African animal trypanosomiasis

PART I. DISEASE AND CHEMOTHERAPY

P. FINELLE *

This is the first of three articles on African animal trypanosomiasis by Dr. Pierre Finelle, who has spent many years in Africa studying this parasitic disease. This first part describes the disease and its occurrence, and the drugs that have been introduced to combat it and their relative merits.

The second part will deal with chemoprophylaxis and the raising of trypano-tolerant livestock, while the third and last article will review vector control as a means of overcoming trypanosomiasis.

Trypanosomiasis is a parasitic disease caused by species of flagellate protozoa belonging to the genus *Trypanosoma* which inhabit the blood plasma and various body tissues and fluids. These parasites are found in many animals but seem to be pathogenic only for mammals, including man.

Animal trypanosomiasis occurs in most of the tropical regions, but only in equatorial Africa does it constitute a major obstacle to the development of animal production. The considerable economic and social repercussions make control of this disease a priority operation for the development of a large part of the African continent.

Trypanosomes

African animal trypanosomiasis can be caused by several species of trypanosomes:

Trypanosoma congolense is found in most domestic mammals: cattle, sheep, goats, horses, pigs, camels and dogs; and also in many wild animals (Figure 1).

T. vivax is a parasite of domestic and wild ruminants and of horses.

T. simiae is found mainly in domestic and wild pigs.

T. brucei is a parasite very close to *T. gambiense* and *T. rhodesiense,* which are the causes of human sleeping sickness. It can be found in practically all domestic and wild animals.

T. evansi is found in Africa only in the Saharan and Sahelian regions where it is primarily a camel parasite, but it may be a parasite of horses, cattle and dogs as well. It also occurs in Asia — where it commonly causes disease in camels and horses, and less commonly in cattle, water buffaloes, elephants and dogs — and in Central and South America. Thus it has a very wide distribution.

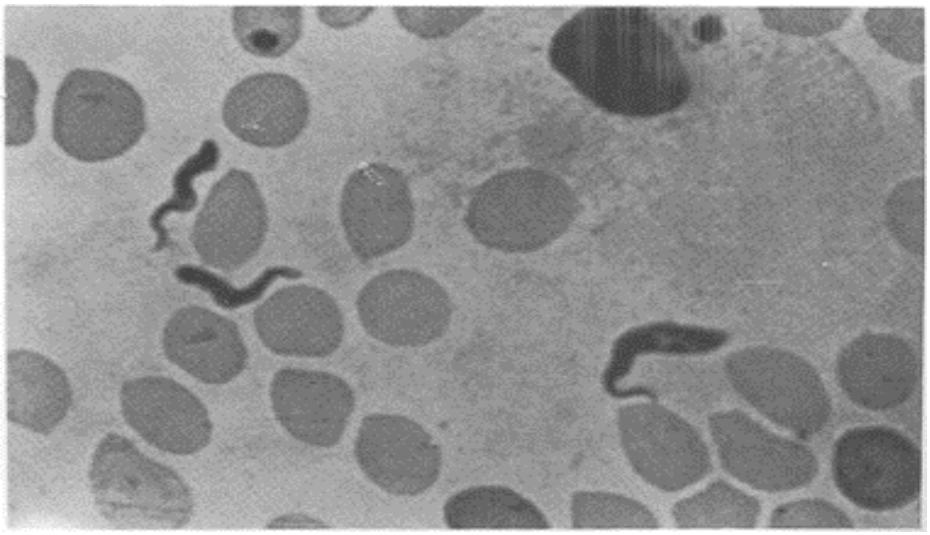


Figure 1. Photomicrograph of a film of blood showing three specimens of *Trypanosoma congolense.*

T. equiperdum is the causal agent of dourine, a contagious equine disease transmitted by coitus, which in Africa occurs only in the north African region and in South Africa. As control of dourine is an entirely different problem from that presented by other forms of trypanosomiasis, it will not be discussed in the present review, which deals only with the African trypanosomiasis transmitted by insects.

Transmission of trypanosomes

Transmission of trypanosomes by insects may be effected by widely different means.

Cyclical transmission, during which the trypanosomes actively multiply in the vectors, occurs through the intermediary of *Glossina* or tsetse flies (Figures 3 and 4). This form of transmission occurs with *T congo-lense, T. vivax, T. simiae, T. brucei,* and the trypanosomes responsible for human sleeping sickness, *T. gam-biense* and *T. rhodesiense. Glossina* spp. are strictly blood feeders living exclusively in tropical Africa. There are about thirty species or subspecies, classified in three groups: *palpalis, morsitans* and *fusca.* Each species has distinct biological characteristics, but in general it may be said that the *palpalis* group consists basically of the species living in forest galleries or in the marginal areas of forests; the *fusca* group consists of large-sized species whose habitat is generally associated with equatorial forests; and the *morsitans* group consists mainly of species living in wooded savanna.

Mechanical transmission is effected by various blood-sucking insects such as flies of the family Tabanidae (horse flies) and *Stomoxys* spp. In the course of a blood meal begun on an infected animal and ended on a healthy one, these insects may carry trypanosomes provided that the interval between the two meals is short. This form of transmission is the rule for *T. evansi,* but may also occur with trypanosomes habitually transmitted cyclically by *Glossina,* particularly *T. vivax* which may therefore be found in regions far from the *Glossina* distribution area (such as Latin America).

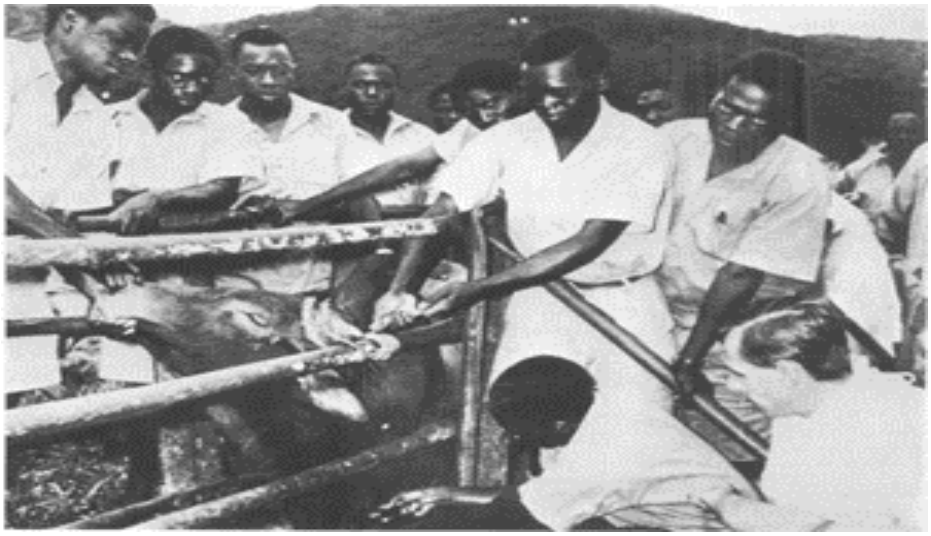


Figure 2. Demonstration of chemotherapeutic treatment against trypanosomiasis by inoculation with a trypanocidal drug in the dewlap.

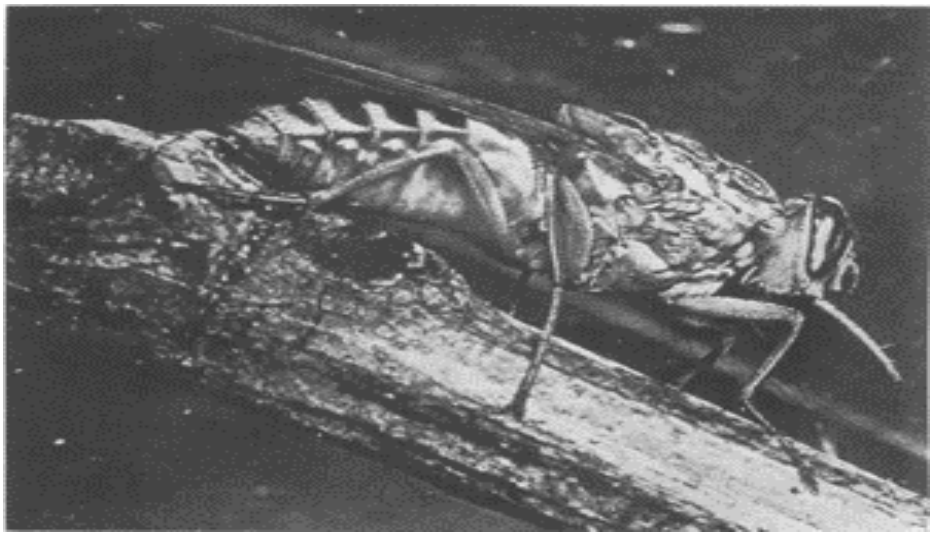


Figure 3. Close-up of a tsetse fly, the insect vector of African trypanosomiasis.

Trypanosomiasis

Trypanosomiasis is generally a chronic evolving disease which is usually fatal if appropriate treatment is not established. It leads to considerable loss of weight and anaemia. Various symptoms are exhibited, including fever, oedema, adenitis, dermatitis and nervous disorders. Because of its protean symptomatology the disease cannot be diagnosed with certainty except through detection of parasites by microscopic examination of blood or by various serological reactions.

The evolution of trypanosomiasis varies widely according to the try-panosome involved and the animal species or breed affected. Trypanosomiasis caused by *T. simiae* in pigs usually assumes a highly acute form leading to rapid death, at least in improved pig strains. *T. brucei* is highly pathogenic for horses and dogs, but in cattle this trypanosome usually causes asymptomatic infection. Zebu cattle are extremely susceptible to infections caused by *T. congolense* and *T. vivax,* but the humpless cattle of west Africa and the Guinean strain of goats show remarkable resistance, enabling these animals to live in areas where other breeds cannot exist.

Biologically-based control of animal trypanosomiasis

In the control of animal trypanosomiasis action is possible on various aspects of the epizootiological cycle of the infection: parasites, host animals and vectors.

ACTION ON PARASITES

This consists of the use of trypano-dal drugs on infected animals. The method aims first at limiting losses caused by the disease, and second at eliminating trypanosome reservoirs. Thus, detection and treatment of infected animals can be considered to be both a curative and a prophylactic procedure.

ACTION ON HOST ANIMALSs

Although immunological responses occur in trypanosomiasis, it has not yet been possible to develop a practical method for immunization. Short of such a method, the use of prophylactic trypanocidal drugs makes it possible in certain conditions to protect animals for several months. Another method consists in raising animals showing natural resistance to trypanosomiasis, such as the humpless cattle of west Africa.

ACTION ON VECTORS

This method applies primarily to *Glossina.* Attempts may be made to (a) destroy the insects, particularly through the use of insecticides; (6) make the environment unsuitable as a habitat, either by altering the vegetation or by eliminating the animal species which constitute the preferred hosts of these insects; (c) reduce their reproductive capacity by the release of sterile males; *{d)* limit their number by using biological control methods. The two latter techniques are still only in the research stage and have not been used so far as a practical control method for *Glossina.*

The various methods will now be considered which can be used in the control of African animal trypanosomiasis, excluding dourine; and the account will be confined to measures for the treatment and protection of cattle, small ruminants, pigs, horses and camels. The measures reviewed include (a) chemotherapy, *(b)* chem-oprophylaxis, (c) breeding of try-panosome-tolerant animals and *{d)* vector control.

Chemotherapy

Since 1938, the date of the discovery of the trypanocidal properties of the phenanthridines, the chemotherapy of animal trypanosomiasis has made great progress and there are several highly active drugs now available which are easy to use. The use of trypanocides has consequently become widespread, and the number of trypanocidal treatments carried out every year in Africa can be estimated at over 6 million, the great majority of them for combating bovine trypanosomiasis.

The trypanocides currently employed are: homidium salts (Ethi-dium-Novidium); quinapyramine sul-fate (Antrycide); diminazene acetu-rate (Berenil); isometamidium (Samo-rin-Trypamidium) and suramin sodium.

Table 1, which gives the data concerning the use of these products, shows that the action of the different trypanocides varies according to the animal species infected and the try-panosomes involved.

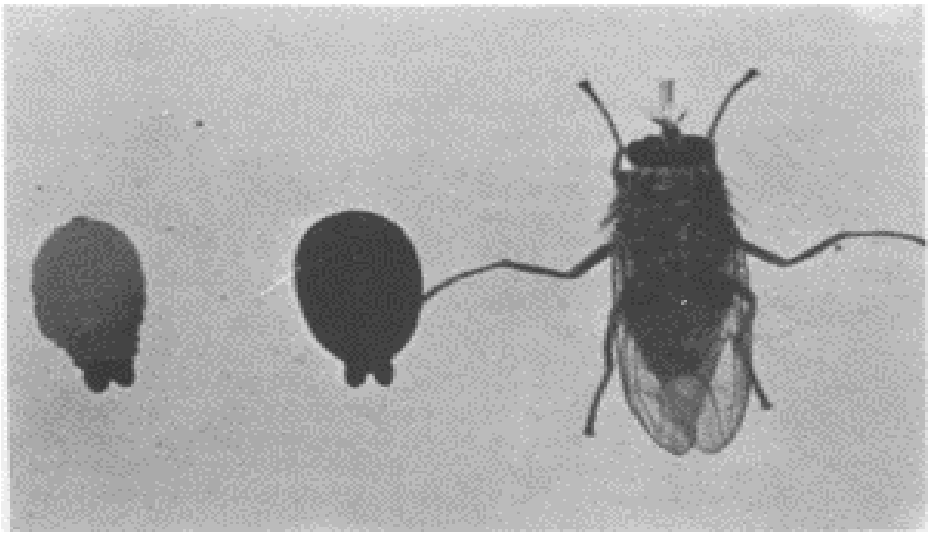


Figure 4. Different stages of evolution of the tsetse fly: larva, pupa and adult fly.

CATTLE TRYPANOSOMIASIS

T. congolense and T. vivax

Cattle infections caused by *T. congolense* and *T. vivax* are by far the most serious, both for frequency and for economic influence. The first really effective trypanocides were the dimidium salts which were widely used during the 1950s. However, their toxic effects, the difficulties involved in their adoption and the frequent appearance of drug-resistant trypanosomes have made the use of more recent trypanocides preferable. Homidium salts have been and are widely used, but a considerable number of cases of drug resistance to homidium have been reported and in many countries it has been necessary to suspend their use. Drug resistance has also been a serious handicap in the employment of quinapyramine sulfate (Antrycide) which is no longer extensively used in cattle for the treatment of either *T. congolense* or *T. vivax* trypanosomiasis.

