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J. Parasitol., 81(1), 1995, p. 1–7
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DEVELOPMENT OF *TRYPANOSOMA CRUZI* IN *TRIATOMA INFESTANS*: INFLUENCE OF TEMPERATURE AND BLOOD CONSUMPTION

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ABSTRACT: This work had 2 objectives. The first was to quantify *Trypanosoma cruzi* development within *Triatoma infestans* maintained at 2 different temperatures, using an experimental design that simulated the natural transmission process and, second, to learn how the vector blood consumption rate modifies the parasite's development. Two hundred and three, fifth-stage nymphs of *T. infestans* were infected with the X-1 strain of *T. cruzi* (about 10^4 trypanosomes/ml blood), maintained at 20 and 28 C, and daily offered the opportunity to feed on uninfected laboratory mice. From 24 hr to the 55th day after the infective meal, the total number of epimastigotes and rectal and fecal metacyclic trypomastigotes were counted. Epimastigote multiplication began on the first day after the infective meal at both temperatures. This parasitic stage developed similar population densities within the vector under both temperature regimes. Trypomastigotes appeared in the rectum and feces at 20 C, 32 and 24 days later, respectively, than at 28 C; however, once they became infective, insects developed similar population densities of fecal metacyclic forms. Blood consumption was related to epimastigote and rectal trypomastigote development at 28 C, but not to the number of trypomastigotes in the feces. A minimum of 120 and 180 mg of fresh blood consumed assured that all bugs developed epimastigotes and trypomastigotes. In spite of the delay in producing metacyclic forms at 20 C, the insect's infective capacity was similar at both temperatures.

The epidemiology of Chagas' disease involves the knowledge of different factors affecting Triatominae vector capacity (WHO, 1983). Among these, temperature seems to play an important role in modifying *Triatoma infestans* population density (Gorla and Schofield, 1989), biting rate (Catalá, 1991), and infectivity of wild, domestic, and experimental populations (Wood, 1976; Giojalas et al., 1990; Catalá, Gorla, and Basombrío, 1992).

The existence of seasonal changes in a vector's infectivity supports the assumption that variations in environmental temperature affect the development of *Trypanosoma cruzi*. The influence of temperature upon *T. cruzi* development within the vector was examined by Neves (1971). He qualitatively characterized the parasite's life cycle in *T. infestans* at temperatures ranging from −5 C to 37 C for those that fed only once. According to his results, the entire *T. cruzi* development occurs between 23 C and 28 C, whereas multiplication is inhibited and metacyclic forms are not produced at the other temperatures. Later, Asin (1992) showed that at temperatures of 12 C, the multiplication of *T. cruzi* declines, but a small population of epimastigotes remains alive within the vector.

Besides having a direct influence, temperature may also regulate *T. cruzi* development by modifying some physiological processes of the vector; the *T. infestans* life cycle occurs normally at 28 C (Perlowagora-Szumlewics, 1969), whereas 16 C is considered the lower temperature limit for its development (Stemp, 1988). On the other hand, temperature changes are closely related to variations in the rate of *T. infestans* blood consumption (Catalá, Giojalas, and Crocco, 1992) and could modify the environment where *T. cruzi* multiplication and differentiation takes place.

Very little is known about the physiological interaction between *T. cruzi* and *T. infestans*, and especially the influence of microecological factors that affect transformation of the parasite to the infective form (Zeledón, 1987; García and Azambuja, 1991). Furthermore, studies on *T. cruzi* development within the vector have been carried out using insects infected as first or second instar and fed every 3 wk (Schaub and Losch, 1988; Schaub, 1989), which are not the normal conditions for vector feeding in nature (Catalá, 1991).

The present study had 2 objectives. The first was to quantify *T. cruzi* development within *T. infestans* maintained at 2 different temperatures using an experimental design that simulated the natural transmission process and, second, to learn how the vector blood consumption rate modifies the parasite's development.

MATERIALS AND METHODS

Uninfected fifth-stage nymphs of *T. infestans* supplied by the Chagas' National Service (Córdoba, República Argentina) were used. Four to 15 days after molting, the insects were fed on mice (BALB/c strain) infected with *T. cruzi* (X-1 strain) (Cano and Rubiolo, 1985). The amount of ingested blood was calculated from the difference in an insect's weight before and after feeding. To estimate the number of *T. cruzi* ingested by each bug, the number of bloodstream trypomastigotes present in the blood of a mouse's tail was determined by counting parasites in a Neubauer chamber. The mouse's blood contained after 10^4 *T. cruzi*/ml.

