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LIFE CYCLE OF *TRYPANOSOMA CRUZI*

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SUMMARY

The course of *T. cruzi* experimental infection in the vertebrate host is higly influenced by the parasite strain peculiarities. Strains with differing percentage of morphologically different trypomastigotes (slender, broad and stout) have been described, a correlation being observed between the predominance of one form and the course of parasitemia. Slender forms are apparently more fitted to penetrate cells of normal hosts and more sensitive to the immune mechanisms than the stout forms.

In some instances, triatomid-bugs proved to be insusceptible do *T. cruzi* infection even after ingestion of large numbers of blood parasites from human or from experimental acute infections. Some possible reasons accounting for this fact, such as genetic factors related to the vector, bacterial flora and the parasite morphology have been discussed.

The *T. cruzi* life cycle may be considered as rather complex, the parasite undergoing several stages of development (amastigote, epimastigote, trypomastigote, "sphaeromastigote") in both vertebrate and invertebrate hosts. Although the general features of the cycle are well known, many aspects concerned with the mechanism responsible for the parasite differentiation in both hosts and with the morphogenesis of those different stages have not been thoroughly studied. As has recently been reported by BRACK [1], even some apparently simple phenomena as the origin of infective metacyclic forms in the vector are still a matter of discussion.

In this paper, some of those points will be discussed, more emphasis, however, being put on the interrelationships between *T. cruzi* life cycle in the vertebrate host, the strain characters and the different morphological patterns exhibited by the parasite in experimental infections.

CYCLE IN THE VERTEBRATE HOST

T. cruzi undergoes discontinuous multiplication in the vertebrate host, dividing forms occurring intracellularly and the emerging non-multiplying trypomastigotes circulating in the blood stream. At the acute phase, plenty of tissue and blood forms are observed whereas, in the slow running chronic phase, the number of parasites is very small and usually undetectable by repeated fresh blood examinations. It is generally accepted that no cystic or resistant intracellular forms occur in the chronic phase of the disease, the very low parasitemia being caused by the building up of immune mechanism and the steady balance between host and parasite ("premunition"). Apparently, no spontaneous exhaustion of the infection occurs since by regular repetition of the laboratory tests it is possible to demonstrate blood parasites in a high percentage of animals and humans in the chronic phase (BRENER [2], SCHENONE et al. [18]).

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Strains, parasite morphology and course of infection

The course of experimental Chagas' disease has been shown to be highly influenced by some peculiarities of the *T. cruzi* strain. BRENER [3], in a comparative study of seven *T. cruzi* strains performed on mice, showed that three parasitemia patterns had occurred: "FL" and "CL" strains had induced gradually increasing parasitemia, most animals having died with a large number of parasites in their blood; strains "ABC" and "PNM" had given origin to slowly ascending parasitemia presenting, later on, a decline in the number of flagellates, most mice having passed into the chronic phase; and, finally, "Berenice" and "Y" strains had caused infections presenting a low parasitemia peak on the 5th day, followed by marked increase on the seventh or eighth day, and irregular decrease thereafter. Those patterns have still been kept after a 5-year period of serial passages in the laboratory. Such study also showed that different trypomastigote forms could be identified and that the following patterns could be observed: slender forms are highly predominant during nearly the whole course of infection in the animals inoculated with "Y" and "Berenice" strains; in animals inoculated with other strains, slender forms predominate only on the first days of the patent period, the number of broad or stout forms increasing and constituting most of the parasites during the ascending phase of the parasitemia. Those patterns were also seen to be constant, no tendency to monomorphism having so far been observed. PEREIRA DA SILVA [13] had also shown that, when two strains ("C" and "L") were inoculated into mice, broad forms predominated during the whole course of infection whereas with "Y" strain, the slender forms were observed to be very frequent on the first days of infection, broad forms increasing only after ten or more days. In general, slender forms are the earliest to appear in the blood stream but, afterwards, the course of infection as regards the morphology of the trypomastigotes seems to depend on the parasite strain. It is worthwhile remarking that no attempt at approaching the polymorphism of *T. cruzi* from a statistical point of view has yet been performed. This method has been used by HOARE [9] who was able to resolve the length distribution of *T. evansi* into three distinct curves. According to HOARE [9], this multi-modal frequency curve crearly demonstrates the co-existence of distinct forms or phases of the parasite.

