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OBSERVATIONS ON *TRYPANOSOMA CRUZI* STRAINS MAINTAINED OVER AN 8-YEAR PERIOD IN EXPERIMENTALLY INOCULATED MICE

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SUMMARY

An 8-year follow-up of three *Trypanosoma cruzi* strains maintained in mice by repeated blood passages showed the curves of parasitemia of each strain to be constant all along this period. After this 8-year period of sequential passages *T. cruzi* keeps its polymorphism as well as its capacity to infect triatoma-bugs and to grow in culture medium. *T. cruzi* then differs from *brucei*-group trypanosomes which usually become monomorphic and unable to develop in the invertebrate host after some months of blood passages. Some of those comparative aspects have also been discussed.

INTRODUCTION

Changes have been observed in strain characteristics of *brucei*-group trypanosomes following some months of mechanical passage (HOARE [8]). The present paper reports observations made over an 8-year period on three strains of *T. cruzi* maintained in albino mice by sequential blood passage. Particular attention has been paid to the persistence of trypomastigote polymorphism and the ability of the parasites to infect triatomids and to grow in culture medium. Some characteristics of the experimental infection produced in mice by those strain have been previously reported (BRENER [2]).

MATERIAL AND METHODS

The following *T. cruzi* strains were used:

1. *F L* strain, isolated from a naturally infected *Triatoma infestans* collected in Rio Grande do Sul, South Brasil.

2. *Berenice* strain, isolated by xenodiagnosis from a woman considered to be the first human case of Chagas' disease described by Chagas (SALGADO et al. [13]).

3. *PNM* strain, isolated by xenodiagnosis from a patient with chronic Chagas' disease.

The three strains have been kept for about 8 years in male albino mice by regular intraperitoneal blood passages performed every 7 days with the *Berenice* strain and every 8-12 days with the *FL* and *PNM* strains. Some morphological and biological characteristics of those strains have been previously reported (BRENER & CHIARI [4]; BRENER [2]).

COURSE OF INFECTION

On four different occasions between 1964 and 1972, groups of 10 male albino mice weighing 18-20 gm were intraperitonealy inoculated with 150,000 bloodstream trypomastigotes of the mentioned strains. The number of trypomastigotes in the inocula and in the blood of the inoculated mice was determined according to the technique described by BRENER [1]. The relative proportion of slender, broad and stout trypomastigotes was determined by examining 300 unselected parasites in Giemsa stained smears.

The course of parasitemia of strain *FL*

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and *Berenice* was also studied in groups of 10 male mice of strains DBA/2 and C57BL/6, weighing 18-20 gm, intraperitoneally inoculated with 150,000 bloodstream trypomastigotes.

INFECTION OF TRIATOMIDS

Groups of three heavily infected mice previously inoculated with, respectively, *FL, Berenice* and *PNM* strains, were anesthetized with Tionembutal and 12 5th-instar *Triatoma infestans* were allowed to feed on the animals for 40 minutes. The insects were kept at 26°C for 30 days and then their feces were microscopically examined for living flagellates. Those experiments have been performed after an 8-year period of consecutive passages of the strains in mice.

HEMOCULTURES

Blood of mice infected with the three strains was inoculated into LIT ("liver-infusion tryptose") liquid culture medium (CAMARGO [6]). The cultures were kept at 28°C and then microscopically examined after 15 days of cultivation. There mice inoculated with each strain were used and the experiments have been also performed after an 8-year period of maintanance in infected mice.

RESULTS

Figures 1, 2 and 3 show the curves of parasitemia obtained in groups of mice inoculated with the three different strains. Animals inoculated on different occasions (June 1964, November 1964, October 1969 and April 1972) with *FL* strain presented a gradually increasing parasitemia (Fig. 1); the successive slopes of the curves suggest a progressive increase in the virulence of the strain but the general pattern of parasitemia was maintained. All mice died from the infection.