After the infective meal, 203 insects were distributed into 2 experimental groups. Group 1 nymphs were maintained at 28 ± 1 C, the optimum temperature for the vector's development (Perlowagora-Szumlewics, 1969), and at 60–70% relative humidity. Group 2 nymphs were maintained at 20 C, a temperature close to the lower limit for *T. infestans* development (Stemp, 1988), and at 60–70% relative humidity.

To simulate the natural transmission process of *T. cruzi*, insects from each experimental group were placed daily in a plastic box containing 1 uninfected mouse (BALB/c strain) that was restricted in movement. For each day up to the 55th day after insect infection, the experiment was interrupted when blood sucking began, the digestive tract was dissected, and the promesenteron (stomach), postmesenteron, and rectal sac were separated. The walls of the postmesenteron, and rectal sac were broken between the tips of dissecting forceps and their contents were well diluted in physiological saline solution and collected in graduated capillary tubes. The total number of living epimastigotes within the postmesenteron and the rectal sac and the total numbers of rectal living metacyclic trypomastigotes were counted using a Neubauer chamber. Trypomastigotes were counted in 85 insects maintained at 28 C and 34 insects at 20 C. Sphaeromastigotes or intermediate stages leading to epimastigotes and trypomastigotes were not counted.

The number of trypomastigotes shed with feces was estimated over

Received 20 September 1993; revised 25 July 1994; accepted 25 July 1994.

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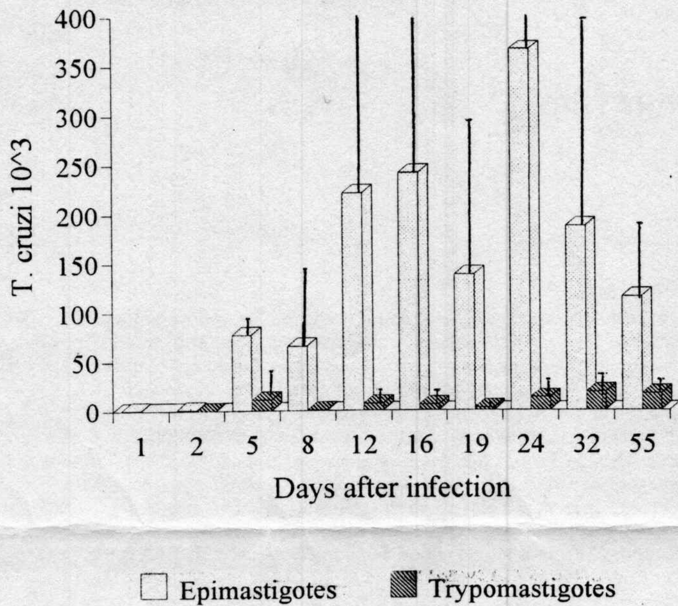

FIGURE 1. Epimastigotes in midgut and rectum (□) and trypomastigotes in rectum (▨) of fifth-stage nymphs of *Triatoma infestans* at 28 C and after infection.

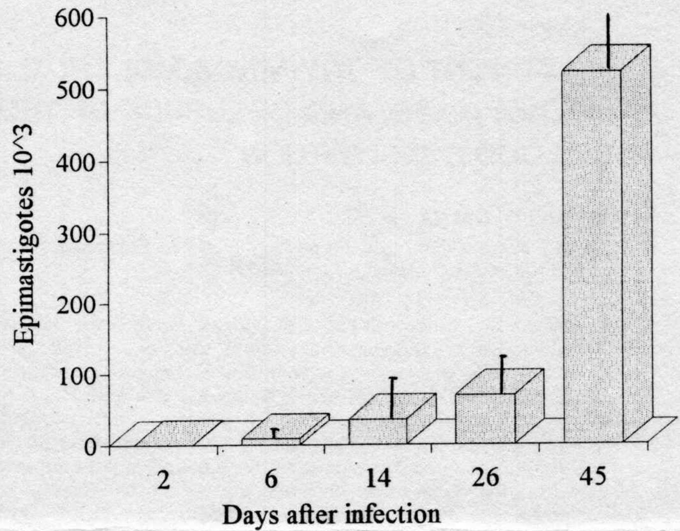

FIGURE 2. Epimastigotes in midgut and rectum of fifth-stage nymphs of *Triatoma infestans* at 20 C and after infection.

a 30-mo period from insects that fed during the first 30 min after being exposed to the host. After the completion of feeding, the bugs were placed in glass capsules and the volume of each drop of feces excreted was measured with graduated capillary tubes. Because the first 30 min after feeding could be the most important in *T. cruzi* transmission, the total number of metacyclic forms emitted with feces spontaneously produced during this period was counted (49 insects at 28 C, and 35 insects at 20 C). Data were analyzed using ANOVA tests and a posteriori multiple comparisons of means for significant *F* values (Sokal and Rohlf, 1979).