Polymorphism of T. cruzi *trypomastigotes*

The term "polymorphism" has been suggested for expressing the different ability, competence or behaviour of the morphologically distinct trypomastigotes occurring during the course of infection. The incapability of *T. brucei* slender monomorphic trypomastigotes to develop in the tse-tse fly (WIJERS & WILLET [21]) — unlikely to what occurs with the stumpy forms-could be a good example of this phenomenon. Moreover, VICKERMAN [19] and VICKERMAN & LUCKINS [20], have lately reported that, in *T. brucei* stumpy forms, the chondriome displays strong NAD diaphorase activity, whereas slender forms from either old monomorphic strains or decent strains do not show such activity.

As regards *T. cruzi*, the presence of slender and broad forms has been observed since Chagas' first description of the parasite, only a few attempts, however, having been made to correlate the different forms with different behaviour. PEREIRA DA SILVA [13] has kept trypomastigotes *in vitro*, using infected citrated blood, and reported that, after 48 hours, practically all broad trypomastigotes develop into epimastigote or round amastigote forms, whereas the slender forms keep their typical movements for 4 or 5 days, and then, degenerate without undergoing further development. BRENER & CHIARI [5] studying, in liquid medium, the early growth of newly-isolated *T. cruzi* blood forms, have shown that trypomastigotes from strains with predominance of stout and broad forms regularly change into amastigotes which divide themselves and then give origin to large colonies of round parasites; on the other hand, trypomastigotes from strains predominating in slender forms soon change into epimastigote forms, round amastigote parasites being rarely detected. BRENER [4] recently studied the behaviour of *T. cruzi* slender and stout forms in the blood stream of normal and immune mice after

intravenous inoculation of samples of infected blood containing a high proportion of either form. Slender forms disappear, within a few hours, from the blood stream of normal animals in order to accomplish their intracellular cycle, whereas stout forms keep circulating in the blood for some days, which clearly shows the former parasites to be apparently more fitted to penetrate the tissues. In immune animals (selected from mice in the chronic phase) that had spontaneously survived the acute phase, the intravenously injected slender forms survive in the blood for just 1 or 2 hours whereas stout forms keep circulating for many days before being eliminated. Tables I, II show the number of parasites, on different days, in the blood of rats (animals considered relatively resistant to *T. cruzi* infections) inoculated, respectively, with strains "Y", "Berenice", "CL" and "MR". The animals infected with "Y" and "Berenice" strains showed slight and transient parasitemia, whilst the other two groups presented higher and more enduring parasitemia. This might be a different response of the slender and stout forms to the host innate and acquired immune mechanisms. PIZZI [15] reported that slender forms could not be detected in the blood of animals with acquired immunity and TALIAFERRO & PIZZI [17] mentioned that slender trypomastigotes were numerous on the first days of experimental infection with "Tulahuen" strain but clearly decreased thereafter. Antibodies would, then, apparently, act as a selective factor clearing the blood of forms probably less competent to develop in the invertebrate host PEREIRA DA SILVA [13].

Intracellular morphogenesis

Some evidence has been produced showing that the different trypomastigote forms

TABLE I

Number of parasites in 5 cmm of blood in rats inoculated by intraperitoneal route with 200,000 blood forms of "Berenice" and "Y" strains

"Berenice" strain

	Days after inoculation												
no.	6	7	8	9	13	14	15	16	17	20	24	30	34
1	700	770	420	630	420	140	0	0	0	0	0	0	0
2	0	140	140	140	210	0	0	0	0	0	0	0	0
3	70	70	70	560	140	0	70	0	0	0	0	0	0
4	70	0	0	210	140	0	0	0	0	0	0	0	0

"Y" strain

no.	5	6	7	8	10	12	15	20	24	30	34
1	840	0	0	70	70	0	0	0	0	0	0
2	140	140	0	70	70	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2800	0	0	0	70	0	0	0	0	0	0
5	280	0	0	0	0	0	0	0	0	0	0

TABLE II

Number of parasites in 5 cmm of blood in rats inoculated by intraperitoneal route with 200,000 blood forms of "CL" and "MR" strain

"CL" strain

	Days after inoculation																	
no.	5	6	7	8	11	12	13	15	16	18	19	20	23	24	26	27	33	34
1	210	140	140	210	560	1050	840	4200	2380	3500	1680	4900	3500	4200	3500	1690	4200	1400
2	420	560	350	350	980	140	280	350	560	840	1260	1400	1050	2100	840	840	560	1400
3	280	140	140	140	840	560	140	980	140	1540	1400	1260	280	980	980	980	420	420
4	70	140	560	840	420	280	280	420	490	70	840	1540	700	1050	1540	1400	560	140
5	140	420	560	630	1260	560	560	3500	2660	7700	13300	7000	4900	7000	7000	9100	7140	7000

