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A HIGH INCIDENCE OF *TRYPANOSOMA CONGOLENSE* STRAINS RESISTANT TO HOMIDIUM BROMIDE IN ETHIOPIA

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SUMMARY

Field investigations at settlements in Didessa valley and Angar valley in Wollega province, Ethiopia, suggested the presence of drug-resistant strains of Trypanosoma congolense.

Laboratory experiments using mice confirmed a high incidence of resistance to homidium bromide, a phenanthridinium drug.

INTRODUCTION

The occurrence of strains of *Trypanosoma congolense* resistant to homidium bromide,[1] a phenanthridinium drug, has been recorded in Nigeria (Williamson and Stephen, 1960). A field trial in Uganda showed that most relapses following homidium bromide treatment were due to *T. congolense* Mwambu, 1967). In Ethiopia resistance to this drug was confirmed in a strain of *T. brucei* from Gambela in the west of the country (Walker and Watts, 1970).

During a trypanosomiasis incidence survey in the Didessa valley, where homidium bromide had been used for some years, cattle showing trypanosomes in thick and thin blood films were treated with this drug at a dosage of 1·0 mg/kg. Repeated thick and thin blood film examinations after treatment revealed that 25 per cent of the animals were again infected within 30 days and the majority of infections were due to *T. congolense.*

At Angar-Gutin, a settlement in the Angar valley, another phenanthridinium drug, Isometamidium,[2] had been administered prophylactically for 6 months at a dose of 1·0 mg/kg at 8-weekly intervals. When the examination of thick and thin blood films from cattle 5 weeks after the third treatment revealed a 66 per cent infection of trypanosomes it was suspected that phenanthridinium-resistant strains were appearing.

In order to confirm the presence of drug-resistant strains at Arjo and Angar-Gutin it was decided to perform drug-resistance tests in mice. This technique has been extensively used at the East African Trypanosomiasis Research Organization (EATRO) to assess drug-resistant strains of *T. brucei, T. congolense* and *T. rhodesiense* (Walker and Watts, 1970; Mwambu, Mayende and Masinde, 1969; Van Hoeve and Grainge, 1965).

DESCRIPTION OF THE TWO LOCALITIES

The Didessa and Angar valleys are situated in Wollega province in western Ethiopia and are two of the many catchment areas of the Blue Nile (see Fig. 1).

Arjo, at an altitude of approximately 1,500 m, is on the periphery of the limits of *Glossina morsitans ugandensis* infestation in the Didessa valley. Survey teams working in the locality were unable to find tsetse flies, although in a trypanosomiasis incidence

[1] Ethidium (Boots Drug. Co.).
[2] Trypamidium (SPECIA).

