CHEMOTHERAPEUTIC CONTROL OF
BOVINE TRYPANOSOMIASIS:
THE PRESENT POSITION AND PROSPECTS FOR THE FUTURE

VICTOR HUGO GUZMAN

M.Sc.
DEPARTMENT OF TROPICAL ANIMAL HEALTH
FACULTY OF VETERINARY MEDICINE
UNIVERSITY OF EDINBURGH
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ABSTRACT

The literature on the chemotherapeutic control of bovine trypanosomiasis has been reviewed in relation to the existing drugs and some of the new drug developments of potential use in veterinary medicine. Emphasis has been given particularly to the chemical control of the disease in cattle due to *T. vivax*, *T. congolense* and *T. brucei* infections.

Brief mention is made of aspects of the disease in cattle which are relevant to the main subject under consideration in this dissertation. This section deals with basic information about the disease itself including its geographic distribution and economic importance, trypanosome species involved and their mode of transmission, pathogenic effects and current diagnostic procedures. It is concluded that pathogenesis and diagnosis play an important role in the chemotherapeutic and chemoprophylactic control of cattle trypanosomiasis.

Undoubtedly, some aspects of the pathogenesis of the disease are influencing the efficacy of antitrypanosomal drug action. In view of the known different body distribution of each trypanosome subgenus, comparative studies of the pharmacokinetics of the existing and new trypanocidal drugs are urgently needed. In relation to
diagnosis a better identification of the disease condition and pathogenic trypanosomes in the field, could contribute to more effective drug treatment and possibly to the avoidance of drug resistance development.

Description of the existing trypanocidal drugs is included with special reference to their historical development, drug characteristics, methods of application and use, costs and benefits, problems of drug resistance and drug use improvement.

It is argued that the small number of trypanocides which have been available for the past forty years for the treatment of cattle trypanosomiasis could also have influenced the widespread development of drug resistance. In addition, the successful build up of immunity in already infected animals when using the available drugs could be exploited in the control of the disease in cattle while alternative drugs are being produced. With regard to drug residues, determination of the distribution and levels of drugs in animal products and carcasses needs to be carried out on the commonly known and used trypanocidal drugs.

With respect to new drug developments, a description is made of the economic implications, new approaches, new leads and prospects for the future. Some outstanding achievements in this complex area of
research have particularly concerned the *T. brucei* group of trypanosomes, considered normally of low pathogenicity for cattle. Thus, the justification for continuing this tendency to research on *T. brucei* in seeking new drugs for animal trypanosomiasis requires reappraisal of the real significance and comparability of the disease in man and the disease in cattle. It might be better to place greater emphasis in drug development work on infections with *T. vivax* and *T. congolense*.

Finally, it is important to emphasize in this review the reduced availability of some trypanocidal drugs such as Antrycide compounds and Samorin, which are not at present being manufactured. To face this problem a strong collaboration between the pharmaceutical industry and the international bodies is needed to develop new drugs for the chemotherapeutic control of cattle trypanosomiasis.
INTRODUCTION

Trypanosomiasis is one of the most important and widespread diseases of domestic animals and is present in almost all tropical and subtropical areas of the world. In Africa it is a major disease in cattle and represents the main obstacle to the agricultural development of parts of this continent. In the control of this disease action has always been taken against the parasites, one of the various components of the epizootiological complex of the infection. This action has consisted of the use of trypanocidal drugs on at risk and infected animals, utilizing both curative and prophylactic methods.

Chemotherapy is the most widely used method of trypanosomiasis control and plays an important role in combatting the threat of the disease to the livestock industry. In Africa, where the cattle population at risk is about 80 million and the disease has great economic importance, nearly 8 million doses of trypanocides are used every year.

However, chemotherapy at present is dependent on a small number of drugs, some of which are of reduced availability and most of which have serious disadvantages of side effects and drug resistance.

During the past two decades, no new drugs have been
introduced, mainly due to the fear of the pharmaceutical industries of uncertain return on the high investments needed in their development. In addition, some developing countries have small drug budgets and the widespread development of drug resistance to the existing drugs has left only one trouble-free drug for the treatment of cattle trypanosomiasis. Thus, there is undoubtedly an urgent need for new and better drugs which can be relied upon to afford effective therapeutic control of this disease.

In this dissertation an attempt is made to present up to date information about the chemotherapy of bovine trypanosomiasis based on a review of existing data relating to the present situation, drug developments and approaches for the future. Preference has been given to more recent publications which deal with the subject in a comprehensive manner and contain also references to earlier work.

It is the author's hope that this information may be useful to the workers interested in this field of animal trypanosomiasis.
CATTLE TRYPANOSOMIASIS

1. The Disease

Trypanosomiasis is a vector borne disease caused by species of flagellated protozoa belonging to the genus Trypanosoma which live in the blood of their mammalian hosts but may also invade other body tissues and fluids.

In trypanosomiasis of domestic animals the principal reservoir is wild game (Stephen, 1975) and Hoare (1972), has pointed out that wild mammals are the source of the pathogenic trypanosomes from which man and animals acquire their infections.

Several species of trypanosomes are responsible for serious diseases in mammals, such as nagana in animals and sleeping sickness in man. The disease in domestic animals is particularly important in cattle. Different varieties of cattle respond differently to the infection, those most severely affected being the improved breeds.

Trypanosomiasis is observed in many countries in Africa and in the northern areas of South America. In the African continent pathogenic trypanosomes for domestic animals follow the distribution of tsetse flies (species of Glossina), which are the main vectors, between latitudes 15°N and 15°S of the equator (Richardson and Kendall, 1963). This area includes over 10 million square
kilometers (Finelle, 1974a). In the absence of Glossina T. vivax has managed to establish itself in West Indies, Central and South America and Mauritius, far beyond its original area of distribution in tropical Africa. T. vivax infections in cattle have been described in several South American countries (Wells, Betancourt and Page, 1970; Clarkson, McCabe and Colina, 1971; Shaw and Lainson, 1972) and Wells, Betancourt and Ramirez (1977) have postulated T. vivax infection as extending from at least 12°N down to the tropic of Capricorn.

