanosomiasis. In: Nuclear Techniques in Animal Production and Health., PP. 463-474. I.A.E.A., Vienna.

Holmes, P.H. (1980). Vaccination against parasites. In: Sympossium of the British Society for Parasitology. Eds. A.E.R. Taylor and R. Muller., pp. 75-105. Blackwell Scientific Publications. Oxford.

Holmes, P.H. and Jennings, F.W. (1976). The effect of treatment on the anaemia of African trypanosomiasis. In: Pathophysiology of Parasitic Infections. Ed. E.J.L. Soulsby., pp. 199-210. Academic Press, New York.

Holmes, P.H. and Torr, S.J. (1988). The control of animal trypanosomiasis in Africa: current methods and future trends. *Outlook on Agriculture.*, **17**, 54-60.

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

Holmes, P.H., Mamo, C., Thompson, A., Knight, P.A., Lucken, R., Murray, M., Jennings, F.W. and Urquhart, G.M. (1974). Immunosuppression in bovine trypanosomiasis. *Vet. Rec.*, **95**, 86.

Homeida, A.M., Amin, E.A., El Adam, S.E.I. and Mohamoud, M.M. (1981). Toxicity of diminazene aceturate (Berenil). to camels. *J. Comp. Path.*, **91**, 355-360.

Houba, V., Brown, K.N. and Allison, A.C. (1969). Heterophile antibodies, M-aglutinins and immunoglobulins in experimental trypanosomiasis. *Clin. Exp. Immunol.* **4**, 113-123.

Hudson, K.M., Byner, C., Freeman, J. and Terry, R.J. (1976). Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis. *Nature*, **36**, 256-258.

Hunter, A.G. (1986). Urine odour in a camel suffering from Surra (*T. evansi* infection). *Trop. Anim. Hlth. Prod.*, **18**, 146-148.

Ikede, B.O. and Losos, G.J. (1972). Pathology of the disease in sheep produced experimentally by *Trypanosoma brucei*. *Vet Path.*, **9** (Suppl.), 278-289.

Jancso, N. von and Jancso, H. von (1934). The role of the natural defence forces in the evolution of drug-resistance. I. A method for the exclusion of the natural defence mechanisms from the chemotherapeutic processes. *Ann. Trop. Med. Parasitol.*, **28**, 419-438.

Jancso, N. von and Jancso, H. von (1935). The role of the natural defence forces in the evolution of the drug-resistance of trypanosomes. II. The rapid production of Germanin-fast *T. brucei* strains in animals with paralysed defence. *Ann. Trop. Med. Parasitol.*, **29**, 95-109.

Jatkar, P.R. and Purohit, M.S. (1971). Pathogenesis of anaemia in *Trypanosoma evansi* infection. Part 1: haematology. *Indian Vet. J.*, **48**, 339-344.

Jennings, F.W., Murray, P.K., Murray, M. and Urquhart, G.M. (1973). Protein catabolism in trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.*, **67**, 277.

Jennings, F.W., Murray, P.K., Murray, M. and Urquhart, G.M. (1974). Anaemia in trypanosomiasis: studies in rats and mice infected with *T. brucei*. *Res. Vet. Sci.*, **16**, 70-76.

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

Jennings, F.W., Whitelaw, D.D. Holmes, P.H., Chizyuka, H.G.B. and Urquhart, G.M. (1979) The brain as a source of relapsing *Trypanosoma brucei* infection in mice after chemotherpy. *Int. J. Parasitol.*, **9**, 381-384.

Jones, T.W. and McKinnell, C.D. (1984). Antigenic variation in *Trypanosoma evansi*. Isolation and characterisation of Variable Antigen Type populations from rabbits infected with a stock of *T. evansi*. *Tropenmed. Parasitol.*, **35**, 237-241.

Jones, T.W. and McKinnell, C.D. (1985). Antigenic variation in *Trypanosoma evansi*: variable antigen type (VAT) composition of first relapse populations in mice. *Trop. Med. Parasitol.*, **36**, 127-130.

Jones-Davies, W.J. (1967). The protection of a small group of Nigerian trade cattle from trypanosomiasis with Samorin. *Bull. Epizoot. Dis. Afr.*, **15**, 323-335.

Kalu, A.U., Edeghere, H.U. and Lawani, F.A. (1986). Comparison of diagnostic techniques during subclinical single infections of trypanosomiasis in goats. *Vet. Parasitol.*, **22**, 37-47.

Kaukha, G.W. and Ramasamy, R. (1981). Haemolytic activity of trypanosomes. *East Afr. Med. J.*, **58**, 907-911.