Diminazene aceturate (Berenil) offers numerous advantages: its high activity against *T. congolense* and *T. vivax,* particularly on those strains resistant to other trypanocides, its very low toxic effects in cattle and its easy utilization make it a practical and safe trypanocide, at least for cattle. Although some cases of resistance were observed early in the use of the trypanocide, it was the accepted view at the time that this was the result of cross-resistance with quinapyramine, and that diminazene did not directly cause resistance because of its rapid elimination through the kidneys, which prevents accumulation of residual subcurative doses. Since 1967, however, strains of trypanosomes directly resistant to diminazene have been found in var-ious countries, notably in the Central African Republic, Chad, Kenya, Nigeria and Uganda, primarily with regard to *T. vivax* but also to *T. congolense.* These strains are fortunately still vulnerable to the phen-anthridine group of trypanocides, particularly isometamidium, leading to the conclusion that in case of failure of a diminazene treatment it is preferable to use another trypano-cide such as isometamidium rather than give further treatment with an increased dose of diminazene.

Table 1. Use of trypanocidal drugs

Trypanocide-laclics	Trade *name*	Method of treatment			Indications		Toxic effects			Treatment of relapses
		Solution	Dosage	Injection 1	Highly active on	Less active on	Good tolerance	Possible local reactions	Possible general reactions	
Homidium bromide	Ethidium 2	*Percent 2* hot	*Mg/kg*	IM	*T. vivax* *T.congolense*		Cattle Sheep Goats	Horses		Diminazene Isometami-dium
Homidium chloride	Novidium 3	2 cold water	1							
Diminazene aceturate	Berenil4	7 cold water	3.5	SC or IM	*T.congolense* *T. vivax*	*T.brucei* *T.evansi*	Cattle Sheep Goats	Horses	Horses Camels	Isometami-dium
Quinapyra-mine sulfate	Antrycide5 (sulfate)	10 cold water	5	SC	*T.congolense* *T. vivax* *T. brucei* *T. evansi*		Cattle Sheep Goats Camels	Horses		Isometami-dium
lsometami-dium chloride	Samorin,3 Trypami-dium6	1 or 2 cold water	0.25 to 1	IM (deep)	*T. vivax* *T.congolense*	*T.brucei*	Cattle Sheep Goats Horses	Cattle		Diminazene
Suramin sodium		10 cold water	10	IM	*T. evansi* *T. brucei*		Camels Horses			Quinapyra-mine

1 im = intramuscular injection: sc = subcutaneous injection.

2 Boots Pure Drug Co. Ltd.

3 May & Baker Ltd.

4 Farbwerke Hoechst A.G.

5 Imperial Chemical (Pharmaceutical) Ltd.

6 Specia.

Isometamidium (Samorin, Trypa-midium) is the most recent of the commonly employed trypanocides. Its main advantage is its effectiveness on trypanosomes resistant to other drugs. At the same time it has the disadvantage of easily creating drug-resistant strains itself; however, these trypanosomes show no cross-resistance with diminazene, which therefore retains *its* effectiveness on such strains. The isometamidium deposit at the injection site can cause a persistent local reaction which may be invisible from outside if deep in-tramuscular injection has been given, as is recommended. This reaction makes the surrounding flesh unfit for consumption and partial

confiscation of the carcass is necessary. It is therefore advisable to choose an inoculation site on a part of the body where the meat is inexpensive; the neck muscles are usually recommended.

The two foregoing drugs, diminazene and isometamidium, are currently the preferred treatments for *T. congolense* and *T. vivax* trypano-somiasis in cattle.

T. brucei and T. evansi

Trypanosomiasis in cattle caused by *T. brucei* is of secondary importance as this trypanosome is only slightly pathogenic for cattle. The most active trypanocide against it is quina-pyramine.

T. evansi trypanosomiasis is extremely rare in cattle in Africa, where the disease occurs mainly in camels. It is encountered more frequently, however, both in cattle and in water buffaloes in southeast Asia. The best treatment is quinapyramine.

TRYPANOSOMIASIS IN SMALL RUMINANTS

Sheep and goats are seldom affected by trypanosomiasis and there is little information on treatment. If necessary, the treatments indicated forcattle, with diminazene and isometa-midium, can be used.

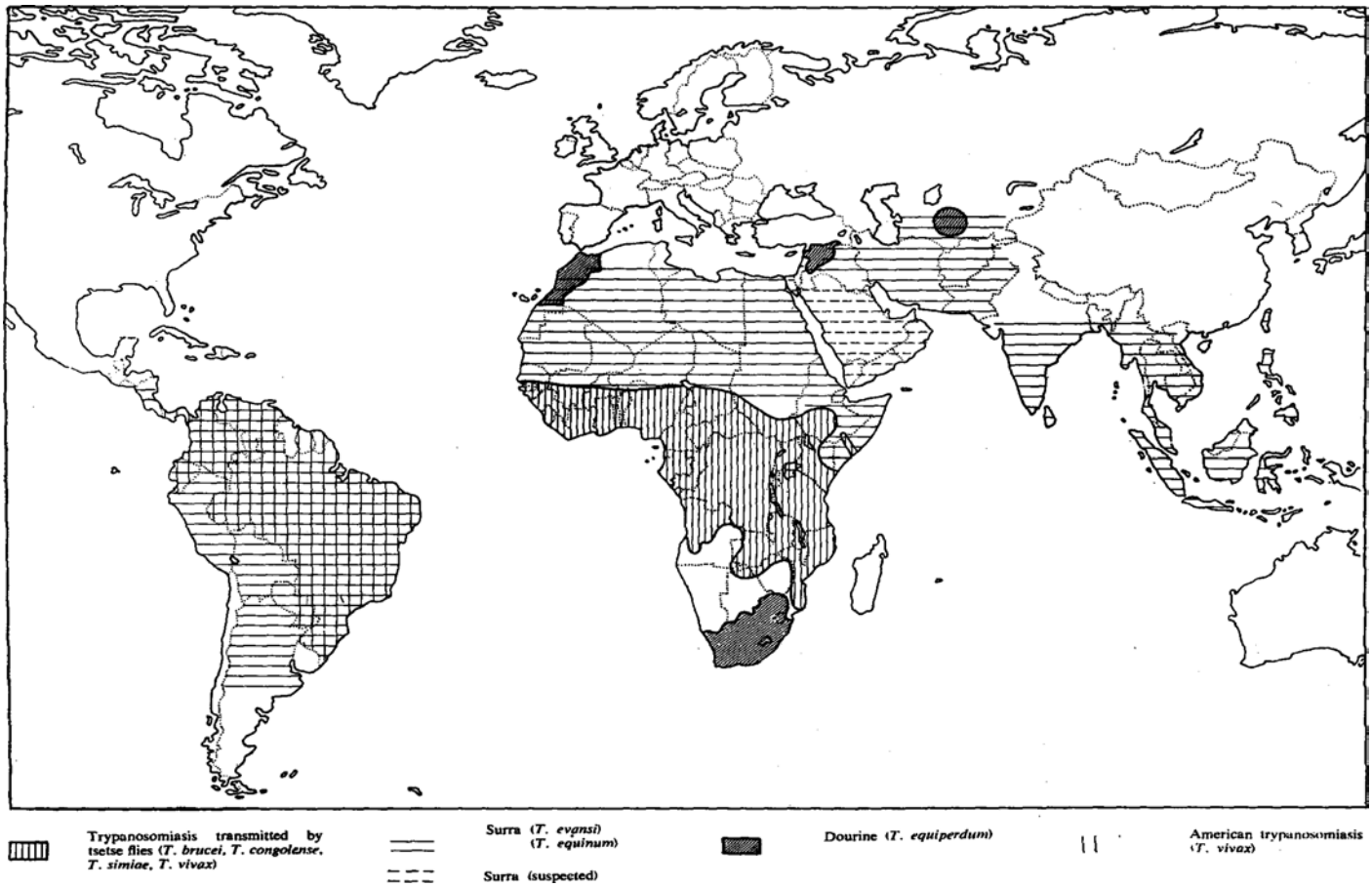


Figure 5. Geographical distribution of animal trypanosomiasis.

TRYPANOSOMIASIS IN PIGS

T. simiae, which is found mainly in domestic and wild pigs, presents a special problem because of its low vulnerability to the various trypano-cides, requiring the application of considerably higher doses than those used

against other trypanosomes. Two treatments appear to be effective: an extremely high dosage of isometamidium (12.5 to 35 mg/kg); or a combination of quinapyramine sulfate (7.5 mg/kg) with diminazene (5 mg/kg).

However, the rapid course of this form of trypanosomiasis usually makes any therapeutic action impossible, so that it is necessary to rely on preventive rather than curative treatment.

EQUINE TRYPANOSOMIASIS

T. congolense and T. vivax

Diminazene is not as well tolerated by horses as by cattle. Local reaction and fatal poisoning, with kidney or brain lesions, have been reported. Homidium and isometamidium can be used on horses, although both drugs often cause local reactions; doses should therefore be divided so as to inject no more than 10 milli-litres per injection site.

T. brucei and T. evansi

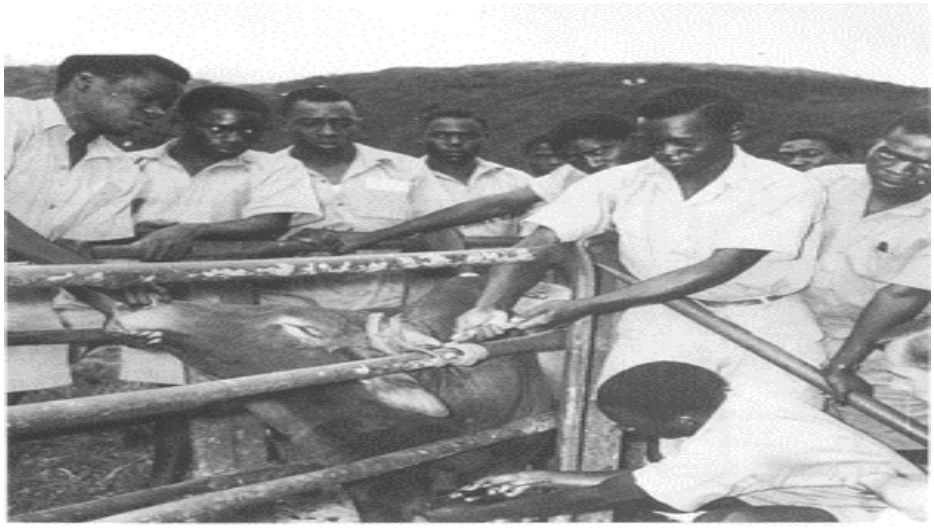
Quinapyramine sulfate is the most effective trypanocide against these two trypanosomes, but this drug is often poorly tolerated and is likely to cause serious local reactions and general disorders. It is therefore advisable to administer the dose in two or three parts at six-hour intervals.

CAMEL TRYPANOSOMIASIS

Quinapyramine sulfate is the preferred treatment for *T. evansi* trypanosomiasis in camels, but suramin sodium is still used in many countries although its cost is markedly higher and cases of drug resistance have been observed. Suramin-resis-tant strains of *T. evansi* remain sensitive to quinapyramine.

Conclusions

Several drugs are now available which are highly effective (except in the case of *T. simiae)* and easy to use; but for each of these products there are specific instructions which must be observed. Care and expert advice must always be taken before any large-scale treatment is started.



Cattle being injected with a drug to control trypanosomiasis

PART II. CHEMOPROPHYLAXIS AND THE RAISING OF TRYPANOTOLERANT LIVESTOCK

P. FINELLE

The first article in this series exam-ined the possibilities afforded by direct action on the parasites responsible for animal trypanosomiasis by the use of trypanocidal drugs on diseased animals. This second paper reviews two other methods of control: protecting susceptible animals by the use of preventive drugs, and making use of the natural resistance of certain breeds of cattle to trypanosomiasis.

Chemoprophylaxis

There are three trypanoprophylactic drugs which can be used: quinapyra-mine prophylactic (Antrycide Prosalt), pyrithidium (Prothidium), and isome-P. Finelle is Animal Health Officer, Animal Production and Health Division, fao, Rome. tamidium (Samorin-Trypamidium). Quinapyramine can also be used in complex forms with suramin, but as this formula is not on the market it must be prepared by the user as follows:

Quinapyramine sulfate	10 g
Suramin anhydrate	8.9 g
Distilled water	q.s. per 200 ml

The methods of using these trypanoprophylactic drugs are shown in Table 1.

CATTLE TRYPANOSOMIASIS

T. congolense, T. vivax, T. brucei

Quinapyramine (Antrycide Prosalt) was the first trypanoprophylactic drug that was sufficiently active for use in common practice. However, it has fallen into disuse because of the frequent appearance of drug-resistant trypanosome strains. Moreover, its prophylactic action, extending over two to three months, is considerably less than that of more recent products. Isometamidium and pyrithidium afford protection ranging from three to six months, depending on the risk. In principle it would be advisable to make a preliminary trial in each case in order to determine the treatment rate. In practice, a four-month cycle may generally be adopted — three injections per year. Isometamidium is most frequently used, particularly because of its lower cost. As in curative treatment, and especially since higher doses are administered for prevention, it is advisable to give the injection in a muscle where the local reaction is not likely to affect the price of the carcass substantially For large animals it is also advisable to divide the dose so as not to inject more than 15 ml per injection site. If trypanosomes reappear before another preventive injection has been given, a curative treatment with diminazene should be administered so as to eliminate theisometamidium-resistant trypanosomes.

Table 1. Use of trypanoprophylactic drugs

Trypanocide-laclics	Trade name	Method of treatment			Indications		Toxic effects		Treatment of relapses
		Solution	Dosage	Injection [1]	Trypanosomes	Length of protection	Good tolerance	Possible local reactions	
Isometamidium chloride	Samorin [2] Trypami-dium[3]	1 to 2 parts per 100 cold water	*Mg/kg* 0.5-1	IM (deep)	*T. vivax* *T.congolense* *T. brucei*	3-6 months	Cattle Sheep Goats Horses	Cattle	Diminazene
Pyrithidium bromide	Prothidium [4]	2 parts per 100 boiling water	2	IM (deep)	*T. vivax* *T.congolense*	3-6 months	Cattle Sheep Goats	Cattle	Diminazene Isometamidium
Quinapyramine chloride and sulfate	Antrycide Prosalt [5]	3.5 g per 15 ml cold water	7.4	SC	*T. brucei* *T.evansi*	2-3 months	Horses Camels Cattle	Horses	Suramin
Quinapyramine-suramin complex		5 parts per 100 cold water	40 (of quinapyramine)	SC	*T. simiae*	young 3 months; adults 6 months	Pigs		Isometamidium 12.5-35 mg/kg

[1] IM = intramuscular injection; sc = subcutaneous injection.

[2] May and Baker Ltd.

[3] Specia.

[4] Boots Pure Drug Co. Ltd.

[5] Imperial Chemical (Pharmaceutical) Ltd.

T. evansi

In areas where *T. evansi* is prevalent, quinapyramine prophylactic (An-trycide Prosalt) can be used.

