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Autor: Otieno, L.H. / Darji, N. / Onyango, P.

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The International Centre of Insect Physiology and Ecology,
P.O. Box 30772, Nairobi, Kenya

Development of *Trypanosoma (Trypanozoon) brucei* in *Glossina morsitans* Inoculated into the Tsetse Haemocoel

L. H. Otieno, N. Darji and P. Onyango

Abstract

Classically, infective development of *Trypanosoma (Trypanozoon) brucei* in tsetse flies is thought to take the route crop-midgut-hindgut proventriculus-hypopharynx-salivary gland, where the parasites reach their infective phase. It has been shown experimentally that *T. (T.) brucei* is capable of developing up to the infective stage in *G. morsitans* following inoculation of bloodstream form trypanosomes into the haemocoel. The rabbit on which flies were maintained became infected 18 days after exposure to the bite of experimentally inoculated flies. The possibility that *T. (T.) brucei* may be transmitted cyclically from tsetse flies to a mammalian host without necessarily following the classical, prescribed route is discussed. Apart from the normal longitudinal binary fission, various modes of multiplication were observed among trypanosomes in the haemocoel, modes which have not been observed previously in the tsetse fly.

Introduction

The classical theory (Taylor, 1932; Yorke et al., 1933) of *Trypanosoma (Trypanozoon) brucei* migration in the tsetse flies is that when a tsetse feeds on a parasitaemic animal, the parasites are ingested with the blood meal, pass into the midgut, and lie together with the blood meal enveloped by the peritrophic membrane. About 4 days later, the parasites may be found in the ecto-peritrophic space. It is suggested (Buxton, 1955, p. 809) that the parasites find their way into this space by passing down the alimentary canal inside the peritrophic membrane as far as the posterior end of the alimentary canal, somewhere in the hindgut. While there, the trypanosomes move from within the membrane into the ecto-peritrophic space, and then migrate forwards along the midgut to the proventriculus. It is further suggested (Lewis & Langridge, 1947; Fairbairn, 1958) that the trypanosomes once more get into the gut lumen from the proventriculus by passing through the base of the peritrophic membrane at the point where it is being freshly synthesized by the epithelial cells. The trypanosomes then advance forwards through the oesophagus into the hypopharynx, and then migrate into the salivary ducts and so to the salivary glands. Until this cycle of migration and development is complete, the tsetse fly is incapable of transmitting trypanosomes that are infective to other mammalian hosts, unless the infection is mechanically transmitted.

It should be noted that the hypothesis outlined above confines the entire cycle of development of the infective trypanosomes to the alimentary canal and the salivary glands of the tsetse fly. Recently (MSHELBWALA, 1972; OTIENO, 1973) there have been reports of *T. (T.) brucei* trypanosomes occurring naturally in tsetse haemolymph. In order to verify whether or not this is a possible site of development of infective trypanosomes we have introduced blood forms of *T. (T.) brucei* into the haemocoel of *Glossina morsitans* Westwood and noted the subsequent events.

Materials and Methods

Trypanosomes

Trypanosome materials used in these experiments were stabilates prepared and stored in dry ice at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. The trypanosomes were originally isolated from *Glossina pallidipes* Austen caught from Lambwe Valley, South Nyanza District, Kenya. *G. pallidipes* from the field were fed on a rabbit which became parasitaemic 9 days after the first fly bite. The blood of the infected rabbit was passaged into 4 mice, 6 days later, one of the inoculated mice was killed, and trypanosome stabilates prepared from the infected mouse blood. These were designated «*T. (T.) brucei* Lambwe strain».

Tsetse Flies

Glossina morsitans bred and maintained at the ICIPE Insectary were used. This colony originated in 1968 from the Tsetse Research Laboratory, Langford, Bristol, England. Teneral flies of both sexes were used in all experiments.

