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The Testing of Proven *Trypanosoma brucei* and *T. rhodesiense* Strains by the Blood Incubation Infectivity Test*

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The authors describe a simple test (the blood incubation infectivity test) by which Trypanosoma brucei *(*sensu stricto*) may be differentiated from* T. rhodesiense *without recourse to human volunteers. The method consists in incubating the strain of trypanosome under test for 5 hours at 37°C* in vitro *in human blood, followed by observation of the effect of this procedure on the strain's infectivity to rats.*

Thirteen strains of T. rhodesiense *were investigated; in each, the ability to infect rats was retained after incubation. In all 6 strains of man-tested* T. brucei, *it was destroyed.*

The consistency of the results with proven strains suggests very strongly that the blood incubation infectivity test provides a valid means of differentiating these parasites.

The differentiation of *Trypanosoma brucei* (*sensu stricto*) from the other morphologically indistinguishable members of the subgroup rests at present on its non-infectivity to man, as demonstrated by inoculation into human volunteers.

There is a considerable amount of experimental evidence, notably that of Lester (1933), Fairbairn (1933a, 1933b, 1937), Willett & Fairbairn (1955) and Ashcroft (1959), to show that *T. rhodesiense* remains consistently infective to man and that *T. brucei* is consistently non-infective.

The successful isolation of a strain of *T. rhodesiense* from game by Heisch et al. (1958) and from cattle by Onyango et al. (1966) confirmed the epidemiological significance of these animals as potential reservoirs of human trypanosomiasis and emphasized the urgency of the need to find a method of differentiating *T. brucei* from *T. rhodesiense* which was more acceptable and simpler than the inoculation of human volunteers.

Although during the past half-century studies have been made on the *in vitro* effect of human serum on trypanosome strains, seldom was the infectivity of the incubated trypanosomes for laboratory animals tested. During an outbreak of sleeping-sickness, in what was then Tanganyika, Fairbairn (1933a) tested the action of human serum against 64 recently isolated strains of *T. rhodesiense*. After incubating them for 24 hours *in vitro* at 37°C in human serum, he found the majority to be still infective to rats.

The investigations described in this paper were carried out to determine whether or not the infectivity to rats of strains of *T. brucei* and *T. rhodesiense* trypanosomes would be differentially affected by exposure *in vitro* to human blood, since this possible method of distinguishing between the two species had apparently been overlooked in the past.

From these studies the " Blood Incubation Infectivity Test " (BIIT) was evolved (Rickman & Robson, 1970).

THE BLOOD INCUBATION INFECTIVITY TEST

Method

When donor rats and mice, inoculated with the strain to be tested, became positive as assessed by microscopical examination, they were sacrificed to provide the samples needed. Parasitaemia, as seen on wet film examination, varied from scanty (±) to massive (++++).

For each strain to be tested paired bottle samples were prepared, bottle A constituting the control sample and bottle B the test.

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For the test sample 0.25 ml of donor cardiac blood was taken aseptically and added to a sterile Bijou bottle containing 2.0 ml of human blood mixed with anticoagulant (0.25 ml of 2.0% potassium oxalate with glucose added to a strength of 3.0 mg/ml of blood).

For the control sample 2.0 ml of sterile phosphate-buffered saline (pH 7.4) replaced the human blood.

The samples were mixed by gentle rotation and incubated in a water-bath at 37°C for 5 hours, and then separately inoculated intraperitoneally into rats. Though the size of animals varied from one experiment to another, it was standardized for any one test; smaller rats, when used, received 1.0 ml and larger ones 2.0 ml of inoculum.

Wet films were made daily from all experimental animals for 40 days, or until they became persistently positive, and were examined for 5 minutes (approximately 200 fields) using a ×40 objective with ×6.3 oculars.

Normal precautions were taken to prevent cross-infection between the experimental animals.

Interpretation of the results

A positive result (BIIT pos.) is given where both test and control animals develop a persistent parasitaemia; a negative result (BIIT neg.) where the control animal alone does so.

Origin of the strains tested

Nineteen strains of *T. brucei* subgroup trypanosomes were tested. Thirteen strains were *T. rhodesiense*, of which 6 came from sleeping-sickness cases in the Lambwe Valley, South Nyanza, Kenya. The remainder were all stabilates obtained from the trypanosome banks at the East African Trypanosomiasis Research Organization (EATRO), Tororo, Uganda, and at the Medical Research Centre, Nairobi, Kenya. Of these stabilates 7 were *T. rhodesiense* and 6 were man-tested *T. brucei*.

Details of the trypanosome strains used in these experiments are given in Table 1.

RESULTS

All 13 strains of *T. rhodesiense* gave positive BIIT results (Table 2), though in almost all instances the prepatent period in the test animal exceeded that in the control.

By contrast, all 6 strains of man-tested *T. brucei* gave a negative BIIT result. One strain (EATRO 795), however, showed a scanty and transient parasitaemia (on the 15th day only; Table 3).

The 13 strains of *T. rhodesiense* were tested a total of 28 times and the 6 *T. brucei* strains a total of 10 times, as shown in Table 1. In all cases the retest results were identical with those of the first test.