PROPHYLACTIC TREATMENT OF SLAUGHTER CATTLE

In Africa, tsetse-free livestock production areas are often located far from the large cities; this means that slaughter animals have to travel a long way to market, often through tsetse-infested zones. These journeys, usually made on the hoof, frequently last for several weeks during which the animals may contract trypano-somiasis that is all the more acute because the cattle come from regions free of infection and therefore have no immunity. Moreover, their

resistance is lowered by travel stress. It is therefore necessary that trypano-prophylactic treatment be administered before livestock intended for slaughter enter tsetse-infested areas. Because *(a)* a large number of animals are to be treated at low cost, *(b)* a comparatively short period of protection is required (about one month), and *(c)* drug resistance is unlikely since the animals are to be slaughtered, the following drugs may be used:

— homidium salts, which in regions where drug resistance to this product has not yet appeared give protection for about one month; or

— isometamidium, which in doses of 0.25 or 0.5 mg/kg makes it possible to obtain protection lasting up to two months.

TRYPANOSOMIASIS IN SMALL RUMINANTS

Chemoprophylactic treatment of trypanosomiasis in small ruminants is rare, but it appears that the measures indicated for cattle can be applied equally to these animals.

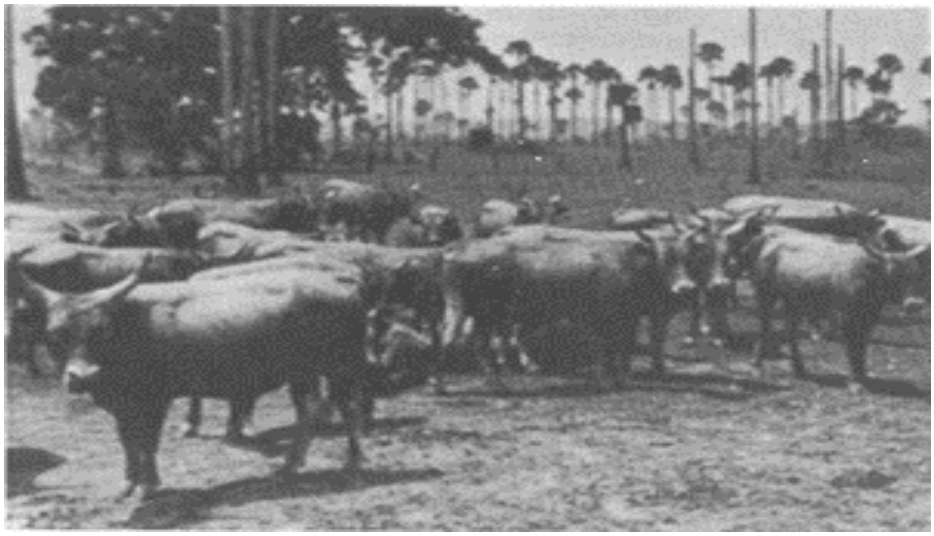


Figure 1. Herd of N'dama trypanotolerant cattle in Ivory Coast. These cattle are humpies s, and their coats are light fawn in colour.

TRYPANOSOMIASIS IN PIGS

For the prevention of *T. simiae* infection in pigs, the following can be used:

— quinapyramine-suramin complex in a dose of 40 mg/kg (quinapyramine sulfate), or 4 ml of suspension for 5 kg liveweight. This product affords protection lasting about three months for piglets and six months for adult pigs;

— isometamidium through deep intramuscular injection into the neck muscles, in doses between 12.5 and 35 mg/kg. This treatment provides protection for about four months.

EQUINE TRYPANOSOMIASIS

T. congolense, T. vivax, T. brucei

Isometamidium and pyrithidium can be used for horses and donkeys under the same conditions as for cattle, although such treatments may cause temporary lameness. It is advisable to administer deep intramuscular injections and to divide the dose if a large amount is to be injected.

T. evansi

Quinapyramine (Antrycide Prosalt) is the most effective, but this product causes serious local reactions in horses. The protection period is from three to four months.

TRYPANOSOMIASIS IN CAMELS

Quinapyramine can also be used to prevent *T evansi* trypanosomiasis in camels.

Drug resistance

The discovery of trypanocidal drugs with preventive action raised high hopes that their use would make it possible to turn subtropical Africa into a flourishing livestock production area. It must be admitted that most of these hopes have not been realized. Although these drugs do provide protection, which in some conditions may last up to six months, all of them frequently give rise to the formation of drug-resistant try-panosome strains. This drug resistance occurs when the'trypanosomes are in contact with a trypanocide administered in a subcurative dose insufficient to ensure the destruction of the parasites. This situation may be due to one or more of the following factors:

a. the application of insufficient doses, due in particular to underestimating the weight of animals;
b. the formation of abscesses followed by partial rejection of the drug;
c. a cyst-forming reaction which prevents the diffusion of the product;
d. preventive treatments at too long or irregular intervals;
e. halting the application of try-panoprophylactics while the animals are still exposed to the risk of infection ;
f. the occasional use of preventive drugs in curative treatments.

Trypanoprophylactic drugs should therefore be used with considerable caution, especially since there is a cross drug resistance between various trypanocides and drug-resistant try-panosome strains which may persist for a long time even after passage through tsetse. In fact, these drugs can be used without danger only on controlled livestock, where it can be certain that the treatment rate and application requirements will be fully observed. These prerequisites sharply limit the possibilities of applying chemoprophylaxis under traditional African livestock production conditions.

Figure 2. Herd of small short-horned humpless cattle in Dahomey. This breed is the second of the trypanotolerant breeds in west Africa, where it is known by different names.

Raising of trypanotolerant livestock

The low susceptibility of some west African cattle breeds to trypano-somiasis has long been known. Early workers observed that such livestock were able to survive and thrive in areas infested with tsetse where other breeds, especially zebu, could not exist.

These trypanotolerant livestock are the small, humpless cattle of west Africa, of which there are two distinct breeds. One is the N'dama, which seems to have originated in the Fouta Djallon massif in Guinea and whose area of distribution covers southern Senegal and Mali, Guinea, northwestern Ivory Coast, and northern Ghana. The horns of these animals are lyre-shaped and the tawny coat is characteristic. The other breed, according to the region in which it is found, is called Baoule, Laguna, Samba, Muturu, Dahomey, and west African short-horned cattle. It is found in Ivory Coast, Ghana, Dahomey, Togo, and in the southern regions of Mali, Upper Volta and Nigeria. These cattle are smaller than the N'dama and more powerfully built; their coats are usually black or piebald black, and they have short pointed horns. The areas of distribution of these two breeds and the zebu are often poorly defined, and many crossbreeds can be found.

There is still very little known about trypanotolerance. It seems to depend on two groups of factors: hereditary and acquired characteristics.

HEREDITARY CHARACTERISTICS

Trypanotolerance is a feature of the small, humpless cattle of west Africa. By studying the behaviour of zebu cios=breds it has been shown that the ujscfjtibility of these animals to trypanosomiasis is intermediate between that of pure humpless and zebu breeds and is approximately proportional to the degree of zebu blood.

ACQUIRED CHARACTERISTICS

Humpless cattle raised in tsetse-free areas have no resistance to trypanosomiasis and behave like those of other breeds; their serum does not contain antibodies and when they become infected the course of the disease is acute and results in death. Trypanotolerance is therefore in part an acquired immunological phenomenon. It is also relative and may break down in certain conditions, particularly in the case of too frequent infections which may succeed in overcoming the animal's immuno-logical defences. Moreover, all the causes capable of affecting the production of antibodies can also reduce it or cause it to disappear. These include malnutrition, overwork, intestinal parasitism and infectious diseases.

The mechanism of trypanotolerance may therefore be explained as follows: the trypanotolerant breeds have a hereditary capacity to produce try-panosome antibodies; but the production of antibodies is set off by infections contracted while the young animal is still protected by the mother's antibodies. Subsequent production of antibodies is maintained and strengthened by subsequent infections, but it can be reduced and even eliminated by all the factors which exert an unfavourable action on the immunological defences.

Introduction of trypanotolerant livestock

The west African trypanotolerant livestock which have been imported into the central African countries have enabled a significant development of livestock production in areas unsuited to the raising of zebu and where there had been no cattle production previously. The number of trypanotolerant livestock in various countries of central Africa can be estimated at about 220 000 head in Zaire, 35 000 in Congo, 15 000 in the Central African Republic, and 5 000 in Gabon.

Two systems for the introduction of trypanotolerant animals can be adopted. In the first, imported breeding stock is assembled on ranches, and the increase in the herd, the offspring, is distributed to the farmers. This permits an effective control of the herd and is perfectly suited to N'dama cattle, which respond well to ranching. The short-horned cattle however, appear to settle better in small herds. In the second system, the breeding stock is distributed directly to the farmers, which has the advantage of immediately involving the village people in the operation. A farmer is given several females and a bull, which are to be repaid in cattle later as the increase in his herd makes this possible. These will be used to start new herds. Whichever system is applied, the operation is faced with technical and human problems.

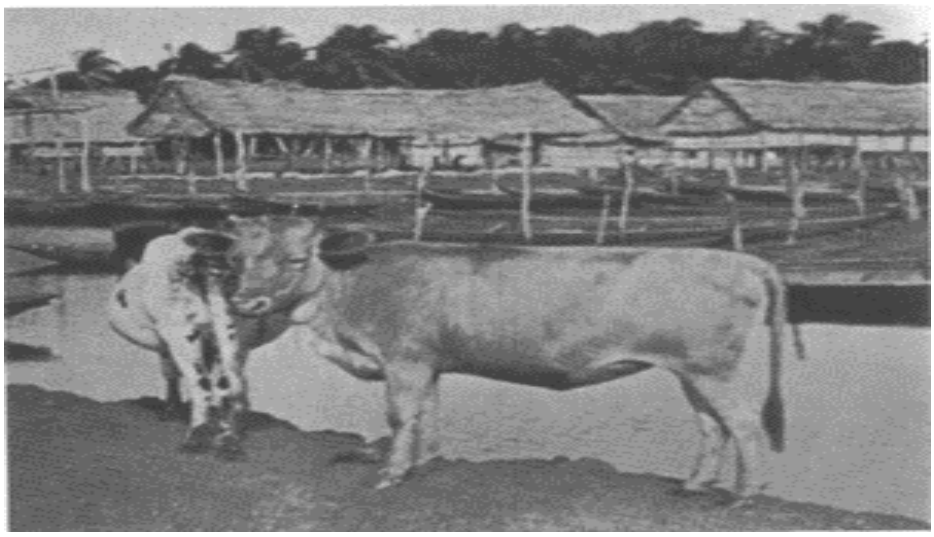


Figure 3. Crossbreeding between N'dama and west African short-horned cattle is frequent. This crossbred Dahomian heifer with short horns and a fawn coat is a good example.

TECHNICAL PROBLEMS

These are primarily the problems connected with any importation of animals, in particular the danger of introducing contagious diseases: rinderpest and contagious bovine pleuro-pneumonia. Since trypanotolerance is related to local strains of trypano-somes, the transferred animals may be susceptible to other strains. It is therefore advisable to ensure strict sanitary inspection during the first *few* months after importation. If necessary, trypanocidal treatments should be given to help the animals overcome trypanosome infections and enable them to adapt their production of antibodies to new strains against which they have no immunity.

HUMAN PROBLEMS

Trypanotolerant livestock are usually distributed in areas where cattle husbandry is a completely new activity. The operation therefore requires considerable organization and resources, at least for the first few years, its success depends on the training of the new stock-raisers. Under prose i': conditions, the introduction of" y anotolerant cattle is one of the most Hxtive methods for developing livestock production in countries where tr/panosomiasis is prevalent. It is costly, requiring considerable personnel, and is slow to start, but these drawbacks are more than offset by the results, which are permanent, whereas the methods considered previously, chemotherapy and *chemoprophylaxis,* must be repeated constantly.

A fourth method, vector control, can also be employed, and will be the subject of the third article in this series on African animal trypano-somiasis.

PART III. CONTROL OF VECTORS

P. FINELLE

Deforestation and bush clearing — an indirect method of tsetse control.

Tsetse flies are the chief vectors of African trypanosomes, and also serve as intermediate hosts in which the parasites multiply actively. Various methods may be used to control these vectors: indirect methods, that attempt to alter the environment so as to make it an unsuitable habitat for tsetse flies, and direct methods — chemical and biological — aimed at destroying the insects or at eliminating their ability to breed.

INDIRECT METHODS

Deforestation

The microclimate that is established by plant cover provides the most suitable combination of temperature and humidity for the tsetse fly because it limits variations in climate to a minimum. The fly concentrates in certain types of vegetation, which vary for the different tsetse species. When this vegetation is cleared, changes occur in the microclimate that may cause the species concerned to disappear. Use of this selective deforestation method therefore requires a very precise knowledge of the biology of the species concerned in the prevailing conditions.

Destruction of the vegetation can be done manually, by felling the trees, or by ringbarking in the case of plant species for which this technique is effective. Mechanical means can be employed with quicker results, but these can only be used in flat country. The use of arboricides has not proved very practical as they are expensive and slow-acting products that do not work well with all plant species.

Regardless of the technique used, the selective destruction of vegetation presents two major drawbacks: it is generally a very expensive operation and it increases soil erosion, which is liable to cause sterility in the cleared land. This method is now seldom employed, except to establish deforested barriers to prevent areas cleared by insecticide spraying from being reinvaded.

Elimination of wild animals

As well as being trypanosome carriers, wild animals are an important source of food for the tsetse fly. Studies of these hosts have shown that the tsetse fly obtains much of its nourishment from a small number of wild animal species, which differ for each species of fly. A control method has therefore been developed with the aim of

eliminating the preferred hosts. This method has been employed to a considerable extent in some countries of east and southern Africa with noteworthy results, but at the cost of the massive destruction of big game.

However, the elimination of wild animals, even if restricted only to host species, is not easy to accomplish, especially when it involves destroying small animals like the warthog, the bush pig and the small antelope which are the favourite hosts of many testse species. It has also been observed that the tsetse is not rigidly dependent on specific animal species, and that when the preferred hosts disappear it can feed on other species.

Because of these difficulties, and the increasing concern for wildlife protection, the control of tsetse fly by the selective elimination of wild animals is not to be recommended at present.

DIRECT METHODS

Insecticides

The treatment of tsetse-infested zones with insecticides is currently the most common method of eradication. Insecticides may be applied from the ground or from the air.

APPLICATION FROM THE GROUND

The method consists in applying a persistent insecticide where it has the most chance of coming into contact with tsetse flies, that is, on their most frequent resting places. The insecticide is applied in the dry season, not only to prevent it from being washed off by rain but also because the severe conditions prevailing in this season force the flies to concentrate on certain types of plant which provide a more favourable microclimate. Only one treatment is applied; the insecticide must therefore have sufficiently long persistence, exceeding the maximum pupation period, to act on the newly hatched insects coming from pupae deposited before the treatment.

The insecticides used are usually chlorinated hydrocarbons, chiefly DDT and dieldrin, both of which persist for several months on vegetation. DDT is more frequently used in regions with a Sudanian climate and a long dry season, while dieldrin is preferred in more humid regions with a Gui-nean climate. DDT is applied in the form of wettable powders or emul-sifiable concentrates, diluted to obtain a final concentration of between 2 and 5 percent according to the needs of the region. Dieldrin is used in concentrations varying between 1.8 and 2 percent, obtained from an emulsifiable concentrate. Insecticides can be applied with pressurized sprayers, or motorized or high-capacity vehicle-mounted sprayers. The choice of equipment is essentially a matter of convenience in use, according to the local vegetation and the physical features and extent of the area to be treated.

