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BIOLOGICAL CHARACTERIZATION OF *TRYPANOSOMA CRUZI* STRAINS FROM DIFFERENT ZYMODEMES AND SCHIZODEMES

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The development in C3H mice of thirteen strains of Trypanosoma cruzi *belonging to different zymodemes and schizodemes was studied. Host mortality, virulence, histiotropism, parasitemia and polymorphism of the parasites were recorded. The strains were grouped into: a) high virulence – causing 100% mortality and characterized by predominance of very broad trypomastigotes in the bloodstream at the end of infection; b) medium virulence – causing no mortality and with a predominance of broad trypomastigotes; c) low virulence – causing no mortality with blood forms not described due to the very low parasitemia. During 18 months maintenance the parasitemia curves were kept constant for all strains except one. A direct correlation between either zymodeme or schizodeme and experimental biological properties of* T. cruzi *strains was not found. However, the parasitemia was subpatent and patent for strains from zymodeme C and the others respectively. Furthermore the high virulence seems to be related to one of two shizodemes found within zymodeme B strains. All strains presenting patent parasitemia independent of shizodeme and zymodeme showed a myotropism towards heart and skeletal muscle with variable inflammatory intensity. The present study confirmed the heterogeneity found by isoenzyme and k-DNA patterns among the strains of* T. cruzi *isolated from chagasic patients in Bambuí, Minas Gerais State, Brasil.*

Key words: *Trypanosoma cruzi* – zymodeme – schizodeme – development in C3H mice

The *Trypanosoma cruzi* life cycle occurs in nature between vertebrate and invertebrate hosts, the biological interaction with man causes Chagas' disease which presents different clinical forms. The existence of *T. cruzi* intraspecific variations have been demonstrated widely by many authors on different aspects, such as, biological, immunological, pharmacological and biochemical (Brener, 1965, 1977; Andrade, 1979, 1985; Miles et al., 1977, 1981; Schlemper Jr, 1982; Andrade et al., 1983; Luquetti et al., 1986). Currently efforts have been directed to demonstrate possible associations between *T. cruzi* strains, infection, epidemiological characteristics and clinical manifestations of the disease. Among the methods used for *T. cruzi* characterization isoenzyme and k-DNA restriction endonucleases patterns were emphasized by Toye (1974), Godfrey (1976), Miles et al. (1977), Matei et al. (1977), Romanha et al. (1979a), Morel et al. (1980) and Gonçalves et al. (1984).

Based on isoenzyme patterns, Romanha et al. (1979b) grouped sixty nine *T. cruzi* strains isolated from chronic chagasic patients from the endemic area of Bambuí, Minas Gerais State, into four groups, the zymodemes A, B, C and D. These same strains were also classified according to the k-DNA patterns generated by digestion with restriction endonucleases, being grouped in schizodemes. The number of schizodemes observed was greater than the number of zymodemes, therefore separating some zymodemes into sub-groups (Morel et al., 1980). In the present study we investigated the behavior of *T. cruzi* strains from different zymodemes and schizodemes in C3H mice.

MATERIALS AND METHODS

Trypanosoma cruzi *strains* – Thirteen strains representing the major zymodeme groups from the sixty nine strains isolated from chronic chagasic patients from Bambuí, MG were studied (Chiari et al., 1989). These strains were characterized by isoenzyme (Romanha et al., 1979b) and k-DNA patterns generated by restriction endonucleases (Morel et al., 1980).

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Mice – Thirty-day-old, males weighing 15 g, isogenic C3H mice were used. In some experiments mice immunossupressed by 650 rad doses of gamma radiation (Gammacel 220, NUCLEBRAS) were also used.

Infection and strain maintenance – On the first infection each mice was injected i.p. with 10 metacyclic trypomastigotes from the 6th day of culture in M16 medium (Chiari et al., 1980). The infectivity of each strain was analyzed via fresh blood slides, after the 4th day of infection. Strains with patent parasitemia were maintained in mice by successive passages with intervals varying from strain to strain according to the parasitemia peak. The passage intervals were: 7 days for the strains 144 and 147; 10 to 15 days for the strains 84, 167, 229 and 239 and 20 days for the strains 138, 150 and 207. Blood of mice infected with strains which did not present a patent parasitemia were submitted to haemoculture on the 30th day after inoculation. Parasites from positive haemoculture were inoculated in mice and the strain maintained by alternate passages (haemoculture-mouse-haemoculture).

Biological behavior of T. cruzi *strains in mice* – For the characterization of *T. cruzi* strains maintained in mice, the curve of parasitemia, mortality of the mice, morphology of the bloodstream forms and histiotropism were recorded. The virulence was defined by parasitemia and mortality and the pathogenicity by histiotropism and the characteristics of the lesions.