Animals inoculated with "Berenice" strain on different occasions (May 1964, March 1965, September 1969, March 1972) showed a small peak of parasitemia on the 5th day of inoculation, then a conspicuous increase in the number of parasites followed by a sharp decrease and an irregular parasitemia thereafter (Fig. 2). A progressive increase in the number of parasites in the successive inoculations could also be observed. All infected mice died before the 15th day of inoculation.

Animals inoculated with PNM strain (July 1964, July 1965, October 1969, October 1972) showed the following patterns (Fig. 3): in the two first experiments, a gradual ascending parasitemia occurred, which was followed by a slow decline in the number of parasites, most animals going into the chronic phase; a similar pattern was observed in the October 1969 inoculation but most animals died after the 25th day of inoculation. In the last experiment, the curve of parasitemia was apparently similar to the previous ones but most animals died around the 15th day of inoculation.

Figures 4 and 5 show the curves of parasitemia obtained in groups of 10 male albino mice as well as groups of 10 male mice from strains DBA/2 and C57BL/6, weighing 18-20 gm, inoculated in November 1968 with strains *FL* and *Berenice*. Mice from strains DBA/2 and C57BL/6 are apparently far more resistant to *T. cruzi* infection than the albino mice; nevertheless, the general configuration of the curves is similar in the three groups of animals.

Table I shows the percentage of the different bloodstream trypomastigotes in mice inoculated with the three strains and examined on the 7th day of inoculation. This observation was carried out after 8 years of maintenance in experimentally infected mice. Figure 6 shows slender and stout trypomastigotes observed in the blood of a mouse inoculated with *FL* strain.

All hemocultures done with blood of mice inoculated with the three strains were positive. Bloodstream forms of strains *Berenice* and *PNM* readily developed into epimastigote and metacyclic trypomastigotes; bloodstream forms of *FL* strain changed into "leishmanoids" (BRENER & CHIARI [5]), which gradually developed into epimastigotes and metacyclics.

All *T. infestans* fed on inoculated mice were positive, presenting living epimastigotes and trypomastigotes in their feces.