Ground spraying with portable prepressurized sprayers.

The preferred resting sites of the tsetse fly vary with the different species, the season and local ecological conditions. It is therefore essential to have accurate knowledge of the biology of the species in the region to determine the types of vegetation to be treated. In Nigeria, for example, in the Sudanian regions infested by *Glossina morsitans submorsitans* and *G. tachinoides,* a 2 percent DDT solution is applied exclusively on the supports where the tsetse flies rest during the hottest hours at the end of the dry season. In the case of *G. morsitans,* these supports are shady tree trunks with a diameter of over 20 centimetres from ground level to a height of about 1.5 metres. For *G. tachinoides* it is necessary to treat all tree trunks, visible roots overhanging the banks of streams and woody vegetation near water, all up to a height of about 1 metre. When forest galleries are relatively narrow and well separated from both banks of streams, only 5-metre strips are treated along each bank in the case of *G. tachinoides* and 10-metre strips for *G. morsitans.* If the forest is broader and the banks are not clear, treatment is effected in strips, about 20 metres wide, along the outside edge of the forest and inside the forest galleries in the direction of the stream, at intervals of about 100 metres. In flood plains, where the forest is divided into thickets, only the edges of the thickets and narrow parallel strips inside them, about 20 metres apart, are sprayed. Operations of this kind in northern Nigeria have given highly satisfactory results, and the zones where tsetse flies have reappeared and require further treatment do not exceed 1 percent of the total area treated.

The technique of selective spraying with persistent insecticides using ground equipment has been and continues to be widely and successfully employed in several countries against various species of tsetse fly. It has been most extensively applied in northern Nigeria, where it has led to the clearing of some 125 000 square kilometres, and where the programme is continuing at the rate of about 12 500 square kilometres a year. It is an attractive method because of its effectiveness, its relatively low cost, and because it results in reduced environmental contamination. It should be stressed, however, that it requires thorough prior studies of tsetse fly ecology to determine the conditions for using insecticide, and large, well-equipped and well-trained spraying teams, as well as a dense network of roads and tracks. The method is of real value only in regions where the habitat of the tsetse fly is relatively restricted, at least for part of the year. These various conditions, which cannot often be met, have led to the adoption of aerial spraying, which gives quick results and requires limited personnel.

AERIAL SPRAYING

The first attempts to control tsetse fly by the aerial spraying of insecticide were made in 1948 in Tanzania, and the first large-scale operation was carried out shortly afterwards in South Africa, leading to the elimination, although at a very high cost, of *G. pallidipes* in the Zululand region. Research, particularly in Tanzania, has led to improvements in this technique and to appreciable reductions in its cost. The .insecticide can be applied either as an aerosol without residual action, distributed over the whole infested zone, or as a deposit, with a persistent effect, on the preferred resting sites only.

Ground spraying with portable motorized sprayers.

Aerial spraying.

Aerosol

The aerosol method has been used in various countries, including Rwanda, Kenya and Tanzania, but has been applied most widely in Zambia.

The insecticide is sprayed in the form of fine droplets with a diameter between 10 and 60 *im,* which because of their lightness remain suspended in the air and can penetrate through the vegetation and reach the adult tsetse flies. However, the insecticide is not deposited on the vegetation and has no persistent action, so the treatment must be repeated to reach the tsetse flies which had been in the pupal stage at the time of the first operation.

DDT, BHC, dieldrin, endosulfan, iso-benzan, fenthion and pyrethrum have been used. Light single-engined aircraft are generally employed for these operations, fitted either with heat generators working off the engine exhaus| pipe or with rotary sprayers. In Zambia, heavier two-engined aircraft, which allow more rapid operation, are used successfully. Recent advances in aerial spraying techniques have enabled considerable reductions to be made in rates of application, with highly concentrated insecticides; in Zambia, endosulfan is sprayed at the rate of 30 grams per hectare. Operations are carried out during the dry season, at a time when many trees lose their leaves,

and at hours when the meteorological conditions are favourable, at dawn and just before sunset. The aircraft are guided either by ground teams using radio or markers, or by the aircraft's own navigating equipment.

Aerial sprayings are repeated four or five times, at three-week intervals. The conclusion to be drawn from the various, operations for controlling tsetse flies by the aerial spraying of nonpersistent insecticides is that total elimination is possible provided the treated area is suitably isplated from other contaminated regions. In Zambia aerial spraying has led to the eradication of *G. morsitans* from nearly 15 000 square kilometres at a lower cost than spraying with ground equipment. However, the method suffers from several disadvantages: it requires total coverage of the tsetse-infested zone and all that this implies from the point of view of environmental pollution; it requires several applications staggered over about 100 days; the insecticide, which is dispersed in the form of fine droplets, is often carried away by the wind to areas outside those to be treated. Because of these disadvantages the application of long-lasting insecticides is sometimes preferred.

Persistent insecticides

Various tests involving the aerial spraying of persistent insecticides have been made, using an inverted-emulsion insecticide or by selective spraying from helicopters.

Inverted emulsions. In these tests, performed in Kenya under a who/ fao project, dieldrin was used as a water-in-oil emulsion instead of the normal oil-in-water formula. With these inverted emulsions, in which the oil phase is the continuous one, evaporation is limited and the droplets are coarser and less sensitive to atmospheric conditions, so that they reach the target zone with greater accuracy. The vegetation of the region chosen was composed of very dense thicket infested by *G. pallidipes.* Applications were made from aircraft and helicopters, but the latter proved to be more expensive. The results of these preliminary tests proved that the technique was effective in the conditions prevailing in the region, and that its cost was competitive with that of the other methods. The tests should be continued and extended to other species and other types of vegetation.

Selective spraying. The principle underlying the selective spraying of persistent insecticides by helicopter is similar to that of selective spraying using ground equipment: the aim is to treat only the vegetation used as a refuge by the tsetse fly, when weather conditions for the insect are most severe. This method has been successfully employed in northern Nigeria in regions infested by *G. morsitans submorsitans.* A helicopter flying at a low speed (about 40 kilometres an hour) 1 or 2 metres above the treetops sprays (in the dry season) 10 percent dieldrin in the form of droplets with a diameter varying between 90 and 200 (xm, over a width of about 20 metres, at the rate of 1.5 kg of active product per hectare. This technique has turned out to be effective, but expensive if the price is related to the area treated. However, if account is taken of the fact that only 10 percent of the total area is effectively treated, the cost of this method calculated in terms of the cleared area is comparable to that of unselective spraying by aircraft.

PROBLEMS OF CHEMICAL CONTROL

Whatever the technique used, chemical control of the tsetse fly raises several general problems.

Isolation of the cleared region

It is obviously necessary to prevent the cleared region from being reinvad-ed by insects from neighbouring contaminated areas. This can be achieved either by establishing barriers which have been deforested or treated with persistent insecticides and which are sufficiently broad to prevent crossing by the tsetse fly, or simply by re-treating the edge of the already treated zone the following year. It is also necessary to check the movements of vehicles, livestock and possibly of game, all of which can transport tsetse flies over great distances.

Pollution

Chemical control of the tsetse fly certainly raises the pollution level of the area concerned. The insecticides used, usually chlorinated hydrocarbons, are toxic to other insects, including useful species such as bees or predatory insects. They are also very toxic to fish. Their toxicity as far as birds and mammals are concerned is still not very clear, but it is known that they can accumulate in fats.

It must be noted, however, that the levels and quantities of insecticides used for tsetse fly control are much lower than those for controlling crop parasites, and hence account for only a very small part of the general pollution caused by pesticides. Nevertheless, it is highly desirable that new insecticides should be tested with the aim of finding a product with a more selective action and with fewer effects on the environment than the organochlorinated compounds currently used. Biological control methods may also provide a solution to this problem.

Biological control methods

The biological control of tsetse flies is as yet only in the experimental stage. Two methods, which have been proved against other insects, may be considered: the use of organisms pathogenic to tsetse flies, and genetic control.

PATHOGENIC ORGANISMS

Little is known about the pathology of tsetse flies. While there is information concerning insect parasites which prey on tsetse pupae and which in nature certainly play a part in limiting the number of flies, so far there has been no success in breeding these parasites in the laboratory.

Likewise, no work has been done on organisms that are pathogenic to tsetse flies, an approach which has been so promising in the control of other insect species. These matters should receive the very close attention of research laboratories.

GENETIC CONTROL

Research on the genetic control of the tsetse fly has made great strides since it became possible to breed these insects in the laboratory on a large scale. It is now possible to consider methods involving induced sterilization or the transmission of lethal genes. The underlying principle is that as the female tsetse fly generally copulates only once at the beginning of its life, it will produce no progeny when inseminated by a male whose spermatozoids have undergone chromosomal modifications that render them incapable of fertilizing the egg. By releasing a sufficient number of sterile males in a region so that they have a greater chance of mating with the females than the existing normal males, a reduction and eventually an extinction of the population through reduced numbers of progeny can be achieved.

Contact with various chemical products can cause sterility in males, and the gamma irradiation of adult males has the same effect. Laboratory studies have shown that although the longevity of the males is reduced, they live long enough to copulate several times and retain their ability to mate. The advantages of this system are obvious. The species is used to destroy itself, without disturbing the natural biological equilibrium. The males seek out the females and can track them down in places inaccessible to man.

The application of this method requires a large number of males, which is now possible through recent improvements in laboratory breeding techniques. One important problem which remains to be solved concerns the behaviour of the artificially bred insects when they are released in a natural environment. First experiments suggest that after some days of adaptation the sterilized males tend to behave like normal insects, although their longevity is significantly curtailed. However, the practical and economic feasibility of this method can be fully established only after pilot tests have been carried out on the most important tsetse fly species.

The main factor governing feasibility is the size of the natural tsetse fly population. It will certainly be effective to release sterile males after the population has been reduced by treatment with a nonpersistent insecticide. From this point of view, the release of sterile males may be regarded as a supplement to chemical control, opening the way to the complete elimination of the tsetse fly after a brief and nonpolluting insecticide treatment.

Conclusions

The methods of controlling animal trypanosomiasis are numerous and varied; each possesses advantages and disadvantages and these must be assessed in the light of local data and the end results that are sought.

In the fourth and final article in this series, the economic problems raised by animal trypanosomiasis and its control will be considered, in order to assess the relative costs and benefits of the various methods.

PART IV. ECONOMIC PROBLEMS

P. FINELLE

In the previous three articles the methods that can be used to control African animal trypanosomiasis and its principal vector, the tsetse fly, have been examined. However, control of the disease is not exclusively a technical problem because, as the Joint fao/who Expert Committee on African Trypanosomiasis has stated: "The problems caused by the disease should be viewed against the wider background of the general social and economic needs of the countries concerned."

Trypanosomiasis control is expensive. Before large-scale operations are undertaken, an economic analysis is necessary for the region in question to show the extent of the socio-economic losses due to the disease, to determine the priority of trypanosomiasis control in development planning, and to furnish the data needed to estimate the economics of possible control methods by a comparison of their cost with the results that can be expected of them.

Socioeconomic consequences

It may therefore be useful to assess the socioeconomic consequences of African animal trypanosomiasis, and the cost of the different control methods.

INFLUENCE ON LIVESTOCK DISTRIBUTION IN AFRICA

The number of cattle in Africa is assessed at around 160 million head, but distribution is very uneven among the various regions. Two phyto-climatic zones are virtually unsuitable for cattle raising, owing to lack of pasture: the desert or semidesert zone roughly corresponding to the regions where annual precipitation is less than 300 millimetres, covering about 12 million square kilometres, and the zone of dense rain forest, estimated to cover 3 million square kilometres. It can therefore be assumed that of the 30 million square kilometres of the African continent only half carry pastures suitable for livestock raising. These are the areas covered by wooded steppe or savanna. Yet even in these 15 million square kilometres, with their obvious suitability for grazing, livestock production is very unevenly distributed. While the number of cattle exceeds 20 per square kilometre in the most favoured places, there are areas which are completely devoid of livestock despite the presence of good-quality grazing and plentiful water supplies. This anomaly is partly due to animal trypanosomiasis, which occurs in tropical Africa over about 10 million square kilometres. As the infected areas include the 3 million square kilometres covered by equatorial forest, the land suitable for grazing in which livestock raising is limited by animal trypanosomiasis can therefore be estimated at 7 million square kilometres.

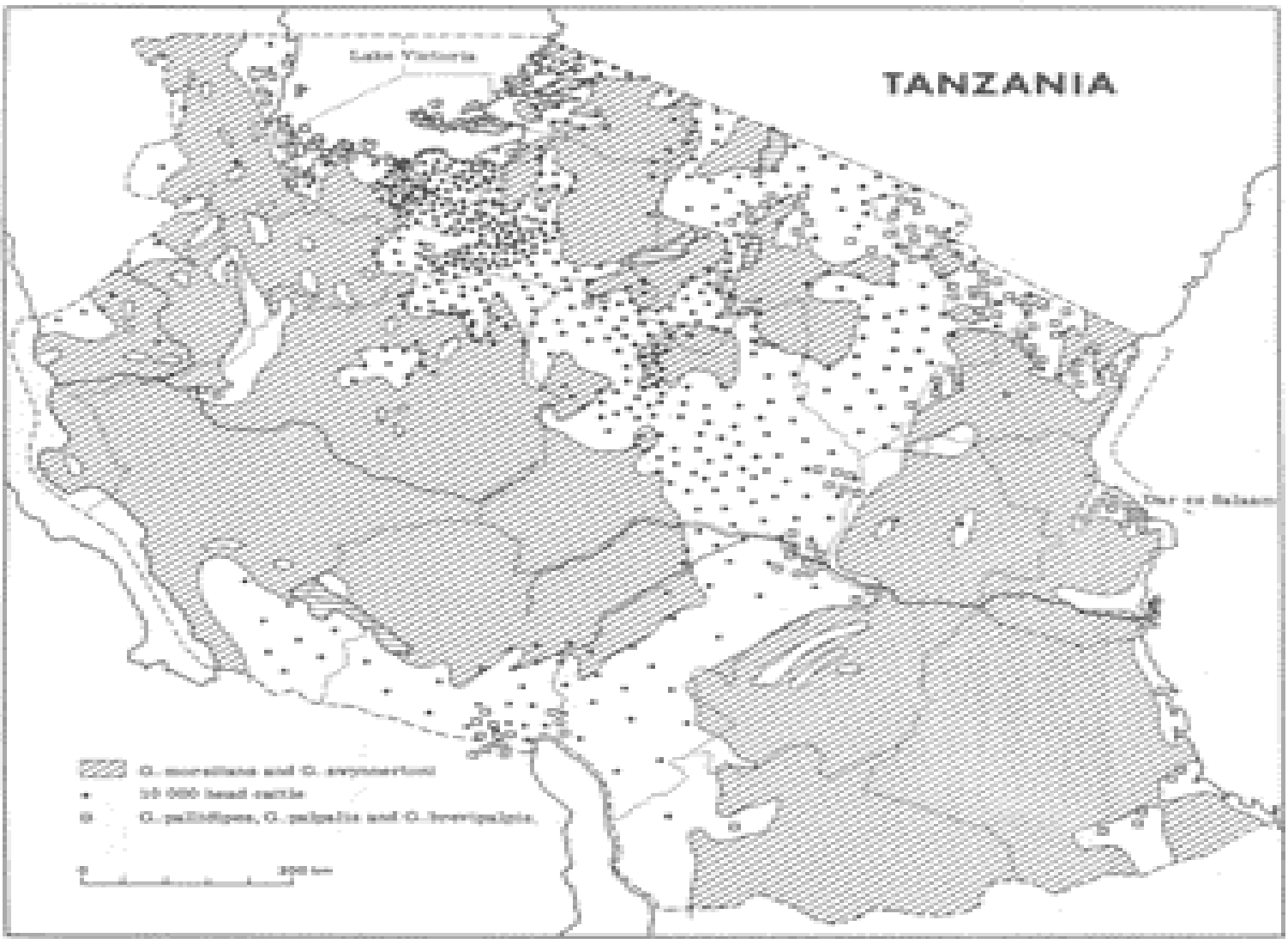
The consequences of animal trypanosomiasis vary in gravity from place to place. Broadly speaking, there are:

— areas where there is virtually no livestock raising;

— areas where only certain livestock breeds, possessing a natural resistance to trypanosomiasis (trypanotolerant breeds), can live;

— areas where, despite the presence of tsetse fly, livestock susceptible to trypanosomiasis can be raised either because of particular local conditions (tsetse flies are limited in number or confined to certain plant types) or because curative or preventive treatment is regularly practised.

