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# The effect of human serum in vitro on Trypanosoma (Trypanozoon) brucei species trypanosomes and its relationship to infectivity in the Blood Incubation Infectivity test

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## SUMMARY

All attempts to identify Trypanosoma (Trypanozoon) brucei species trypanosome strains by their sensitivity or resistance to human serum in vitro have so far been unsuccessful. Wet-film examination of T.(T.) b. brucei, T. (T.)b. rhodesiense and T. (Nannomonas) congolense trypanosome strains, both before and after in vitro incubation in fresh human blood for 5 hours at 37°C showed that serum-sensitivity or serum-resistance is not a constant and reproducible property of a trypanosome strain and is totally unrelated to infectivity.

## INTRODUCTION

The trypanolytic action of human serum in vitro at 37°C on different strains of salivarian trypanosomes, was first demonstrated by Yorke et al (1), although Laveran (2a, b) had earlier shown that human serum, when inoculated into infected mice, temporarily inhibited the course of Trypanosoma (Trypanozoon) brucei brucei but not that of T.(T.)b. gambiense.

On the basis of both in vivo and in vitro evidence, Yorke et al (1) suggested that T.(T.)b. brucei and T.(T.)b.rhodesiense were in fact identical and that, perhaps when dietary deficiency or illness reduced the protective power of human serum, T.(T.)b.brucei strains from animals might become established in man as T.(T.)b.rhodesiense; conversely, when such strains were transmitted back to animals they would lose their serum resistance and revert back to T.(T.)b. brucei.

This hypothesis was tested and refuted by the classical, "Tinde" experiment in which it was shown that a single, pure strain of T.(T.)b.rhodesiense from man, when cyclically passaged for more than 20 years through a variety of game and domestic animals, retained its infectivity for man. (Corson, 3; Fairbairn and Burtt, 4; Willett & Fairbairn, 5 and Ashcroft, 6.).

Attempts to reverse this property of infectivity for man, by changing the serum sensitivity pattern, were unsuccessful. Collier (7) and Lester (8) made strains of T.(T.)b.brucei resistant to human serum in vivo but they remained non-infective for man. By contrast, Fairbairn (9) infected himself with a T.(T.) b.rhodesiense strain that he had made sensitive to his own serum in vitro and from his findings with 64 freshly isolated strains of T.(T.)b.rhodesiense, he later concluded that . . . "the microscopic appearance of trypanosome morphology and motility in wet-films made from samples cultured in vitro in human serum at 37°C is often totally unrelated to infectivity; motile trypanosomes often failing to infect rats and mice and, conversely, inoculated animals becoming infected from samples in which no intact trypanosomes could be detected by wet-film examination" (Fairbairn, 9).

More recently, Rickman & Robson (10) showed that the lack of correlation between wet-film appearance and infectivity applied equally to T.(T.)b. brucei strains; in addition they demonstrated that in vitro incubation in human serum or freshly drawn blood at 37°C in some way inhibited the infectivity of T.(T.)b. brucei to rats and mice, but not that of either T.(T.)b.rhodesiense or T. (T.) gambiense. From this they evolved the Blood incubation Infectivity Test (BIIT).

## MATERIALS AND METHODS

T.(T.)b.brucei strains were all stabilates from wild or domestic animals which had been tested in man; 3 of the T.(T.)rhodesiense strains were fresh isolates from humans in Lambwe Valley, Kenya, another was a stabilate of a strain from a bushbuck

