Tokai J Exp Clin Med., Vol. 23, No. 6, pp.335-340, 1999

Development of *Trypanosoma cruzi* after Starvation and Feeding of the Vector - A Review

Astrid H. KOLLIEN* and Günter A. SCHAUB

Department of Special Zoology, Ruhr-University Bochum, Bochum, Germany
(**Department of Zoology, University of Aberdeen, Aberdeen, U.K.*)

Trypanosoma cruzi **develops in the intestinal tract of reduviid bugs and may be affected by changes in the nutritional state of the vector. In regularly fed *Triatoma infestans* the population of *T. cruzi* in the rectum consists mainly of equal amounts of epi- and trypomastigotes. Starvation of the bug reduces the total number of flagellates and the number and percentage of trypomastigotes. The number and the percentage of drop-like forms and of resulting spheromastigotes, however, increases up to 30% 60 days after the last feeding (daf). Feeding of starved bugs (60 daf) reduces the original population density, which then increases again. In starved bugs 1 daf spheromastigotes (including intermediate forms) have almost disappeared and epimastigotes dominate. In addition "giant cells" (a multiple division stage) comprise about 10% of the population and in the following two days this form represents on average 30-50% of the total population, before disappearing nearly completely. Feeding the vector at 40 daf; a) induces the appearance of pure populations of trypomastigotes in immediately deposited drops of bug urine; b) induces metacyclogenesis in epimastigotes, and c) reduces metacyclogenesis in spheromastigotes. Incubating isolated recta together with the Malpighian tubules in *Drosophila* Ringer's solution and initiating the excretion with 5-hydroxy-tryptamine also induces metacylogenesis in epimastigotes.**

Keywords : *Trypanosoma cruzi*, Development, Vector, Starvation, Feeding

INTRODUCTION

Trypanosoma cruzi, the causative agent of Chagas' disease, is transmitted by triatomine bugs. In the bug's intestinal tract a variety of different developmental stages of the parasite appear [3]: the three main stages (epi-, trypo- and spheromastigotes) and many intermediate stages, which can be summarized as flagellates with either a drop-like shape (intermediates between sphero- and epi- or trypomastigotes) or a slender shape (intermediates from epi- to trypomastigotes) [11]. The biological importance of the developmental stages of *T. cruzi* is known only for epi- and trypomastigotes. Both stages dominate in an established *T. cruzi* population of regularly fed bugs, in which spheromastigotes rarely occur. Epimastigotes are, in contrast to trypomastigotes, able to multiply and colonize the whole intestinal tract of the vector. The metacyclic trypomastigotes develop in the rectum and are infectious for mammals.

Since *T. cruzi* lives in the intestinal tract of the bugs, the flagellate may be affected by changes in the nutritional supply, i.e. by the ingestion of blood or by starvation. A quantitative effect of starvation of the reduviids on *T. cruzi* is a reduction in the number of parasites and the appearance of dead flagellates in starved bugs [summarized by 12, 14]. In these investigations the population of the entire intestinal tract or of the rectum only was considered, and quantitative comparative investigations of the effect of starvation on development in different regions of the intestinal tract are lacking. Both the population density and the stages of the flagellate seemed to be affected by short periods of starvation [11, 14]. Ingestion of blood by the bug is followed by excretion and this affects the population of the flagellates in the rectum. The number of parasites is

Astrid H. KOLLIEN, Department of Zoology, University of Aberdeen, Aberdeen AB24 2TZ, Scotland, U.K. TEL: 44-1224-272880 FAX: 44-1224-272396

reduced because flagellates are deposited with feces and urine. Parallel to these quantitative changes the distribution of the different developmental stages also changes. The percentage of trypomastigotes is higher in the urine than in the feces [literature summarized in 11]. This seems to be caused by a selective "washing out" of the unattached trypomastigotes in contrast to the attached epimastigotes [13-15] and an increased metacyclogenesis rate during the defecation period [14].

Since these previous investigations only indicated that starvation and feeding induced the development of sphero- and trypomastigotes, we performed detailed studies of the induction of three developmental stages [6-8].

MATERIAL AND METHODS

The strain of *Trypanosoma cruzi* ("Chile 5") was isolated from *Triatoma infestans* which originated from Cachiyuyu, Chile [4]. *T. infestans* strain "Chile" originated from the same locality in Chile [6]. Bugs were maintained at 27 ± 1℃, 60-70% relative humidity and on a 16-h/8-h day/night rhythm.