Trypanosoma evansi, the causative agent of the disease known as surra is widely distributed in countries with hot and warm climates. Surra occurs in a number of domestic animals, as well as in some wild ones. It is common in bovines (cattle and buffaloes) but the infection is usually subclinical particularly in those countries where surra has been enzootic for a long time. For this reason, cattle may play an important epizootiological role as a source of infection for more susceptible animals, especially in South America (Hoare, 1972; Ramirez, Wells and Betancourt, 1979).

Trypanosomiasis is a restraint on the livestock industry in several countries of Africa, the Middle East, India and Central and South America. In Africa, animal trypanosomiasis affects 7 million square kilometers of the continent which would otherwise be suitable for the
production of cattle and other livestock (Finelle, 1974a). Trypanosomiasis results in poor cattle health and condition, loss of meat, milk, manure, hides, transport and labour (Stephen, 1975). These factors limit food production at a time when human populations and animal protein requirements in Africa are increasing. However, Ormerod (1976) has claimed that trypanosomiasis is a factor in the preservation of African soil against erosion, preventing in this way large scale cattle ranching throughout the tsetse fly belt.

Colombia is the best documented of all South American countries in relation to the effect of trypanosomiasis in cattle, mainly due to T. vivax infections. This disease, known as "Secadera" in Colombia and Venezuela, produces losses because of deaths of untreated animals, suppression of milk flow in lactating animals, deaths of calves directly from the disease or from lack of milk, abortions and loss of weight (Betancourt, 1977). However, the relationship of Old and New World T. vivax isolations, the true geographic distribution of the infection in the absence of tsetse, and the economic significance to the livestock industry have yet to be assessed (Clarkson, 1976).

2. The Trypanosomes

The trypanosomes of importance in cattle have been
classified on the basis of their morphology, life cycle, and other biological characteristics (Hoare, 1972). Three subgenera have been assigned to the Salivaria (inoculative infection): Duttonella, Nannomonas and Trypanozoon. In the subgenus Duttonella the important species is *T. vivax*. In the subgenus Nannomonas it is *T. congolense*. The subgenus Trypanozoon contains *T. brucei*, one subspecies of which, *T. b. brucei*, produces disease in cattle and other animals, and *T. evansi* which infects cattle and other animals. Within the Stercoraria (contaminative infection) only the subgenus Megatrypanum is important from the cattle viewpoint since it includes *T. theileri*, a species infecting cattle, but which is normally non-pathogenic.

In most cases of trypanosomiasis in cattle the infection is due to either *T. congolense* or *T. vivax*; occasionally *T. brucei*, *T. theileri* and *T. evansi* are encountered. Multiple infections (*T. congolense*, *T. vivax* and *T. brucei*) in the field have also been reported (Willett, 1970).

3. Transmission of Trypanosomes

The epizootiology of bovine trypanosomiasis is very complex, for it depends on three biological elements: the host, the vector and the parasite. As already mentioned, whereas only one species of Salivarian trypanosomes affects cattle in South America there are four species of
importance in cattle in Africa and all of them are transmitted by insects in different ways.

Cyclical transmission occurs through tsetse flies (Glossina spp.) that are strictly blood feeders living exclusively in tropical Africa south of the Sahara within the latitudes 15°N and 29°S (Soltys and Woo, 1977). There are about 22 species of the genus Glossina classified in three groups, namely Fusca, Palpalis and Morsitans, broadly associated with their preferred habitats: forest, riverine terrain and savannah. Animals are infected with trypanosomes by the bite of an infected tsetse fly. Tsetse flies become infected when they feed on an infected animal host. The parasite survives and multiplies in the fly, which becomes capable of transmitting trypanosomes to susceptible animals. This form of transmission occurs in cattle with T. congoense, T. vivax and T. brucei.

Mechanical transmission is effected by both tsetse flies and other blood-sucking insects such as biting flies of the family Tabanidae (horse flies) and Stomoxys spp. In this case the parasite is transferred from one host to another without undergoing multiplication and the parasite survives for only a few hours in the vector. This form of transmission is common for T. evansi but may also be possible for trypanosomes usually transmitted cyclically by Glossina. Wells (1972), for example, reviewed the role of mechanical transmission in relation to T. vivax and has
questioned its importance in the epidemiology of the disease in Africa and South America. Hoare (1972), has noted that there is evidence for T. congolense in Africa to be transmitted mechanically in tsetse-free areas lying either within or outside the tsetse-belt, where the vectors are chiefly Tabanid flies.

4. Pathogenesis

Trypanosomiasis in cattle is characterized by its chronicity, intense anaemia and emaciation. The clinical picture depends upon several factors, such as the strain of parasite, its virulence and the resistance of the host. Most information, however, is about symptoms and little is understood of the pathogenesis which varies from parasite to parasite, host to host, individual to individual (Cox, 1979).

Mild chronic infections or acute, fatal disease are attributed to T. congolense and T. vivax. T. brucei is usually considered a low grade pathogen for cattle although this generalization is now questioned by Moulton and Sollod (1976). T. theileri and T. evansi are not pathogenic trypanosomes for cattle and Ilemobade (1971), draw attention to the fact that cattle can harbour T. evansi and consequently may act as a reservoir host.

According to Losos and Ikede (1972), the different species of trypanosomes induce different syndromes related
9.

to the distribution of the parasites in the animals. They have considered two groups: T. congolense and T. vivax constitute the hematic group being plasma parasites and affecting the host by causing anaemia, an important clinical feature of trypanosomiasis in cattle attributed to inhibition of haemopoiesis and/or to haemolysis. T. brucei and the related trypanosomes constitute the humoral group dwelling in the organic fluids other than the blood, without relationship between the level of parasitaemia and severity of clinical signs. Invasion of different organs and accompanying inflammatory reactions are the features of T. brucei infections (Ikede, 1975).

However, there is now ample evidence showing that T. vivax (Büngener and Mehlitz, 1977) and T. congolense (Luckins and Gray, 1978), considered to be strict plasma parasites, have also an extravascular localization. This feature is very important when thinking in the diagnosis, immunology and chemotherapeutic control of bovine trypanosomiasis.

5. Diagnosis

A provisional diagnosis of bovine trypanosomiasis is sometimes possible based on the history of the affected animals, the geographical incidence of the disease and the clinical signs of the infection. Definite diagnosis can be established only when pathogenic trypanosomes are found in
the host's blood or other fluids and identified.

