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The Penetration of the Peritrophic Membrane of the Tsetse Flies by Trypanosomes

JOAN C. FREEMAN *

Abstract

The presence of trypanosomes in the ectoperitrophic space from 30 minutes after the ingestion of the infective blood meal is best explained by trypanosomes penetrating the membrane at its anterior end, where it is freshly secreted.

Introduction

There are three possible ways in which trypanosomes could reach the ectoperitrophic space of the tsetse fly:

1. The classical theory is that trypanosomes can travel along the length of the gut and pass round the open, distal end of the peritrophic membrane within the first 24 hours after the eclosion of the fly (TAYLOR 1932).

2. Trypanosomes could cross this membrane if it ruptures, in the anterior, mid or hind gut regions, during engorgement.

3. The trypanosomes might also reach the ectoperitrophic space by forcing their way through the peritrophic membrane at the anterior end, where it is soft and freshly secreted.

The present study was designed to examine these possibilities, particularly with regard to the infection of young flies. Preliminary results have been reported (FREEMAN, 1970a, b).

Materials and methods

The strains of trypanosomes used in this work were: *T. b. brucei*, Lugala I and EATRO 1099, *T. congolense*, EATRO 187, *T. vivax*, Dr. Clarkson's Liverpool strain, originally from W. Africa. The vertebrate hosts were outbred guinea pigs, at the London School of Hygiene and Tropical Medicine (LSHTM); outbred Theiler original albino mice at LSHTM and Swiss white mice at EATRO; outbred albino rats at LSHTM, and a calf at the Department of Veterinary Medicine, Liverpool. The tsetse flies were derived from colonies bred at the Tsetse Research Laboratory, Langford, Bristol (*Glossina morsitans orientalis* and *G. austeni*) or at EATRO (*G. m. morsitans*, *G. palpalis fuscipes*, *G. pallidipes* and *G. brevipalpis*).

All animals were inoculated intraperitoneally except for the calf which received trypanosomes intravenously. *T. b. brucei* infections were established in white mice and guinea pigs, *T. congolense* in rats and *T. vivax* in the calf.

Tsetse flies were fed, within 24 hours after their eclosion, on animals with heavy infections (i.e. greater than 100 trypanosomes per microscope field \times 1,000).

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Table 1. To show the relationship between trypanosomes and the peritrophic membrane in *G. morsitans* from European colonies at intervals after the infective blood meal

time after blood meal	<i>T. b. brucei</i>						<i>T. vivax</i>					
	site 1		site 2		site 3		site 1		site 2		site 2	
	a	b	a	b	a	b	a	b	a	b	a	b
15 minutes	3	3	0	3	0	3	–	–	–	–	–	–
30 minutes	27	27	3	27	1	27	15	15	1	15	1	15
1 hour	19	19	0	19	0	19	–	–	–	–	–	–
2 hours	2	2	0	2	0	2	–	–	–	–	–	–

Key to Tables 1 and 2: Site 1: in lumen; Site 2: in peritrophic membrane; Site 3: in ectoperitrophic space; a: numbers of trypanosome-infected flies; b: numbers of flies sectioned.

The flies were killed at various intervals after their engorgement, ranging from 30 minutes to 20 days. Guinea pigs provided the maintenance blood meals for the older flies.

Flies were killed, by a ten second exposure to osmium tetroxide vapour, at 30 minutes, 1 hour, 2 hours, 3, 6 and 20 days after the infective blood meal. They were fixed in Schaudinn's fluid for 24 hours, double embedded in celloidin and 1% methyl benzoate and paraffin wax. 5 μ m sections were stained with Giemsa-colophonium (BRAY & GARNHAM, 1962) with the following modifications of Nesbitt (personal communication): slides were stained for 24 hours in Merck's Giemsa, rinsed briefly in distilled water and then in dilute acetic acid (1 drop of glacial acid: 50 ml distilled water) before adding the acetone-colophonium solution. This gave better staining of the peritrophic membrane and the trypanosomes. Sections were then examined. Trypanosomes were only positively identified as such when the nucleus and kinetoplast of the same organism were seen in the same section. In a number of sections organelles closely resembling nuclei or kinetoplasts were seen, but these have been excluded in the results because it was impossible to make unequivocal identification.

Results

Trypanosomes were found:

1. In the lumen of the gut.
2. In association with the peritrophic membrane.
3. In the ectoperitrophic space completely dissociated from the peritrophic membrane, in flies killed by 30 minutes after the infected blood meal. (At this time all flies had trypanosomes in the lumen of the gut.)

Tables 1 and 2 show that with *T. b. brucei*, a total of 12 out of 50 flies had trypanosomes associated with the peritrophic membrane. Three of these also had trypanosomes in the ectoperitrophic space.

None of the flies fed on *T. b. brucei* infections which were killed later than 30 minutes after the infective blood meal was found to have trypanosomes in the peritrophic membrane or in the ectoperitrophic space.