Blood ingested by triatomid bugs is initially stored within the promesenteron, with little or no digestion. The main digestive process occurs in the postmesenteron. The amount of ingested blood transferred from the promesenteron to the postmesenteron represents the blood consumption (Montenegro and Pasina, 1984).

To determine if blood consumption (BC) affects epimastigote and trypomastigote development, the relationship between these parasitic populations and blood consumption was examined by regression analysis. BC was estimated as BC = IB + I − RB (modified from Catalá, Giojalas, and Crocco, 1992), where IB (initial blood) = the amount of blood within the insect's promesenteron before the infective meal and RB (residual blood) = the blood amount within the insect's promesenteron at different days after infection; I (intake) = the total blood intake during the experimental period.

RESULTS

When *T. infestans* were maintained at 28 C, 100% of the epimastigotes were kept in the postmesenteron from the first to the fifth day after infection. By the sixth to the seventh day after infection, the epimastigotes appeared in the rectal sac and constituted 3–19% of the epimastigote population present in the vector. From the 7th to the 55th day after infection 74% (±13%) of the total epimastigotes were observed in the postmesenteron while 26% (±13%) were counted in the rectum.

By 24 hr after infection, the bugs had a mean population of 450 ± 490 epimastigotes per insect, which increased significantly ($P < 0.05$) by the 12th day after infection, reaching a mean value of 220,000 (±224,000) epimastigotes per insect. From that time to the 55th day, the epimastigote population density did not show any further changes (Fig. 1).

Among the insects at 20 C, the epimastigotes appeared in the postmesenteron at the same time as at 28 C. However, the time of first rectal appearance and the peak population size was delayed by 8 and 24 days, respectively. Between the 14th and the 54th day after infection, the epimastigotes were most abundant in the postmesenteron, representing 89 ± 6% of the epimastigote population present in the vector.

Analysis of the population densities at various times after infection showed that by 24 hr, the insects had a mean population density of 550 (±800) epimastigotes per bug. The density increased significantly by the 36th day after infection, reaching a mean value of 520,000 (±339,000) epimastigotes per bug (Fig. 2).

During the experimental period, the epimastigotes developed similar population densities under both thermal regimes ($F = 0.38$, $P > 0.05$, n = 97). The 63 insects living at 28 C had a mean population of 137,000 (±226,000) epimastigotes per bug, which was not statistically different from the population density developed in the 34 insects maintained at 20 C (102,000 ± 305,000 epimastigotes per bug).

Rectal trypomastigotes appeared later than epimastigotes. At 28 C, 12% of insects had metacyclic forms in the rectum from the sixth day after infection. The percentage of infective bugs increased significantly until the 16th day. At this time, 86% of the insects dissected showed metacyclic forms within the rectum. From that time to the 55th day, the percentage of infective bugs did not show any further change. By the sixth day after infection, the rectal trypomastigotes developed a mean population density of 11,000 (±31,000) trypomastigotes per insect. There were no further changes during the experiment ($F = 1.28$, $P = 0.27$, n = 63) (Fig. 1).

Trypanosoma cruzi differentiation to metacyclic form was not inhibited at 20 C; however, the appearance of this form in the rectum was delayed until up to the 37th day after infection. A failure in temperature control killed the insects on the 38th day and the experiment was stopped.