Showing the wet-film appearances of trypanosome strains before and after in vitro incubation in freshly drawn human blood for 5 hours at 37°C

| Trypanosome Strain | Strain Number or Name | Origin of Strain | WET-FILM APPEARANCES | | | | | | BIIT Result |
|---|---|---|---|---|---|---|---|---|---|
| | | | Test 1 | | Test 2 | | Test 3 | | |
| | | | Before | After | Before | After | Before | After | |
| T.(T.)b. brucei (man-tested) | EATRO 795 | Bovine, Kenya Stabilate. | +A | O | +++A | O | +++A | +++A | All neg. |
| " | EATRO 1093 | Sable, Tanzania Stabilate. | ++A | O | ++A | +S | ++++A | ++ A | All neg. |
| " | EATRO 1094 | Sable, Tanzania Stabilate. | +A | O | +++A | +S | +++A | O | All Neg. |
| " | EATRO 1097 | Reedbuck, Tanzania Stabilate. | ++++A | +++A | ++++A | ++A | +++A | O | All neg. |
| " | EATRO 1126 | Waterbuck, Tanzania Stabilate. | ++++A | +++A | ++A | O | ++++A | O | All neg. |
| T.(T.)b. rhodesiense | GAUDENSIA | Human, Kenya Fresh isolate. | ++A | ++A | ++A | O | +++A | +A | All pos. |
| " | OKELLO | Human, Kenya Fresh isolate | +++A | O | +++A | ++A | +++A | O | All pos. |
| " | OGWELA | Human, Kenya Fresh isolate | +++A | O | +++A | +A | ++++A | O | All pos. |
| T.(T.) b.rhodesiense (man-tested) | EATRO 247* | Bushbuck, Kenya Stabilate | +++A | O | ++++A | +++A | +++A | ++A | All pos. |
| " | MAGUNGA 16/3** | Bovine, Kenya Fresh isolate | ++A | +A | +++A | O | ++++A | O | All pos. |
| T.(N). congolense | MAGUNGA 127/5 | Bovine, Kenya Fresh Isolate | +A | O | – | – | – | – | Pos. |
| " | WIGA 371/6 | Bovine, Kenya Fresh Isolate | ++++A | +S | – | – | – | – | Pos. |
| " | SCARP 1/8 | Bovine, Kenya Fresh isolate | +A | O | – | – | – | – | Neg. |

Notes: O – no trypanosomes seen; + – less than log 5.3 orgs./ml. (approx.); ++ – log 5.6 orgs./ml
+++ – log 6.5 – 7.4 orgs./ml.; ++++ – log 7.7 orgs./ml. and above; A – actively motile
S – sluggishly motile; – the "Heisch" bushvuck strain.
** – later proved infective for man (Robson et al, 13)

which had been man-tested and the last was a fresh strain from a bovine in Lambwe Valley. Three freshly isolated strains of T.(Nannomonas) congolense from bovines in Lambwe Valley were also tested.

## Incubation

Trypanosome samples were exposed to freshly drawn human blood in vitro for 5 hours at 37°C during the course of repeated BII tests, carried out in the manner described by Rickman & Robson (11a,b).

## Microscopy

Using a X 40 objective, wet-film samples were examined, both before and after in vitro incubation and parasite numbers and motility were noted.

## RESULTS

Reference to the Table shows that the wet-film appearance of T. (T.)b.brucei and T.(T.)b.rhodesiense strains, both before and after incubation in human blood in vitro at 37°C were found to differ considerably for each strain from test to test.

Even with T.(N.) congolense, of 2 BIIT – positive strains, freshly isolated from naturally infected bovines, only one showed trypanosomes in the wet-film after standard test exposure to human blood in vitro, although both infected the indicator animals.

It has been suggested that, when using the BIIT to identify T.(T.)b.brucei species strains isolated from non-human hosts, the possibility of T.(T.)b.rhodesiense isolates becoming serum-sensitive should always be considered. (Targett & Wilson, 12).

From the results that we have obtained during our experiments with the BIIT and the few examples shown here, we conclude that not only is "serum-sensitivity" in this sense not a constant and reproducible property of trypanosome strains, but also, as our experience and that of others has shown, is totally unrelated to infectivity. We therefore suggest that the terms "serum-sensitivity" and "serum-resistant", when applied to the wet-film appearance of trypanosomes after in vitro incubation in human blood or serum

have little or no significance and should no longer be used without precise qualification.

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