Since the experiments described above were completed, an additional 29 strains of *T. brucei* subgroup trypanosomes have been tested and mention must be made of 2 strains which have given equivocal results.

One was a derivative of a man-tested *T. brucei* (EATRO 795) which, after 13 passages, was inoculated into a bovine at EATRO; 97 days later it was passaged again 4 times in mice before preservation as stabilate EATRO 999. The test rat inoculated with this derivative showed persisting parasitaemia after a prepatent period of 15 days, which is outside the range of that for test animals inoculated with *T. rhodesiense* (mean 4.6 days, range 1–12 days). The saline-control rat was sacrificed to provide the positive blood for a retest, the result of which was BIIT negative.

The other strain to give an ambiguous result was an unknown *T. brucei* subgroup strain, isolated from cattle in the Lambwe Valley. This behaved in exactly the same way under test and retest as EATRO 999; parasitaemia became apparent on the 15th day in the first test and the test animal remained negative on retest.

Results from some preliminary supplementary experiments suggest that the test works equally well at ambient temperature (24°C–25°C), with refrigerated blood (outdated samples from the Nairobi blood bank were used) and, also, when *in vitro* incubation is dispensed with and the samples are inoculated immediately intraperitoneally into rats.

It has also been found that mature rats are more reliable indicator animals than mice, since the higher mortality in the latter, during the prepatent period, could invalidate the test.

DISCUSSION

There has always existed a need for a method of differentiating *T. brucei* from *T. rhodesiense* that does not rely upon human volunteers. This need was stressed by the discovery of Wilde & French (1945), who showed that cattle could be infected with *T. rhodesiense*, and latterly, more forcibly, by Onyango et al. (1966), who succeeded in isolating a strain of *T. rhodesiense* from cattle at Alego, Central Nyanza, Kenya, at the time of the 1964

TABLE 1

SUMMARIZED DETAILS OF TRYPANOSOME STRAINS TESTED

Trypanosome species	Strain No. or name	Isolation			No. of small animal passages	No. of times tested	Results [a]	Remarks
		Host	Area	Date				
T. rhodesiense	Gaudensia	Man	South Nyanza, Kenya	July 1969	>10	3	All+	
	Okello	Man	South Nyanza, Kenya	Nov. 1969	3	5	All+	
	Owino	Man	South Nyanza, Kenya	April 1969	>10	1	+	
	Julius	Man	South Nyanza, Kenya	March 1969	>10	3	All+	
	Ogalo	Man	South Nyanza, Kenya	April 1969	>10	1	+	
	Francis	Man	South Nyanza, Kenya	April 1970	1	2	Both+	
	MRC 163	Man	South Nyanza, Kenya	Oct. 1967	>10	1	+	Stabilate
	EATRO 116	Man	Central Nyanza, Kenya	Aug. 1961	3	1	+	Stabilate
	EATRO 247	Bushbuck	Central Nyanza, Kenya	July 1958	31	2	Both+	Stabilate (Heisch strain)
	EATRO 846	Man	Central Nyanza, Kenya	Dec. 1964	6	1	+	Stabilate
	EATRO 941	*Glossina morsitans*	Tororo, Uganda	May 1965	3	1	+	Stabilate
	EATRO 1084	Man	Bukedi, Uganda	Aug. 1966	8	1	+	Stabilate
	EATRO 1293	Man	Lango, Uganda	June 1969	3	6	All+	Stabilate
Man-tested *T. brucei*	EATRO 795	Bovine	Central Nyanza, Kenya	Sept. 1964	3	2	Both–	Stabilate
	EATRO 1066	*Glossina pallidipes*	Lugala, Uganda	April 1955	27	1	–	Stabilate (Lugala 1 strain)
	EATRO 1070	Bovine	Central Nyanza, Kenya	Sept. 1964	8	1	–	Stabilate
	EATRO 1093	Sable antelope	Ulanga, Tanzania	Oct. 1966	3	3	All–	Stabilate
	EATRO 1097	Reedbuck	Ulanga, Tanzania	Nov. 1966	3	2	Both–	Stabilate
	EATRO 1100	Sable antelope	Ulanga, Tanzania	Nov. 1966	3	1	–	Stabilate

[a] + = BIIT pos.; – = BIIT neg.

TABLE 2

BLOOD EXAMINATION RESULTS FOR RATS INOCULATED WITH 13 STRAINS OF *T. RHODESIENSE* AFTER *IN VITRO* INCUBATION IN HUMAN BLOOD FOR 5 HOURS AT 37°C COMPARED WITH CONTROL RATS INOCULATED WITH THE SAME SALINE-INCUBATED STRAINS [a]