Curves of parasitemia – After an adaptation period of three to five passages, trypanosome strains were inoculated in mice, i.p., with 5x10 blood trypomastigotes. The parasitemia was monitored every day after the 4th day of infection by fresh blood slides (Brener, 1962). The mean parasitemia was obtained from six infected mice.

Morphology of bloodstream forms – The morphology of parasites was studied on fresh blood slides with immersion objective and phase contrast (Ormerod et al., 1963). The result is expressed as percentage of slender, broad and very broad forms among 300 randomly observed forms (Brener & Chiari, 1963).

Mortality – The mouse mortality rate is expressed as the accumulated percentage of dead animals during 120 days after the infection.

Histiotropism – Mice were sacrificed at the acute phase of the infection (parasitemia peak). Mice with subpatent parasitemia were sacrificed on the 30th day of infection. Mice with ascendant parasitemia were sacrificed at two day intervals after the infection. At least two histological slide sections of the following organs were examined: brain, heart, spleen, liver and skeletal muscle. The slides were stained by haematoxilin-eosine and examined with a compound microscope (400x). The level of inflammatory infiltration and preferential localization of amastigotes were recorded.

Isolation of T. cruzi *from mice* – At different period of maintenance in mice the strains were isolated by haemoculture in LIT medium. The positive hemocultures were established and bulk cultures grown in the same medium. The parasite culture forms were harvested and washed three times by centrifugation, 2,000 g, 4 °C, 10 min, in KRT (Krebs Ringer Tris, pH 7.3) buffer. The parasite pellet was stored at –70 °C until used for isoenzyme and k-DNA pattern determination.

Isoenzyme patterns – Parasite enzymatic extract and isoenzyme pattern determination was made according to Carneiro et al. (1990). The isoenzyme used for zymodeme classification were: alanine aminotransferase [E. C. 2.6.1.2], aspartate aminotransferase [E. C. 2.6.1.1], malic enzyme [E. C. 1.1.1.40], 6-phosphogluconate dehydrogenase [E. C. 1.1.1.44], phosphoglucomutase [E. C. 2.7.5.1], glucose-6-phosphate dehydrogenase [E. C. 1.1.1.49], glucose phosphate isomerase [E. C. 5.3.1.9] and malate dehydrogenase [E. C. 1.1.1.37].

K-DNA patterns – The washed culture form pellet stored at –70 °C was trawed at room temperature and submitted to a k-DNA extraction according to Gonçalves et al. (1984). The k-DNA was digested by the enzyme Eco RI and the fragments generated separated by vertical electrophoresis in acrilamide gel. The electrophoretic pattern was visualized by ethidium bromide fluorescence (Gonçalves et al., 1984).

RESULTS

Culture forms infectivity – Nine out of the thirteen strains studied presented a patent parasitemia, whereas the remaining four presented a subpatent parasitemia in mice (Table). The strains producing patent para-

TABLE

Zymodeme and biological behavior of *Trypanosoma cruzi* strains in C3H mice

Strain	Zymodeme	Biological behavior		
		Parasitemia	Morphology	Virulence
138	A	Patent	–	Medium
229	A	Patent	Broad	Medium
239	A	Patent	–	Medium
84	AB*	Patent	Broad	Medium
144	B	Patent	Slender and very broad	High
147	B	Patent	Slender and very broad	High
167	B	Patent	Broad	Medium
182	C	Subpatent	–	Low
222	C	Subpatent	–	Low
231	C	Subpatent	–	Low
254	C	Subpatent	–	Low
150	D	Patent	–	Medium
207	D	Patent	Broad	Medium

AB* = intermediate zymodeme.

sitemia were maintained via successive blood passages, whereas the strains producing subpatent parasitemia, demonstrated only by haemoculture, were maintained via alternate passages. Eight alternate passages were carried out for 222 and 182 strains and three passages for the strains 231 and 254. They all kept their subpatent parasitemia throughout passages, except strain 222 which, chaged from a subpatent to a patent parasitemia on the 8th passage. In an attemp to increase the parasitemia two out of four strains producing subpatent parasitemia were inoculated in irradiated mice, however this was unsuccessful.

Parasitemia curve – Two parasitemia profiles were recorded in nine strains. Seven strains produced parasitemia curves with a long duration, irregular peaks, varying in level and the period of maximum circulating parasites (Figs 1 and 2). Only two strains (144 and 147) presented ascendant parasitemia, with rapid parasite multiplication and short duration (Fig. 3). The parasitemia curves were not altered throughout passages in mice carried out during about eighteen months of strains maintenance.