The parasitological techniques used for the diagnosis of trypanosomiasis have been reviewed and discussed by Molyneux (1975). Standard trypanosome detection methods (STDM) combine wet, thin and thick blood films with rodent inoculation and concentration methods. If a thick blood film is found positive, then the equivalent stained thin blood film should be examined carefully for parasites to identify the species (Killick-Kendrick, 1968). It seems, however, that the most reliable approach for the diagnosis of trypanosomiasis is to use a combination of parasitological techniques (Wilson, Paris and Dar, 1975). At the present time there are no serological tests for routine diagnosis of trypanosomiasis but many of them are under study and development.

Wells et al. (1970) and Betancourt (1977), have pointed out that the diagnosis of cattle trypanosomiasis mainly in South America has been hindered by the lack of appropriate diagnostic techniques. Leeflang (1978), has claimed that diagnosis is an essential tool especially under field conditions, where it contributes to evaluation of the clinical condition of the cattle and enables more accurately directed drug treatment.
CONTROL OF TRYPANOSOMIASIS

In the control of animal trypanosomiasis action is possible on three biological elements: parasites, host animals and vectors. It is intended to deal here only with the therapeutic control of the parasites in cattle.

1. Historical Background

The story of trypanocides evolution began over a century ago with the treatment, without knowledge of the causative agent, of diseased domestic animals with inorganic arsenical compounds such as potassium arsenite, arsenious oxide or sodium arsenite (Williamson, 1970a). These drugs were suggested because of their tonic effect rather than for their parasiticidal effect.

Prior to 1938 and the discovery of the phenanthridinium compounds the only satisfactory drugs available were suramin for T. brucei group infections and tartar emetic for T. vivax and T. congolense. Although tartar emetic did not induce drug resistance, it had limitations for use on a large scale because intravenous injections were necessary to avoid local dermonecrotic reactions and repeated dosages were required.

Mass treatment of cattle trypanosomiasis, however, became possible after the discovery of the trypanocidal action of phenanthridinium salts in 1938. The first two
drugs introduced into field use in the 1940's were Phenidium chloride and Dimidium bromide. Both of them were later discarded because of resistance development, photosensitization and delayed toxicity. Ethidium (homidium), a much less toxic version of Dimidium bromide appeared in 1952. At the same time several diaminoquinaldines were investigated and chemical manipulations of members of this group led to the discovery of Antrycide (quinapyramine) that came into use in Africa in 1949. Antrycide in the form of the "pro-salt" (quinapyramine chloride plus quinapyramine methyl sulphate) was the first active prophylactic drug available for cattle infections of T. vivax and T. congolense. The diamidines came on the scene in 1955, Berenil (diminazene aceturate) being the most important curative drug of this group. Examples of trypanocidal hybrid molecules with curative and prophylactic characteristics are Prothidium (pyrithidium bromide) and Samorin (isometamidium chloride) which appeared in 1956 and 1960, respectively.

2. **Drugs Available**

The trypanocides currently available for the treatment of bovine trypanosomiasis are listed below. Although there are recent indications that ICI (Imperial Chemical Industries Ltd.) have discontinued the manufacture of Antrycide compounds, they have been included in this list and following discussion because residual
stocks of drugs are still in use.

(a) Phenanthridinium compounds
   (i) Homidium bromide B. vet. C. (Ethidium)
   (ii) Homidium chloride (Novidium)
   (iii) Pyrithidium bromide B. vet. C. (Prothidium)
   (iv) Isometamidium chloride (Samorin, Trypamidium)

(b) Antrycide (Quinapyramine B. vet. C.) salts
   (i) Quinapyramine methyl sulphate (Antrycide methyl sulphate)
   (ii) Quinapyramine chloride (Antrycide chloride)
   (iii) Quinapyramine (prophylactic) B. vet. C. (Antrycide pro-salt R. F.)

(c) Diamidine compounds
   (i) Diminazene aceturate B. vet. C. (Berenil, Ganaseg)

   Essentially, the trypanocidal drugs available are curative or prophylactic and it is intended to give here a brief account only of the major compounds which are in use at the present time.

(a) Phenanthridinium compounds

   This important class of trypanocides shows high activity against T. vivax, T. congolense and T. brucei infections in cattle.
(i) Ethidium and Novidium

As already mentioned, in 1952 Dimidium bromide was replaced by Ethidium bromide (Wilde and Robson, 1953) which is a much less toxic compound. Ethidium bromide is readily soluble only in hot or boiling water, but this practical disadvantage was overcome by the use of the chloride salt which is soluble in cold water (Williamson, 1970a). These two salts constitute the homidium compounds namely Ethidium (Boots Pure Drug Co. Ltd.) and Novidium (May and Baker Ltd.), which differ only in the nature of the salt ion being bromide and chloride, respectively. Soltys and Woo (1977), have pointed out that the mode of action of Ethidium bromide is by inhibition in the trypanosome of lysosomal and DNA synthesis. They are used as curative or prophylactic drugs at a dose rate of 1.0 mg/kg given as a 1 to 2% w/v aqueous solution intramuscularly (Stephen, 1970), but subcutaneous injection into the dewlap is also recommended. They may cause local tissue damage if given subcutaneously and liver damage only at high dosage (Williamson, 1970a). Ethidium has been in extensive use as a curative drug for *T. vivax* and *T. congolesense* in cattle but its protective action lasts only about 5 weeks (Whiteside, 1962a).

Homidium salts have been used in Nigeria for the therapeutic control of trypanosomiasis in cattle since 1954 (MacLennan, 1968). They were introduced soon after
Antrycide and rapidly became the drugs preferred, being widely used in the field. However, *T. congolense* (Jones-Davies and Folkers, 1966), *T. vivax* (Ilemobade and Buys, 1970) and also *T. brucei* (Mwambu, 1975) developed resistance against both, homidium bromide and homidium chloride. Because of this resistance a change from the use of these compounds to Berenil was made in the chemotherapy of bovine trypanosomiasis in Northern Nigeria in 1965. Soltys and Woo (1977), have noted that Ethidium bromide presents cross-resistance to Prothidium and partly to Antrycide and Samorin. Whiteside (1960, 1962a), has proposed a possible alternating use with Berenil as "sanative pair" to reduce incidence of drug resistance.