Kelly, S. and Schillinger, D. (1983). Improved field diagnostic technique for trypanosomiasis by the use of a minicentrifuge. *Vet. Rec.*, **113**, 219.

Kershaw, W.E. (1970). The African trypanosomiases. Ed. H.W. Mulligan. pp. vii-ix. Allen & Unwin. London.

Killick-Kendrick, R. (1964). The apparent loss of the kinetoplast of *Trypanosoma evansi* after treatment of an experimentally infected horse with Berenil. *Ann. Trop. Med. Parasitol.*, **58**, 481-490.

Killick-Kendrick, R. (1968). The diagnosis of trypanosomiasis of livestock; a review of current techniques. *Vet. Bull.*, **38**, 191-196.

Kirkby, W.W. (1964). Prophylaxis and therapy under continuous exposure to the risk of natural infection with trypanosomiasis by tsetse flies. *Bull. Epizoot. Dis. Afr.*, **12**, 321-329.

Knowles, R.H. (1925). Treatment of camels affected with *Trypanosoma soudanense* with "Bayer 205" and further observations on the formol-gel test. *J. Comp. Path. Ther.*, **38**, 42-46.

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

Kobayashi, A. and Tizard, I.R. (1976). The response to *Trypanosoma congolense* infection in calves: determination of immunoglobulins IgG1, IgG2, IgM and C3 levels and the complement fixing antibody titres during the course of infection. *Tropenmed. Med. Parasitol.*, **27**, 411-417.

Lanham, S.M. and Godfrey, D.G. (1970). Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Exp. Parasitol.*, **28**, 521-534.

Leach, T.M. (1961). Observations on the treatment of *Trypanosoma evansi* infection in camels. *J. Comp. Path.*, **71**, 109-117.

Leach, T.M. and Roberts, C.J. (1981). Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the eastern hemisphere. *Pharmac. Ther.*, **13**, 91-147.

Leach, T.M., Karib, A.A., Ford, E.J.H. and Wilmshurst, E.C. (1955). Studies on ethidium bromide . VI. The prophylactic properties of the drug. *J. Comp. Path.*, **65**, 130-142.

Leese, A.S. (1927). A Treatise on the One-Humped Camel. Haynes & Sons, Maiden Lane, Stamford, Lincolnshire.

Levine, N.D. (1973). Protozoan parasites of domestic animals and of man. 2nd ed. Burgess Publishing Company, Minneapolis.

Lewis, A.R. and Thompson, J.W. (1974). Observations on an isometamidium resistant strain of *Trypanosoma congolense* in Rhodesia. *Rhod. Vet. J.*, **4**, 62-67.

Lohr, K.F., Pholpark, S., Siriwan, S., Leesirucul, N., Srikitjakarn, L. and Staak, C. (1986). *Trypanosoma evansi* infection in buffaloes in North East Thailand. II. Abortion. *Trop. Anim. Hlth. Prod.* **18**, 103-108.

Lohr, K.F., Pholpark, S., Siriwan, P., Sirikul, L., Srikitjakarn, N., Upatoom, N., Leidi, K. and Staak, C. (1988). *Trypanosoma evansi* infection, a frequent cause of abortion in buffaloes. *Protzool. Abst.*, **12**, [2952]

Losos, G.J. (1980). Diseases caused by *Trypanosoma evansi*, a review. *Vet. Res. Commun.*, **4**, 165-181.

Losos, G.J. (1986). Trypanosomiasis. In: Infectious Tropical Disease of Domestic Animals., pp. 183-318. The Bath Press, Avon.

Luckins, A.G. (1972). Studies on bovine trypanosomiasis. Serum immunoglobulin levels in Zebu cattle exposed to natural infection in East Africa. *Brit. Vet. J.*, **128**, 523-528.

Luckins, A.G. (1988). *Trypanosoma evansi* in Asia. *Parasitol. Today.*, **4**, 137-142.

Luckins, A.G., Gray, A.R. and Rae, P. (1978). Comparison of the diagnostic value of serum immunoglobulin levels, an enzyme-linked immunosorbent assay and a fluorescent antibody test in experimental infections with *Trypanosoma evansi* in rabbits. *Ann. Trop. Med. Parasitol.*, **72**, 429-441.

Luckins, A.G., Boid, R., Mahmoud, M.M., El Malik, K.H. and Gray, A.R. (1979). Serodiagnosis of infection with *Trypanosoma evansi* in camels in the Sudan. *Trop. Anim. Hlth. Prod.*, **11**, 1-12.