Inoculation of Flies

Two mice heavily infected with *T. (T.) brucei* Lambwe strain were anaesthetized with ether and bled through the heart. The heparinized blood was pooled and centrifuged to separate trypanosomes from blood cells and plasma. The trypanosome sediment was then suspended in phosphate-buffered saline (PBS) at pH 7.2. The concentration of trypanosomes in the suspension was adjusted to 1×10^7 organisms/ml. Aliquots (1 to 2 μl) of this suspension was sucked by capillarity into glass micropipettes drawn out to a sharp point with an opening measuring 4 μm. These aliquots were then inoculated into the ventro-lateral side of the mesothorax just beneath the integument of young unfed *G. morsitans*. The method used to inoculate the flies was a slight modification of that described by WEATHERSBY (1952). The flies were later fed on a clean rabbit, and then transferred to the insectary maintained at a temperature of 25 °C and a relative humidity of 60 to 80%. They were fed daily on the same rabbit throughout the experimental period.

Table I. The ability of *G. morsitans* to host *T. (T.) brucei* inoculated into the haemolymph. Bloodstream forms of *T. (T.) brucei* were inoculated into the flies, and the latter examined for trypanosome infection about three weeks later

Trial No.	Number of flies inoculated	Number of flies examined	Number of flies with infection in				% of flies infected (No. of flies)
			Proboscis	Salivary glands	Gut	Haemolymph	
1	89	43	1	–	1	–	2.33 (1)
2	76	59	1	–	2	–	3.39 (2)
Total	165	102	2	–	3	–	2.92 (3)

Results

(a) Infection of the mammalian host

The rabbit used to maintain the inoculated flies was found infected 18 days after its first exposure to the bite of the tsetse fly. At this time, 43 of the 89 inoculated flies remained alive; and, of these one (2.33%) was found to have trypanosomes both in the proboscis and the gut.

The experiment was repeated using the same stabilate of *T. (T.) brucei*. After inoculation, the flies were separated into two groups:

Group I tsetse flies were fed daily on a clean rabbit for 10 days after which the host was changed every second day. The rabbits used to maintain these flies started dying from an unknown cause 16 days after the beginning of the experiment; the flies which survived up to day 20 were therefore dissected and examined for trypanosome infection. 59 out of 76 inoculated flies were dissected; 2 (3.39%) of them had gut infection, one of which had proboscis infection as well. Table 1 summarizes the results of these experiments.

Group II tsetse flies, in batches of 5 or 6 flies, were examined daily for haemolymph infection over a period of 10 days. Haemolymph was collected from each fly using the method described by MSHELBWALA (1972). The fluid collected from each fly was mixed with two drops of PBS, and successively sucked into 1-ml syringes. The entire sample was then inoculated intraperitoneally (IP) into a clean mouse. The inoculated mice were examined for trypanosome infection 5 days later, and thereafter twice a week for one month. Parasitaemic mice, and any mouse not infected by the end of the experiment, were destroyed. Table 2 summarizes the results of this experiment.

Infection (or non-infection) of the experimental mice was used as the criterion for determining which flies were harbouring infective trypanosomes.

Table 2. The ability of *G. morsitans* inoculated with bloodstream forms of *T. (T.) brucei* to retain infective trypanosomes. The flies were examined in groups of 5 or 6 over a period of 10 days