(ii) Prothidium and Samorin

More recent additions to the phenanthridinium compounds include Prothidium (Boots Pure Drug Co. Ltd.) and Samorin (May and Baker Ltd.) or Trypamidium (Specia). Prothidium combines a pyrimidine ring of Antrycide with an amino group of a phenanthridinium molecule and Samorin is a cross between a diamidine and a phenanthridinium compound, both molecular hybrids having curative and prophylactic characteristics (Williamson, 1970a). Their mode of action on the trypanosomes is commented on by Soltys and Woo (1977). Prothidium interferes with nucleic acid synthesis and Samorin has an effect on the lysosomes.

Prothidium is soluble only in hot water and is given
as a 2% solution at the rate of 0.2 to 0.4 mg/kg by intramuscular or subcutaneous injection as a curative drug, and at a dosage of 2 mg/kg intramuscularly as a prophylactic drug (Soltys and Woo, 1977). It is indicated against *T. vivax* and *T. congoense* infections.

Samorin is soluble in cold water and it is given as a 1 to 2% aqueous solution at a dose rate of 0.25 to 1.0 mg/kg intramuscularly as a curative drug, and at a dose of 0.5 to 1.0 mg/kg by intramuscular injection as a prophylactic drug (Finelle, 1973a,b). It is indicated against *T. vivax*, *T. congoense* and *T. brucei* infections.

The toxic effects of Prothidium and Samorin are similar to those described above for Ethidium bromide (Williamson, 1970a). The dermonecrotic properties of the drugs have to be avoided by deep intramuscular injection. However, Touré (1973) has reported that intravenous injection of Samorin (0.5 mg/kg) is well tolerated in cattle infected with *T. vivax* and *T. brucei*. In addition, Prothidium and Samorin do not induce severe local reactions at the injection site when used for prophylaxis in cattle complexed with suramin (Williamson and Desowitz, 1956; Braide, 1974). Prothidium and Samorin provide a protection between 3 and 6 months, depending on the intensity of tsetse-fly challenge (Finelle, 1973b). According to Touré (1973), when Samorin is used intravenously the protection does not last longer than 98
days, but this method is still adequate for the protection of trade cattle.

Disadvantage of these drugs is the appearance of chemoresistant trypanosomes. Prothidium and Samorin show direct resistance and cross-resistance to one another and also to Ethidium bromide and partly to Antrycide (Soltys and Woo, 1977). Finelle (1975), has described the associated use of Samorin and Berenil, which do not display cross-resistance, like chemoprophylactics. Williamson (1976a), has drawn attention to the fact that since 1967 Samorin has been the sole prophylactic in areas of high challenge.

(b) **Antrycide salts**

Three forms of Antrycide (quinapyramine B. vet. C.) with curative and prophylactic characteristics have been used in cattle against *T. vivax*, *T. congolense*, *T. brucei* and *T. evansi*. They are Antrycide methyl sulphate, Antrycide chloride and Antrycide pro-salt R.F., a mixture of the chloride and methyl sulphate salts (Imperial Chemical Industries Ltd.). The mode of action of Antrycide on trypanosomes has been recently described by Soltys and Woo (1977). It interferes with nucleic acid synthesis and produces inactivation of ribosomes. The main disadvantage of Antrycide is that it develops direct and cross-resistance to the currently available trypanocides used in
cattle. Cross-resistance to Prothidium, Samorin and partly to Ethidium and Berenil has been noted recently (Soltys and Woo, 1977).

(i) Quinapyramine methyl sulphate

This salt is freely soluble in cold water forming a stable solution which reaches a therapeutic level rapidly after injection. It is, however, readily excreted and it has been used essentially as a curative drug in cattle against *T. vivax*, *T. congolense*, *T. brucei* and *T. evansi*. In the original trials it was found that *T. vivax* needed to be controlled a higher dose compared to *T. congolense*. The drug is excellent for the control of *T. evansi* in cattle and at the present is mainly used to control humoral trypanosomes (*T. brucei* group) in other domestic animals. The recommendations for Antrycide methyl sulphate are 2.2 to 4.4 mg/kg given as a 10% w/v aqueous solution, subcutaneously (Gray and Roberts, 1971a).

After treatment Antrycide methyl sulphate possibly allows build up of antitrypanosomal immunity for up to two weeks. The therapeutic dose is free of side effects but may produce a painful local reaction at site of injection and occasionally systemic toxicity with curare-like properties: salivating, restlessness and muscle tremors (Williamson, 1970a). Antrycide methyl sulphate was used in Northern Nigeria from 1951 to 1963. As a result of widespread development of resistance its use and
effectiveness declined and it was officially withdrawn from field use in 1963 (MacLennan, 1968).

(ii) Quinapyramine chloride

Antrycide chloride is relatively insoluble in cold water, and after subcutaneous injection is retained in the tissues, being poorly absorbed. It has been used for prophylaxis in pigs and in cattle is used usually combined with the methyl sulphate under the common name of Antrycide pro-salt. Antrycide chloride because of its low solubility is virtually non-toxic (Williamson, 1970a).

(iii) Quinapyramine prophylactic

Antrycide pro-salt appeared as a third compound because it became clear that neither the methyl sulphate nor the insoluble chloride alone would be useful for prophylaxis. The former was excreted too rapidly and the latter, although persistent, did not give blood levels high enough to cure an established infection. Since 1958 the current formulation was issued for field trial, being a mixture 2:3 w/w of the chloride and methyl sulphate salts in cold water.

Antrycide pro-salt is used in cattle at a dose of 7.4 mg/kg, subcutaneously (Williamson, 1970a). The solution for treatment contains 3.5g of Antrycide pro-salt per 15 ml of cold water (Finelle, 1973b). Its prophylactic use against T. vivax, T. congolense and T. brucei depends on
the slow rate of absorption of the chloride, which forms depots of drug in the subcutaneous tissues. Later on, these pockets of drug become encapsulated with fibrous and eventually calcified tissues. The length of protection is considered to be from 2 to 3 months, depending on challenge (Finelle, 1973b). Extensive residual immunity was claimed to develop in infected cattle after Antrycide pro-salt prophylaxis (Soltys, 1955).

(c) Diamidine compounds

Within the aromatic diamidine compounds Pentamidine and Stilbamidine have not so far been used very extensively as trypanocides in veterinary medicine. The only compound in this class which has come into accepted field use is Berenil.