First instars (L1) of *T. infestans* were infected with blood from mice (ca. 3,000,000 trypomastigotes/ml blood) and fed on hens every 3 to 5 weeks [14]. After feeding of fourth instar larvae, only totally engorged insects were selected. At 20, 30, 60, 90 and 120 days after this feeding ten bugs were dissected in the fifth instar to investigate the effects of starvation. To investigate the effects of feeding after long starvation periods, larvae which had starved for 60 days were fed on hens and ten L5 were dissected 1, 2, 3, 5 and 10 days later. For investigations into the effect of feeding after short starvation periods ten L5 were dissected 40 days after the last feeding of the L4 and another 10 L5 were fed on hens. The deposited feces and urine were collected, and the bugs were dissected 3 hours after blood uptake. In the last group of 10 bugs the rectal content was collected initially by careful pressing the abdomen of the insect with forceps. The rectum and the unharmed Malpighian tubules were then dissected and put under paraffin oil in Glucose-*Drosophila*-Ringer with 10^{-5} mmol/l 5-hydroxytryptamin. After 4 hours incubation the recta were dissected.

The population density was determined for the rectal wall and the rectal lumen in Neubauer hemocytometers. Smears were made of each sample [10] and after Giemsa staining more than 150 flagellates were classified [11].

RESULTS

Starvation

After a short-term starvation of 20 days the small intestine contained only about 17% of the total population of small intestine and rectum. This population was reduced enormously between 20 and 30 daf, and at 60 daf onwards no flagellates were present in the small intestine. The total rectal population density was reduced between 30 and 60 daf by one third, followed by a much stronger decrease after an additional 30 days. This density did not change substantially after another month, when some bugs had already died. Bugs which died of starvation after up to 200 daf always contained living flagellates in the rectum.

Parallel to the change in the total number of flagellates, the numbers of flagellates in most of the different developmental stages in the rectum changed, i.e. they initially increased and then decreased. The mean percentage of trypomastigotes in the rectal lumen varied in individual bugs between 43 and 61%, and seemed to be unaffected by a bug's starvation period and a similar trend occurred on the rectal wall. The percentage of epimastigotes decreased in the rectal lumen and also on the rectal wall from about 50% at 20 daf to 15-30%, finally reaching 50% at 90 daf on the rectal wall. The percentages of slender intermediate forms varied slightly for the different starvation periods, usually being less than 8%. The percentages of drop-like intermediate forms showed a continuous significant increase during the starvation process from 1% to about 15%. The percentages of spheromastigotes increased from 2% and 1% in the lumen and on the wall at 20 daf to 22% and 18% at 60 daf, but on no occasion more than 61% of the population transformed (Fig. 1). Another 30 days later, these percentages were still at approximately the same level.

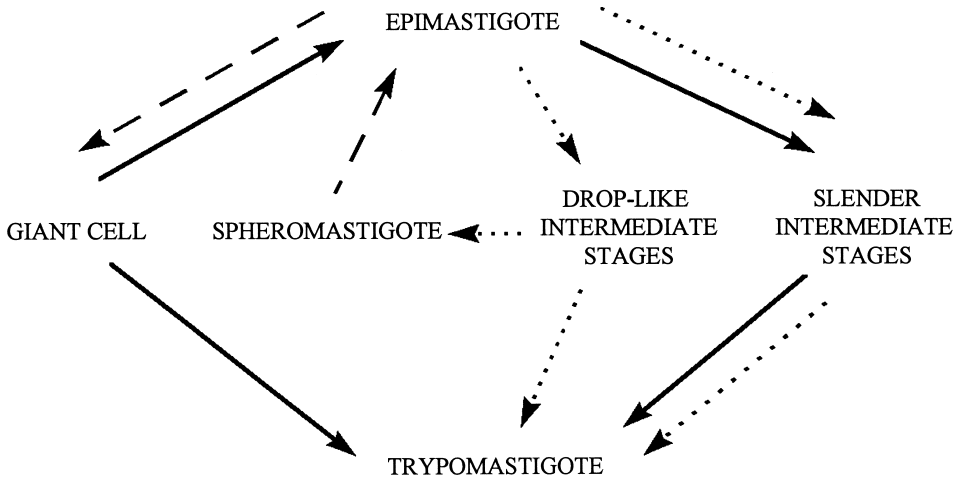

Fig. 1 Development of the different stages of *Trypanosoma cruzi* in the rectum of *Triatoma infestans* after starvation (···►) and a short (–►) or long (—►) period of time after feeding the starved bugs.