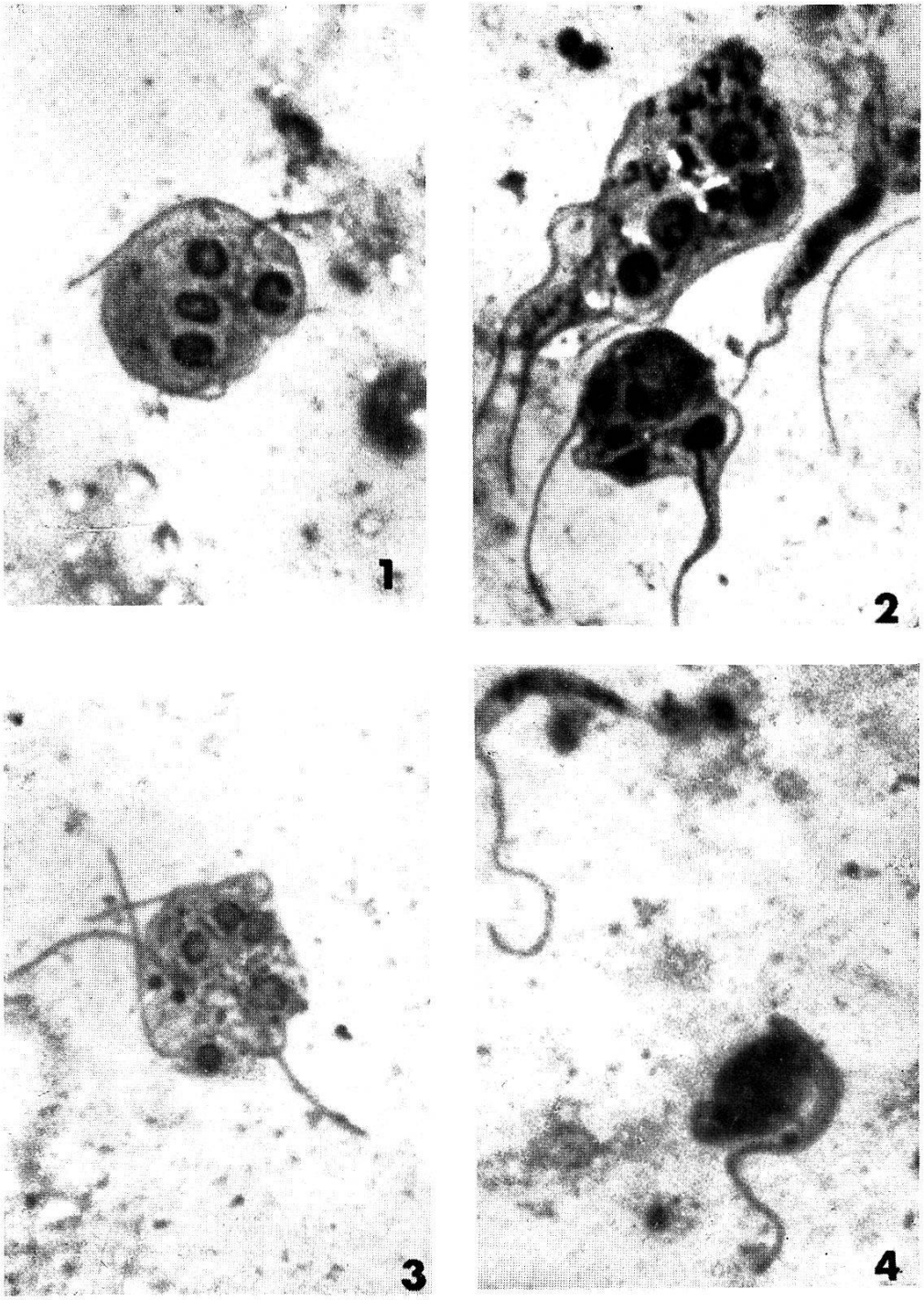
Days after inoculation	Number of tsetse flies examined	Flies with trypanosomes in the haemolymph	Tsetse flies with infective trypanosomes
1	5	5	5
2	6	6	6
3	5	4	4
4	5	3	3
5	5	1	1
6	5	1	1
7	5	–	–
8	5	–	–
9	5	–	–
10	5	–	–

It may be seen from Table 2 that infective trypanosomes were detected from inoculated flies up to day 6 after inoculation. Up to this time, bloodstream form trypanosomes were detected in the haemolymph (Table 2).

(b) Morphology of inoculated trypanosomes

Trypanosomes were demonstrated in the haemolymph of inoculated flies up to day 6. Table 2 shows the proportions of the various morphological forms observed in the haemolymph. It may be seen from the Table that the ratio of bloodstream forms to other forms was highest on day 1, but thereafter their number slowly declined as they changed into the typical vector form (so-called midgut form) and also into forms (which we have called 'bizarre form') not usually associated with tsetse flies. The bizarre forms were mostly large multi-nucleated trypomastigotes or sphaeromastigotes; occasionally, amastigotes were also observed.