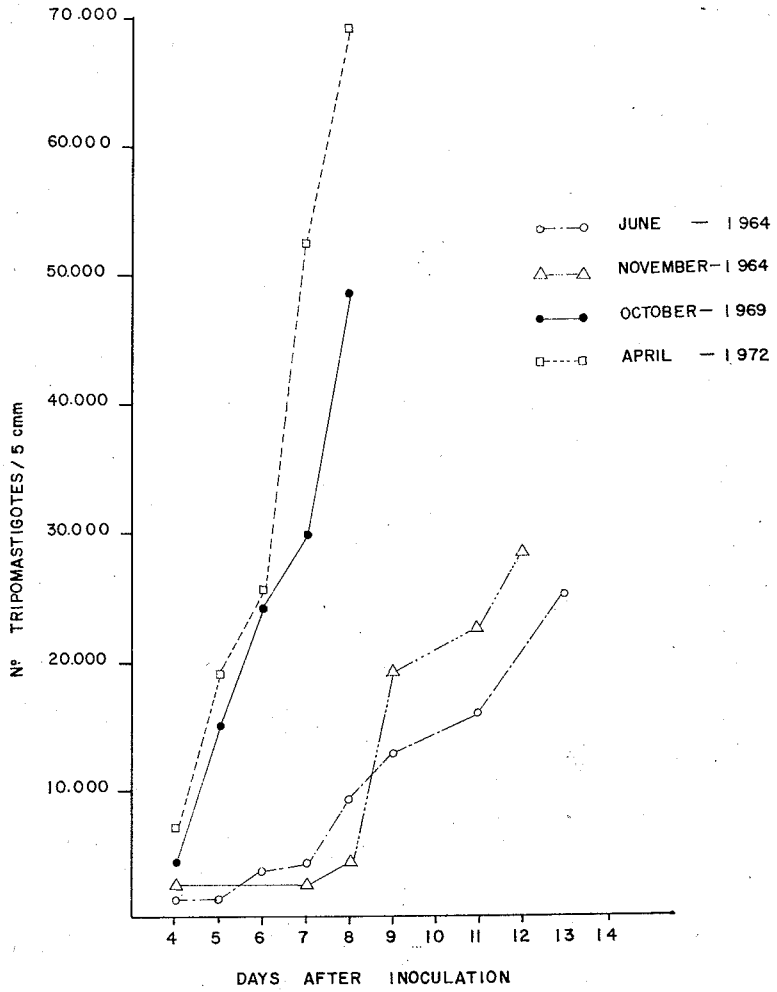

Fig. 1 — Curves of parasitemia in groups of mice inoculated on different occasions with *FL* strain (150,000 bloodstream forms, intraperitoneal route)

TABLE I

Percentage of slender, broad and stout trypomastigotes in mice experimentally infected with different *T. cruzi* strains and examined on the 7th day of infection

Strain	Slender forms	Broad forms	Stout forms
Berenice	63.1%	35.6%	1.3%
F L	33.3%	30.1%	36.6%
P N M	22.7%	63.6%	13.7%

DISCUSSION

Different *T. cruzi* strains show in experimentally inoculated mice a relative predominance of different bloodstream trypomastigote forms (slender, broad and stout) (BRENER [2]). The relative predominance of such forms was apparently responsible for some characteristics of the experimental infection. In mice inoculated, by intraperitoneal route, with parasites from strains showing predominance of stout forms, trypomastigotes could be detected in the bloodstream a few hours after inoculation and a certain proportion