Lumsden, W.H.R. (1974). Leishmaniasis and trypanosomiasis: the causative organisms compared and contrasted. In: Trypanosomiasis and Leishmaniasis with Special Reference to Chagas' Disease. Ciba Foundation Symposium 20 (new series)., pp. 3-27. Associated Scientific Publishers. Amsterdam. London. New York.

Lumsden, W.H.R., Herbert, W.J. and Hardy, G.J.C. (1965). *In vivo* prophylactic activity of Berenil against trypanosomes in mice. *Vet. Rec.*, **77**, 147-148.

Lyttle, C.N. (1960). Field trials of Prothidium as a prophylactic in cattle trypanosomiasis. *J. Comp. Path.*, **70**, 18-35.

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

MacKenzie, P.K.I. and Cruickshank, J.G. (1973). Phagocytosis of erythrocytes and leucocytes in sheep infected with *Trypanosoma congolense* (Broden 1904). *Res. Vet. Sci.*, **15**, 256-262.

MacLennan, K.J.R. (1957). A staining technique for the identification of trypanosomes in thick blood films. *Tran. R. Soc. Trop. Med. Hyg.*, **51**, 301-302.

MacLennan, K.J.R. (1970). The epizootiology of trypanosomiases in livestock in West Africa. In : The African Trypanosomiases. Ed. H.W. Mulligan., pp. 751-773. Allen & Unwin. London.

McNeillage, G.J.C. and Herbert, W.J. (1968). Infectivity and virulence of *Trypanosoma* (*Trypanozoon*) *brucei* for mice. II. Comparison of closely related trypanosome antigenic types. *J. Comp. Path. Ther.*, **78**, 345-349.

Mahmoud, M.M. and Gray, A.R. (1980). Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885) Balbiani, 1888. A review of recent research. *Trop. Anim. Hlth. Prod.*, **12**, 35-47.

Mamo, E. and Holmes, P.H. (1975). The erythrokinetics of Zebu cattle chronically infected with *Trypanosoma congolense*. Res. *Vet. Sci.*, **18**, 105-106.

Mansfield, J.M. and Wallace, J.H. (1974). Suppression and cell-mediated immunity in experimental African trypanosomiasis. *Infec. Immun.*, **10**, 335-339.

Metcalf, B.W., Bey, P., Danzin, C., Jung, M.J., Casara, P. and Ververt, J.P. (1978). Catalytic irreversible inhibition of mammalian ornithine decarboxylase by substrate and product analogues. *J. Am. Chem. Soc.*, **100**, 2551-2553.

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

Molyneux, D.H. (1975). Diagnostic methods in animal trypanosomiasis. *Vet. Parasitol.*, **1**, 5-17.

Molyneux, D.H. and Ashford, R.W. (1983). The Biology of *Trypanosoma* and *Leishmania*, Parasites of Man and Domestic Animals. Taylor & Francis, London.

Morrison, W.I., Murray, M. and Akol, G.W.O. (1985). Immune responses of cattle to African trypanosomes. In: Immunology and Pathogenesis of Trypanosomiasis. Ed. I. Tizard., pp. 103-131. CRC Press, Boca Raton, Florida.

Murray, M. (1974). The pathology of African trypanosomiasis. In: Progress in Immunology. Ed. L. Brent, J. Holborow., pp. 181-192. North Holland publishing Company, Amsterdam.

Murray, M. and Urquhart, G.M. (1977). Immunoprophylaxis against African trypanosomiasis. In: Immunity to Blood Parasites of Animals and Man. Eds. L.H. Miller, J.A. Pino and J.J. Mc Kelvey., pp. 209-241. Plenum Press. New York.

Murray, M., Murray, P.K. and McIntyre, I.M. (1977). An improved parasitological technique for the diagnosis of African trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.*, **71**, 325-326.

Murray, M., Murray, P.K., Jennings, F.W., Fisher, E.W. and Urquhart, G.M. (1974a). The pathology of *Trypanosoma brucei* infection in the rat. *Res. Vet. Sci.*, **16**, 77-84.

Murray, P.K., Jennings, F.W., Murray, M. and Urquhart, G.M. (1974b). The nature of immunosuppression in *Trypanosoma brucei* infections in mice. I. The role of the macrophages. *Immunol.*, **27**, 815-824.

Murray, P.K., Jennings, F.W., Murray, M. and Urquhart, G.M. (1974c). The nature of immunosuppression in *Trypanosoma brucei* infections in mice. II. The role of the T and B lymphocytes. *Immunol.*, **27**, 825-840.

Murray, M., Barry, J.D., Morrison, W.I., Williams, R.O. Hirumi, H. and Rovis, L. (1983). A review of the prospects for vaccination in African trypanosomiasis. Part 1. In: African Animal Trypanosomiasis. *World Animal Review*, **37**, 23-27. FAO, Rome.