In every case, however, trypanosomiasis leads to considerable under-exploitation of natural resources, and to a lower level of animal production than could be achieved if the disease were eliminated.



The influence of the tsetse fly on animal production is nowhere more clearly illustrated than in Tanzania, where the geographical pattern of cattle distribution is almost exactly the opposite of that of tsetse distribution.

ANALYSIS OF SOCIOECONOMIC CONSEQUENCES

The socioeconomic importance of African animal trypanosomiasis is extremely difficult to assess, as the data available are fragmentary and frequently very approximate. In the Present state of knowledge it is possible only to enumerate the various consequences of trypanosomiasis, in the hope that this list can serve as a base for assessments at the local level. Two sets of consequences, direct and indirect, can be identified:

1. *The direct consequences,* represented by the economic losses due to the disease and to the various expenditures incurred in controlling it.

They comprise:

a. mortality;
b. disease, which manifests itself in emaciation, retarded growth, abortion, temporary sterility and various organic lesions;
c. the cost of detection and treatment of infected animals (veterinary service personnel, trypanocidal drugs, equipment, operating expenses);
d. the cost of preventive operations (chemoprophylaxis, tsetse fly control, development of trypanotolerant livestock);
e. the cost of research on animal trypanosomiasis control.

2. *The indirect consequences* of animal trypanosomiasis affect:

a. human health, as the shortage of meat and milk causes protein deficiencies which are particularly harmful to children;
b. agriculture, because the lack of draught animals and manure reduces agricultural output;
c. livestock production: (i) trypanosomiasis limits the possibilities of introducing improved breeds, which are highly sensitive to this disease, thus preventing the upgrading of local livestock by crossing with imported sires; (*ii*) the presence of trypanosomiasis causes livestock to be concentrated in limited grazing areas, which results in their overuse and deterioration; *(Hi)* seasonal variations in the incidence of trypanosomiasis prevent some pastures from being grazed throughout the year and compel herdsmen to practise transhu-mance, which holds them back from integration in the national community;
d. the economy: the deficit in animal production compels countries where trypanosomiasis is rife to resort to imports of meat and dairy products, a practice harmful to their balance of trade.

TENTATIVE ESTIMATE OF DEFICIENCY TO BE MADE UP

While it is impossible to make even a rough assessment of the various socioeconomic consequences of tiy-panosomiasis, one can nevertheless try to estimate the meat production of the regions concerned, if the disease were controlled, on the basis of the following criteria:

— area of the tsetse-infected zone which could be used for livestock raising: 7 million square kilometres;

— average potential density: 20 cattle per square kilometre;

— total potential population of infected zone: 140 million cattle;

— present population: 20 million cattle;

— possibility of increasing the cattle population: 120 million head;

— average productivity in Africa: 12.5 kg per head per year;

— additional meat production: 1.5 million tons per year;

— value of additional meat production (on the basis of 50 cents per kg): US$750 million.

Although very approximate, this estimate shows how animal trypanosomiasis control could contribute to the development of animal production at a time when demand for animal protein, especially beef, is constantly growing and when projections indicate a serious shortfall in the years to come.

Cost of controlling African animal trypanosomiasis

Estimating the cost of controlling trypanosomiasis is no easier than the other estimates of the cost of the disease, as some expenses cannot be accurately quantified. The figures available are not always comparable because they are calculated according to criteria that vary with each country. Therefore, the data given here aim only at supplying an order of magnitude, which will allow an assessment and comparison of the average costs of the possible control techniques.

CHEMOTHERAPY

Approximate cost of a curative dose for a 300-kg bovine animal:

Diminazene (3.5 mg/kg) 15 cents Isometamidium (0.5 mg/kg) 19 cents

The cost of application is difficult to calculate, as it must include a proportion of the costs of the veterinary service and of its budget (peisonnel, equipment and operation) devoted to trypanosomiasis detection and treatment. However, this can be estimated to be around 50 cents. It may therefore be assumed that the cost of curative treatment for a 300-kg bovine animal varies between 65 and 70 cents.

CHEMOPROPHYLAXIS

Approximate cost of a preventive dose for a 300-kg bovine animal:

Isometamidium (1 mg/kg) 38 cents Cost of the opetation 50 cents

As preventive treatments must be repeated on average every four months, the annual cost of chemo-prevention for a 300-kg bovine animal would be about US$2.65.

INTRODUCTION OF TRYPANOTOLERANT LIVESTOCK

An analysis of the cost of importing trypanotolerant livestock was made in 19661 following the import into the Central African Republic of 254 animals from Upper Volta and Ivory Coast. The expenditure was broken down into the following percentages:

Purchase of animals	17.0
Transport	35.3

Salaries of purchasing mission personnel	25.3
Miscellaneous	22.4

The average purchase price of an animal was US$40, and its total average imported cost was US$247 (1966).

TSETSE FLY CONTROL

Data on the costs of tsetse fly control are numerous but difficult to compare because they depend on many factors, especially: the evaluation of the area of *the* cleared zone and its relation to the area actually treated, the accounting system used for certain expenses (management personnel, equipment amortization), the expenses involved in preliminary surveys, subsequent surveillance and conservation measures, and the utilization of the cleared zone.

Variations in the parity of the different cunencies involved also make comparisons difficult.

Deforestation

It is impossible to state average costs for deforestation operations because of their great variation. An example is the cost of deforested barriers in northern Nigeria in 1970, which varied between $3 500 and $4 200 per square kilometre.

Ground spraying

Costs vary greatly with the region and the species of tsetse fly. Some recent examples are given in Table 1. *Air spraying*

Table 2 gives the average cost of the various methods based on recent operations.

These data show how difficult it is to forecast the cost of tsetse control operations. Consequently, it would be hazardous to advise which technique would be the most economic in a given area. Recommendations can be made only after pilot trials have been carried out.

Table 1. Cost of ground spraying

Country	Tsetse fly	Average cost per		Remarks
		Treated	Cleared	
		..... *US. dollars*.....		
Nigeria, 1971	*G. tachinoides* *G. morsitans submorsitans*	20-50	10-25	Not including management personnel and equipment amortization
Botswana, 1971	*G. morsitans centralis*	170	18	Insecticide 29 percent; personnel 54 percent; miscellaneous 17 percent
Zambia, 1971	*G. morsitans morsitans*	300	300	

Table 2. Cost of air spraying

Method	Country	Tsetse fly	Average cost per square kilometre, 1971		Remarks
			Treated	Cleared	
			... U.S. dollars ...		
Nonpersistent aerosol	Zambia	*G. morsitans morsitans*	210	210	Insecticide 56 percent; aircraft 29 percent; miscellaneous 15 percent
Persistent insecticide	Nigeria	*G. morsitans submorsitans*	2000	200	Insecticide 52-54 percent; helicopter 36-41 percent
Inverted emulsion of persistent insecticide	Kenya	*G. pallidipes*	218	218	This cost covers only: insecticide 67 percent, flying hours 33 percent

Conclusions

This attempt to analyse the economic problems raised by African animal trypanosomiasis shows that accurate data are so limited that it is almost impossible at present to draw up even an approximate report.

Aware of these significant limitations, fao plans to undertake a two-year study which will include a number of local surveys in carefully selected regions. The study will furnish the basic data for an assessment of the socioeconomic importance of trypanosomiasis and the costs of the various methods used to control it, and should draw the attention of interested governments and assistance organizations to the necessity for a very substantial increase in funds for field operations if the disease is to be controlled to an extent that would allow a significant expansion in animal production.

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* Animal Health Officer, Animal Production and Health Division, fao, Rome.

P. Finelle Animal Health Officer, Animal Production and Health Division, fao, Rome.

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A review of the prospects for vaccination in African trypanosomiasis

Part I

M. *Murray, J.D. Barry,* W.I. Morrison, R.O. *Williams,* H. *Hirumi and* L. Rovis

This review of the prospects for developing an immunization procedure against trypanosomiasis explores a number of promising avenues of research. Part I covers variable antigen types (VATs); metacyclic antigens; *In vivo* and *in vitro* attenuation; and molecular and genetic engineering.

Part II, to be included in the next issue of this journal, will cover immunogenicity of subcellular fractions; immunological intervention against the tsetse fly; induction of increased resistance by immunostimulants; trypanotolerance; and infection and treatment. This will be followed by a brief statement of the conclusions reached.

The present methods available for the control of African trypanosomiasis, namely, systematic case detection and treatment, and tsetse control, do no more than limit the disease although both these approaches have been shown to be effective where they have been vigorously applied. The disadvantages attending the use of trypanocidal drugs include lack of availability of effective drugs, drug resistance and, in heavy tsetse fly challenge areas, the frequency with which treatment has to be applied, often to economically unacceptable levels. In the same way, while tsetse flies may be completely eradicated in certain areas by insecticide control, few regions of tsetse infestation have circumscribed boundaries and, unless cleared areas are defended (a costly exercise), reinvasion by the tsetse fly inevitably occurs. Thus there is little doubt that the introduction of an effective vaccine, if used strategically along with established control methods, would make an enormous contribution to the control of African trypanosomiasis, not only by increasing productivity in endemic trypanosome areas but also by opening up for exploitation the vast areas of the African continent largely devoid of livestock because of trypanosomiasis.

The major constraint to developing a trypanosome vaccine is the ability of the parasite to undergo antigenic variation. Murray and Urquhart (1977) reviewed the various attempts made to vaccinate both domestic livestock and laboratory animals and it was obvious from the reported studies that complete protection was readily achieved only if the same variable antigen type (vat) was used for immunization and challenge. When a distinct vat was used for challenge no protection occurred. Therefore, it would appear that an effective vaccine would have to contain all vats, possibly an insurmountable task as the number of vats, although as yet undetermined, is likely to be large. The result is that many workers in trypanosomiasis research consider the possibility of vaccination to be remote. It should be borne in mind, however, that many of these conclusions have been drawn from work on laboratory animals, which invariably succumb to massive parasitaemia. There is evidence to show that under certain circumstances cattle can control parasitaemia and then clinically recover. While this is particularly true for trypanotolerant breeds such as the N'Dama, it can also occur in the more susceptible zebu (Stewart, 1951; Chandler, 1958; Desowitz, 1959; Wilson, 1971; Wilson and Cunningham, 1971 and 1972; Murray *et al,* 1979). The greater capability of the bovine to control parasitaemia creates a new perspective on the question of vaccination. Furthermore, advances in scientific knowledge and technology have opened up several different avenues of research and the present article attempts to explore these.

Variable antigen types (VATs).

Antigenic variation, the major obstacle to developing a trypanosome vaccine, is the process whereby trypanosomes sequentially express a series of surface antigens; it is these antigens that are capable of inducing protective immunity. The immune response against each variant, although rapid and highly effective in destroying any trypanosomes that possess that particular antigen, is invariably *too* late *to* affect that proportion of the population that has altered its antigenic identity. Thus, parasitaemia rises and falls in waves with each parasite population carrying different surface antigens (reviewed by Cross, 1978; Vickerman, 1978). This picture of successive waves of a specific antibody chasing variant trypanosomes has been likened by Goodwin (1970) to a "Tom and Jerry cartoon with a monstrously inept cat pulling the place down in its efforts to pulverize a diminutive and highly resourceful mouse".

What would appear to be required is as complete as possible an understanding of antigenic variation in order that, eventually, it might be possible to produce an effective vaccine by the strategic use of certain trypanosomes or their components. At the population level, the authors' knowledge has been increasing over the past few years, thanks mainly to the concept of multiple cloning in which bloodstream populations are divided into their component parts, namely single trypanosomes, each of which gives rise, in a fresh host, to a defined population that can be frozen as reference material. It is essential that as large a number of clones as possible be isolated, since only then will it be possible to detect some of the subtle immunological and biological differences within and between populations.

This approach has begun to reveal what occurs within a parasitaemic peak, to the level of the individual parasite. It appears that a peak is usually a mixture of vats (Van Meirvenne, Janssens and Magnus, 1975a) with the switch to expression of another type, probably occurring before the appearance of antibody, which is thought to act merely as a selective agent (Van Meirvenne, Janssens and Magnus, 1975a; Le Ray *et al.,* 1977). Examination of sequence of appearance of vats arising within cloned infections has confirmed and extended the observation of Gray, 1965) that there is a tendency for certain types to occur preferentially in the early parasitaemic peaks. Thus, it would appear that vats can be divided into these early "predominant" types and other groups of vats that occur later (Van Meirvenne, Janssens and Magnus, 1975a; Capbern *et al.* 1977).