(i) Berenil

Diminazene aceturate (Berenil, Farbwerke Hoechst A.G.; Ganaseg, Squibb), has been used since 1955 in Africa like a curative drug against *T. vivax*, *T. congolense*, *T. brucei* and *T. evansi* infections in cattle. Its trypanocidal effect is probably due to the inhibition of DNA synthesis acting also on cell division (Soltys and Woo, 1977). Berenil is soluble in cold water and at a dose rate of 3.5 mg/kg of a 7% solution is given to cattle by intramuscular or subcutaneous injection (Finelle, 1973a). *T. brucei* infections are less susceptible to Berenil and require a
higher dose (7 mg/kg).

At present Berenil appears to be the drug of choice in cattle and has the following remarkable characteristics: freedom from local or systemic toxicity, high therapeutic ratio, activity against many resistant strains and apparently low potential for the induction of drug resistance.

Berenil is degraded metabolically and excreted rapidly, usually within twenty four hours, and this probably explains its lack of prophylactic activity and its small tendency to give rise to resistant trypanosome strains (Williamson, 1970a). Although this drug has not yet induced any serious degree of drug resistance, resistant strains of both *T. congolense* (MacLennan and Jones-Davies, 1967) and *T. vivax* (Jones-Davies, 1967) have been reported in Northern Nigeria. Cross-resistance in Berenil resistant trypanosomes, however, has not yet been described (Soltys and Woo, 1977).

It is important to include some notes about the distribution and residues in animal products for human consumption of the drugs described earlier, for the treatment of trypanosomiasis in cattle. There are indications that these aspects are closely related to the pharmacology and toxicity of any particular drug. In addition, the drugs more prone to be retained in the carcases are those used for prophylactic purposes. This is
true for the phenanthridinium compounds and Antrycide, which after injection become either distributed in several organs and tissues or are localized at the injection site (Williamson, 1970a). On the contrary, Berenil is rapidly excreted through the kidneys, but it has been mentioned (Williamson, 1970a) that some degree of tissue retention, as yet unlocalized, must occur.

Records of the trypanocides used in South America against cattle trypanosomiasis are scarce, and the information included here is confined to Colombia. Since 1931 drugs such as tartar emetic, Antrycide and Berenil at the known therapeutic doses have been used against T. vivax infections in cattle (Wells et al., 1970).

In relation to the future availability of the existing drugs already mentioned, the homidium compounds and mainly Antrycide are currently less extensively used because of widespread drug resistance (Finelle, 1973a). The latest developed prophylactic drugs, Prothidium and Samorin, have not been as successful in the field as it was originally hoped (Williamson, 1970a) and Samorin is not at present being manufactured although it is still available as Trypamidium. Thus, Berenil is the only drug still fully available without some drawbacks for the treatment of bovine trypanosomiasis.
3. **Drug Treatment**

For more than forty years the use of trypanocidal drugs has been the most important way to control animal trypanosomiasis. As already mentioned the use of trypanocides has become widespread since the discovery of the phenanthridinium compounds. According to estimates nearly 8 million trypanocidal treatments are carried out every year in Africa, the greater number of them for combating trypanosomiasis in cattle (FAO, 1974).

(a) **Chemotherapy and chemoprophylaxis**

Under field conditions the control of bovine trypanosomiasis is chiefly dependent on two methods: chemotherapy and chemoprophylaxis. The former refers to the use of a trypanocide as a curative drug in infected animals, and the latter includes the treatment of animals at regular intervals to prevent infection. Animals under these control methods should be closely observed to detect the occurrence of serious adverse side effects and relapse infections.

Extensive reviews of the chemotherapy of trypanosomiasis have been given by Williamson (1970a, 1976a,b) and Newton (1974). The data concerning the use of the trypanocidal drugs described in detail earlier, are summarised in Table 1. Arguments for and against
### TABLE 1

Curative and prophylactic use of trypanocidal drugs in cattle.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Aqueous solution (% w/v)</th>
<th>Dose rate (mg/kg)</th>
<th>Route (activity)</th>
<th>Trypanosome species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethidium²</td>
<td>1-2 hot water</td>
<td>1</td>
<td>IM(C&amp;P)</td>
<td>T. vivax T. congolense T. brucei</td>
</tr>
<tr>
<td>Novidium³</td>
<td>1-2 cold water</td>
<td>1</td>
<td>IM(C&amp;P)</td>
<td>T. vivax T. congolense T. brucei</td>
</tr>
<tr>
<td>Prothidium²</td>
<td>2 hot water</td>
<td>0.2-0.4</td>
<td>IM(SC(C) IM(P)</td>
<td>T. vivax T. congolense T. brucei</td>
</tr>
<tr>
<td>Samorin³</td>
<td>1-2 cold water</td>
<td>0.25-1.0</td>
<td>IM (C) IM (P)</td>
<td>T. vivax T. congolense T. brucei</td>
</tr>
<tr>
<td>Antrycide methyl sulphate²</td>
<td>cold water</td>
<td>2.2-4.4</td>
<td>SC (C)</td>
<td>T. vivax T. congolense T. brucei T. evansi</td>
</tr>
<tr>
<td>Antrycide pro-salt⁴</td>
<td>3.5g/15ml cold water</td>
<td>7.4</td>
<td>SC (P)</td>
<td>T. vivax T. congolense T. brucei</td>
</tr>
<tr>
<td>Berenil⁵</td>
<td>7 cold water</td>
<td>3.5</td>
<td>IM(SC)</td>
<td>T. vivax T. congolense T. brucei T. evansi</td>
</tr>
</tbody>
</table>

1 IM= intramuscular injection; SC= subcutaneous injection.
3 May and Baker Ltd., Dagenham, England.
5 Farbwerke Hoechst A.G., Frankfurt, Germany.
C= curative.
P= prophylactic.
chemoprophylaxis and chemotherapy for the control of trypanosomiasis in animals including cattle, have been presented by Stephen (1970), MacLennan (1970) and Finelle (1973a,b). They have also made recommendations on the use of trypanocides and their implications and limitations.

(b) Costs and benefits

The presence of Glossina and the occurrence of trypanosomiasis in cattle are the main barriers to economic and agricultural development in large areas of tropical Africa. Eradication of the vector is essential to eliminate the risk of the disease, but technical difficulties in this field have led to the use of trypanocides as the only means to enable cattle to survive in these areas. Thus, chemotherapy is still the more practical and economical approach.