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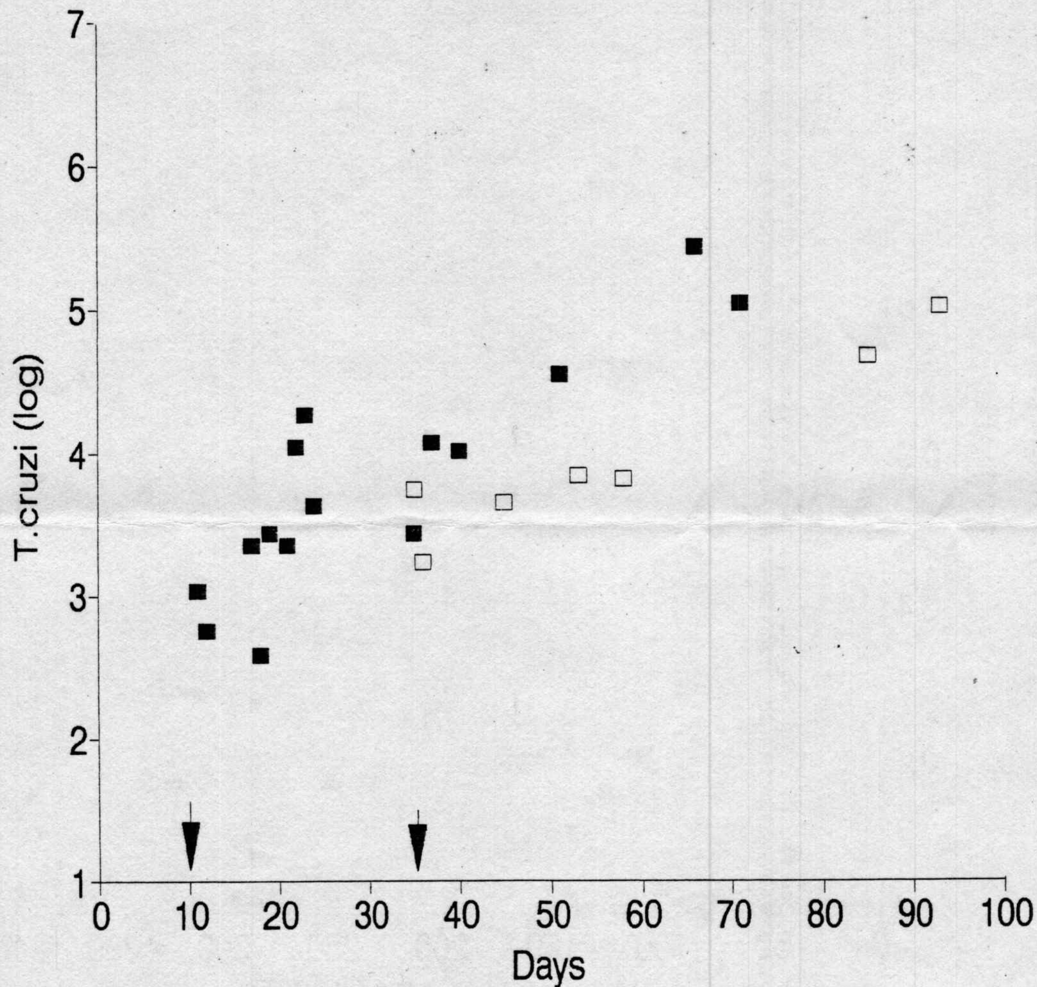

FIGURE 3. Trypomastigotes in feces of fifth-stage nymphs of *Triatoma infestans* at 28 C (■) and 20 C (□). The arrows point to the time of trypomastigote appearance.

During the experimental period under both temperatures, the number of *T. cruzi* (epimastigotes + rectal trypomastigotes) that developed within the vector was not related to the number of bloodstream forms present in the infective meal ($r = 0.16$, n = 62, $P = 0.20$ and $r = -0.08$, n = 36, $P = 0.64$ at 28 C and 20 C, respectively).

At 28 C, metacyclic trypomastigotes were present within feces from the 12th day after infection. Three percent of the rectal trypomastigote population (500 ± 400 trypomastigotes per insect) were excreted with the feces during the first 30 min after feeding. By the 16th day, the percentage of rectal trypomastigotes excreted increased to 68% and remained at a high level (88%) by the 24th day after infection.

Insects maintained at 20 C excreted infective forms with feces 24 days later than at 28 C; however, there was no significant difference in the number of trypomastigotes between the 2 temperatures ($F = 2.37$, $P = 0.13$, n = 20). The increase in trypomastigotes over time was exponential under both temperature regimes ($r = 0.89$, $P < 0.00001$, n = 15 and $r = 0.95$, $P < 0.0001$, n = 7 at 28 C and 20 C, respectively) (Fig. 3).

As the *T. cruzi* developed within the vector (epimastigotes + trypomastigotes) were not related to the number of blood trypomastigotes present in the infective meal, the influence of another factors such as blood consumption for the establishment of the infection was studied. During the experimental period insects maintained at 28 C had a mean blood consumption of 180 ± 90 mg, which was the same as at 20 C (190 ± 100 mg) ($P = 0.12$, n = 81).