"MR" strain

no.	5	6	7	8	11	14	15	16	17	18	19	20	21	22	25	26	27	32	34
1	210	280	70	70	70	70	560	70	70	70	140	140	140	0	140	210	560	700	0
2	140	210	140	70	560	70	280	250	420	630	140	140	420	700	560	840	700	560	210
3	70	140	70	70	140	350	2240	2940	1400	1400	2240	700	3010	2660	5600	2940	2100	700	2240
4	560	140	70	490	420	560	210	0	70	280	70	140	140	70	560	140	70	210	0
5	0	0	280	280	70	770	2590	4760	2800	6300	6300	7000	13300	15400	98000	70000	70000	77000	65000

come out of the parasite harbouring cells already displaying their typical morphology. Some Authors (MAYER & ROCHA LIMA [11]; WOOD [22, 23]; PEREIRA DA SILVA [13]) studied the development of the intracellular stages through tissue culture preparations and tissue impression techniques. A double transformation mechanism of amastigote into trypomastigotes forms is believed to occur: in one process ("direct"), the amastigotes tend to elongate following the flagellar line of growth (WOOD [22]) and, through this simple elongation, give origin to trypomastigotes; in the other ("indirect"), the round-shaped amastigote forms develop close to the kinetoplast, a vacuole which afterwards ruptures and gives origin to trypomastigotes by means of an unfolding mechanism. WOOD [22] describes, instead of a vacuole, a V-shaped splitting appearing between the kinetoplast and the anterior area. According to PEREIRA DA SILVA [13] the "direct" process of transformation would originate slender forms, broad forms developing through the "indirect" or unfolding mechanism.

Some aspects in tissue culture (PEREIRA DA SILVA [13]; BRENER, unpublished data) suggest that trypomastigotes of the same morphological pattern are often found in parasites harbouring cells. A study of the para-

TABLE III

Nineteen *Triatoma infestans* which fed on mice in the acute phase of Chagas' disease and failed to get infected

		Number of parasites ingested by the insects		
no.	Strain	no. slender forms	no. broad forms	Total number of parasites
1	Berenice	186,000	9,500	196,000
2	Berenice	476,000	52,000	528,000
3	Berenice	706,100	35,300	741,400
4	Berenice	1.203,000	88,500	1.291,000
5	MR	28,200	449,200	477,400
6	Berenice	1.530,000	259,000	1.789,000
7	Berenice	1.013,000	112,000	1.125,000
8	Y	1.148,400	23,400	1.171,800
9	Y	699,700	14,280	713,980
10	Y	316,900	6,420	323,320
11	Y	1.296,700	211,092	1.507,792
12	MR	107,800	872,700	980,500
13	FL	52,400	471,760	524,160
14	Y	266,000	14,000	280,000
15	Berenice	1.155,186	73,734	1.228,920
16	Berenice	472,141	35,539	507,680
17	Berenice	2.693,250	141,750	2.835,000
18	Berenice	1.064,000	56,000	1.200,000
19	Berenice	2.019,584	175,616	2.195,200

The number of parasites was determined by counting them in the blood and weighing the insects before and after feeding

sites phenotypic characters in infections produced by single trypanosomes and of the influence of environmental factors on the parasite polymorphism, would be highly desirable. TREJOS et al. [18], showed, for instance, that, in tissue culture, temperature may influence the percentage of slender and broad forms: at 26°C, a predominance of slender forms is observed whereas at 37°C, the broad forms predominate.

Cycle in the invertebrate host

According to DIAS [8], *T. cruzi* life cycle in the invertebrate host is accomplished by irreversible morphological transformations taking place along the digestive tract of the insect: in the stomach, there can be seen trypomastigotes forms developing into epimastigote and round forms, with or without flagella; in the intestine, most forms are epimastigotes, which multiply intensively; finally in the rectum, the epimastigotes differentiate into infective, metacyclic trypomastigotes.

BRACK [1] suggested, based on stained preparations and ultrastructure studies, some changes in this cycle. According to BRACK a varying number of round flagellate forms ("sphaeromastigote") have always been observed in experimentally infected *Rhodnius prolixus*; those forms, apparently a very important stage of the parasite, develop either into metacyclic trypomastigotes or into epimastigote stages. These findings strongly suggest that epimastigote forms do not develop into metacyclic trypomastigotes and that two parallel cycles occur in the vector. In our experience, round amastigote forms and "sphaeromastigotes" have, indeed, always been found in the vector, for some days, after infective blood meal and seem to be an obligatory stage, at least as concerns a certain percentage of the blood forms (BRENER, unpublished data).