The costs and benefits of the chemotherapeutic control of African animal trypanosomiasis have been considered by Whiteside (1962b), and more recently by Finelle (1974a).

In a valuable contribution, Finelle (1974a) has considered the cost of chemotherapy and chemoprophylaxis within the direct consequences of trypanosomiasis. According to this author, for a 300-kg bovine animal the cost of a curative treatment varies between 65 and 70 cents, and the annual cost of the preventive treatments
repeated on average every four months, would be about US$2.65. He has claimed, however, that these estimates are very approximated as some expenses cannot be accurately quantified, and the figures available are not always comparable because they vary with each country.

A significant expansion in animal production in the African continent would be the final result of the control of cattle trypanosomiasis, in which chemotherapy and chemoprophylaxis are important factors to be considered.

(c) **Drug resistance**

Drug resistance is the property ascribed to a trypanosome strain which allows it to withstand therapeutic doses of trypanocides normally effective on it. Direct drug resistance can occur to all of the available trypanocidal drugs and development of resistance to one compound is often accompanied by cross-resistance to others (Whiteside, 1960, 1962a) (Table 2).

Even Berenil, one of the most promising trypanocides has got this problem and resistant strains of *T. congolense* and *T. vivax* have been reported in Nigeria (MacLennan and Jones-Davies, 1967; Jones-Davies, 1967). In addition, Gray and Roberts (1971a,b) have shown that *T. congolense* and *T. vivax* strains resistant to Ethidium, Samorin, Antrycide and Berenil may retain this property over a period of 16 months after transmission through the tsetse fly and also
Cross-resistance analysis of trypanocidal drugs used in cattle.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Response of strain resistant to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethidium</td>
<td>Ethidium  Prothidium Samorin Antrycide Berenil</td>
</tr>
<tr>
<td>Prothidium</td>
<td>C D C P P O</td>
</tr>
<tr>
<td>Samorin</td>
<td>C C D P O</td>
</tr>
<tr>
<td>Antrycide</td>
<td>P C C D P</td>
</tr>
<tr>
<td>Berenil</td>
<td>O O O O D</td>
</tr>
</tbody>
</table>

D = direct resistance
C = cross resistance
P = partial cross resistance
O = no cross resistance
Dependence on regular drug treatment for trypanosomiasis control has led to the major risk of drug resistance development. Trypanosomes develop resistance when trypanocides are used for prophylaxis or when administered in inadequate dosage (Stephen, 1975). The immunosuppressive action of the infections themselves and antigenic variation in some Salivarian trypanosome infections, may also play a role in the emergence of drug resistance (Peters, 1974).

Drug resistance has handicapped the chemical control of trypanosomiasis, which is actually dependent on a limited number of drugs. Thus, a new approach to chemotherapy and chemoprophylaxis is the best alternative to this problem.

4. Drug Use Improvement

(a) Immunity

According to Peters (1974), in trypanosomiasis drugs provide a sort of assistance to the host and the host's immune process ultimately decides the elimination of the infection. Soltys (1955) at first postulated that residual immunity was developed in cattle after Antrycide pro-salt prophylaxis. Residual immunity of this type was first noted by Ehrlich and Shiga (1904, quoted by Williamson,
1970a), and in the field had in fact been observed in early years with tartar emetic by Bevan (1928, quoted by Williamson, 1970a). From the time of these observations to the present day several workers have been trying to take advantage from the development of immunity using available trypanocidal drugs.

Attention has been given to the study of the development of immunity under field conditions in drug treated cattle maintained in tsetse areas (Smith, 1958; Whiteside, 1962a; Boyt, Lovemore, Pilson and Smith, 1962; Wilson, Le Roux, Paris, Davidson and Gray, 1975; Wilson, Paris, Luckins, Dar and Gray, 1976). Wilson et al. (1976), have found partial development of immunity to trypanosomiasis after two years in cattle with mixed infections (T. vivax, T. congolense, T. brucei) treated with Berenil (5mg/kg) and Samorin (0.5mg/kg), on a group and individual basis. According to these authors, Samorin used on a group basis was the most suitable for the maintenance of beef cattle in a tsetse infested area.

(b) Complexes

Another aspect of drug use improvement concerns suramin, an anionic drug which forms insoluble complexes by mutual precipitation with a number of cationic trypanocides (Williamson, 1970a). The use of these complexes which form depots of drug has suggested the
possibility of very long term prophylaxis. Unfortunately under field conditions sloughing of the depot occurs with a consequent termination of the period of protection.

Suramin complexes are likely to be restricted to prophylaxis and treatment of *T. simiae* in pigs, and of *T. evansi* in equines (Williamson, 1970a). Williamson and Desowitz (1956) have reported the use of Metamidium (Samorin (Isometamidium)) by itself and complexed with suramin, particularly against *T. congolense* and *T. vivax* in original field trials in cattle. Besides, Braide (1974) has claimed that Prothidium-suramin complex (10 mg/kg) protects cattle for as long as 280 days. However, this author concluded that procedures for prophylaxis that depend on depot formation at injection sites frequently cause necrosis and lameness.

(c) **Synergism**

The urgent need for a reappraisal of the possibilities of combination chemotherapy in trypanosomiasis has been recently emphasized by Williamson (1976a,b). He has stated (1976a), that "a restricted choice of active drugs forces exploration of combined treatment, as in cancer, malaria and other infections".

The prophylactic suramin complexes described above and the demonstration of synergism between suramin and tryparsamide, are the only two previous examples in
relation to this approach (Williamson, 1966a).

Williamson (1970a), has made the suggestion that the development of hypersensitivity to one trypanocide in a trypanosome strain already resistant to a different drug appears to be related to a synergistic activity.

With the exception of arsenicals and suramin, all trypanocides are potent inhibitors of nucleic acid or protein synthesis, or both. Phenanthridinium compounds and Berenil combine with and prevent replication of DNA (Braide, 1974). On this basis, combinations of the two kinds of drugs would be expected to be synergic (Williamson, 1976a).