A significant potential relationship was observed between blood consumption and epimastigotes at 28 C ($r = 0.60$, n = 63, $P < 0.00001$) (Fig. 4). A blood consumption of <120 mg produced a reduced development of epimastigotes at this temperature. There was a linear relationship between blood consumption and the number of epimastigotes developed for insects maintained at 20 C ($r = 0.47$, n = 34, $P < 0.005$) (Fig. 5).

On the other hand, there was a significant exponential relationship between blood consumption and number of rectal trypomastigotes ($r = 0.64$, n = 63, $P < 0.0001$) for insects maintained at 28 C (Fig. 6). A blood consumption of <180 mg produced a reduced development of rectal metacyclic forms. Insects without rectal trypomastigotes had a mean blood consumption of 81 ± 2 mg, which was significantly lower than the blood consumption for insects with rectal metacyclic forms at 28 C ($P < 0.0001$, n = 45, $F = 16.52$). Also, the mean population

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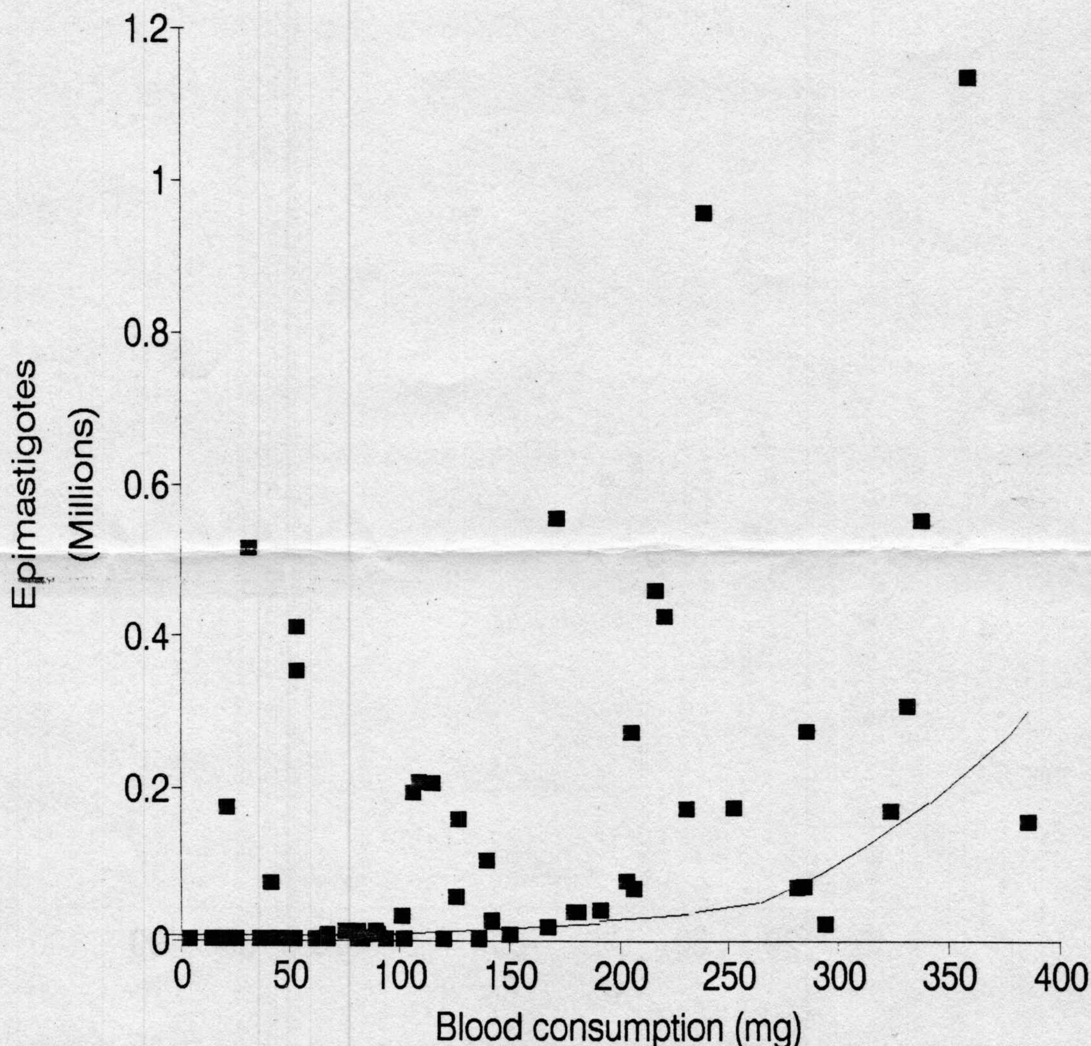