Feeding after starvation

The recta of bugs which had starved for 60 days contained about 90,000 *T. cruzi* and the rectal lumen contained about 3/4 of the total population. At 1 daf the total rectal population had not changed. On subsequent days the rectal population was reduced. Within the first 3 daf the number of flagellates in the lumen and on the wall was similar. Subsequently the population increased by more than three times at 5 daf and again at 10 daf. This increase was mainly due to the increase of the attached flagellates, about 2/3 of the total population.

Comparing the percentages of the different developmental stages in the population of bugs that had starved for 60 days, trypomastigotes dominated in the rectal lumen and on the wall with about 50%, followed by 20% epimastigotes. Spheromastigotes were more frequent in the rectal lumen (23%) than on the wall (7%), drop-like intermediate stages were more common (11 and 15%) than slender intermediate stages (2%). At 1 daf of starved bugs epimastigotes dominated (70-80%) (Fig. 1). The percentages of trypomastigotes had decreased below 10%, and spheromastigotes and drop-like intermediate stages were below 6% for both lumen and wall and a previously unobserved stage, "giant cells" , appeared with 9%. These "giant cells" possessed a high cytoplasmic mass, many nuclei, kinetoplasts and free flagella. Between 1 and 2 daf the percentages of "giant cells" increased to about 38% in the rectal lumen and 22% on the rectal wall. At 3, 5 and 10 daf the percentages of epimastigotes and spheromastigotes did not change. The percentage of trypomastigotes, however, increased and the percentage of "giant cells" decreased beween 3 and 5 daf in the lumen and on the wall from 30 and 47% to 13 and 6%, respectively and had disappeared 10 daf.

Feeding

The population density of *T. cruzi* in unfed bugs was about 200,000 flagellates/rectum, the rectal lumen containing about half of the population. The attached populations on the anterior and the posterior part of the wall consisted of approximately equal numbers. In fed bugs the deposited feces and urine contained about 100,000 flagellates and the remaining rectal population about 240,000. After feeding less flagellates were present in the rectal lumen (40%) than on the rectal wall (60%), and more flagellates were attached to the anterior than the posterior part of the wall. In the third group of bugs, the number of parasites in the rectal contents, which were pressed out before the recta were incubated, was 40,000 on average. After 4 hours of incubation, the rectal lumen and wall contained about 140,000 and 130,000 flagellates/bug, respectively.

In the rectal lumen of unfed bugs (40 daf) ca. half of the population were epimastigotes, ca. 1/3 trypomastigotes and spheromastigotes, and the percentages of the two different intermediate stages were below 5%. Percentages of spheromastigotes and slender intermediate stages in the lumen and on the wall were quite similar, but a preference for the rectal wall was evident for drop-like intermediate forms. After feeding ca. 10% of the original total epimastigote and 45% of the original total trypomastigote population were deposited within the first 3 hours with the feces and urine. In feces and urine the trypomastigotes dominated with 80%, epimastigotes 12% and spheromastigotes and intermediate forms were below 2%. The remaining population consisted mainly of epimastigotes (ca. 60%), trypomastigotes were fewer (ca. 40%) and spheromastigotes and drop-like stages appeared only in low numbers. Comparing the percentages in the feces/urine with that in the rectal lumen, the latter population possessed lower percentages of trypomastigotes but higher percentages of epimastigotes; spheromastigotes and drop-like stages were still at about 1%. However, percentages of slender intermediate stages were ca. 10% after feeding in the rectum - higher than in feces/urine which contained only 2%. In the rectal content, which was pressed out of those bugs used for the *in vitro* diuresis experiments, trypomastigotes dominated with 56% followed by epimastigotes with 28%, the two different intermediate stages with 6 and 7% and spheromastigotes with 3%. After incubation the percentages of most stages in the rectal lumen were similar to those in the rectal content; only the percentage of drop-like intermediate stages decreased after the incubation from 6 to 1%. On the two parts of the rectal wall the distribution of epimastigotes was different; a lower percentage of epimastigotes was present on the rectal pads. Spheromastigotes and drop-like stages were only 1% on the rectal wall. Intermediate stages from epi- to trypomastigotes occurred in similar percentages to the population in the lumen.