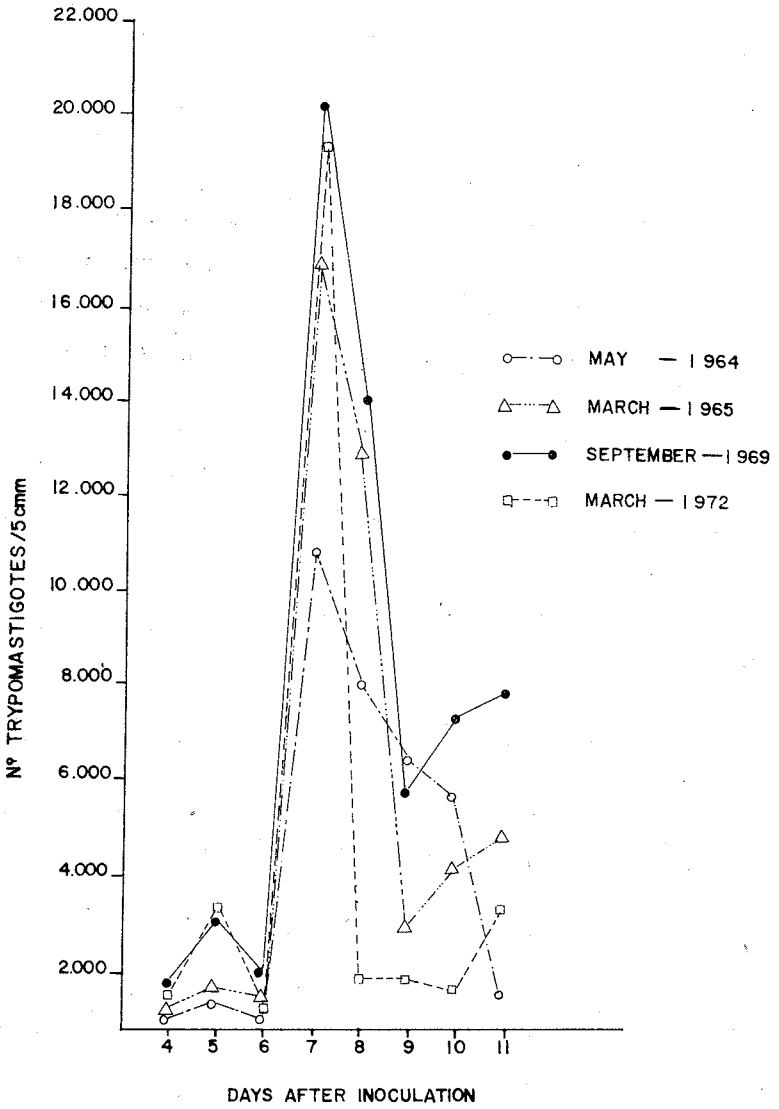

Fig. 2 — Curves of parasitemia in groups of mice inoculated on different occasions with *Berenice* strain (150,000 bloodstream forms, intraperitoneal route)

kept circulating for a few days without penetrating tissues; a gradually ascending parasitemia was observed. In mice inoculated with strains presenting predominance of slender forms, parasites could hardly be detected during the first days of infection; two peaks of parasitemia followed by a sharp decrease in the number of parasites were observed (BRENER [2]). When samples of blood with high proportion of either slender or stout forms were intravenously inoculated in normal mice, slender forms rapidly disappeared from the bloodstream, probably in order to develop intracellularly whereas stout forms were able to keep circulating for some days without accomplishing the tissue cycle (BRENER [3]). Slender forms are apparently better equiped to penetrate cells. Moreover, stout

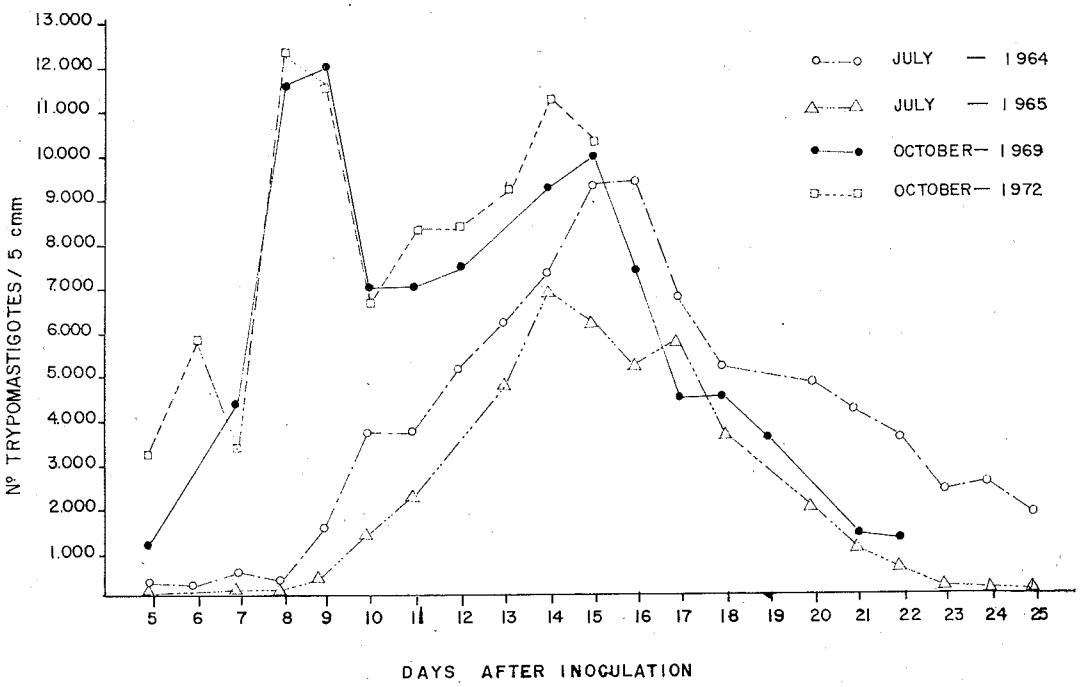

Fig. 3 — Curves of parasitemia in groups of mice inoculated on different occasions with *PNM* strain (150,000 bloodstream forms, intraperitoneal route)

forms seen to be far more resistant than the slender forms to the host's immune mechanisms: when injected intravenously in mice in the chronic phase, stout forms persist in the bloodstream for many days whereas slender forms disappear within 1-2 hours (BRENER [3]).