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

Na'Isa, B.K. (1969). The protection of 60 Nigerian trade cattle from trypanosomiasis using Samorin. *Bull. Epizoot. Dis. Afr.*, **17**, 45-54.

Nantulya, V.M. and Lindqvist, K.J. (1989). Antigen-detection enzyme immuoassays for the diagnosis of *Trypanosoma vivax*, *T. congolense* and *T. brucei* infections in cattle. *Trop. Med. Parasitol.*, **40**, 267-272.

Nash, T.A.M. (1969). Africa's bane: the tsetse fly. Collins. London.

Newton, B.A. (1974). The chemotherpy of trypanosomiasis and leishmaniasis: towards a more rational approach. In: Trpanosomiasis and Leishmaniasis with Special Refrence to Chagas Disease. Ciba Foundation Symposium 20 (new series). pp. 285-305. Elsevier, Amsterdam.

Nielson, K., Sheppard, J., Holmes, W. and Tizard, I. (1978). Experimental bovine trypanosomiasis: changes in serum immunoglobulins, complement and complement components in infected animals. *Immunol.*, **35**, 817-826.

Ng, B.K.Y. and Vaneslow, B. (1978). Outbreak of surra in horses and the pathogenesis of the anaemia. *Kajian Vet.*, **10**, 88-98.

Noble, N.M. (1958). Antrycide suramin complex as a prophylactic against trypanosomiasis in pigs. A field trial in Sierra Leone. *Bull. Epizoot. Dis. Afr.*, **6**, 379-383.

Ormerod, W.E. (1970). Pathogenesis and pathology of trypanosomiasis in man. In: The African Trypanosomiases. (Ed.) H.W. Mulligan. pp. 587-601. Allen & Unwin., London.

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

Peck, E.F. (1936). Annual Report of the Veterinary and Agricultural Department for 1935, Hargeisa, British Somaliland.

Pegram, R.G. and Scott, G.M. (1976). The prevalence and diagnosis of *Trypanosoma evansi* infection in camels in southern Ethiopia. *Trop. Anim. Hlth. Prod.*, **8**, 20-27.

Peregrine, A.S., Ogunyemi, O., Whitelaw, D.D., Holmes, P.H., Moloo, S.K., Hirumi, H., Urquhart, G.M. and Murray, M. (1988). Factors influencing the duration of isometamidium chloride (Samorin) prophylaxis against experimental challenge with metacyclic forms of *Trypanosoma congolense*. *Vet. Parasitol.*, **28**, 53-64.

Peters, W. (1974). Drug resistance in trypanosomaisis. In: Trypanosomiasis and Leishmaniasis with Special Reference to Chagas' Disease. Ciba Foundation Symposium 20 (new series)., pp. 308-334. Associated Scientific Publishers. Amsterdam. London. New York.

Phillips, R. S. and Wakelin, D. (1976). *Trichuris muris*: effects of concurrent infections with rodent piroplasms on immune expulsion from mice. *Exp. Parasitol.*, **39**, 95-100.

Rae, P.F. and Luckins, A.G. (1984). Detection of circulating trypanosomal antigens by enzyme immunoassay *Ann. Trop. Med. Parasitol.*, **78**, 587-596.

Raina, A.K., Gupta, S.L. and Singh, R.P. (1985). Comparison of indirect haemagglutination and capilary tube agglutination tests for detection of *Trypanosoma evansi* antibody in buffalo calves. *Indian J. Parasitol.*, **9**, 191-192.

Raisinghani, P.M. and Lodha, K.R. (1980). Chemotherapeutic levels of Berenil (Hoeschst) in experimental surra in camels. *Indian Vet. J.*, **57**, 891-895.

Raisinghani, P.M. and Lodha, K.R. (1986). Comparative efficacy of different diagnostic tests for surra in camels (*Camelus dromedarius*). *Indian J. Anim. Sci.*, **56**, 1142-1143.

Raisinghani, P.M., Lodha, K.R., Bhatia, J.S. and Dwarakanath, P.K. (1981). Variation in haematological and serum electrolyte levels during first 20 bouts of experimental surra in camels. *Indian J. Anim. Sci.*, **51**, 724-729.

Raynaud, J.P., Sones, K.R. and Friedheim, E.A.H. (1989). A review of Cymelarsan^{R} - a new treatment proposed for animal trypanosomiasis due to *T. evansi* and other trypanosomes of the *T. brucei* group. International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). 20th meeting. Mombasa, Kenya. Pub. No 115.