At present, it is possible to provide some examples of combination therapy from the new drugs developed. According to Williamson and Scott-Finnigan (1975), some RNA synthesis inhibitors may serve as helping potentiating agents. Relevant information has been provided previously by Williamson (1966b), who has shown additive synergism of Cordycepin (antitumour antibiotic) with suramin on *T. rhodesiense*. In addition, combined treatment with inhibitors of RNA synthesis, other than Cordycepin, and antiprotozoal agents has been worth investigation (Williamson and Scott-Finnigan, 1978).

Lastly, some information is available from the new approaches to chemotherapy of trypanosomiasis with regard
to the improvement in the knowledge of trypanosome biochemistry. Clarkson and Brohn (1976) have demonstrated synergistic inhibitory activity of salicylhydroxamic acid (SHAM) and glycerol on the glycolysis of *T. brucei*. More recently heme, organic arsenicals (melarsenoxide, tryparsamide) and naphthoquinones have been found to act mutually in synergy *in vitro* and *in vivo* causing lysis of bloodstream forms of *T. b. brucei* (Meshnick, Blobstein, Grady and Cerami, 1978). So there is an increased susceptibility of this parasite, unable to decompose hydrogen peroxide ($\text{H}_2\text{O}_2$), to be killed by agents that promote the homolytic cleavage of intracellular $\text{H}_2\text{O}_2$.

5. **Drug Developments**

The optimal drug requirements for a trypanocide in domestic animals have been described by Williamson (1970a). It would be cheap, administered preferably on a single occasion, active therapeutically against all stages of the disease, free of side effects, incapable of inducing drug resistance and active against strains already resistant to other drugs. In the case of the curative drugs, the majority of these requirements had been fulfilled by Berenil to which resistance has developed since 1967. Similar requirements would apply to prophylactic drugs which, as well as acting for a longer time would be free from local damage, chronic toxicity and problems of residues in animal products. None of the
available prophylactic drugs satisfy these requirements.

Up to now there are no alternative drugs to replace those in general use, whenever it is necessary. Efforts should be made to find new curative and prophylactic drugs which do not produce local reactions and preferably they should differ structurally from existing compounds. So they can be employed against trypanosomes that are now developing resistance to currently employed drugs (Finelle, 1974b).

(a) Economic implications

In the last years no new drugs have been introduced for the treatment of animal trypanosomiasis. Braide (1974) has claimed that the reason for this fact is two-fold. Firstly, there has been a lack of interest and secondly, developments of new drugs do not represent benefits for the pharmaceutical industry. The following extract from Finelle (1974b) illustrates this situation most succinctly: "pharmaceutical laboratories who developed products which were generally found to be satisfactory do not deem it necessary to undertake risky and certainly very expensive further research to attempt to discover new products".

(b) New approaches

Newton (1977) has pointed out that the success for a
more rational approach to chemotherapy has been delayed by a lack of knowledge of the trypanosomes, attributed to small investments in research in this area.

Nevertheless, this situation has changed because now it is possible to grow in vitro culture forms (Cross and Manning, 1973) and bloodstream forms (Hirumi, Doyle and Hirumi, 1977) of T. brucei-group trypanosomes. These achievements have led the way to make clear the physiology, biochemistry and immunology of this trypanosome species.

A rational approach to the development of new trypanocides would include comparative studies of the biochemistry of the trypanosome and mammalian host, and also a knowledge about the mode of action of the existing trypanocidal drugs (FAO/WHO, 1969; Braide, 1974). Consequently, these studies would indicate important differences in biochemical pathways between host and trypanosome, which can be used strategically for the development of new compounds against the parasites (Borst, 1977; Newton, 1977). The biochemical basis of trypanosome chemotherapy has been reviewed in detail by Williamson (1976a), Newton (1976) and Peters (1976).

(c) New leads

Most of the earlier description of the chemical control of cattle trypanosomiasis has been mainly devoted in this dissertation to the available trypanocidal drugs
and their precursors. From now onwards, it is intended to mention briefly a number of therapeutic compounds which, although not developed beyond their experimental phase, have widely contributed to a better understanding of the chemotherapy of trypanosomiasis.

(i) New diamidines

Important work has been done on the chemotherapeutic activity of new diamidines. Three compounds identified as 98/202, 102/198 and 150/49 have been preliminary tested (Dann, Walker, Kaddu and Watts, 1971). Two of them (102/198 and 150/49) appeared to be promising for the control of *T. congolense* and *T. rhodesiense* infections, respectively. Additional observations (Dann, Bergen, Demant and Volz, 1971) have shown that DAPI (102/198) (4',6-diamidino-2-phenylindole) was the most effective, displaying antiparasitic activity which could be suitable for veterinary purposes.

Investigations on trypanocidal diamidines have included the study in mice of their distribution, formation and excretion (Fink, Kaliwoda, Dann and Schmidt, 1978), the pathomorphology of their side effects (Schmidt, Kaduk and Fink, 1978), and the effects of DAPI on the ultrastructure of *T. rhodesiense* (Williamson and McLaren, 1978).
Adenine nucleoside trypanocides

One of the targets for selective drug design has been the competitive inhibition of metabolism via chemical analogy to essential metabolites within susceptible trypanosomes.

Mammalian pathogenic trypanosomes have seemed, unlike the cells of their hosts, to have a limited ability to synthesize adenine. So this has been a good opportunity for selective chemotherapy by suitable chemical analogues of adenine. Cordycepin (3'-deoxyadenosine), an antitumour antibiotic, was found to have high trypanocidal activity against *T. rhodesiense* and *T. congo**lense* (Williamson, 1966b). Also three adenine analogues Puromycin, Nucleocidin and Puromycin aminonucleoside have been shown to possess trypanocidal activity against *T. rhodesiense* in mice (Jaffe, 1967).

Williamson (1970b) has extended the study of the effects of Cordycepin and analogous nucleosides on *T. congo**lense* in vitro. According to Williamson (1970a), Puromycin has been tested against sleeping sickness in man and Nucleocidin has been used to treat trypanosome infected cattle in West Africa. Aiyedun, Williamson and Amodu (1973), have studied the trypanocidal properties of Cordycepin on *T. vivax* infections in sheep. However these authors did not have consistent success.
More recently Williamson and McLaren (1974), have described in trypanosomes the disturbance of long-chain fatty-acid uptake and disposal by adenine nucleoside analogues. Williamson and Macadam (1976) have found indications of interference with RNA synthesis affecting the fine structure of T. rhodesiense induced by four trypanocidal adenine nucleocides.