DEANE [7] suggested that the absence of genetic transfer among trypanosomes of the *lewisi* group should not be taken for granted in spite of the lack of evidence supporting this phenomenon and more work in this field seems to be necessary.

Factors which might influence the course of infections in the invertebrate host have not so far been thoroughly investigated. In some instances, large numbers of blood trypomastigotes are known to have been ingested by the vector with no consequent infection. Table III shows a number of *Triatoma infestans* which fed on experimentally inoculated mice in the acute phase and failed to get infected. The number of ingested forms has been determined by counting the parasites in the blood of the infected animals (BRENER [2]) and weighing the insects before and just after the infective meals. All insects were individually dissected and examined for flagellates after 30 days. MARSDEN et al. [10] reported that 6 out of 70 *Rhodnius prolixus* fed on patients in the acute phase and with patent parasitemia, failed to show flagellates when examined, by dissection, 30 days later. PHILLIPS & BERTRAM [14] reported that the feces of some bugs fed on infected rats persisted negative even after 4 or 5 infective blood meals. The reasons for such insusceptibility are no yet known, but the following items may prove to be promissing leads for investigation:

Genetic basis — PHILLIPS & BERTRAM [14], when using *Rhodnius prolixus* and experimentally infected rats, observed that the progeny of negative parent bugs from a group of insects with a high infection rate (80.5%) presented a comparetively low infection rate of only 57.0%. In spite of the data reported, the Authors emphasized the need for further investigation on the possible genetic basis the vector susceptibility to *T. cruzi*.

Bacterial flora — Studies on the bacterial flora and symbionts of the reduviid-bug digestive tract have been performed (MÜHLPFORDT [12]; CAVANAGH & MARSDEN [6]) no correlationship, however, having so far been established between bacterial flora and its susceptibility to *T. cruzi* infection.

Strains and morphology of T. cruzi — Based on the fact of *T. brucei* slender forms being considered less competent to develop in the tse-tse flies than the stumpy ones, some Authors suggested that a similar phenomenon might occur concerning *T. cruzi*. PEREIRA DA SILVA [13], reported that, in the digestive tract of triatomid bugs, most slender

forms degenerate after 72 hours, although no direct evolution into amastigote forms could be excluded. In our experience, slender unchanged forms can still be found in the vector's gut when, apparently, all stout forms had already developed into amastigote parasites. On the other hand, 86 *Triatoma infestans*, fed on mice inoculated with "Berenice" and "Y" strains with slender forms predominating, presented 18.6% of negative bugs, whereas 57 fed on animals inoculated with "CL", "FL" and "MR" strains, richer in stout forms, displayed only 5.2% of negativity. The role played by the relative distribution of slender, broad and stout forms in the ingested population of parasites could not, however, de surely disclosed since, as in the case reported by PHILLIPS & BERTRAM [14], pure unmixed populations of each form had not been provided to the reduviid-bugs.

RESUMO

Ciclo de vida do Trypanosoma cruzi

As características da infecção experimental pelo *T. cruzi* dependem, em alto grau, da cepa do parasita, as quais dão origem a curvas de parasitemia e taxas de mortalidade bem definidas e constantes. Diferentes cepas apresentam, durante a infecção, predominância de diferentes formas de tripomastigotes sanguíneos (delgadas, intermediárias, largas), havendo evidências de uma correlação entre a tendência para a predominância de uma dessas formas e certas características da infecção tais como período pré-patente, parasitemia, etc. Estudos recentes demonstraram que as formas delgadas diferem biològicamente das largas no sentido de que são mais aptas a penetrar nos tecidos e menos resistentes aos mecanismos imunitários. Diferenças de comportamento em cultura foram também assinaladas em relação a essas duas formas.

Quanto à evolução no vector, foi recentemente sugerido que a transformação das formas sanguíneas em amastigotes, no tubo digestivo dos triatomíneos, constitui um estágio extremamente importante na evolução subseqüente para epimastigote e tripomastigotes.

Em algumas circunstâncias, triatomíneos tem se revelado insuscetíveis à infecção pelo *T. cruzi*, mesmo após ingestão de milhares de formas sanguíneas. Entre as razões lembradas para essa insuscetibilidade foram discutidas aquelas relacionadas à constituição genérica dos triatomíneos, natureza da flora intestinal dos mesmos e o papel do diferente potencial evolutivo apresentado pelas várias formas de tripomastigote do *T. cruzi*.

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