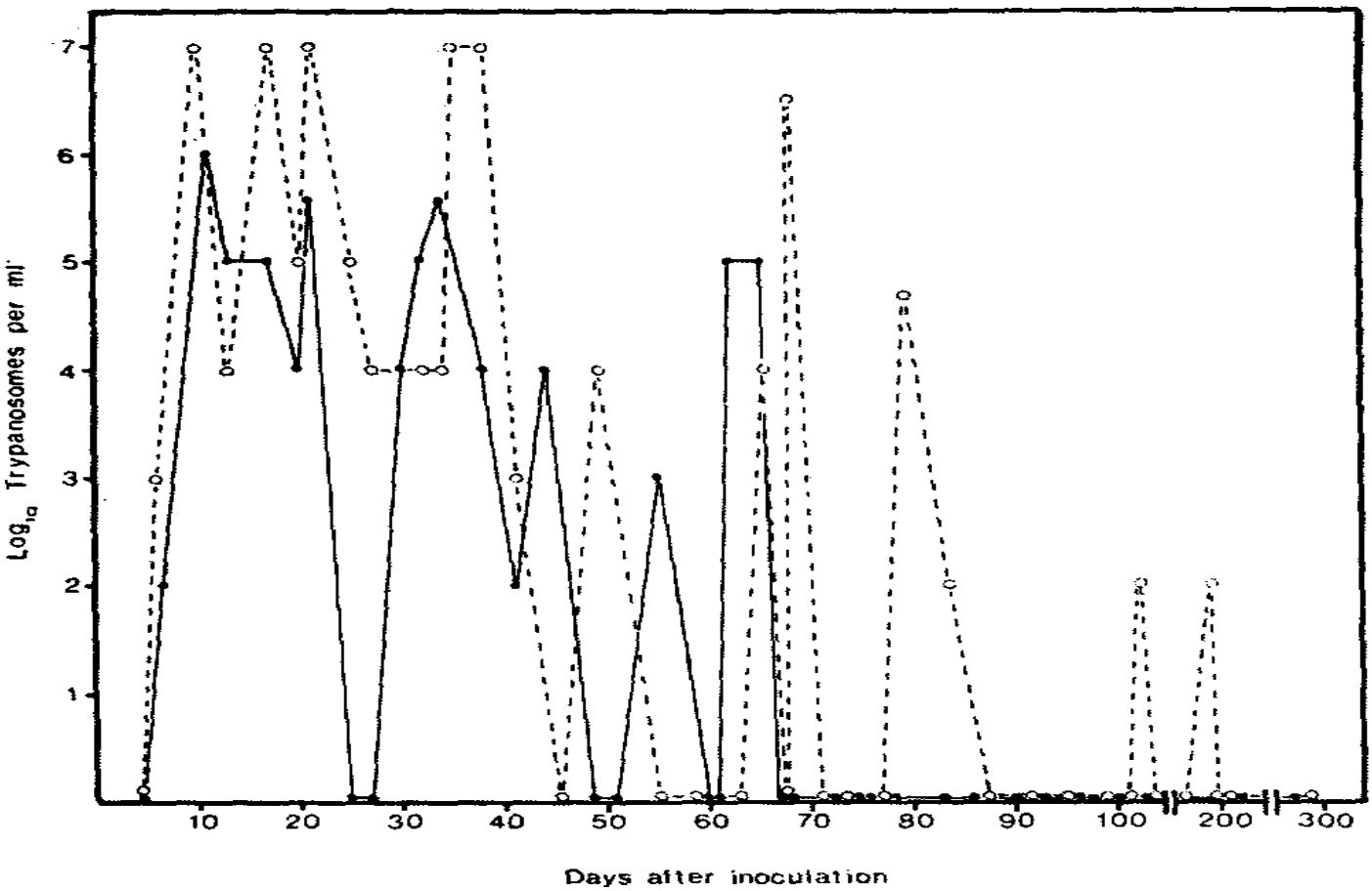

Figure 1 *Parasitaemia profile in an individual four-year-old N'Dama* (•) *and a four-year-old zebu* (o) *inoculated with* Trypanosoma congolense. *Note that the level of parasitaemia is lower in the N'Dama as is the duration of parasitaemia. Both animals were negative for detectable parasites for several months prior to the termination of the experiment and both made a clinical recovery.*

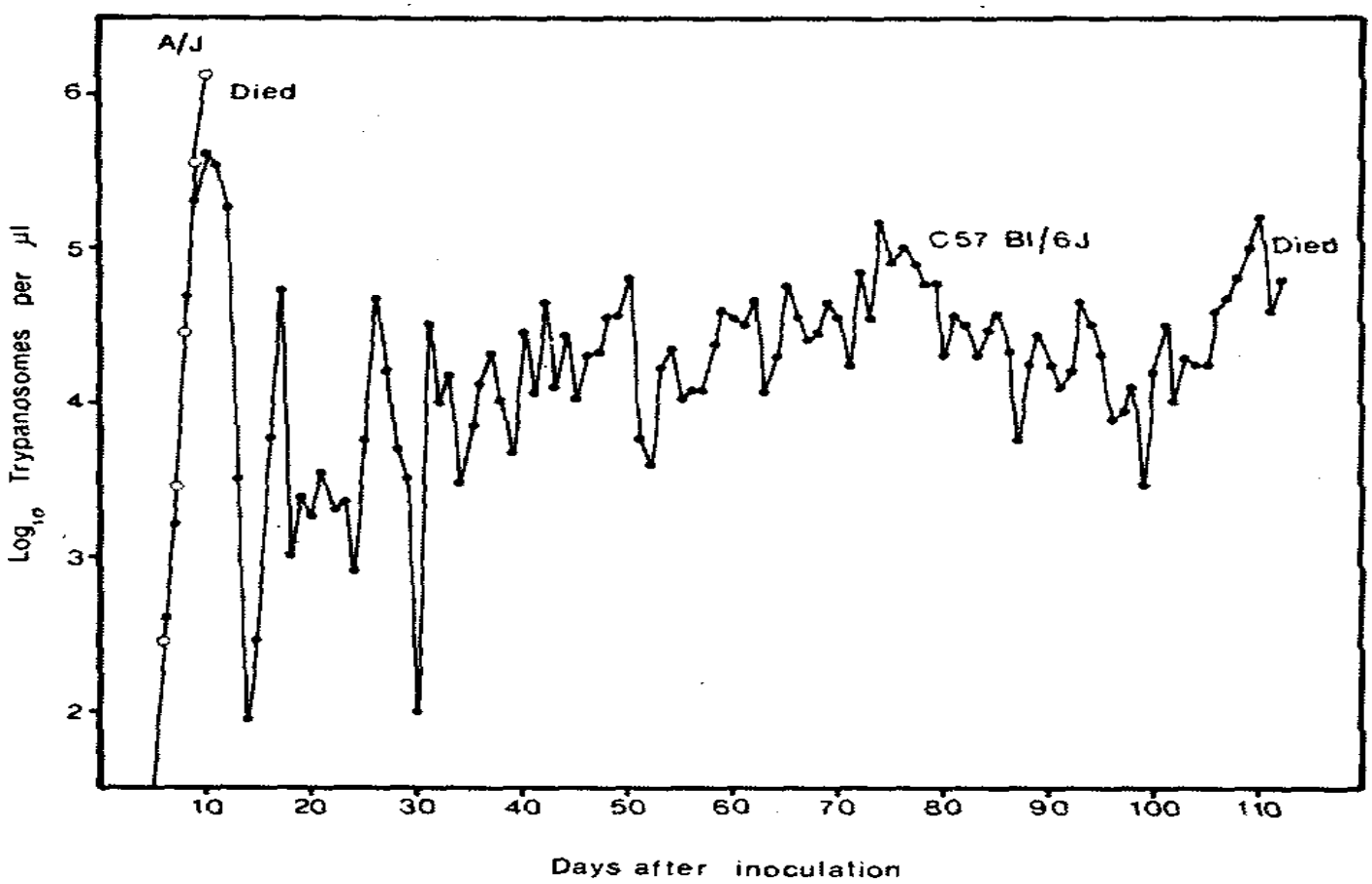

Figure 2 *Parasitaemia profile in an individual C57BI/6J mouse* (•) *and A/J mouse* (o) *inoculated with* Trypanosoma congolense. *The CS7BI/6J was able to control and reduce parasitaemia levels to a significantly greater extent than the A/J and as a result was able to survive for over 100 days. Irrespective of breed or strain, cattle were able to control and reduce parasitaemia to a much greater extent than mice. Following infection in mice, death was inevitable, whereas in cattle recovery may occur, particularly in N'Dama animals.*

The total number of vats that a trypanosome can express is known as its "vat repertoire," the full extent of which is as yet unknown although Capbern *et al.* (1977) have been able to isolate 101 vats from one clone of *Trypanosoma equiperdum.* Comparison of vat repertoires from different clones has been initiated (Van Meirvenne *et al.,* 1975b; Van Meirvenne, Magnus and Vervoort, 1977) and has revealed a surprisingly high degree of similarity; in fact, some vats have been found in every repertoire examined. In addition, there is now indirect evidence from serological studies that during an infection certain vats may recur, in some cases within a few weeks of one another. This has been described in cattle infected with *Trypanosoma congolense* (Wilson and Cunningham, 1971) and with *T. brucei* (Nantulya, Musoke, Barbet and Roelants — unpublished results).

As regards vaccination, a rational approach may be successful. Immunization against individual vats is highly effective using such regimes as infection and treatment; irradiated organisms; killed organisms; crude emulsions containing released soluble antigens; formalized whole infected blood or plasma and purified variable antigen glycoprotein (reviewed by Murray and Urquhart, 1977). As little as 3 µg of variable antigen can give protection in mice (Baltz *et al,* 1977). A cocktail vaccine based on predominant vats is likely to be effective against with that repertoire. Investigation of the feasibility or such an approach requires complete analyses of the number of vats, both predominant and otherwise, within a repertoire, of the extent of crossreaction between repertoires and, eventually, of the number of vats that exist within and without given geographical areas.

A word of warning regarding studies on antigenic variation: it is necessary to define not only the parasite but also the host. The parasitaemic patterns produced by a trypanosome will vary with species of host, breed or strain, age, sex, etc. (Figures 1 and 2). In this regard, there is little doubt that exploitation of the *in vitro* culture system, which supports the growth of animalinfective forms of trypanosomes (Hirumi, Doyle and Hirumi, 1977) by eliminating the variable effects of the host, must yield new information on the basis and mechanisms of antigenic variation. Since much of the above work has been carried out with *T. brucei* the authors believe that it is essential that similar efforts be made with *T. congolense* and *T. vivax,* which are regarded as the major pathogens of bovine African trypanosomiasis.

Metacyclic antigens.

Following ingestion by the tsetse fly, *T. brucei* loses its surface coat, which contains the variable antigen. It eventually regains the coat in the fly's salivary gland in becoming the mammalianinfective metacyclic stage (Vickerman, 1969). It has been suggested that all trypanosomes of a particular clone revert to a common "basic" antigen type in the salivary gland (Jenni, 1977, for *T. brucei;* Nantulya, Doyle and Jenni, 1979, for *T. congolense)* akin to the "basic" type arising in the bloodstream after cyclical transmission (Gray, 1965). Vaccination against such types would obviously be of importance. However, there is now evidence to suggest that this is not the case and that *T. brucei* metacyclics arising from the passage of a clone through the tsetse are antigenically heterogeneous (Figure. 3) (Le Ray, Barry and Vickerman, 1978; Barry and Hajduk, 1979; Barry *et al,* 1979b), although it is still the case that there may be only a limited number. A drawback to the potential use of metacyclic populations for vaccination is that they are antigenically unstable (Le Ray *et al,* 1977; Le Ray, Barry and Vickerman, 1978), preventing mass production of antigen and mRNA (see later, molecular and genetic engineering) for potential vaccine preparation. However, these *dfficulties* may be overcome by a recently devised protocol (Barry *et al,* 1979b) whereby antigenically more stable mammalian bloodstream forms with the same vat as metacyclics can be identified and cloned giving rise to populations suitable for bulk preparative procedures. This approach could be pursued to define the vat complement of metacyclic populations with a view to vaccination against trypanosomes of that vat repertoire. Furthermore, it is essential to determine the degree of crossreaction between metacyclics of different repertoires.

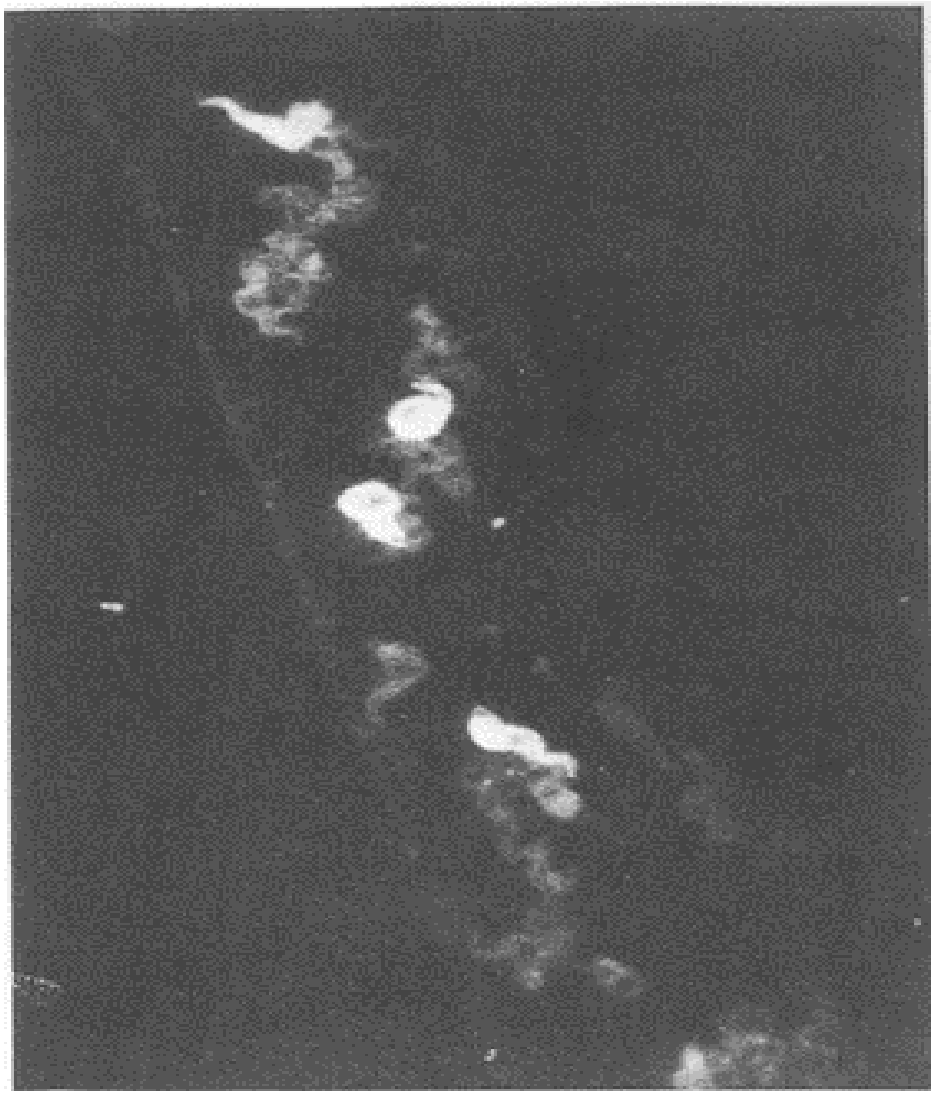

Figure 3 *Antigenic heterogeneity among mammalianinfective metacyclic forms in the saliva of a tsetse fly. The fly was allowed to salivate onto a heated glass slide, to which immunofluorescence was applied using specific antiserum against a characterized bloodstream form trypanosome* vat. *Metacyclics with trypanosome* vat *fluoresce strongly, while those of other* vat *display the weak fluorescence of the counterstrain.*

The *in vitro* culture system would also appear to have potential in this area. It has now been shown that "bloodstream forms" of *T. brucei* in culture (Figure 4) can be induced to undergo morphological changes similar to those that occur in the fly, including the eventual production of metacyclic types, by appropriate manipulation of the culture conditions (Hirumi, Hirumi and Doyle, 1978a). As it has now become possible to clone parasites in culture (Hirumi, Hirumi and Doyle, 1978b) this approach might offer a source of metacyclic types of defined antigenic identity.