Those reported findings fit with the occurrence, in mice inoculated with strains presenting a predominance of stout forms, of a gradual ascending parasitemia (Fig. 1) which could result from a resistance to the host's immune mechanisms and a relative inability to penetrate cells. The marked and sudden decrease of parasitemia observed in animals inoculated with "Berenice" strain could be explained by massive destruction or increased cellular penetration of the slender forms.

The 8-year follow-up of the course of infection in experimentally infected mice showed the patterns of parasitemia to be fairly constant all along this period. The tendency to increase in parasitemia and decrease of survival time suggests that a gradual selection of forms better adapted to the vertebrate host might occur. The curves of parasitemia in mice of strains DBA/2 and C57BL/6, which are more resistant to *T. cruzi* infection, show that in spite of the lower number of parasites observed in these hosts, the patterns of infection are similar to those obtained in the susceptible albino mice. This suggests that although influenced by the host strains the parasitemia seems to be markedly dependent on the parasite strain peculiarities.

As demonstrated in this paper, *T. cruzi* mantains its polymorphism even after a long period of repeated passages in animals; in this respect it differs from *T. brucei*-group trypanosomes which become monomorphic after some months of blood passages (HOARE [8]). Those *T. brucei* slender monomorphic forms are unable to grow in culture medium and develop in the invertebrate host. There is some evidence that those forms are not able to adopt a cytochrome-mediated respiratory metabolism which seems to be essential for their heteroxenous development (NEWTON [11]; VICKERMAN [14, 15]). *T. cruzi*, however, is supposed to use Krebs cycle en-