[illegible]

	23	23	9	23	2	23	16	16	1	16	1	16	19	19	1	19	1	19
30 minutes	23	23	23	23	2	23	16	16	1	16	1	16	19	19	1	19	1	19
1 day	35	36	0	36	0	36	22	22	0	22	0	22	0	30	5	30	1	30
3 days	0	8	0	8	0	8	3	11	0	11	0	11	0	15	0	15	0	15
6 days	0	25	0	25	0	25	0	6	0	6	0	6	0	31	0	31	0	31
20 days	0	4	0	4	0	4	—	—	—	—	—	—	0	12	0	12	0	12

30 minutes	16	16	0	16	0	16	-	-	-	-	-	-
<i>G. pallidipes</i>												
30 minutes	21	21	0	21	0	21	-	-	-	-	-	-
<i>G. brevipalpis</i>												
30 minutes	5	5	0	5	0	5	-	-	-	-	-	-

[illegible][illegible]

30 minutes

Similar results were obtained with flies fed on blood meals containing *T. congolense* and *T. vivax* (Tables 1 and 2) although *T. vivax* was also found in the ectoperitrophic space 24 hours after the infected feed.

Plate 1 shows the highly convoluted peritrophic membrane in the proventriculus. Trypanosomes were seen in various positions with relation to this membrane. Some appeared to be lying actually within the membrane (Plate 2). Others were closely applied to the membrane but were lying within the ectoperitrophic space (Plate 3). In a few instances trypanosomes were found in the ectoperitrophic space, completely free from the peritrophic membrane (Plate 4a, b). In the last plate 4b it may be seen that red blood corpuscles are found in the ectoperitrophic space, with the trypanosomes. Their significance in this site is discussed below.