Razzaque , A. and Mishra, S.S. (1977). Comparative efficacy of Berenil, Antrycide Prosalt and acriflavine in the treatment of experimental surra in buffalo-calves. *Bull. Anim. Hlth. Prod.* **25**, 409-414.

Richardson, U.F. and Kendall, S.B. (1963). Veterinary Protozoology, 3rd ed. Oliver & Boyd, Edinburgh.

Roelants, G.E., Pearson, T.W., Morrison, W.I., Mayor-Withey, K.S. and Lundin, L.B. (1979). Immune depression in trypanosome-infected mice. IV. Kinetics of suppression and alleviation by the trypanocidal drug Berenil. *Clin. Exp. Immunol.*, **37**, 457-469.

Rogers, L. (1901). The transmission of the *Trypanosoma evansi* by horseflies and other experiments pointing to the the probable identity of surra in India and nagana or tsetse fly disease in Africa. *Proc. Roy. Soc.*, **68**, 163-170.

Rollo, I.M. and Williamson, J. (1951). Acquired resistance to "Melarsen", Tryparsamide and amidines in pathogenic trypanosomes after treatment with "Melarsen" alone. *Nature*, **167**, 147-148.

Ruchel, W.-M. (1975). Chemoprophylaxis of Bovine Trypanosomiasis. Ed. GTZ, Eschborn.

Ruttledge, W. (1928). Tsetse-fly (*Glossina morsitans*) in the Koalib hills, Nouba Mountains Province, Sudan. *Bull. Ent. Res.*, **19**, 309-316.

Sabah, A.A., Fletcher, C., Webbe, G. and Doenhoff, M.J. (1985). *Schistosoma mansoni*: reduced efficacy of chemotherapy in infected T-cell deprived mice. *Exp. Parasitol.*, **60**, 348-354.

Sabanshiev, M.S. (1988). Efficacy of a mixture of Naganine and azidine against trypanosomiasis in horses. *Protozool. Abst.*, **12**, [2022].

Sacks, D.L. and Askonas, B.A. (1980). Trypanosome-induced suppression of anti-parasite responses during experimental African trypanosomiasis. *Eur. J. Immunol.*,**10**, 971-974.

Schillinger, D. and Rottcher, D. (1986). Treatment of camels for *Trypanosoma brucei evansi* infection (surra). *World Animal Review.*, **60**, 26-32.

Schnitzer, R.J. and Grunberg, E. (1957). Drug resistance of microorganisms. Academic Press Inc. London. New York.

Schnitzer, R.S., Lafferty, L.C. and Buck, M. (1946). The role of antibodies in experimental drug resistance of *Trypanosoma equiperdum. J. Immunol.*,**54**, 47-57.

Seiler, R.J., Omar, S. and Jackson, R.B. (1981). Meningoencephalitis in naturally occurring *Trypanosoma evansi* infection (surra) of horses. *Vet. Path.*, **18**, 120-122.

Sen, H.G., Dutta, B.N. and Ray, H.N. (1955). Effects of splenectomy on "Antrycide" therapy of *T. evansi* infections in rats. *Nature*, **209**, 309-310.

Shaw, J.J. (1977). The epizootiology of American surra with special reference to the lower Amazon region. *Protozool.*, **3**, 119-128.

Shien, Y.S. (1977). Clinico-pathological studies on canine surra. *Protozool. Abst.* **1**, [3784].

Shien, Y.S. (1980). Immunosuppression in experimentally induced surra goats. I. Serological responses to *Brucella abortus* vaccinations before and after administerations of Naganol. *Protozool. Abst.*, **4**, [1173]

Shien, Y.S., Liou, P.P. and Fung, H.P. (1977). Clinico-pathological studies on experimental canine surra. *Protozool. Abst.* **1**, [3776].

Shen, J., Fang, W.M., Sung, J.L., Zengh, R.J., Wu, K.K., Wang, X.G. and Tao, Z.F. (1986). Diagnosis of *Trypanosoma evansi* infection in buffaloes with ELISA. *Protozool. Abst.*, **10**, [3398].

Silayo, R.S. and Marandu, W.P. (1989). The stability of drug resistance and relative apathogenicity of a strain of *Trypanosoma congolense* maintained in mice. International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). 20th meeting. Mombasa, Kenya. Pub. No 115.

Sileghem, M., Hamers, R. and Baetselier, P. de. (1986). Active suppression of interleukin 2 in mice infected with *Trypanosoma brucei* AnTat 1.1. E. *Parasite Immunol.*, **8**, 641-649.

Simpson, L. (1972). The kinetoplast DNA of haemoflagellates. *Int. Rev. Cytol.*, **32**, 139-207.