FIGURE 4. Relationship between blood consumption (mg) and epimastigotes in midgut and rectum of fifth-stage nymphs of *Triatoma infestans* at 28 C.

of epimastigotes developed in insects that were not able to support metacyclogenesis was significantly lower ($P = 0.008$, n = 40, $F = 7.87$).

Blood consumption was not related to the number of trypomastigotes present in insects' feces at either temperature. Neither was the number of trypomastigotes present in insects' feces related to the amount of ingested blood at each feeding at either temperature ($P = 0.35$, n = 58, $r = 0.12$; and $P = 0.52$, n = 9, $r = -0.25$ at 28 C and 20 C, respectively).

DISCUSSION

Early laboratory work by Neves (1971) and the existence of seasonal changes in the infectivity of *T. infestans*'s domestic and experimental populations (Giojalas et al., 1990; Catalá, Gorla, and Basombío, 1992) support the assumption that variations in environmental temperature affect the development of *T. cruzi* within the vector. Besides having a direct influence, this environmental factor could regulate some physiological processes of the vector by modifying the environment where *T. cruzi* multiplication and differentiation take place.

Our results describe how temperature and blood consumption affect *T. cruzi* development when *T. infestans* has daily opportunities to take blood. Epimastigote multiplication was weakly affected by temperature. Epimastigotes began to multiply immediately at both temperatures; however, at 20 C the epimastigote population density increased more slowly than at 28 C. Also, there was a significant delay in the appearance of epimastigotes within the insect's rectum; this is probably reflected in the high number of epimastigotes found within the postmesenteron for insects maintained at 20 C (90% of the entire epimastigote population developed within the vector).

The appearance of metacyclic forms within the insect's rectum and feces was also delayed at 20 C. Once the infective stage was reached, however, insects supported a similar population density of fecal trypomastigotes at both temperatures. Consequently, suboptimal temperature retards *T. cruzi* differentiation within the vector. Comparison of these data with other authors (Neves, 1971; Giojalas et al., 1990; Catalá, Gorla, and Basombío, 1992) is not easy because of the differences in the experimental design. However, an inverse relationship between environmental temperature and appearance of metacyclic forms

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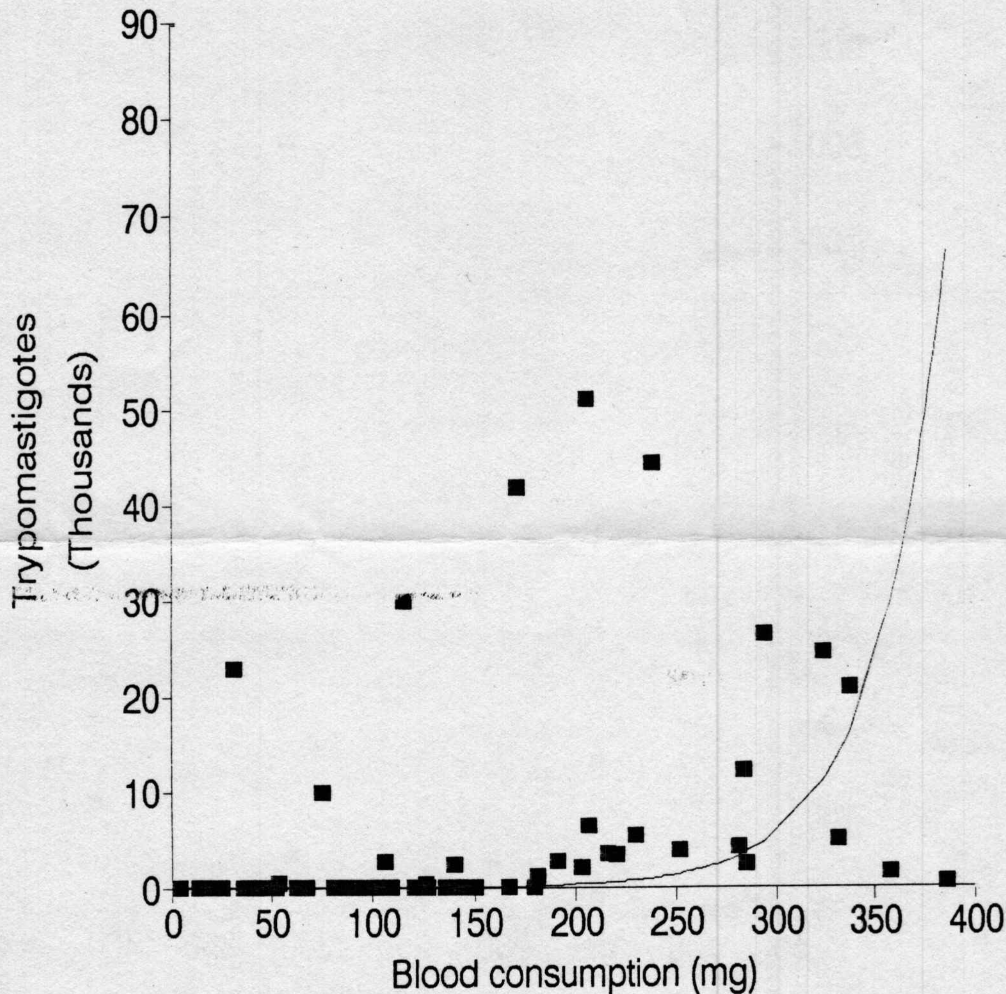