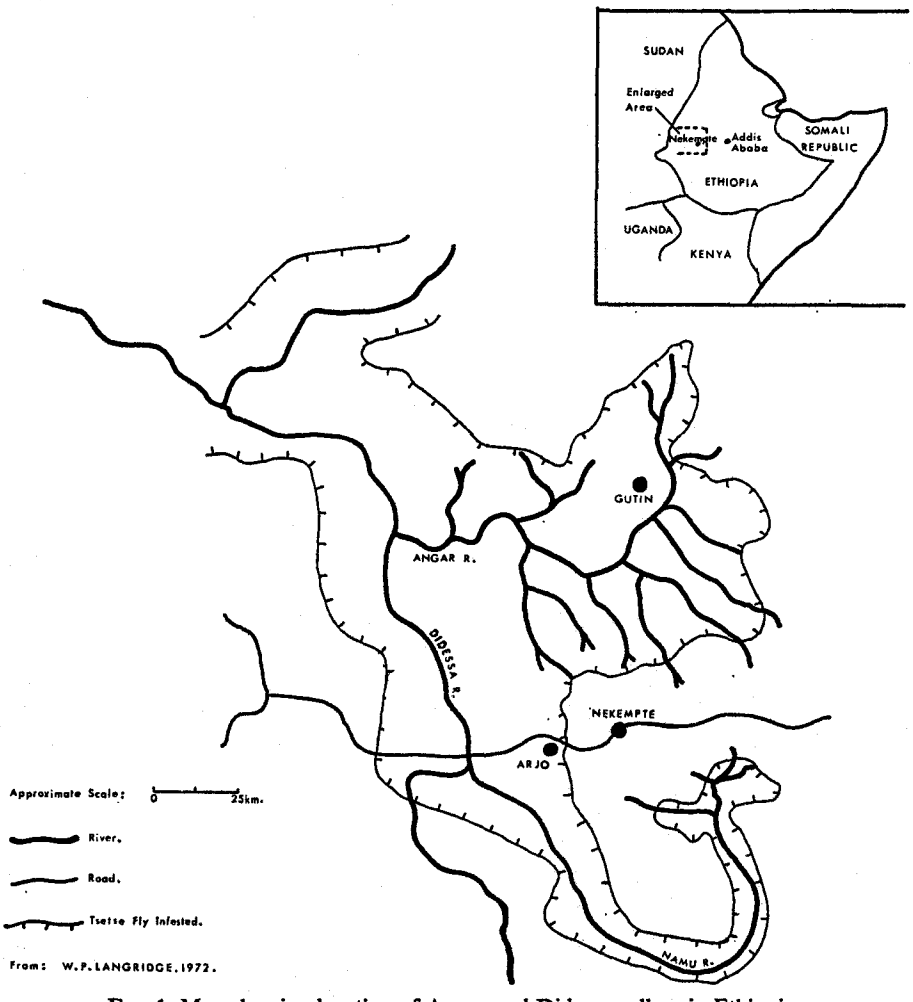

FIG. 1. Map showing location of Angar and Didessa valleys in Ethiopia.

survey involving 321 cattle, 23 per cent were found to be positive on thick and thin blood film examination with *T. congolense* determined in 80 per cent of cases. During the past 6 years clinical cases of trypanosomiasis in the area had been treated with homidium bromide but at irregular intervals.

Angar-Gutin at an altitude of 1,400 m is situated in the Angar valley, an area of heavy infestation with *G. morsitans ugandensis* and *G. tachinoides*. Langridge (pers. comm., 1972) found an apparent density of 150 and considered that this fly density made the area completely unsuitable for keeping cattle. However, a settlement scheme had been started and in view of the possibility of a future tsetse fly eradication programme in the area it was decided to monitor the herd of plough oxen already working on the settlement farms. Originally diminazene aceturate[3] was administered to these cattle but an examination of thick and thin blood films from oxen 27 days after one treatment with this drug revealed a 96 per cent infection rate. As a result of these findings prophylactic treatment with Isometamidium was instituted.

[3] Berenil (Hoechst).

LABORATORY METHODS

Isolation and diagnosis

Blood for thick and thin films and mouse inoculation was taken from the ear veins of cattle. The thick films were dehaemoglobinised and both thick and thin films were fixed in methanol and stained with Giemsa stain as described by Killick-Kendrick (1968).

Trypanosomes were isolated by the intraperitoneal inoculation of 1 ml of blood in sodium citrate into mice which were subsequently bled from the tail and wet smears were examined on alternate days. Mice were destroyed if negative for trypanosomes after 60 days.

Minimum Curative Dose (MCD) of homidium bromide for *T. congolense* strain TREU 938 in Swiss white mice

The MCD of homidium bromide was determined using *T. congolense* strain TREU 938.[4] Sixty white mice were inoculated intraperitoneally with this known sensitive strain. Wet smears were examined daily from the third day after inoculation until all mice showed a parasitaemia. They were graded according to parasitaemia and placed 10 in a cage, the various degrees of parasitaemia being equally distributed throughout the cages. The mice were numbered with crystal violet and carbol fuchsin and weighed on a flat pan balance. Each group was treated intraperitoneally with approximately equal volumes of homidium bromide at doses of 1×10^{-3}, 5×10^{-4}, 1×10^{-4}, 5×10^{-5} and 1×10^{-5} mg/g respectively. Ten mice were left untreated as controls.

Mice were bled from the tail and a wet smear examined daily for 5 days after treatment and then on alternate days, those remaining free from trypanosomes for 30 days being considered cured.

Maximum Tolerated Dose (MTD) of homidium bromide for Swiss white mice

Approximately equal volumes of homidium bromide at doses ranging from 2×10^{-2} to 1×10^{-1} mg/g in steps of 1×10^{-2} mg/g were injected intraperitoneally into weighed and numbered groups of five or ten Swiss white mice. The mice were inspected daily for 30 days and any deaths recorded.

Homidium bromide sensitivity of field *T. congolense* strains

When a moderate and rising parasitaemia occurred in mice used for the isolation of field strains a thin smear was stained with Giemsa stain to confirm that the infection was *T. congolense*. Infected mice were slaughtered and 1 ml bled by cardiac puncture into anticoagulant (0·25 per cent sodium citrate solution).