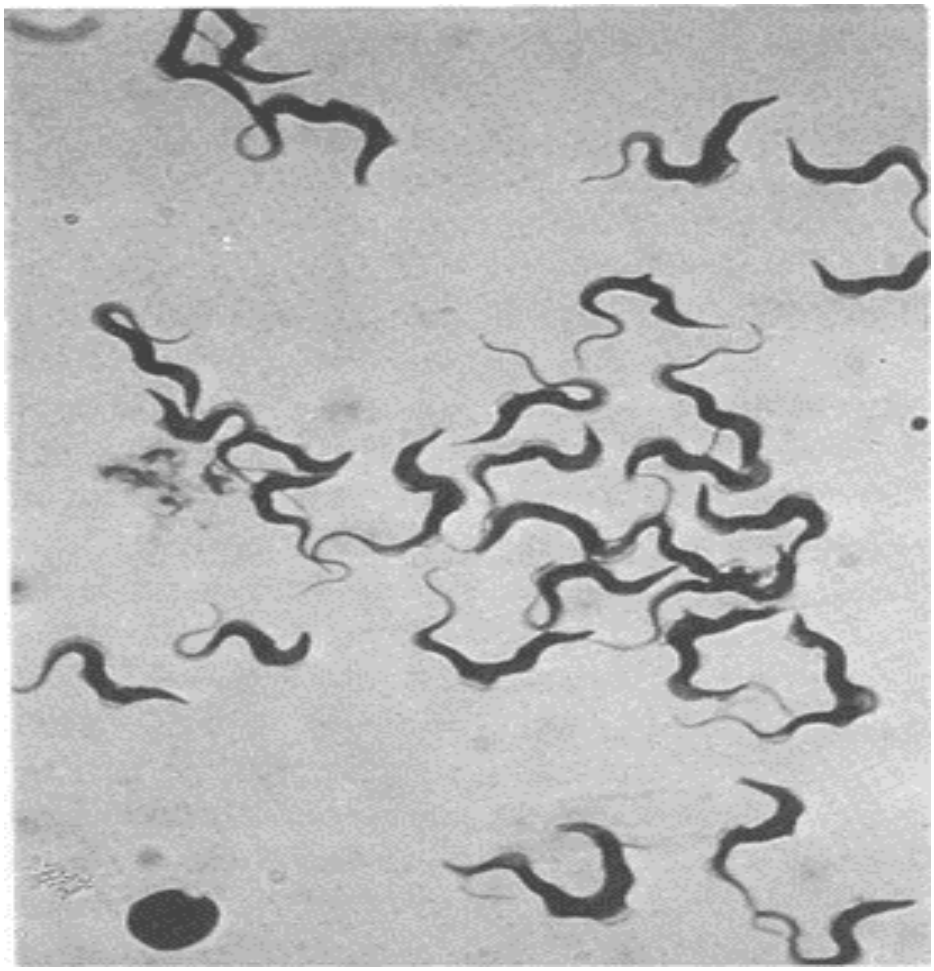

Figure 4 *Bloodstream forms of* Trypanosoma brucei (*ILR-TbC-221*) *grown* in vitro *for over 31 months. Giemsa's stain.*

In vivo **and** *in vitro* **attenuation.** Another facet of the problem is that, despite the authors' steadily increasing knowledge of antigenic variation, very little is known of how it is linked to the biology of the trypanosome and the hostparasite interaction, apart from the fact that it allows the trypanosome to evade the host's immune response and thus survive. For example, an association between vat and virulence has been proposed (McNeillage and Herbert, 1968; Van Meirvenne, Janssens and Magnus, 1975a) although it is essential that the precise circumstances of such a link are fully investigated (Barry, Le Ray and Herbert, 1979a). It is a common mistake to equate the vat of a clone with all the characteristics displayed by that clone; the vat is just one phenotypic marker. Confirmation of a link between vat and virulence, and the observation that trypanosomes of different vat may interfere with the expression of each other at the population level (Herbert, 1975) conceivably could be exploited to decrease the number of variable antigens required in a vaccine. At a later stage of infection, after expression of predominant vats, it appears that trypanosomes are in some way biologically altered as evidenced by their decreased infectivity and virulence in fresh hosts. The basis of this and whether it is linked to vat or some other characteristic of the parasite remains to be investigated.

Can these changes in behaviour be induced artificially and incorporated into a vaccination protocol ? The possibility now exists of attenuating trypanosomes by continuous passage in culture. In preliminary studies, it has been found that mice infected with parasites maintained *in vitro* by serial subcultivation over 12 months have shown alteration in pathogenicity when compared with noncultured organisms or organisms that have been

maintained *in vitro* for less than three months (Hirumij unpublished data). The potential protective effect of attenuated protozoa has already been demonstrated in the control of babesiosis in cattle in Australia (Callow, 1977).

Molecular and genetic engineering.

There is little doubt that the basis of understanding antigenic variation will come from investigations of the molecular biology of the trypanosome. *In vitro* cultivation techniques and recently developed tools in biochemistry and genetic engineering have opened up new horizons. Thus studies of the type carried out by Williams *et ah* (1978) on trypanosomal RNA will provide much essential information on trypanosome biology. Reannealing studies on the nucleic acid coding for the vat repertoire should give an insight into the size of the repertoire, the extent of similarity between different repertoires and the molecular nature of the genes involved. The genetic control of expression of antigenic variation should be studied; artificial restriction of a trypanosome population to expression of only a limited number of its vats might allow effective vaccination.

In any discussion of vaccination, consideration must be given not only to the obvious application of these newly developed techniques to antigen production but also to novel approaches to vaccination. It is possible that in the near future many protein vaccines will be produced from largescale bacterial cultures that contain the gene sequences coding for the appropriate proteins. Recombinant dna technology has already been applied to the largescale production of human somatomammotropin (growth hormone) (Shine *et al.,* 1977), a precious substance that has traditionally been isolated from human placenta.

A further example of the application of recombinant dna technology to vaccine production is the development of a bacterial strain that is capable of producing the native chickalbumin protein at a level of 10 percent by weight of the bacterial cell (Mercereau-Puijalou *et al,* 1978). Thus bacterial strains can be developed to produce proteins for vaccines that normally would be either too expensive to isolate or impossible to purify because of limited amounts of starting material.

In addition to vaccine production in bacteria recent reports describe new techniques that possibly could find application in vaccination procedures. The transfer of specific genes from one genome to another has now been achieved. An example of such a transfer was reported by Wigler *et al.* (1978) where a specific viral gene coding for the enzyme thymidine kinase was purified by electrophoresis and introduced into a thymidine-kinasedeficient tissueculture cell line. Many of the tissueculture cells were able not only to incorporate the dna sequence into their genome but also were able to produce the enzyme at apparently normal levels. It may be possible, therefore, to modify certain tissues during a proliferative stage so as to yield a gene product to correct a genetic deficiency or possibly to produce a foreign protein for use in vaccination.

In other novel procedures recently reported by Dimitriadis (1978) and Ostro *et al.* (1978), differentiated tissue cultures were modified to produce a specific protein for a limited time. In each of these reports, a specific purified messenger RNA (rabbit globin) sequence was encapsulated in a lipid micelle called a liposome. The liposome was introduced to tissueculture cells with the membrane of which the liposome presumably fused. The purified messenger RNA was thereby introduced into the cytoplasm of the cells where it was translated into rabbit globin protein. The messenger RNAS used in such a procedure are degraded at a normal rate and can be modified to delay the cell's normal messenger RNA degradation processes. The normal cell's genome is not permanently modified and would produce the desired protein only for a limited time. In this manner, one could presumably use specific messenger RNAS and specific target tissues to produce the protein required for immunization. The inherent appeal of such a system would be that target tissues could produce sufficient quantities of a specific protein for a limited amount of time, thus allowing immunization to occur. Although such applications of molecular biology to vaccination are presently a dream, there is little doubt of their being a reality in the future. ■

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Part II

M Murray, *J.D. Berry, W.I. Morrison, R.O. Williams, H. Hirumi and L. Rovis*

In Part I of this review of the prospects of vaccination against African trypanosomiasis, contained in the previous issue of World Animal Review, the constraints in developing a vaccine were discussed and current knowledge on the molecular biology of the trypanosome and on antigenic variation with respect to possible future vaccines was evaluated. In this part other possible regimes for immunological intervention, including the immunogenicity and cross reactivity of trypanosome subcellular fractions, reduction of host susceptibility to African trypanosomes by the nonspecific use of immunostimulants or chemotherapy and also the role of trypanotolerant livestock in such approaches, are discussed.

Immunogenicity of subcellular fractions.

Modern biochemical technology has allowed the isolation, purification and characterization of a whole range of trypanosomal subcellular fractions. Thus, the variable antigen, which is responsible for induction of protective immunity, has been shown to be a glycoprotein with a molecular weight of 60 000 to 65 000 daltons, depending on species (Cross, 1975 and 1977 with *T. brucei;* Baltz, Baltz and Pautrizel, 1976, and Baltz *et al.,* 1977 with *T. equiperdum;* Rovis, Barbet and Williams, 1978 with *T. congolense).* While (at least with *T. brucei)* the N minerals of different variable antigen types (vats) differ in amino acid sequence (Bridgen, Cross and Bridgen, 1976), there is now evidence that different vats of the same and also of different species of trypanosomes *(T. brucei/T. congolense)* may have cross-reacting determinants (Barbet and McGuire, 1978). Although it would seem likely that these are hidden from the host's immune response the possibility of vaccination against these determinants must be pursued.

Crossreaction may occur at different levels. For instance, some crossreacting determinants may be found only on vats within one vat repertoire (the total number of vats that a trypanosome can express), while others may be universal. Complete characterization of all determinants, using such techniques as monoclonal antibody production (Kohler and Milstein, 1975), should reveal the structural and functional significance of any crossreacting components. Hyperimmunization against such components may prove effective if manipulated properly.

Most biochemical and immunological studies to date relating to immunogenicity of subcellular fractions have been aimed at analysing the variable antigen of the surface coat It is possible, however, that at some time in the trypanosome's complex lifecycle "weak spots" amenable to immunological control might be exposed. Thus, recent investigations have been made into the purification of a range of subcellular fractions of the trypanosome such as flagellum, membranes and kinetoplast. The biological characteristics and immunogenicity of these fractions have been investigated and compared with those of variable antigen. What the authors have found in studies on *T. brucei* in the mouse is that flagellum and membrane fractions stimulate protection against homologous vat challenge to the same degree as variable antigen (Table 1). It is likely that this is the result of the presence of variable antigen in these subcellular fractions although it is interesting that, per unit weight protein, flagellum is more effective than the purified variable antigen. No protection was achieved on challenge with a different vat

although with the membrane and kinetoplast preparations there was significant prolongation of survival accompanied by an alteration in the parasitaemic profile. This was possibly a result of a nonspecific stimulant effect of these fractions (see below, the section on "Induction of increased resistance by immunostimulants").

Using a subcellular fraction of *T. brucei* or *T. rhodesiense* that probably contained a mixture of variable antigen, mitochondrion and kinetoplast to immunize mice, Powell (1976; 1978) found increased survival times and reduced parasitaemias in mice challenged with *T. brucei.* Using *T. brucei* in *C57BI/6J* mice and a similar fraction for immunization, the authors were able to stimulate protection only if trypanosomes of the same vat were used for challenge (Table 1). When another vat was used for challenge protection was not achieved although there was a significant increase in survival time. Of considerable interest is the report of Powell (personal communication) that the use of the above fraction in aluminium hydroxide protected across trypanosome species. Three sheep were immunized in the "feet" with three doses of 1-mg protein fraction of *T. rhodesiense* in aluminium hydroxide. On subsequent challenge with *T. vivax* each of the three sheep developed a transient parasitaemia and then made a complete recovery. All three challenge control sheep became infected and died. These observations now await confirmation.

TABLE 1. **Immunization with various subcellular fractions of Trypanosoma brucei**

Fraction	**Challenge**	
	Same VAT	**Different VAT**
Variable antigen	Complete protection	No effect
Flagella	Complete protection	No effect
Membrane	Complete protection	Prolonged survival
"Powell" fraction	Complete protection	Prolonged survival
Kinetoplast	Increased resistance	Prolonged survival

Immunological intervention against the tsetse fly.

It should be borne in mind that trypanosomes have a complex lifecycle in which there may be "weak spots" susceptible to immunological intervention. For example, stimulation of the mammalian host's response to the tsetse bite or saliva may be such a method. Also, the trypanosomes in the midgut of the tsetse fly are uncoated (Vickerman, 1969) and possess a common surface antigenic identity (Seed, 1964; Barry and Vickerman, 1979). As ingested antibody can retain specific activity for up to four days in the tsetse midgut (Cunningham *et ah,* 1962) it would be of interest to study the effect on fly infection of uptake of high levels of antibody against these common surface antigens (Barry and Vickerman, 1979). Once again, the antigen could be supplied by *in vitro* culture techniques.

Induction of increased resistance by immunostimulants.

The host's immune response to the trypanosome is still poorly understood but there are indications that it is defective. For example, a feature of African trypanosomiasis is the development of a state of immunosuppression (reviewed by Murray *et al.,* 1974) and hypergammaglobulinaemia — involving mainly *igM* (Mattern *et al.,* 1961;

Luckins, 1972), a large proportion of which would appear not to be specific for the trypanosome (Freeman *et al.,* 1970; Corsini *et al.,* 1977). It is possible that the capacity of the trypanosome to survive may be related to the immunologically compromised state of the host. Thus, a complete understanding of the basis of immunosuppression and the relevant immunological effector mechanisms that kill the trypanosomes might allow some form of intervention so that effector mechanisms are stimulated and the host is able to control or eliminate the parasite.

TABLE 2. **Effect of Bordetella pertussis on survival of A/J and C57BI/6J mice challenged with Trypanosoma congolense**

Percentage survival

Days after challenge.	**A/J** 1		**C57BI/6J** 1	
	Control	**B. pertussis**	**Control**	**B.pertussis**
10	68	96	100	100
15	0	43	88	100
20		43	88	96
30		43	88	96
40		39	80	96
50		35	80	91
100		8	26	64
150		0	0	24
Average time to death, in days	11.2±1	26.4±24.6 2	75.4±35.4	113.3±47.8 2

[1] 25 mice per group.

[2] Significant to controls (arithmetic mean ± one standard deviation).