Singh, B.P. and Misra, S.K. (1986). Haematological changes in *Trypanosoma evansi* infection in calves. *Indian J. Vet. Med.* **6**, 108-109.

Smith, I.M. (1958). The protection against trypanosomiasis conferred on cattle by repeated doses of Antrycide, alone or with *Trypanosoma congolense*. *Ann. Trop. Med. Parasitol.*, **52**, 391-401.

Soltys, M.A. (1955). Studies on resistance to *Trypanosoma congolense* developed by Zebu cattle treated prophylactically with Antrycide Pro-salt in an enzootic area of East Africa. *Ann. Trop. Med. Parasitol.*, **49**, 1-8.

Soltys, M.A. (1957a). Immunity in trypanosomiasis. I. Neutralization reaction. *Parasitol.*, **47**, 375-389.

Soltys, M.A. (1957b). Immunity in trypanosomiasis. II. Agglutination reaction with African trypanosomes. *Parasitol.*, **47**, 390-395.

Sones, K.R., Holmes, P.H. and Urquhart, G.M. (1989). Interference between drug-resistant and drug-sensitive stocks of *Trypanosoma congolense* in goats. *Res. Vet. Sci.*, **47**, 75-77.

Srivastava, R.V.M. and Ahluwalia, S.S. (1973). A note on the efficacy of homidium bromide and pyrithidium bromide against *Trypanosoma evansi* infection in the dog. *Vet. Rec.*, **92**, 260.

Stephen, L.E. (1958). Suramin complexes. IV. Ethidium bromide complex: a large-scale laboratory trial of its prophylactic activity in cattle. *Ann. Trop. Med. Parasitol.*, **52**, 417-426.

Stephen, L.E. (1962). Suramin complexes. X. Comparison of prophylactic activity of Prothidium-suramin complex and Prothidium bromide in West African Zebu cattle. *Ann. Trop. Med. Parasitol.*, **56**, 406-414.

Stephen, L.E. (1966). Pig trypanosomiases in tropical Africa. Commonwealth Bureau of Animal Health, Review Series No. 8, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks., U.K.

Stephen, L.E. (1970). Clinical manifestations of the trypanosomiasis in livestock and other domestic animals. In: The African Trypanosomiases. Ed. H.W. Mulligan., pp. 774-794. Allen & Unwin, London.

Stephen, L.E. (1986). Trypanosomiasis a Veterinary prespective. Pergamon Press. Oxford. New York. Toronto.

Stephen, L.E. and Gray, A.R. (1960). Suramin complexes. VI. The prophylactic activity of Antrycide-suramin complexes and Antrycide chloride against *Trypanosoma simiae* in pigs. *Ann. Trop. Med. Parasitol.*, **54**, 493-507.

Stephen, L.E. and Williamson, J. (1958). Suramin complexes. V. Ethidium complex. Attempts to overcome the injection site reaction in cattle. *Ann. Trop. Med. Parasitol.*, **52**, 427-442.

Strickland, G.T., Voller, A., Pettit, L.E. and Fleck, D.J. (1972). Immunodepression associated with concomitant toxoplasma and malarial infections in mice. *J. Infec. Dis.*, **126**, 54-60.

Stuart, K. (1983). Mini-review. Kinetoplast DNA, mitochondrial DNA with a difference. *Mol. Biochem. Parasitol.*, **9**, 93-104.

Suliman, H.B. and Feldman, B.F. (1989). Pathogenesis and aetiology of anaemia in trypanosomiasis with special reference to *T. brucei* and *T. evansi*. *Protozool. Abst.*, **13**, 37-45.

Suryanarayana, C., Gupta, S.L. and Singh, R.P. (1986). Studies on clinico-haematological changes in donkeys experimentally infected with *Trypanosoma evansi*. *Indian Vet. Med. J.*, **10**, 199-203.

Tager-Kagan, P., Itard, J. and Clair, M. (1989). Essai de l'efficacite du CymelarsanND sur *Trypanosoma evansi* chez le dromedaire. *Rev. Elev. Med. Vet. Pays Trop.*, **42**, 55-61.

Terry, R.J. (1976). Immunity to African trypanosomiasis. In: Immunology of Parasitic Infections. Eds. S. Cohen & E.H. Sadun., pp. 203-221. Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne.

Tobie, E.G. and von Brand, T. (1953). The development of arsenic resistance in *T. gambiense* and its influence on parasitaemia. *J. Infec. Dis.* **92**, 132-138.

Toure, S.M. (1973). Notes on the trypanocidal activity of isometamidium administered intravenously. *Bull. Epizoot. Dis. Afr.*, **21**, 1-3.

