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Colonization of the rectum of *Triatoma infestans* by *Trypanosoma cruzi*: influence of starvation studied by scanning electron microscopy

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Summary

The colonization of the different regions of the rectum of *Triatoma infestans* by a *Trypanosoma cruzi* strain (zymodeme I) originating from the same locality as the bugs was studied by scanning electron microscopy after different periods of starvation of the bugs. Throughout the first 16 weeks no changes in colonization pattern could be observed. Parasite density was always minimal at the midgut/rectal junction and highest on the rectal pads; it was at a similar level in the other three regions of the rectum. Twenty weeks after feeding, a proportion of the bugs had died and in the surviving larvae a decreasing colonization of the cuticle occurred. Nonetheless, despite other regions being flagellate-free, a residual *T. cruzi* population always remained attached to the rectal pads. No changes in the proportion of trypomastigotes to epimastigotes were observed as starvation progressed.

Key words: scanning electron microscopy; *Trypanosoma cruzi; Triatoma infestans;* starvation.

Introduction

Chagas' disease is the only tropical parasitic infection, in which the aetiologic agent, *Trypanosoma cruzi,* was found in the vector before the disease itself was recognized (Chagas, 1922). Chagas investigated parasite/vector-interrelationships, but many important aspects of parasite behavior, including metacyclogenesis, are still unknown (Zeledón, 1976; Zeledón et al., 1977). The influence of bug starvation on *T. cruzi* has only been cursorily investigated (Dias, 1934; Phillips and Bertram, 1967; Piesman and Sherlock 1985; Vargas and

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Zeledón, 1985), and a need for further studies on this topic has been emphasized (Zeledón, 1976). Using scanning electron microscopy our observations on the effect of starvation on the *T. cruzi*-population attached to the rectal wall are presented here. This study is based on our previously published results on the rectal cuticle of the vector *Triatoma infestans,* its colonization by *T. cruzi* at different times post infection (p.i.) and the morphology of the stages of *T. cruzi* in the rectum (Böker and Schaub, 1984).

Materials and Methods

Parasite and vector originated from Cachiyuyu, Chile (Schaub and Schottelius, 1984; Böker and Schaub, 1984). The *T. cruzi* strain "Chile 5" was isolated from *Triatoma infestans,* is cyclically maintained in mice and bugs and belongs to zymodeme I (Ebert and Schaub, 1983). Colonies of *T. infestans* strains "Chile 11" and "Chile 12" were both initiated in 1979 from a single infected female. Bugs were fed on chickens and reared at ca. 26 °C, 50–60% relative humidity and a 16 h/8 h day/night rhythm.

Third instar larvae were fed on infected mice (10,000 flagellates/ml blood; 3–4 weeks p.i.). They were allowed to feed on one further occasion on chickens 3 or 4 weeks later. Fifth instar larvae were studied at monthly intervals, beginning 4 weeks after the last feed. The larvae were fed on uninfected mice before dissection to obtain a clean rectal surface devoid of feces. As this was not always achieved if feeding occurred 2 h prior to dissection, bugs studied 16 and 20 weeks p.i. were fed 12 h earlier. Optimal fixation of the inner rectal surface was obtained by injecting glutaraldehyde through a fine capillary tube inserted into the anus. Details of the procedure, dissection of bugs, fixation, dehydration, critical-point drying and gold coating of recta have been described previously (Böker and Schaub, 1984). The specimens were examined in a Nanolab 7 SEM (Zeiss/Semco, Oberkochen, FRG).

Results

Colonization density was recorded for the five rectal regions (Böker and Schaub, 1984), which were identified on the basis of their cuticular architecture (region A: around the midgut/rectal junction; region B: rectal pads; region C: adjacent narrow zone; region D: main rectal wall; region E: surrounding the anus). Density of flagellates was assessed as follows: ∅ = no flagellates attached throughout the whole region; + = individual flagellates, not arranged in groups; ++ = dense groups of flagellates; +++ = "carpet" of flagellates (Fig. 1).

A comparison of the colonization of the different regions indicated a clear preference of *T. cruzi* for the rectal pads (region B) (Tab. 1). In regions C, D and E the density was similar throughout; region A was slightly less heavily colonized. Starvation caused only slight differences during the first 16 weeks after feeding. After 19 weeks of food deprivation some of the bugs had died, but dissections of surviving larvae, 20 weeks after feeding, demonstrated a dramatic change in region A, C, D and E. In one bug single trypanosomes were found in regions A and E but a "carpet" of flagellates in region B remained. Three larvae had dense colonies on the rectal pads and one of these 3 larvae single flagellates in region E. In 2 other bugs only some individual parasites could be detected in region B and in one of these also in region C. In region D, the main rectal sac, all trypanosomes had detached.

Fig. 1. A "carpet" of flagellates covering totally region B (rectal pads) in the rectum of *Triatoma infestans*. ×1200.

In almost all bugs epimastigotes and trypomastigotes occurred throughout the rectum. It was not possible, therefore, to infer any correlation between duration of food deprivation and the occurrence of developmental stages in this investigations.