FIGURE 6. Relationship between blood consumption (mg) and trypomastigotes in rectum of fifth-stage nymphs of *Triatoma infestans* at 28 C.

after feeding, whereas fewer flagellates were counted in this region after a starvation period of 3–4 wk. Asin (1992) observed that 38 days after infection, *T. infestans* fourth-instar nymphs that fed only once did not possess rectal trypomastigotes and that the total parasitic population had declined. This decrease could be related to the reduction of specific stimulating factors present in *T. infestans* intestine and hemolymph (Isola et al., 1981, 1986).

Based on our results, temperature may modify the availability of nutrients affecting the *T. cruzi* life cycle. A minimum amount of blood consumption is necessary to support *T. cruzi* reproduction and differentiation. In insects maintained at 28 C, a significant difference was observed in the mean population density of epimastigotes and the mean blood consumption between insects that were able, and those that were unable, to support differentiation. These results suggest that blood consumption could be regulating the epimastigote population density and then influencing epimastigote differentiation. The relationship between blood consumption and the beginning of *T. cruzi* differentiation found in *T. infestans* maintained at 28 C (Asin, 1992) would reinforce this assumption. These results confirm the importance of the nutritional status of insects in *T. cruzi* development. Blood consumption as a regulating factor of population density of *T. infestans* (Schofield, 1980; Gorla and Schofield, 1989) seems also to limit *T. cruzi* development within the vector.

ACKNOWLEDGMENTS

Our special thanks go to Servicio Nacional de Chagas for supplying insects and to Dr. R. Rovasio for his suggestions. This work received financial support from Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba (CONICOR) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