In this regard, the authors attempted to improve the host's immune response, and thus host resistance, by using the immunostimulants *Bordetella pertussis, Corynebacterium parvum* and Bacillus Calmette-Guérin (BCG) prior to or at the time of challenge (Murray and Morrison, 1979). So far this strategy has been successful, at least in mice. It was possible to increase survival times in both susceptible (*A /J)* and more resistant *(C57BI/6J)* strains of mice (Table 2). Thus, following challenge with *T. congolense,* the treated *A/J* strain behaved in a manner much more akin to the more resistant *C57BI/6J.* It should be emphasized, however, that complete protection was never induced by this method. The reduced susceptibility appeared to be ? related to the ability of these immunostimulants, particularly *B. pertussis* and *C. parvum,* to delay the onset of parasitaemia or to reduce the level of parasitaemia (Figures 1 and 2). The best results were achieved when both of these parameters were affected. The possibility that these immunostimulants acted by improving the immune response is being investigated at present.

The strategy of increasing host resistance by nonspecifically acting immunostimulants offers an attractive alternative or additional approach to the complex undertaking of a breeding programme for trypanotolerant livestock. However, whether immunostimulants can be employed effectively in this way in domestic livestock remains to be determined.

Trypanotolerance.

As trypanotolerance was the subject of an earlier review in this journal (Murray *et al.,* 1979), the authors will limit their remarks.

There is now a substantial body of evidence to indicate that certain breeds of cattle, sheep and goats are able to survive and be productive without the aid of treatment in areas of tsetsefly challenge, where other breeds cannot. This attribute is known as trypanotolerance although, as this state is not absolute, it would be better termed as reduced susceptibility. These trypanotolerant breeds are of considerable interest and importance. Not only is there evidence that they are economically exploitable in their own right but they also provide an excellent experimental system for evaluating the important factors that influence host susceptibility to trypanosomiasis. If it is confirmed, as the results of Desowitz (1959) strongly indicate, that the basis of trypanotolerance is the ability to mount a more effective immune response to the trypanosome, it might well be that any immunotherapeutic strategy that may be developed would be more effectively employed if used in trypanotolerant breeds of animals.

Infection and treatment.

Bevan (1928; 1936), working in Southern Rhodesia, was perhaps the first worker to note that bovines that recovered from clinical trypanosomiasis after treatment frequently remained in good health despite reinfection, suggesting that it might be possible to create a "nonsterile" form of immunity. That infection and treatment regimes can achieve this has been confirmed more recently by Wilson and his colleagues working in East Africa (1957 a and b; 1976). Since the method developed is immediately applicable, the authors would like to describe these studies in some detail.

Wilson attempted to evaluate the use of different drug strategies in the development of immunity in young cattle over a period of two to three years. "Immunity" was assessed by trypanocidal drug requirement, development and duration of parasitaemia, ability to maintain normal blood values in the presence of parasites, calving rates in breeding stock and growth rates in a beef herd.

In one experiment a breeding herd located in a high tsetsefly challenge area was managed under the following drug regime. Animals were treated with Berenil, treatment being determined not by the presence of the parasite but on the basis of the development of clinical signs of disease and of packed red cell volume (pcv) below 20 percent (Wilson, Paris and Dar, 1975a).

During the first and second years requirement for treatment did not change; an average of eight treatments was used and animals became parasitaemic about 30 to 40 days after each treatment. However, during the second year there was indirect evidence of reduced susceptibility to trypanosomiasis: for example, the number of live calves born increased and subsequent mortality decreased; abortions, a not uncommon occurrence in bovine trypanosomiasis, also decreased.

Even more promising results were achieved in a later series of experiments in which steers were introduced into an area of medium trypanosome challenge (Wilson *et al.,* 1975b; Wilson *et al.*. 1976). As before, a treatment regime based onappearance of clinical signs or pvc below 20 percent was used in a first group of cattle over a period of 29 months. The period between drug treatments, which was initially between 50 and 60 days, increased to around 130 days by the ninth treatment; at the same time the periods when trypanosomes were present in the blood without great adverse effect had increased from a mean of 11.7 days prior to the first drug treatment to 30.9 days by the ninth treatment. When the drugs were withdrawn from a number of steers six months before the end of the experiment, all survived and the growth rate and pvc values were the same as in the steers with access to therapy, showing that the resistance that developed was not drag dependent.

In contrast, a second group of steers, all of which were treated with Berenil whenever blood infection rather than clinical signs was detected, showed no evidence of developing immunity and they required treatment every 26 days throughout the course of the experiment. When treatment was withdrawn from some of the steers six months before termination of the experiment, their mean weight gains were 58 kg less than those steers in which treatment continued and, in addition, one animal died.

With a third group of steers, Samorin (isometamidium) was used in the same way as Berenil in Group 2, and there was some evidence of the development of immunity. While the need for therapy did not decrease throughout the experiment, pvc values and growth rates were maintained in the animals in which drug treatment was withdrawn six months prior to termination of the experiment, despite the more frequent presence of parasites in this group.

In terms of weight gain there was no doubt that the use of drugs prophylactically on a group basis, particularly Samorin, gave by far the best results. Nevertheless, as Wilson *et d.* (1976) pointed out, the particular advantage in encouraging the development of nonsterile immunity by infection and treatment might lie in the development of lesssusceptible breeding herds over periods of several years, particularly in areas of low to medium trypanosome challenge. This procedure might be even more successful if used with trypanotolerant breeds of livestock. It should be emphasized that drug resistance was not experienced in these studies.

The basis of this form of tolerance or "nonsterile" immunity to the trypanosome awaits investigation. It may be that the host has built up a whole battery of immune responses to the range of metacyclic antigens and vat repertoires that occur in that particular location, or alternatively there might exist a common priming antigen that allows the host to make a series of secondary responses to each vat, thus controlling the infection in the manner of the carrierhapten effect proposed for malaria by Brown (1971). However, it might be related to some nonspecific effect such as expansion and activation of the mononuclear phagocytic system.

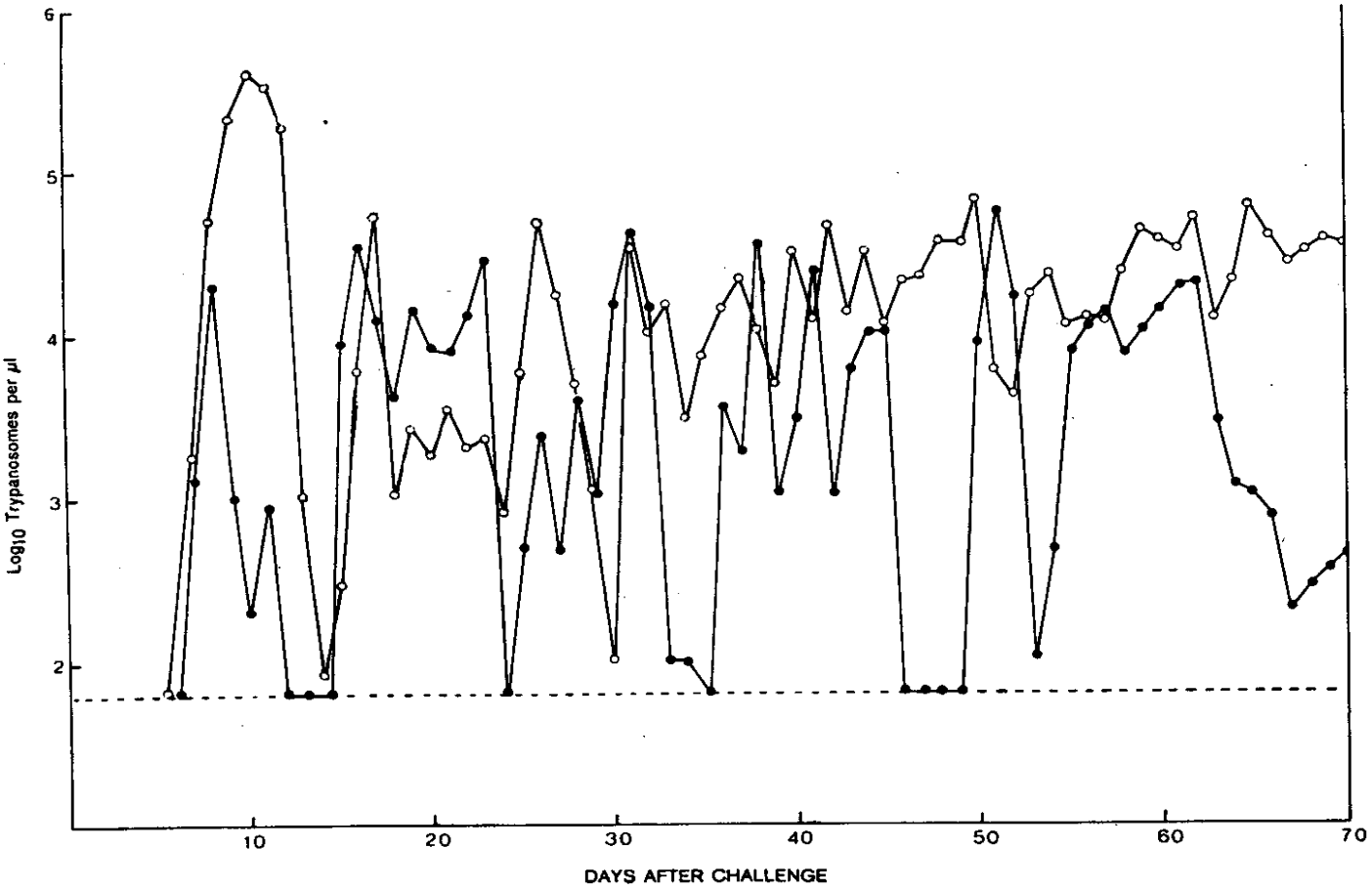

Figure 1.*The parasitaemia profile of a* Bordetella pertussisfreated *C57BI/J61 mouse (•) and a control C57BI/6J mouse (o) challenged with* Trypanosoma congolense. *The broken line just below 2 logio trypanosomes per \il indicates the level of sensitivity for detection of trypanosomes with the haemocytometer technique.*

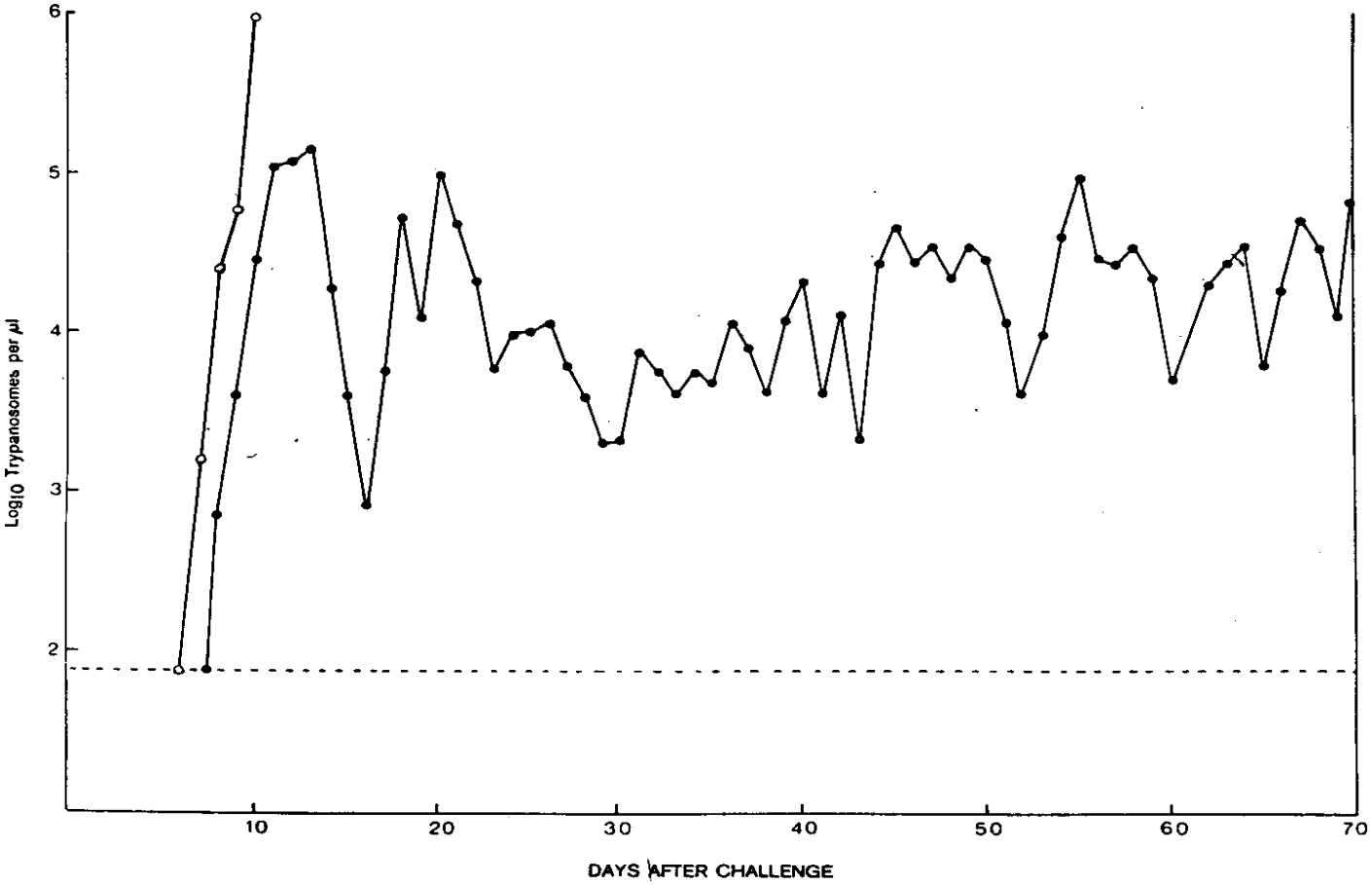

Figure 2.*The parasitaemia profile of a* Bordetella pertussistreated *A/J mouse (•) and a control A\J mouse (o) challenged with* Trypanosoma congolense. *The broken line just below 2 logio trypanosomes per* (i/ *indicates the level of sensitivity for detection of trypanosomes with the haemocytometer technique.*

Conclusions.

While a vaccine against trypanosomiasis is not an immediate prospect, what the two parts of this article have attempted to show is that there are several promising avenues for immunological exploration, namely vat cocktails, trypanosomes attenuated in *in vitro* culture systems, genetic engineering, crossreacting subcellular fractions, intervention against the tsetse, nonspecific induction of increased resistance by immunostimulants, and infection and treatment regimes. It is likely, if any one of these areas is rewarding, that the resulting vaccine will be more successfully exploited, at least initially, in trypanotolerant animals.

The authors would like to emphasize that any immunotherapeutic solution for trypanosomiasis control can come only through a thorough knowledge of the lifecycle of the trypanosome and its basic biology coupled with a comprehensive understanding of the immune response of the finite host. ■

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The authors are with the International Laboratory for Research on Animal Diseases (ILRAD), PO Box 30709, Nairobi, Kenya.

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