Fig. 1

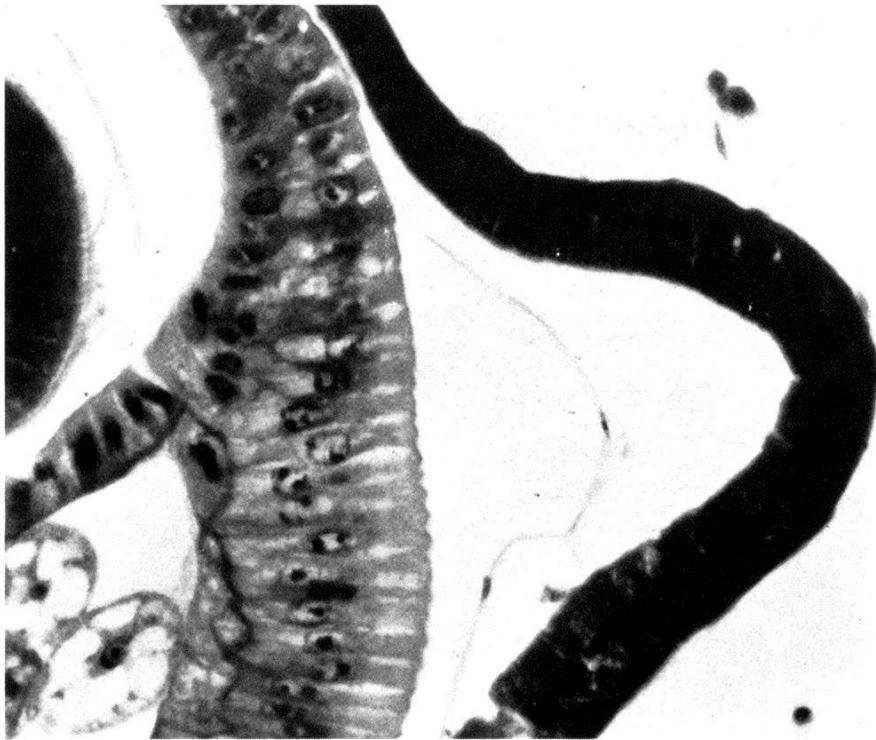


Fig. 2

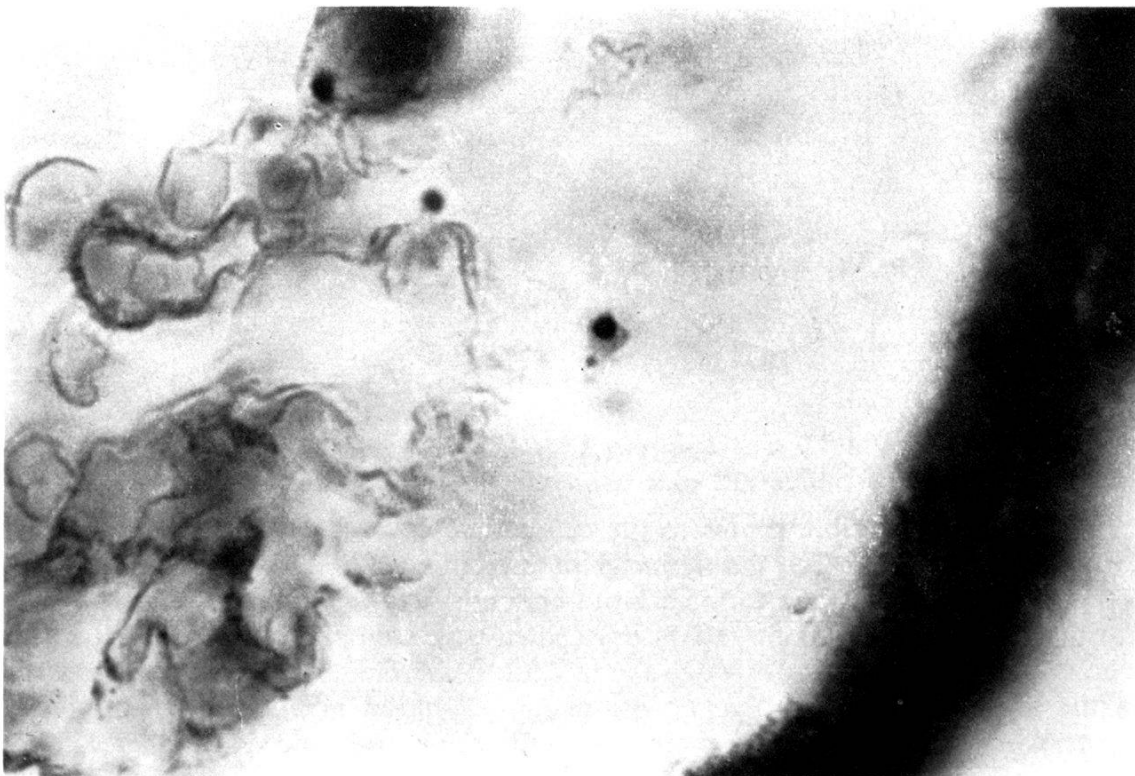


Fig. 3

*Fig. 4a*

Discussion

Of the three possible routes to the ectoperitrophic space the first is not likely to be a common one for the majority of infections. WILLETT (1966) shows that the peritrophic membrane forms a blind ending sac in the anterior midgut before the first blood meal. HOARE (1931) has shown that after the blood meal, it forms a continuous tube running through the midgut. If the membrane becomes ruptured in the hind gut, by the force of the oncoming blood meal, then, theoretically the trypanosomes could make their way to the ectoperitrophic space albeit against the peristaltic movement of the gut.

However, BURSELL & BERRIDGE (1962) demonstrated that the content of the hind gut has a pH of 5.8 and that this is immediately lethal to trypanosomes.



Fig. 4b

Unless the tsetse fly feeds on infected blood within 24 hours after eclosion the peritrophic membrane will have extended into the acid medium of the hind gut.

The rapid appearance in the ectoperitrophic space means that they would avoid the toxic environment of trypanosomes in the hind regions of the fly. Whereas the midgut is pH 7.2.

In only one of about 800 flies examined has the peritrophic membrane been seen to be frankly ruptured. This is in agreement with the observations of HOARE (1931) and WILLETT (1966) and suggests that infection of flies by this, the second possible route, only occurs rarely.

The present experiments support the concept that trypanosomes actually penetrate the membrane in the anterior region. It is difficult to explain their appearance within the membrane (Plate 2) by any other hypothesis. Furthermore,

it is known that they are certainly capable of penetrating the membrane in the reverse direction after about 6 days of infection (YORKE, MURGATROYD & HAWKING, 1933; FAIRBAIRN, 1958). Support for this hypothesis is also provided by the demonstration of red blood cells lying within the ectoperitrophic space in the absence of rupture of the membrane (Plate 4). If the red blood cells can be forced across the apparently intact membrane, then trypanosomes are likely to travel the same route, aided by their own mobile powers and it is most likely that penetration can take place when and where the membrane is softest and freshly secreted.

This may explain the greater susceptibility of younger flies to infection with trypanosomes (WIJERS, 1958) although it must be admitted that this can be explained equally well by postulating that trypanosomes pass round the open end of the membrane. The red blood cells found in the ectoperitrophic space must have been forced through the membrane. The impetus for this could have been provided by the pulsatile emptying of the crop into the anterior part of the gut (MOLOO & KUTUZA, 1971). It is likely that this mechanism provides at least the initial impetus for penetration by the trypanosomes.

Trypanosomes were only found in association with the peritrophic membrane and within the ectoperitrophic space of *G. morsitans*. It is possible that they would have been seen here in other tsetse species had more flies been examined. Table 2 shows the numbers of the other species of *Glossina* which were used. These are small, as are the numbers of *G. morsitans* killed at each time interval.

The apparent paucity of trypanosomes in the ectoperitrophic space may be explained by the difficulties in identification of the trypanosomes in section. *T. b. brucei* causes a low rate of infection in the salivary glands both in the field and experimentally; therefore many flies must be examined to find the few with trypanosomes in the ectoperitrophic space. Nevertheless trypanosomes have been found within the ectoperitrophic space in as little as 30 minutes of the fly taking its blood meal and this suggests that penetration occurs more rapidly than has hitherto been supposed.

In the light of this and other evidence such as that of MSHELBWALA (1972) who found trypanosomes in the haemocoel of 20% of infected tsetse flies, it may be necessary to modify present views of the life cycle of trypanosomes in tsetse flies by the addition of another possible route of trypanosomes across the peritrophic membrane.

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