Trail, J.M.C., Sones, K., Jibbo, J.M.C., Durkin, J., Light, D.E. and Murray, M. (1985). Productivity of Boran cattle maintained by chemoprophylaxis under trypanosomiasis risk. International Livestock centre for Africa (ILCA). Addis Ababa, Ethiopia. Research Report No **9**.

Umeda, M., Sugiyama, M. and Isoda, M. (1988). Experimental *Trypanosoma evansi* infection in Japanese field vole (*Microtus montebelli*) - histopathological study. *Protozool. Abst.*, **12**, [2315].

Urquhart, G.M. and Holmes, P.H. (1987). African trypanosomiasis. In: Immunology, Immunopathology and Immunoprophylaxis. (Ed.) E.J.L. Soulsby. Vol.III: Protozoa. pp. 1-23. Boca Raton, Florida, U.S.A., CRC Press Inc.,

Vale, G.A., Bursell, E. and Hargrove, J.W. (1985). Catching-out the tsetse fly. *Parasitol. Today.*, **1**, 106-110.

Van Der Ploeg, L.H.T., Valerio, D., De Lange, T., Bernards, A., Borst, P. and Grosveld, G.F. (1982). An analysis of cosmid clones of nuclear DNA from *Trypanosoma brucei* shows that the genes for variant surface glycoproteins are clustered in the genome. *Nucleic Acids Res.*, **10**, 5905-5923.

Van Hoeve, K. and Cunningham, M.P. (1964). Prophylactic activity of Berenil against trypanosomes in treated cattle. *Vet. Rec.*, **76**, 260.

Van Hoof, L.M.J.J. (1947). Observations on trypanosomiasis in the Belgian Congo. *Trans. R. Soc. Trop. Med. Hyg.*, **40**, 728-754.

Verma, B.B. and Gautam, O.P. (1979). Observations on pathological changes in experimental surra in bovines. *Indian Vet. J.*, **56**, 13-15.

Verma, B.B., Gautam, O.P. and Malik, P.D. (1973). Diminazene aceturate in the treatment of experimental *Trypanosoma evansi* infection in buffalo calves. *Vet. Rec.*, **93**, 465-467.

Vickerman, K. (1969). On the surface coat and flagellar adhesion in trypanosomes. *J. Cell Sci.*, **5**, 163-193.

Vickerman, K. and Barry, J.D. (1982). African trypanosomiasis. In: Immunology of Parasitic Infections. Eds. S. Cohen & K.S. Warren., pp. 204-260. Blackwell Scientific Publication. Oxford. London.

Vickerman, K. and Tetley, L. (1979). Biology and ultrastructure of trypanosomes in relation to pathogenesis. In: Pathogenicity of Trypanosomes. Eds. G.J. Losos, A. Chouinard, pp. 23-31. IDRC (Ottawa),

Watkins, T.J. and Woolfe, G. (1952). Effect of changing the quaternizing group on the trypanocidal activity of dimidium bromide. *Nature*, **169**, 506.

Weitz, B.G.F. (1970). Infection and resistance. In: The African Trypanosomiases. Ed. H.W. Mulligan., pp. 95-124. Allen & Unwin., London.

Wellems, T.E. (1991). Molecular genetics of drug resistance in *Plasmodium falciparum* malaria. *Parasitol. Today* **7**, 110-112.

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

Whitelaw, D.D., Bell, I.R., Holmes, P.H., Moloo, S.K., Hirumi, H., Urquhart, G.M. and Murray, M. (1986). Isometamidium chloride prophylaxis against *Trypanosoma congolense* challenge and the development of immune responses in Boran cattle. *Vet. Rec.*, **118**, 722-726.

Whiteside, E.F. (1960). The control of animal trypanosomiasis in Kenya. Symposium on Animal Trypanosomiasis, Luanda, pp. 81-116. Pub. 45, Commission for Technical Co-operation South of the Sahara, London.

Whiteside, E.F. (1962a). The control of cattle trypanosomiasis with drugs in Kenya: methods and costs. *East Afr. Agric. Fores. J.*, **28**, 67-73.

Whiteside, E.F. (1962b). Interaction between drugs, trypanosomes and cattle in the field. In: Drugs, Parasites and Hosts (Eds. L.G. Goodwin, R.H. Ninimo-Smith.), pp. 117-132. Churchill (UK).

Whiteside, E.F. (1963). A strain of *Trypanosoma congolense* directly resistant to Berenil. *J. Comp. Path.*, **73**, 167-175.

Wilde, J.K.H. and Robson, J. (1953). The effect against *T. congolense* in Zebu cattle of three new phenanthridine compounds. *Vet. Rec.*, **65**, 49-51.