(iii) Metabolic inhibitors

As already mentioned a detailed knowledge of trypanosome metabolism and nutrition compared to the mammalian host, have been essential for the design of new leads in the chemotherapy of trypanosomiasis.

In relation to trypanosome metabolism, Ryley (1956) has shown that for energy production the parasites metabolize glucose both aerobically and anaerobically. Grant and Sargent (1960) have found that blood forms of T. brucei-group trypanosomes depend for energy production on aerobic glycolysis, mediated by a parasite specific L-α-glycerolphosphate oxidase system (GPO). They have claimed that GPO is also present but considerably less active in bloodstream forms of T. congolense, T. vivax and T. lewisi. In addition, Evans and Brown (1973a,b) have demonstrated that GPO is inhibited by benzhydroxamates such as salicylhydroxamic acid (SHAM).

An alternative pathway to GPO, which is inhibited by
glycerol has also been reported (Opperdoes, Aarsen, van der Meer and Borst, 1976; Opperdoes, Borst and Fonck, 1976). In fact the possibility for an effective trypanosomicidal drug combination was claimed by several workers, being verified by Clarkson and Brohn (1976). According to these authors SHAM inhibits aerobic respiration and glycerol inhibits anaerobic glycolysis in bloodstream forms of T. brucei, causing cell death in vitro.

This observation has been used to devise regimens of treatments with these two drugs against experimental infections of T. brucei, T. rhodesiense and T. vivax in laboratory animals. Evans and Holland (1978) have used successfully this combination against T. vivax infections in mice. These authors have suggested that the drug combination should now be tested in cattle and sheep infected with T. vivax.

There are, according to Brohn and Clarkson (1978) with regard to the mammalian host metabolism, radical differences in the pathways described above. Furthermore, the nature of the alternative pathway has not yet been elucidated (Borst, 1977).

(d) Prospects for the future

New leads like those mentioned above represent actual opportunities when seeking for novel trypanocidal drugs. In view of the prolonged absence of new compounds to control
the disease, drug companies should take advantage from the support of the new WHO Special Programme for Research and Training in Tropical Diseases (WHO, 1978). The Scientific Working Group on African Trypanosomiasis which forms part of the Special Programme, has held its first meeting in Geneva in October 1977 to select overall priorities for research. They have taken into account the research priorities recommended by the Joint WHO Expert Committee and FAO Expert Consultation on the African Trypanosomiasis, held in Rome in November 1976. One out of two research areas selected for top priority was drug development.

Earlier on, the Consultation on the FAO Programme for the Control of African animal Trypanosomiasis (FAO, 1976) have considered among the measures a liaison with the pharmaceutical industries, to look for new insecticides and trypanocidal drugs. FAO has established a special Working Group (FAO/Industry Task Force on Trypanosomiasis Control) to maintain contacts with industries and orientate research.
SUMMARY AND CONCLUSIONS

The literature on animal trypanosomiasis has been reviewed particularly in relation to the chemical control of the parasites in cattle, regarding the present situation and looking forward into the future.

In the introductory section a brief account is made of aspects of the disease in cattle which, as seen by the writer, would contribute to a better consideration of the subject matter. Undoubtedly, pathogenesis and diagnosis play an important role in the chemotherapeutic and chemoprophylactic control of the disease, methods currently employed in cattle.

In relation to pathogenesis, it is clear that one of the causes of failures of specific trypanocidal action could be explained by differences in the body distribution of both the drugs and the different trypanosome species. To be certain of that, pharmacokinetic studies of the existing and new drugs being developed, compared to the actual distribution of each trypanosome subgenus, are urgently needed.

With regard to diagnosis, the lack of accuracy in identifying the condition and its causative agent make difficult the proper drug treatment of cattle trypanosomiasis in the field. This appears to be true in Africa as well as in South America where anaplasmosis and
babesiosis are the main causes of misdiagnosis of clinical trypanosomiasis. The extend to which this factor is influencing the development of drug resistance has yet to be assessed in field work.

Special emphasis has been given to the review of the most relevant and available information about the trypanocidal drugs, with respect to the earlier and some of the latest drug developments.

With relation to the available drugs, it is argued that the treatment of cattle trypanosomiasis has relied upon a small number of drugs during more than forty years. This, added to irregular drug treatments and inadequate dosage could give reasons for the widespread development of drug resistance. One of the practical points concerning drug use improvement is the development of immunity when using the existing drugs in already infected animals. This aspect, previously observed in bovine babesiosis and named chemoimmunisation, could also be exploited in the control of cattle trypanosomiasis while new and better alternative drugs are being produced. With respect to drug residues, determination of the distribution and levels of drugs in animal products and carcases has yet to be assessed for the drugs currently used.

Remarkable achievements have been described regarding new drug developments. However, it is also true
that most of the research done has concerned the development of drugs against trypanosomes of the *T. brucei* group, considered normally of low pathogenicity for cattle. The justification for continuing this tendency requires a reappraisal of the real significance of the disease in man compared to the disease in cattle.

Finally, it will suffice for a main conclusion to give an extract of the recommendations of the Second Consultation on the FAO Programme for the Control of African animal Trypanosomiasis held in Lusaka, Zambia in December 1978 (FAO, 1978):

The Consultation:

- **considering** that in many countries it will not be possible to eradicate tsetse flies in the foreseeable future and the only means of combating this scourge available to livestock owners is chemotherapy and chemoprophylaxis,

- **is of the opinion** that:

  i) FAO include chemotherapy in the expert consultation programme in order to review the situation and advise on the implementation of field trials.

  ii) Training courses for veterinarians include instruction on the rational use of chemotherapy.

  iii) FAO also make an inventory and assess the potential market for new trypanocidal drugs. In the light of the results of these assessments, the different
assistance agencies will consider the possibility of providing appropriate funds to the pharmaceutical companies that will develop these drugs. This information may stimulate assistance agencies to provide financial support for development of new drugs.
I wish to express my sincere appreciation to Dr. A. R. Gray for his direction, assistance and constructive criticism in the preparation of this dissertation.

My gratitude must also be given to all members of the staff for the invaluable teaching and guidance in the course of my studies at the Centre for Tropical Veterinary Medicine.

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