Discussion

In studies on the influence of starvation on the *T. cruzi*-population in the vector short- and long-term effects have to be distinguished. As starvation capacity of the bugs may vary for different stages, species and strains (Perlowagora-Szumlewicz, 1969; Mello, 1980; Feliciangeli et al., 1980; further literature in Hase, 1932; Zeledón and Rabinovich, 1981) such data are necessary for comparison. Short-term effects, after a 7-week period of starvation, are reported by Phillips and Bertram (1967), but the loss of *T. cruzi* infections in 55% of *Rhodnius prolixus* they observed is contrary to our observations on long-term effects and was possibly due to the use of an old laboratory strain of *T. cruzi*, maintained for over 30 years by serial mouse passage and thus apparently attenuated. After the same period of starvation (7 weeks) Piesman and Sherlock (1985) found a statistically significant reduction in the number of metacyclics per bug (*Panstrongylus megistus*), but such a difference could not be detected in SEM-studies.

After a prolonged period of starvation a loss of *T. cruzi* in naturally infected *Triatoma dimidiata* was recently reported by Vargas and Zeledón (1985). How-

Table 1. Influence of starvation on the colonization density in the five rectal regions A–E

Weeks after feeding	Weeks p.i.	Region A					Region B					Region C					Region D					Region E				
		n	colonization				n	colonization				n	colonization				n	colonization				n	colonization			
			∅	+	++	+++		∅	+	++	+++		∅	+	++	+++		∅	+	++	+++		∅	+	++	+++
4	8	0	–	–	–	–	4	0	1	2	1	4	0	4	0	0	4	0	3	1	0	3	0	2	1	0
8	12	2	2	0	0	0	3	0	1	0	2	2	0	2	0	0	2	0	2	0	0	2	0	2	0	0
12	15	3	1	2	0	0	8	0	0	2	6	7	1	6	0	0	8	0	8	0	0	8	0	8	0	0
16	19	4	1	3	0	0	6	0	0	2	4	5	0	5	0	0	6	0	6	0	0	6	0	3	3	0
20	23	4	3	1	0	0	6	0	2	3	1	6	5	1	0	0	6	6	0	0	0	6	4	2	0	0

Colonization density: ∅ = no flagellates detected; + = few flagellates; ++ = dense groups of flagellates; +++ = "carpet" of flagellates
n = number of recta in which the colonization of the region could be studied. Differences in n of the five regions are because:
1. region A was often covered by the rectal gland;
2. some regions (often the narrow region C) were destroyed when the fixed recta were dissected;
3. feces in the rectum were not entirely defecated.

ever, in the same study some of the bugs maintained with regular feeding also showed such a loss. The lower susceptibility of this species of *Triatoma* to *T. cruzi* is consistent with previous findings which demonstrated a restricted colonization of the rectum (Zeledón et al., 1984). Moreover, this system is one of the few cases, where a *T. cruzi* strain infected a lower percentage of the local bug species than non indigenous bugs (Zeledón and Vieto, 1957).

Apparent long-term effects of starvation were noted by Dias (1934); after prolonged starvation, parasites in the feces were weakly active or immobile. Similar results were obtained by us in studies of starvation capacity of infected and uninfected bugs (unpubl.). Bugs dissected within 24 h after death always revealed some living flagellates in the rectum, but more than 90% were dead. A higher proportion of living trypanosomes was found on the rectal wall than in the lumen. The stomach and small intestine often contained only limited blood residues, but dense populations of active flagellates. Preliminary results indicate that in bugs starved for long periods and then fed on chickens the complement lyses the stomach epimastigotes but the populations in the small intestine and rectum flourish.

As data on the influence of starvation on the *T. cruzi*-subpopulation which is attached to the rectal wall were totally lacking, our results can only be compared with previous SEM studies of *T. cruzi*. This method has been used previously by Zeledón et al. (1984) and Böker and Schaub (1984). In a naturally infected *Triatoma dimidiata* with an unknown feeding history parasites were restricted to the rectal pads (Zeledón et al., 1984). In previous transmission electron microscope studies flagellates were also attached to other parts of the rectum, but preferentially to the rectal pads (Zeledón et al., 1977). This was also found in our dissection at different times p.i., where the colonization density in different regions of the rectum could be classified as follows: low in region A, highest in region B (rectal pads), high in C and D and slightly higher than D in E (Böker and Schaub, 1984). Colonization densities in bugs dissected in the study here were always slightly lower and regions C, D and E possessed almost similar densities of flagellates, mainly dense groups. Only small areas of these regions were covered by a "carpet" of trypanosomes.

The pronounced preference for the rectal pads, which has also been demonstrated in *Triatoma infestans* infected with *Blastocrithidia triatomae* (Schaub and Böker, 1986), remained 20 weeks after the last feed. However, at this time greater variation in parasite number and distribution between individual bugs was found. If the assumption is made that density of *T. cruzi* can be influenced by the remaining life span, the data can be interpreted as follows. After prolonged starvation, parasite density in the regions A, C, D and E is reduced and only individual *T. cruzi* remain, but the rectal pads are still covered by a "carpet" of flagellates. Later the "carpet" breaks down into isolated groups of trypanosomes and the last flagellates disappear in the other regions. Finally, shortly before the death of the bug only single flagellates remain on the rectal pads.

Further studies should elucidate the population development after feeding of these starved bugs. Also different zymodemes should be studied, as preliminary results (unpubl.) indicate a reduced developmental capacity for a strain of zymodeme II.

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