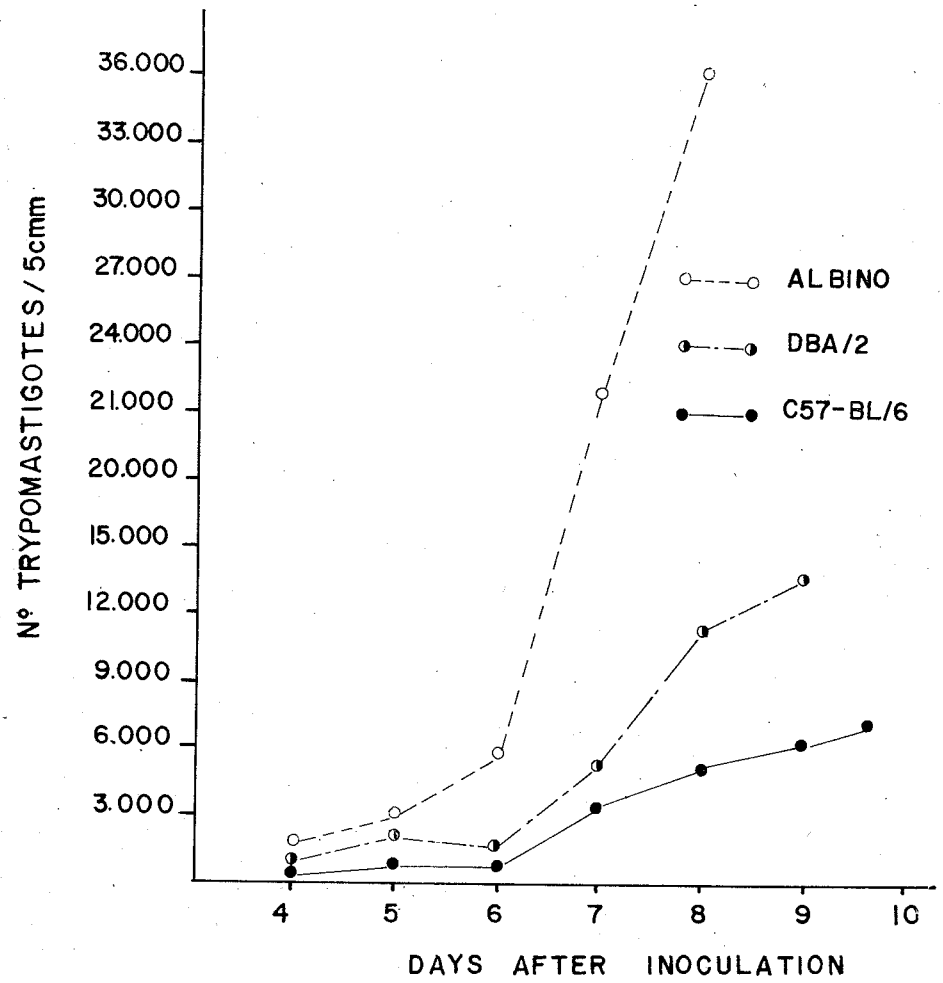

Fig. 4 — Curves of parasitemia in groups of albino, DBA/2 and C57-BL/6 mice inoculated with *FL* strain (150,000 bloodstream forms, intraperitoneal route)

zymes and cytochrome pigments during the whole development in both hosts (KALLINIKOVA [9]). A participation of such enzymes in culture forms respiratory metabolism has been demonstrated by cyanide inhibition and direct espectroscopic characterization of cytochrome pigments (RYLEY [12]; FULTON & SPOONER [7]). The persistence of those respiratory enzymes in *T. cruzi* bloodstream forms, even after long-term mechanical transmission, would explain their ability to develop in the vector regardless of the "respiratory switch" which has been described in the stumpy *T. brucei* trypomastigotes. Nevertheless, a selective study of the mitochondrial respiratory enzymes in the different *T. cruzi* trypomastigotes has not so far been performed. It is difficult, therefore, to assume whether the coding of mitochondrial enzymes proceeds in all bloodstream forms or, likewise in *brucei*-group, only in the stout and broad forms which would be, then, the sole forms with competence to develop in the vector.

MÜHLPFORDT [10], has suggested that an occasional contact between nucleus and kinetoplast, disintegration of the membranes and exchange of genetic material between nucleus and kinetoplast, disintegration of the