Multi-dose test

The resultant blood/anticoagulant mixture was diluted to 3 ml with Ringer-lactate solution and injected intraperitoneally into 30 Swiss white mice, which were bled daily from the third day after inoculation until all showed a parasitaemia. They were graded according to parasitaemia and placed five in a cage, the various parasitaemias being equally distributed throughout the cages. The mice were numbered, weighed and injected intraperitoneally with homidium bromide at doses of $2{\cdot}5 \times 10^{-2}$, 2×10^{-2}, $1{\cdot}5 \times 10^{-2}$, 1×10^{-2} and 5×10^{-3} mg/g respectively. Five mice remained as untreated controls.

[4] Supplied by the CTVM, University of Edinburgh.

Single dose test

In this test the blood/anticoagulant mixture was injected into eight mice which were treated similarly to those in the multi-dose test. When all the mice were parasitaemic, six were inoculated with homidium bromide at a dose of $1{\cdot}5 \times 10^{-2}$ mg/g and two were left as untreated controls.

In both these tests mice were examined by wet smear daily for 5 days after treatment and then on alternate days for 60 days. Mice which became consistently positive after an apparent cure were deemed to have relapsed.

RESULTS

Isolation of *T. congolense*

Seven of the eight strains isolated from Arjo and five of the six strains from Angar-Gutin were used to determine their sensitivity to homidium bromide.

MCD of homidium bromide for *T. congolense* strain TREU 938 in Swiss white mice

All mice were permanently cured at doses of 5×10^{-4} mg/g and above. At 1×10^{-4} mg/g 50 per cent of mice relapsed and at lower doses mice relapsed or were not effectively cured.

Thus, the MCD of homidium bromide for *T. congolense* strain TREU 938 within the range tested was 5×10^{-4} mg/g.

MTD of homidium bromide in Swiss white mice

Mortality following intraperitoneal injection of homidium bromide occurred at two distinct time intervals. Acute mortality within 24 hours and chronic mortality in 4 to 14 days.

The acute MTD was 9×10^{-2} mg/g. All mice inoculated with doses of 1×10^{-1} mg/g died within 24 hours. The chronic MTD followed a less satisfactory pattern: no ill effects were seen at doses of less than 3×10^{-2} mg/g but increasing chronic mortality occurred from 20 per cent at a dose of 3×10^{-2} mg/g to 80 per cent at 8×10^{-2} mg/g. The experiment was repeated on three occasions with similar results.

Homidium bromide sensitivity of field *T. congolense* strains

Five of the six strains examined using the multi-dose test were at least partially resistant to homidium bromide at $2{\cdot}5 \times 10^{-2}$ mg/g. The other strain was sensitive at all doses used.

Four of the six strains examined using the single dose test were fully resistant to homidium bromide at $1{\cdot}5 \times 10^{-2}$ mg/g. One strain was partially resistant and one strain sensitive.

These results are summarised in Table I.

DISCUSSION

The results obtained for the acute MTD of homidium bromide in mice when treated by the intraperitoneal route, are in agreement with Hawking (1963) who gave the MTD as 1×10^{-1} mg/g. Watkins and Woolf (1952) obtained an acute LD_{50} of $1{\cdot}1 \times 10^{-1}$ mg/g when the drug was given by the subcutaneous route. In the present investigation chronic deaths occurred at doses as low as 3×10^{-2} mg/g.

The MCD for a known sensitive strain was 5×10^{-4} mg/g. Doses used in the present drug sensitivity tests were evaluated on the MCD and MTD of homidium bromide and the example quoted by Hawking (1963) of five times the MCD in assessing resistance to curative doses. The present doses compare with those used at EATRO (Mwambu,

TABLE I
Summary of results of drug-resistance tests in mice

Reference number*	Mouse experimental data	Dose of "Ethidium" (mg/g) 0·025	0·020	0·015	0·010	0·005	Results
A1.2	Percentage relapse	67	100	100	†	N.T.	Resistant to
	Average no. of days	36·5	25·5	22·5			0·02 mg/g.
	Range of days	36–37	17–36	18–21			Partially resistant to 0·025 mg/g
A1.6	Percentage relapse	0	0	0	0	N.T.	Sensitive to
	Average no. of days						0·01 mg/g
	Range of days						
A2.2	Percentage relapse	80	80	100	100	100	Resistant to
	Average no. of days	13·0	12·25	12·0	10·6	9·0	0·015 mg/g.
	Range of days	13	9–14	11–13	9–11	9	Partially resistant to 0·025 mg/g
A2.5	Percentage relapse	100	100	100	100	100	Resistant to
	Average no. of days	14·25	13·3	14·0	12·67	11·0	0·025 mg/g
	Range of days	11–18	11–16	14	11–21	11	
A2.9	Percentage relapse	80	100	100	100	100	Resistant to
	Average no. of days	16·25	15·25	14·8	13·4	10·8	0·02 mg/g.
	Range of days	12–23	10–18	10–21	10–21	10–14	Partially resistant to 0·025 mg/g
A2.14	Percentage relapse			100			Resistant to
	Average no. of days			27·8			0·015 mg/g
	Range of days			13–49			
A2.15	Percentage relapse			67			Partially resistant to
	Average no. of days			20·5			0·015 mg/g
	Range of days			19–24			
G2.3	Percentage relapse			100			Resistant to
	Average no. of days			13·0			0·015 mg/g
	Range of days			13			
G2.4	Percentage relapse			0			Sensitive to
	Average no. of days						0·015 mg/g
	Range of days						
G2.6	Percentage relapse			100			Resistant to
	Average no. of days			15·0			0·015 mg/g
	Range of days			11–23			
G2.7	Percentage relapse			100			Resistant to
	Average no. of days			22·4			0·015 mg/g
	Range of days			13–45			
G2.10	Percentage relapse	100	100	100	100	100	Resistant to
	Average no. of days	11·25	10·8	10·2	10·8	7·5	0·025 mg/g
	Range of days	10–13	10–14	7–12	10–14	5–10	