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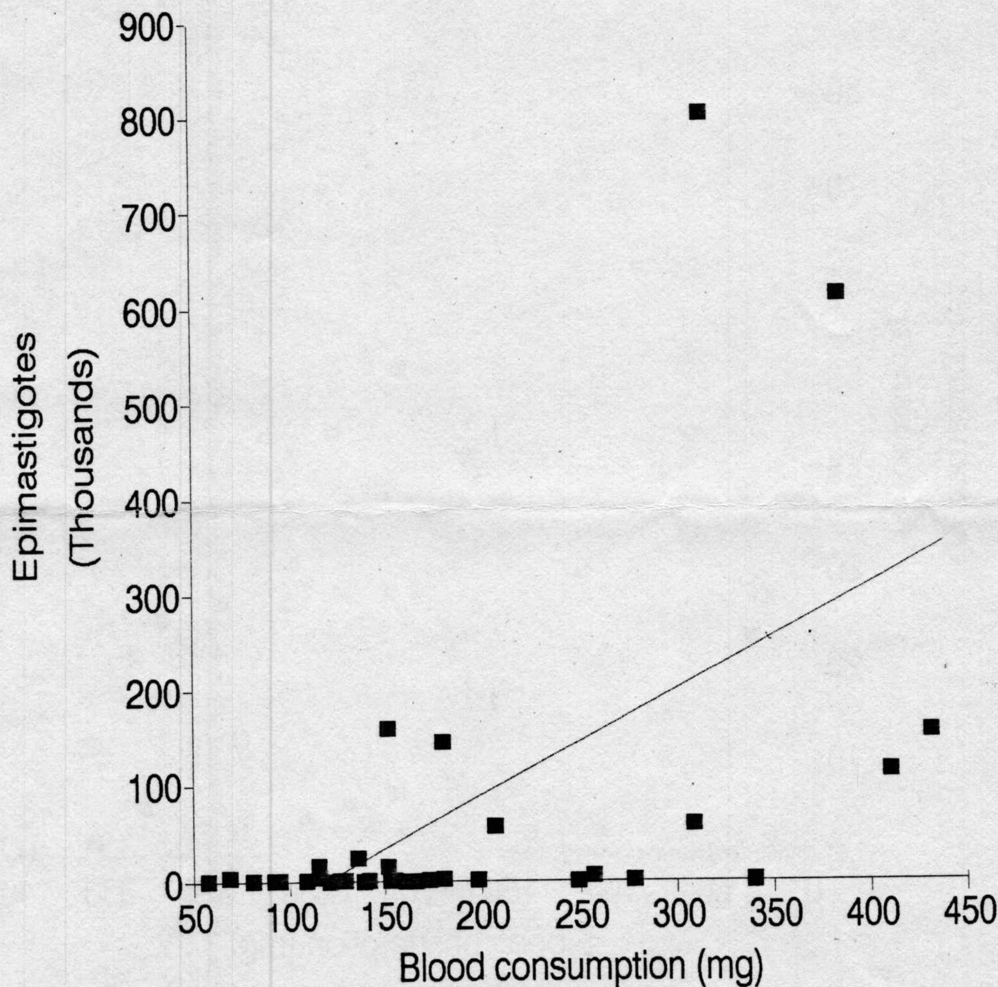

FIGURE 5. Relationship between blood consumption (mg) and epimastigotes in midgut and rectum of fifth-stage nymphs of *Triatoma infestans* at 20 C.

in the insect has been observed for *T. protracta*. During summer temperatures, the number of metacyclic trypomastigotes tends to increase in succeeding fecal droplets from the 15th day after infection. However, at temperatures ranging from 22 C to 23 C, the fecal samples examined 36 days after the infective meal revealed few metacyclic forms (Wood, 1954). On the other hand, Phillips (1960) pointed out that in *Rhodnius prolixus*, trypomastigotes can appear after an infective meal as early as the second day at 30 C, whereas at 20 C the appearance occurs on the seventh day after the infective meal.

Trypomastigotes in feces increased exponentially over time at both temperatures, particularly from the 45th day after infection. An exponential increase was also reported for 2 clones of *T. cruzi* in *Dipetalogaster maximus* over 30 days after the infective meal (García and Dvorak, 1982). This could be related to the physiological changes that occur in the insect before molting and would explain the high infectivity in adults of natural populations of *T. infestans* (Giojalas et al., 1990).

In insects maintained at 28 C, a high percentage of the trypomastigotes that had been found in the rectal content of the unfed nymphs was excreted during the first 30 min after feeding. This percentage increased to about 70% and 80% by the 16th and 24th days after infection, respectively. According to Schaub and Losch (1988), the fifth instar of *T. infestans* infected as first instar defecated in the first drop of feces 5 min after feeding about 40% of the trypomastigotes that were found in the rectal lumen. The high percentage that we observed could be related to the experimental design because a high number of parasites and a high percentage of trypomastigotes were counted in drops that were excreted 30 min after feeding.

On the other hand, nutrition appears to be another factor that affects *T. cruzi* persistence over time, with a total or partial absence of key nutrients within an insect's digestive tract. The influence of fasting upon *T. cruzi* development was studied by several authors. Immobility of parasites (Dias, 1934), a reduction in the number of metacyclic forms per bug (Piesman and Sherlock, 1985), and the loss of experimental (Phillips and Bertram, 1967) and natural (Vargas and Zeledón, 1985) *T. cruzi* infection by vectors have been reported. The influence of food supply upon *T. cruzi* growth within *T. infestans* was also studied by Schaub and Boker (1986). Based on their results, all *T. infestans* fifth-instar nymphs were infected after death by starvation and had dense populations of active flagellates in the stomach and the small intestine. Schaub (1989), studying the development of 2 strains of *T. cruzi* in *T. infestans*, reported that the number of flagellates in the small intestine increased

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