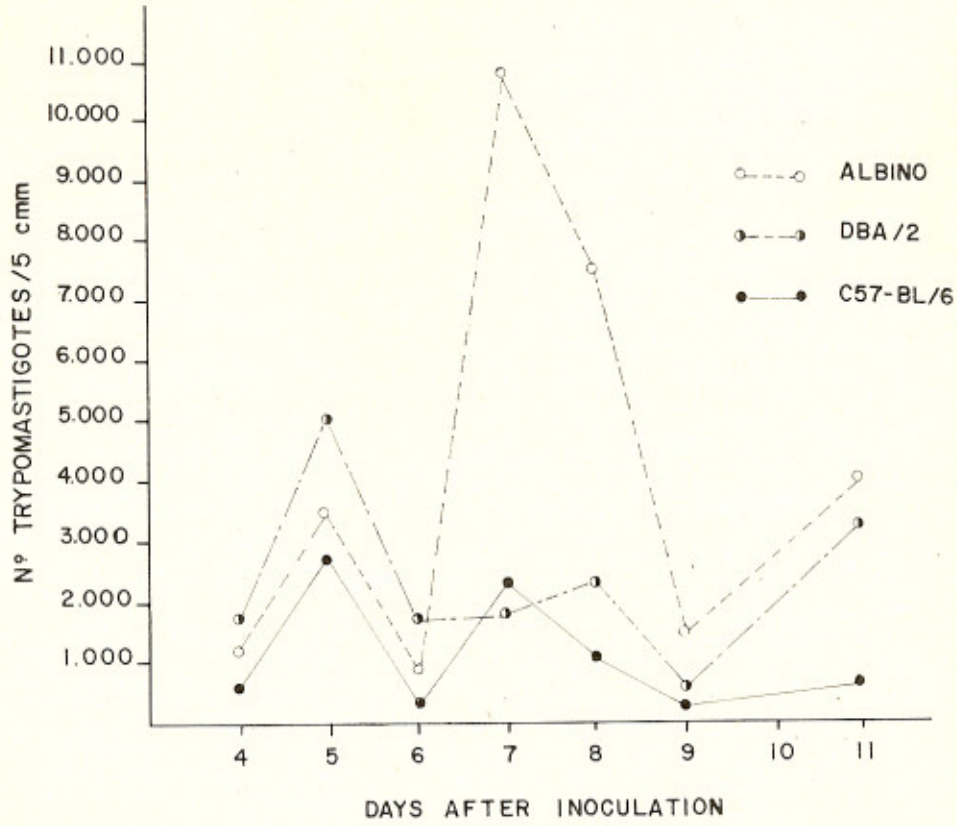

Fig. 5 — Curves of parasitemia in groups of albino, DBA/2 and C57-BL/6 mice inoculated with *Berenice* strain (150,000 bloodstream forms, intraperitoneal route)

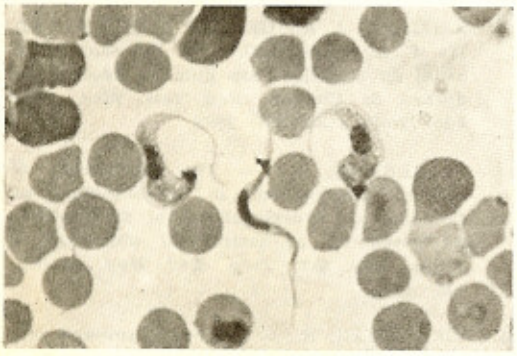

Fig. 6 — Slender and stout *T. cruzi* bloodstream forms in a mouse inoculated with *FL* strain after 8 years of repeated blood passages

membranes and exchange of genetic material between both organelles is apparently essential for the morphogenesis and normal function of trypanosome mitochondria. This phenomenon is more likely to occur during the development into epimastigote stages, when both organelles have a chance to contact each other. The repeated blood passages of *T. brucei*-group trypanosomes would prevent the normal transformation into epimastigotes which takes place in the vector; as a consequence, the DNA interchange and the normal biosynthesis of mitochondrial enzymes, essential for the survival of the bloodstream forms in the invertebrate would also be prevented. As hereby demonstrated, *T. cruzi* bloodstream trypomastigotes are still able to infect triatomids after long periods of maintenance in the vertebrate host. Contact between nucleus and kinetoplast has been described in *T. cruzi* culture forms but never in bloodstream trypomastigotes (MÜHLPFORDT [10]). This would suggest that in *T. cruzi* the coding of mitochondrial enzymes proceeds, in the trypomastigotes, regardless

of any presumptive contact between the DNA-containing organelles occurring during cyclical transmission. Another possibility not yet demonstrated would be the suggested contact taking place in the tissue stages present in the vertebrate host.

RESUMO

Observações sobre linhagens de Trypanosoma cruzi *mantidas durante oito anos em camundongos inoculados experimentalmente*

São relatadas as observações, realizadas durante 8 anos, em camundongos experimentalmente inoculados com três cepas do *Trypanosoma cruzi* (*FL, Berenice* e *PNM*). A configuração geral das curvas de parasitemia de cada uma das cepas se manteve constante, sendo ainda demonstrada, após esse período, a persistência do polimorfismo do parasita assim como a sua capacidade de infectar triatomíneos e se desenvolver em cultura. São discutidos alguns aspectos comparativos com a evolução dos tripanosomas do grupo *brucei* que, quando mantidos por longos períodos através de passagens sucessivas em animais, tornam-se monomórficos e perdem a capacidade de evoluir no vector.

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