Mortality – Mortality was observed only in animals infected with the strains 144 and

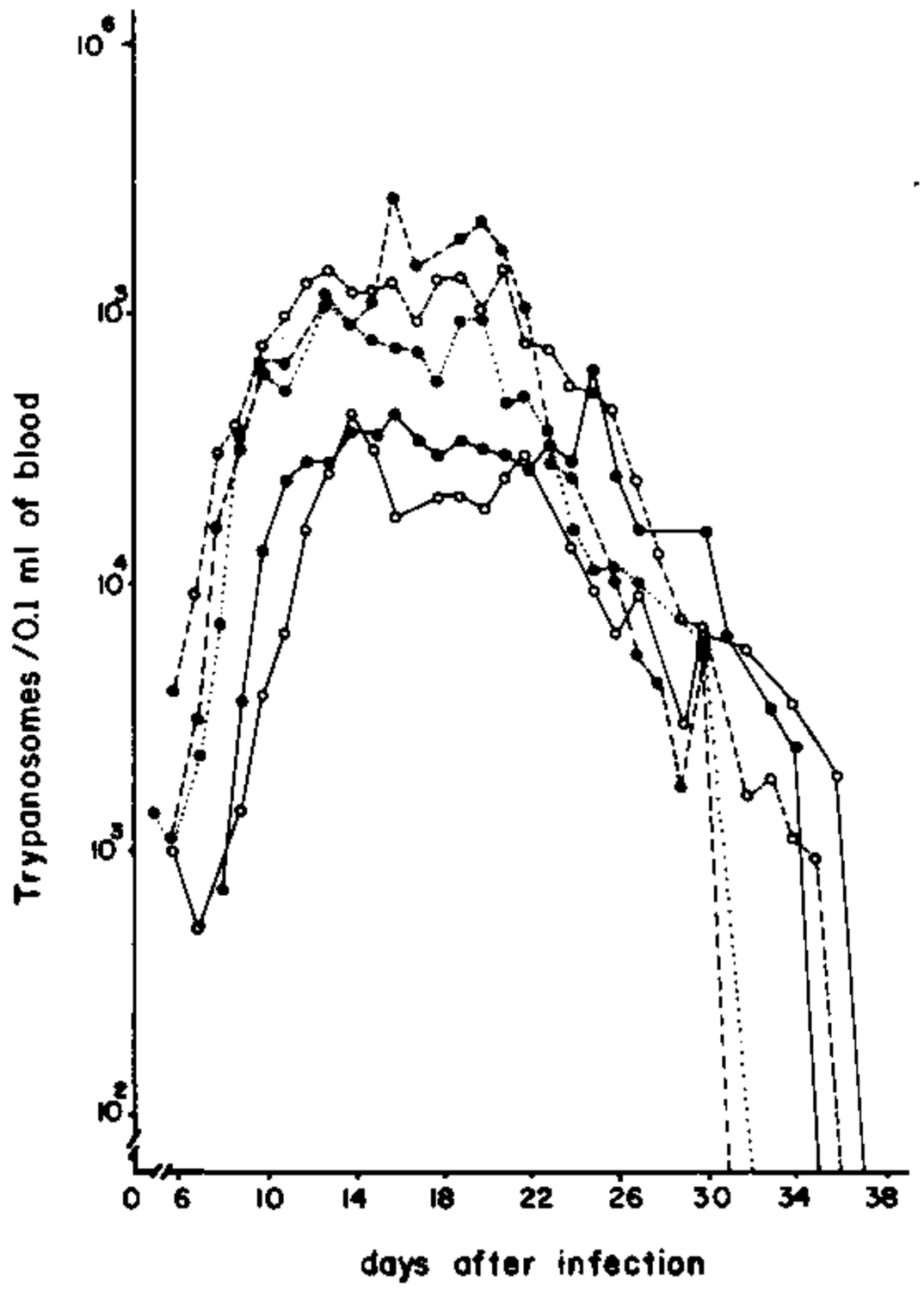

Fig. 1: parasitemia of *Trypanosoma cruzi* strain 167 at different passages in C3H mice.
Passages: 7th (o—— o), 18th (• —— •), 25th (o– – –o), 32nd (• – – – •) and 38th (• . . . •).