Days after inoculation	Gaudensia		Okello		Owino		Julius		Ogalo		Francis		EATRO 116		EATRO 247		EATRO 846		EATRO 941		EATRO 1084		EATRO 1293		MRC 163	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±	−	−	−	−	−	−	−	−	−	+	−
2	−	−	±	−	−	−	−	−	−	−	+	+	−	−	+	−	−	−	±	−	−	−	+	−	+	−
3	±	−	++	−	+	−	−	−	−	−	+++	++++	−	++	+	−	−	−	+++	−	−	−	++	−	+++	−
4	++	−	+++	−	+	−	+	−	−	−	++++	+++	+	++	++++	±	−	−	D	±	−	+	++++	−	++++	+
5	+++	−	D	−	++	−	++	−	−	−	D	D	+	++	D	+++	±	−		+++	±	+++	D	±	D	++
6	++++	−		−	+++	−	++	−	±	−			++	+		++++	+++	+		+++	+++	+++		−		++
7	D	±		+	D	+	D	±	+	−			+++	++		D	+++	+		D	D	D		−		++
8		++		+++		+		+	++	−			D	+++			D	+++						+		D
9		++		+++		±		+	++	−				D				D						+		
10		+++		D		++		D	D	−														++		
11		D				+++				−														+++		
12						D				±														D		
13										++																
14										++																
15–40										D																

[a] A = control rat; B = test rat; D = blood examination discontinued.

TABLE 3

BLOOD EXAMINATION RESULTS FOR RATS INOCULATED WITH 6 STRAINS OF MAN-TESTED *T. BRUCEI* AFTER *IN VITRO* INCUBATION IN HUMAN BLOOD FOR 5 HOURS AT 37°C COMPARED WITH CONTROL RATS INOCULATED WITH THE SAME SALINE-INCUBATED STRAINS [a]

Days after inoculation	EATRO 795		EATRO 1066		EATRO 1070		EATRO 1093		EATRO 1097		EATRO 1100	
	A	B	A	B	A	B	A	B	A	B	A	B
1	−	−	−	−	+	−	±	−	±	−	−	−
2	+	−	±	−	++	−	+	−	++	−	−	−
3	++	−	++	−	+++	−	+++	−	++++	−	++	−
4	+++	−	D	−	D	−	D	−	D	−	++++	−
5	D	−		−		−		−		−	D	−
6–14		−		−		−		−		−		−
15		±		−		−		−		−		−
16		−		−		−		−		−		−
17–40		−		−		−		−		−		−

[a] A = control rat; B = test rat; D = blood examination discontinued.

sleeping-sickness epidemic. These discoveries drew attention to the danger that such livestock could act as reservoirs of human trypanosomiasis.

In his review of the epidemiology of human trypanosomiasis in Africa, Ashcroft (1959) pointed out that few attempts have been made to prove the existence of *T. rhodesiense* in wild animals, as these require the use of human volunteers. In fact, the first and only isolation of *T. rhodesiense* from game was made by Heisch et al. (1958), when they obtained a strain from a bushbuck in a *Glossina pallidipes* area of Kenya. This work has not yet been followed up either in Kenya or elsewhere in Africa, because of the difficulties involved in distinguishing *T. brucei* from *T. rhodesiense*, but there can be little doubt that animals are of fundamental significance in the epidemiology of this form of the disease (Nelson, 1965).

The conflicting results given by two, more-recently isolated, non-proven strains of *T. brucei* subgroup trypanosomes cannot, at this stage, be explained. It is possible that these are intermediates, the test results reflecting the variability of their infectivity after incubation in human blood. These results could equally well be due to some unknown factor in the test animals. Clearly more work needs to be done on this aspect.

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RÉSUMÉ

DIFFÉRENCIATION DE SOUCHES DE *TRYPANOSOMA BRUCEI (SENSU STRICTO)* ET DE *T. RHODESIENSE* PAR L'ÉPREUVE D'INFECTIVITÉ APRÈS INCUBATION EN PRÉSENCE DE SANG HUMAIN

Les auteurs décrivent une méthode simple permettant de différencier *Trypanosoma brucei (sensu stricto)* de *T. rhodesiense* sans devoir recourir à l'inoculation des parasites à des volontaires. Elle est l'aboutissement de recherches expérimentales visant à déterminer si le pouvoir infectieux pour le rat de ces deux espèces de trypanosomes est affecté dans une mesure différente après une exposition *in vitro* à du sang humain.

Dix-neuf souches de trypanosomes du sous-groupe *T. brucei*, comprenant 13 souches de *T. rhodesiense* et 6 souches de *T. brucei (sensu stricto)* ont été incubées en présence de sang humain, pendant 5 heures à 37°C. Les résultats de l'épreuve d'infectivité pour le rat indiquent clairement que *T. brucei*, après ce traitement, a perdu son pouvoir infectieux pour l'animal d'expérience, alors que *T. rhodesiense*, après une exposition semblable, l'a conservé. L'expérience a été répétée à 28 reprises avec *T. rhodesiense* et à 10 reprises avec *T. brucei*, avec des résultats identiques.

Des épreuves similaires, pratiquées plus récemment sur 29 souches du sous-groupe *T. brucei*, ont donné dans deux cas des résultats douteux.

Des essais complémentaires donnent à croire que l'épreuve d'infectivité après contact des parasites avec du sang humain peut être également pratiquée à la température ambiante (24°C–25°C), avec du sang conservé, et sans incubation préalable *in vitro*.

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