Williams, R.O. (1979). The genetic basis of antigenic variation. In: Pathogenicity of Trypanosomes. Eds. G. Losos and A. Chouinard., pp. 46-48. IDRC Ottawa, Canada.

Williamson, J. (1957). Suramin complexes I. Prophylactic activity against *Trypanosoma congolense* in small animals. *Ann. Trop. Med. Parasitol.*, **51**, 440-456.

Williamson, J. (1962). Chemotherapy and chemoprophylaxis of African trypanosomiasis. *Exp. Parasitol.*, **12**, 274-322.

Williamson, J. (1970). Review of chemotherapeutic and chemoprophylactic agents. In: The African Trypanosomiases, ed. H. W. Mulligan. pp. 125-221. Allen & Unwin (London),

Williamson, J. (1976). Chemotherapy of African trypanosomiases. *Trop. Dis. Bull.*, **73**, 531-542.

Williamson, J. and Desowitz, R.S. (1956). Prophylactic activity of suramin complexes in animal trypanosomiasis. *Nature*, **177**, 1074-1075.

Wilson, A.J. (1969). Value of the indirect fluorescent antibody test as a serological aid to diagnosis of *Glossina*-transmitted bovine trypanosomiasis. *Trop. Anim. Hlth. Prod.*, **1**, 89-95.

Wilson, A.J. and Cunningham, M.P. (1970). Immunological aspects of bovine trypanosomiasis. II. Antigenic variation in a strain of *Trypanosoma congolense* transmitted by *Glossina morsitans*. *Trans. R. Soc. Trop. Med. Hyg.*, **64**, 812-821.

Wilson, A.J., Le Roux, J.G., Paris, J. Davidson, J. and Gray, A.R. (1975). Observations on a herd of beef cattle maintained in a tsetse area. I. Assessment of chemotherapy as a method for the control of trypanosomiasis. *Trop. Anim. Hlth. Prod.*, **7**, 187-199.

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

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

Wilson, S.G. and Fairclough, R. (1953). A preliminary note on treatment of *T. congolense* infections with ethidium bromide in cattle in Kenya colony. *Vet. Rec.*, **65**, 201-202.

Woo, P.T.K. (1970). The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Trop.*, **27**, 384-386.

Woo, P.T.K. (1977). Salivarian trypanosomes producing disease in livestock outside Sub-Saharan Africa. In: Parasitic Protozoa. Vol. 1., Taxonomy, Kinetoplastids, and Flagellates of Fish. Ed. Julius P. Kreier, Academic Press, New York, San Francisco, London.

Ye, P.Z. (1989). Study of immunosuppression in *Trypanosoma evansi* infections in mice. *Protozool. Abst.*, **13**, [378].

Yorke, W., Murgatroyd, F. and Hawking, F. (1931). Studies in chemotherapy. V: preliminary contribution on the nature of drug resistance. *Ann. Trop. Med. Parasitol.*, **25**, 351-358.

Yorke, W., Murgatroyd, F. and Hawking, F. (1932). Studies in chemotherapy. VIII: Comparison of strains of *T. rhodesiense* made resistant to various arsenicals and antimonials, to Bayer 205, and acriflavine, respectively. *Ann. Trop. Med. Parasitol.*, **26**, 577-586.

Yorke, W., Murgatroyd, F. and Hawking, F. (1933). Studies in chemotherapy. X: Further observations on the transmissibility of tryparsamide-resistance by Glossina. *Ann. Trop. Med. Parasitol.*, **27**, 157-178.

Zakrzewski, S.F. (1973). Biochemical bases of drug resistance in protozoa. In: Drug Resistance and Selectivity., pp. 185-469. Academic Press. New York. London.

Zelledon, R. (1974). Epidemiology, modes of transmission and reservoir hosts of Chagas' disease. In: Trypanosomiasis and Leishmaniasis with Special Reference to Chagas' Disease. Ciba Foundation Symposium 20 (New series)., pp. 51-76. Associated Scientific Publishers. Amsterdam. London. New York.

Zelleke, D., Kassa, B. and Abebe, S. (1989). Efficacy of RM110, a novel trypanocide, in the treatment of *Trypanosoma evansi* infections in camels. *Trop. Anim. Hlth. Prod.*, **21**, 223-226.

Zweygarth, E. and Kaminsky, R. (1990). Evaluation of an arsenical compound (RM110, mel Cy, Cymelarsan^{R}) against susceptible and drug-resistant *Trypanosoma brucei* and *T.b. evansi*. *Trop. Med. Parasitol.*, **41**, 208-212.