DISCUSSION

Larvae of triatomines usually need one full engorgement to develop to the next instar. The ingested blood is stored nearly undigested in the stomach, before it is transported in small portions into the small intestine, the digestive part of the gut. Nearly all blood in the stomach is used until the molt and then starvation begins. Therefore, the development of parasites in the digestive tract of triatomines should be affected by feeding, digestion and starvation [14].

Comparison of results of different investigations is difficult, since colonization densities of *T. cruzi* are also influenced by several other parameters, e.g. the strain of *T. cruzi*, species and instar of bugs and the dose and duration of infection [5]. The development of *T. cruzi* in different regions of the bug's intestine has only been investigated in our system in which *T. cruzi* and the vector strain originate from the same village. Using a susceptible strain of *T. infestans* and regular feeding at intervals of 21 or 28 days, the population density in the small intestine was reduced before feeding [11] but was always higher than in fifth instar larvae in a recent study [7]. At about 30 daf the recta of fifth instar larvae of the susceptible strain contained 1,450,000 parasites, about three times more than the small intestine. Using the unselected strain and starvation periods of 40 days, the rectum contained 350,000 parasites [14]. A similar number was found in both groups in recent investigations after starvation periods of 20 to 30 daf [7, 8]. It should be emphasized that in nearly all investigations the rectum and not the small intestine is the region most preferred by *T. cruzi*.

Increasing **starvation** periods caused a total loss of *T. cruzi* in the small intestine and a strong reduction in the rectum starting after 30 daf. Starvation effects on the population density were not only caused by a reduced multiplication of the flagellates but also by their death. Starvation not only affects the distribution of *T. cruzi* in the vector, it also acts differently on different developmental stages of *T. cruzi*. Whereas the number of metacyclics per bug was reduced [7, 9] the percentage of trypomastigotes was unaffected by starvation. In epimastigotes, both the numbers and percentages were reduced, correlated with an early increase in the number of intermediate flagellates between sphero- and epi- or trypomastigotes and followed by an increase in the number and percentage of spheromastigotes. According to previous studies [11, 14] the

increase in the rectum occurs after starvation periods >30 to 40 days. The spheromastigote seems to be a stage which develops under stress conditions, i.e. in the initial development of ingested blood-trypomastigotes [2] or during starvation.

Feeding of starved bugs initiates an increase in population density within 1 week [10] and a four-fold increase within 10 days [8]. The population composition of *T. cruzi* in the rectum was changed substantially by feeding of bugs starved for 60 days: 24 h later the percentage of epimastigotes had increased from 20% to 70-80%, whereas the percentage of spheromastigotes and the two intermediate stages had decreased [8]. At this time about 10% "giant cells" resembling a multiple division form, appeared. Within 1 to <10 daf mean percentages of "giant cells" up to 38% and up to 70% in individual bugs developed. Previously, two pathways in the development of "giant cells" have been proposed: both originating from spheromastigotes, which should either develop directly to "giant cells" [1] or initially to stumpy epimastigotes, which then multiply or by a multiple division [2]. Our data support the latter pathway. The development of "giant cells" seems to be induced by feeding conditions either after an infection of the vector [1, 2] or after long starvation periods.

Whereas the previous investigation considered the development of *T. cruzi* in daily intervals after feeding of long-term starved bugs, additional effects are evident within hours after feeding. **Feeding** primarily affected the population in the rectal lumen, because ca. 30-40% of the total population of parasites in the rectum of unfed bugs is deposited in faeces and urine [8, 14]. However, not only is the population density affected, but the percentages of the different stages also changed initially in feces and urine. Trypomastigotes, which appeared in high numbers in the urine, seem to originate from the rectal wall [13-15]. Changes in the percentages of intermediate stages also occur, especially after starvation periods of 6 weeks, after which the *T. cruzi* population consisted of many spheromastigotes (about 10%) and drop-like stages (about 20%) [6, 14]: a decrease of drop-like intermediates to trypomastigotes but an increase of percentages of slender intermediates originating from epimastigotes occurred in the course of excretion [6, 14]. These important changes in the composition of the *T. cruzi* population also appeared after incubation of the rectum together with the Malpighian tubules in saline and the induction of diuresis, excluding the influence of factors originating from the hemolymph or the small intestine. Most recently an effect similar to the present one could be induced by incubating the population attached to the rectal wall in urine of bugs (Kleffmann, personal communication).