Nuclear division was a common feature among many trypanosomes recovered from the haemolymph, consequently suggesting that these organisms succeeded in multiplying in this environment. Apart from the normal longitudinal binary fission, many trypanosomes adopted various ways of multiplication. Typical examples are shown in Figures 1–4.



Figures 1–4. Examples of various morphological forms of *T. (T.) brucei* observed in the haemolymph of *G. morsitans* inoculated with bloodstream forms of this trypanosome. 1. multinucleated sphaeromastigote; 2. multinucleated trypomastigotes; 3. daughter cells acquiring flagella; 4. filiform trypomastigotes. 1,800 ×.

Discussion

There are records of attempts to infect insects with pathogenic trypanosomes. WENDELSTADT & FELMER (in WENYON, 1926, p. 514) showed that *T. (T.) brucei* could survive in the tissues of beetles for at

Table 3. The proportions of different morphological forms of *T. (T.) brucei* observed in the haemolymph smears of *G. morsitans* inoculated with bloodstream forms. The trypanosomes were enumerated from 5 microscope fields at × 1,000 magnification. Bizarre forms were those which could not be classified as either bloodstream or midgut-like forms

Day after inoculation	Flies with trypanosomes in haemolymph	Morphological types of trypanosomes in the haemolymph		
		Bloodstream forms	midgut-like forms	bizarre forms
1	5	64	29	14
2	6	40	74	33
3	4	16	16	11
4	3	10	20	61
5	1	6	5	7
6	1	1	1	1

least 7 days. Unfortunately, there is no mention of the morphology of these organisms and their infectivity to mammalian hosts. Nevertheless, beetles are unlikely to be of any importance in the life cycle of *T. (T.) brucei* trypanosomes.

We have shown in the experiments reported in this paper, that *T. (T.) brucei* introduced into *G. morsitans* through the haemocoel can survive in this environment for at least 6 days. The failure to demonstrate any trypanosomes in these flies after day 6 made it impossible to assess how long the bloodstream forms were capable of retaining their infectivity to the mammalian host and also to study their morphology while residing in this environment. However, it was interesting to note that the trypanosomes inoculated directly into the haemolymph were able to invade the midgut and proboscis; and, in one case, the host on which such flies were maintained developed *T. (T.) brucei* infection. No salivary-gland infection was observed in any of these cases.

Similar observations have been made by other workers. Recently, DIPEOLU & ADAM (1974) found that among 39 *G. morsitans* which carried infective *T. (T.) brucei* to mice, only 15 had trypanosomes in the salivary glands. In all the flies with infective organisms, they found trypanosomes in the midgut and proventriculus, and in some cases the labrum-epiharynx complex was invaded. This observation prompted them to ask, 'Where, then do the metacyclics develop, or is it only the metacyclic form which establish in a mammal?' WARD & BELL (1971) also found that the infection rates of *T. (T.) brucei* in *G. morsitans* was low (2.2%) when they based their observations on infected salivary glands; but, when they fed flies individually on normal mice, the

transmission rate was approximately 5 times higher than revealed by the examination for tsetse salivary gland infections. They considered it possible that the proventricular forms might be infective to the vertebrate host. It may be noted that LUMSDEN (1972) has criticized the classical theory of *T. (T.) brucei* infective development in the tsetse fly on the ground that it lacks experimental evidence to support it.

These observations suggest strongly that *T. (T.) brucei* may be transmitted cyclically from tsetse flies to a mammalian host without necessarily following the classically prescribed route. It may, therefore, be necessary to reconsider the present views of the life cycle of *T. (T.) brucei* in tsetse flies. The classical view is clearly stated by HOARE (in MULLIGAN, 1970, p. 14) that the intermediate host (tsetse fly) is incapable of transmitting *T. (T.) brucei* trypanosomes to the mammalian host until the cycle (from the bloodstream form, through the epimastigote stage, to the meta-trypanosome stage) is completed. The present findings, although preliminary, challenge this view.

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