* Prefix A=Arjo, G=Gutin.
† All mice died without remission of trypanosome parasitaemia.
N.T. Not Tested.

Mayande and Masinde, 1969). Walker and Watts (1970) considered a strain of *T. brucei* for which the MCD of homidium bromide in mice was $1{\cdot}25 \times 10^{-2}$ mg/g to be resistant.

Homidium bromide was used to determine the drug sensitivity of *T. congolense* strains from Arjo, where this drug had been used for treatment and from Angar-Gutin where another phenanthridinium drug, isometamidium, had been used prophylactically. Stephen (1960, cited by Whiteside, 1960) found that homidium bromide failed to cure metamidium resistant strains of *T. congolense*. In the same paper, Whiteside demonstrated that metamidium resistant strains of *T. congolense* are also resistant to

high doses of homidium bromide (2 mg/kg in cattle). Hawking's (1963) observations using mice agreed with those of Whiteside using cattle. Thus it was decided to use homidium bromide for laboratory work.

The laboratory results obtained with strains of *T. congolense* from Arjo and Angar-Gutin indicate a high incidence of drug resistance. Whiteside (1960) recorded that some of the circumstances tending to produce resistant strains in the field include underdosing with anti-trypanosomal drugs, the irregular use of prophylactics or their discontinuation while cattle remain at risk, and the high incidence of trypanosomiasis. In this study the reasons for the development of resistant strains are considered to be different in the two localities.

CONCLUSION

Homidium bromide had been used for several years to treat clinical cases of trypanosomiasis at Arjo. Because of the isolation of the area treatment could not be adequately supervised and it seems probable that at some stage resistance had developed because of underdosing with the drug. The field observations showed that 25 per cent of treated cattle developed parasitaemia within 30 days of treatment. This occurred during a long dry spell associated with the absence of tsetse fly and therefore suggested that many of the cattle may have relapsed rather than have been re-infected. The laboratory findings confirmed this.

At the other locality, Angar-Gutin, it is considered that the extremely high tsetse fly challenge and the incidence of trypanosomiasis led to the rapid appearance of phenanthridinium resistant strains. This is supported by the field examinations which showed a 66 per cent incidence 5 weeks after the third treatment with isometamidium at prophylactic doses. The herd at Angar-Gutin is still being monitored and despite the high challenge and the incidence of infection, the oxen remain in good working condition.

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SOUCHES DE *TRYPANOSOMA CONGOLENSE* HAUTEMENT RÉSISTANTES AU BROMURE D'HOMIDIUM EN ETHIOPIE

Résumé—Des investigations en brousse, dans des établissements de Didessa et d'Angar Vallée, dans la province de Wollega, en Ethiopie, ont suggéré l'existence de souches de *Trypanosoma congolense* résistantes aux trypanocides.

Les recherches en Laboratoire sur souris ont confirmé leur haut degré de résistance au bromure d'homidium, dérivé de la phenanthridine.

ALTA INCIDENCIA DE CEPAS DE *TRYPANOSOMA CONGOLENSE* RESISTENTES AL BROMURO DE HOMIDIO EN ETIOPÍA

Sumario—Se sospechó la presencia de cepas resistentes de *Trypanosoma congolense*, a las drogas utilizadas en el campo en investigaciones llevadas a cabo en los caceríos del valle de Didessa y Angar en la provincia de Wollega, Etiopía.

Experimentos con ratones en el laboratorio confirmaron una alta incidencia de la resistencia del tripanosoma al bromuro de homidio y al fenantridinio.