These data emphasize the importance of the feeding state of the vector on the development of stages of *T. cruzi*. Starvation conditions of the vector induce the development of spheromastigotes and a subsequent feeding induces a further development to epimastigotes and "giant cells". Feeding after short-term starvation induces metacylogenesis in epimastigotes. Further investigations of the physiological conditions in the rectum and urine (osmolarity, pH, ions, amino acids and proteins) can elucidate the factors responsible for the development of the different stages of *T. cruzi*.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. A.J. Nisbet for proof reading the manuscript.

REFERENCES

1) Alvarenga NJ : Evolução do *Trypanosoma cruzi* no trato digestivo de *Triaoma infestans*. PhD Dissertation, Universidade Minas Gerais, Brasil, 1974.
2) Brack C : Elektronenmikroskopische Untersuchungen zum Lebenszyklus von *Trypanosoma cruzi*. Acta Trop 25: 289-356, 1968.
3) Chagas C : Nova tripanozomiaze humana. Über eine neue Trypanosomiasis des Menschen. Mem. Inst. Oswald Cruz 1: 159-218, 1909.
4) Ebert F & Schaub GA : The characterization of Chilean and Bolivian *Trypanosoma cruzi* stocks from *Triatoma infestans* by isoelectrofocusing. Z Parasitenk 69: 283-290, 1983.
5) Garcia ES & Azambuja P de : Development and interaction of *Trypanosoma cruzi* within the insect vector. Parasitol Today 7: 240-244, 1991.
6) Kollien AH & Schaub GA : *Trypanosoma cruzi* in the rectum of the bug *Triatoma infestans*: effects of blood ingestion of the vector and artificial diuresis. Parasitol Res 83: 781-788, 1997.
7) Kollien AH & Schaub GA : The development of *Trypanosoma cruzi* (Trypanosomatidae) in the reduviid bug *Triatoma infestans* (Insecta): influence of starvation. J Euk Microbiol 45: 59-63, 1998.
8) Kollien AH & Schaub GA : *Trypanosoma cruzi* in the rectum of the bug *Triatoma infestans*: effects of blood ingestion by the starved vector. Am J Trop Med Hyg

59: 166-170, 1998.
9) Piesman J & Sherlock IA : *Trypanosoma cruzi*: kinetics of metacyclogenesis in adult nymphal *Panstrongylus megistus*. Exp Parasitol 59: 231-238, 1985.
10) Schaub GA : *Blastocrithidia triatomae* (Protozoa, Flagellata) - *Triatoma infestans* (Insecta, Reduviidae). Nachweis der verschiedenen Entwicklungsstadien. *In*: Böckeler W & Wülker W (ed.), Parasitologisches Praktikum. Verlag Chemie, Weinheim. 1-6, 1983.
11) Schaub GA : *Trypanosoma cruzi*: quantitative studies of development of two strains in the small intestine and rectum of the vector *Triatoma infestans*. Exp Parasitol 68: 260-273, 1989.
12) Schaub GA & Böker CA : Colonisation of the rectum of *Triatoma infestans* by *Trypanosoma cruzi*: influence of starvation studied by scanning electron microscopy. Acta Trop 43: 349-354, 1986.
13) Schaub GA & Böker CA : Colonization of the rectum of *Triatoma infestans* by *Trypanosoma cruzi* studied by scanning electron microscopy: influence of blood uptake of the bug. Parasitol Res 73: 417-420, 1987.
14) Schaub GA & Lösch P : *Trypanosoma cruzi*: origin of metacyclic trypomastigotes in the urine of the vector *Triatoma infestans*. Exp Parasitol 65: 174-186, 1988.
15) Zeledón R, Bolaños R, Espejo N & Rojas M : Morphological evidence by scanning electron microscopy of excretion of metacyclic forms of *Trypanosoma cruzi* in vectors'urine. Mem Inst Oswaldo Cruz 83: 361-366, 1988.