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Development of *Trypanosoma cruzi* in the vector in the absence of blood

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Summary

First stage nymphs of *Triatoma infestans* and *Dipetalogaster maximus* which had never fed after egg hatching, were allowed to ingest by artificial feeding a blood-free suspension of *Trypanosoma cruzi* trypomastigotes collected from experimentally infected mice. A high percentage of the vectors acquired a normal infection and produced infective stages. The parasite's development in the vector did not require blood but may have needed the presence of unknown factors secreted and/or excreted in the insect's digestive tract.

Key words: T. cruzi; T. infestans; D. maximus; development in vector; development in absence of blood; artificial feeding.

Introduction

In most studies dealing with the life-cycle of *T. cruzi* no reference has been made to the requirements for its development in the digestive tract of triatomine bugs. Blood is apparently considered as an element which cannot be dispensed with by the parasite. By using a simple apparatus to feed artificially new-born first stage nymphs of *T. infestans* and *D. maximus*, it has been demonstrated that *T. cruzi* bloodstream forms can develop and differentiate in the gut of the insects in the complete absence of blood.

Material and methods

The feeding apparatus used to infect the bugs was based on that of Garcia et al. (1975), with some modifications. The strains of *T. cruzi* used in the experiments were *Y* (Silva and Nussenzweig, 1953) and *FL* (Brener and Chiari, 1963), maintained by weekly blood passages in mice. The triato-

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mine bugs used were first stage nymphs of *T. infestans* and *D. maximus* bred in the insectary; they had not been previously fed.

The following technique was used to obtain bloodstream trypomastigotes uncontaminated with blood: heparinized blood from the orbital sinus of infected mice was collected in plastic tubes, centrifuged at 100 *g* for 10 min and then incubated for 15 min at 37° C. The supernatant was diluted 1:3 with 0.85% saline, centrifuged at 1000 *g* for 10 min and the material incubated at 37° C for 30 min. During the last incubation a large percentage of the parasites actively leave the centrifuged deposit at the bottom of the tube and can be collected, free of blood cells, from the supernatant. Such parasites were then washed three times by centrifugation at 1000 *g* for 10 min using either saline (NaCl 0.85%), Hanks solution or 199 medium (Difco). Washed parasites suspended in these media were injected into the internal chamber of the apparatus used for artificial feeding; the insects kept in wooden boxes were fed for 1 h through a parafilm membrane on the infective liquid maintained at 37° C. Engorged insects were kept at 23–27° C for 10, 15, 20, 30 and 60 days, and then their feces were examined. In some instances they were fed on normal mice and their naturally produced faeces or urine collected for examination. Metacyclic trypomastigotes obtained from the insects were inoculated into mice previously immunosuppressed by irradiation with 500 r from a RT Mueller 250 apparatus (250 kV, 15 MA), or into normal mice.

Results

From 27 *T. infestans* and 14 *D. maximus* first larval stages that had ingested *Y* strain trypomastigotes in saline, the following results were observed: on the 15th day after the infective meal, 5 out of 7 *T. infestans* were positive and all 4 examined *D. maximus* were infected with *T. cruzi*. Thirty days after infection the remaining nymphs were fed on normal mice and the pool of urine examined for the presence of flagellates. The infective stages were inoculated into 2 X-irradiated mice which nine days later showed parasites in their blood. On the 35th day after the infective meal, dissection of the insects showed infection in 10 out of 20 *T. infestans* and in 3 out of 10 *D. maximus*.

Another 32 *T. infestans* first stage larvae were infected similarly with the *FL* strain of *T. cruzi*. Thirty days later 18 insects were examined and 16 showed infection. Five normal mice acquired infection after being inoculated with their faeces. After 60 days of infection, the remaining 14 triatomines were examined and 10 of them showed *T. cruzi* infection. Results are given in Table 1.

Discussion

By means of simple experiments it was possible to demonstrate that *T. cruzi* is able to multiply, in the absence of blood, to differentiate and produce infective stages in triatomine-bugs. Probably the content of the digestive tract supplies the requirements for the parasite to complete its life-cycle. Participation of symbionts from the natural flora from the insects digestive tract is possible. However, Mühlpfordt (1959) and Geigy et al. (1953) demonstrated that *T. cruzi* do develop in germ-free triatomines fed on whole blood. The possibility of this flora providing essential nutrients for *T. cruzi* in the absence of blood has not yet been investigated. A definite demonstration that only materials secreted

Table 1. Infection of *D. maximus* and *T. infestans* first larval stages with *T. cruzi Y* and *FL* strains in absence of blood

T. cruzi strain	Days after infection	*D. maximus*	*T. infestans*
		No. infected/examined	No. infected/examined
Y	15	4/4 (100%)	5/7 (71%)
Y	35	3/10 (30%)	10/20 (50%)
FL	30	–	16/18 (89%)
FL	60	–	10/14 (71%)

and/or excreted by the digestive tract and not symbionts are supplying the factors required for the development of *T. cruzi* in the vector (ex: hemin, that is essential for "in vitro" cultivation [Lwoff, 1951]) could be obtained using germ-free triatomine-bugs fed with blood-free suspensions of *T. cruzi*.

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