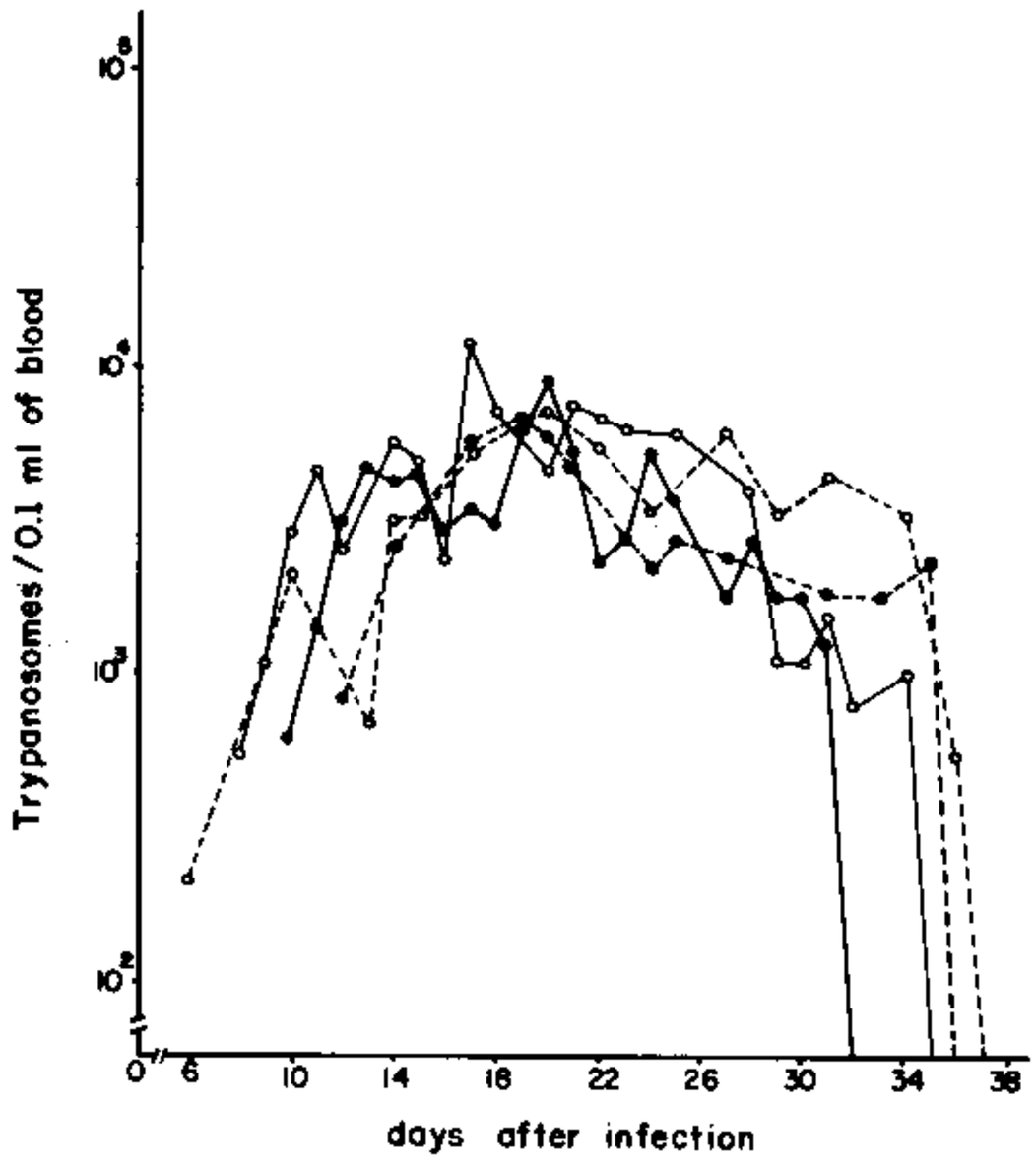

Fig. 2: parasitemia of *Trypanosoma cruzi* strain 138 at different passages in C3H mice.
Passages: 14th (o —— o), 20th (• —— •), 27th (o – – – o) and 32nd (• – – – •).

147 which presented high parasitemia levels. Mortality occured mainly between the 14th and 16th day after infection.

Bloodstream form morphology – Only strains which presented high parasitemia were examined. The morphology of the bloodstream forms predominant during the infection is shown on the Table.

Histiotropism – The nine strains (138, 229, 239, 84, 144, 147, 167, 150 and 207) evaluated presented a preferential tropism for both heart and skeletal muscle cells. The parasitism was followed by inflammatory infiltration with variable intensity.

Regard to the virulence three distinct groups of strains were observed: a) high virulence strains with 100% animal mortality and the predominance of very broad trypomastigotes at the end of the infection; b) medium virulence strains with no mortality and with the predominance of broad forms at the end of infection, and c) low virulence strains with absence of mortality and blood forms not determined due to a subpatent parasitemia.

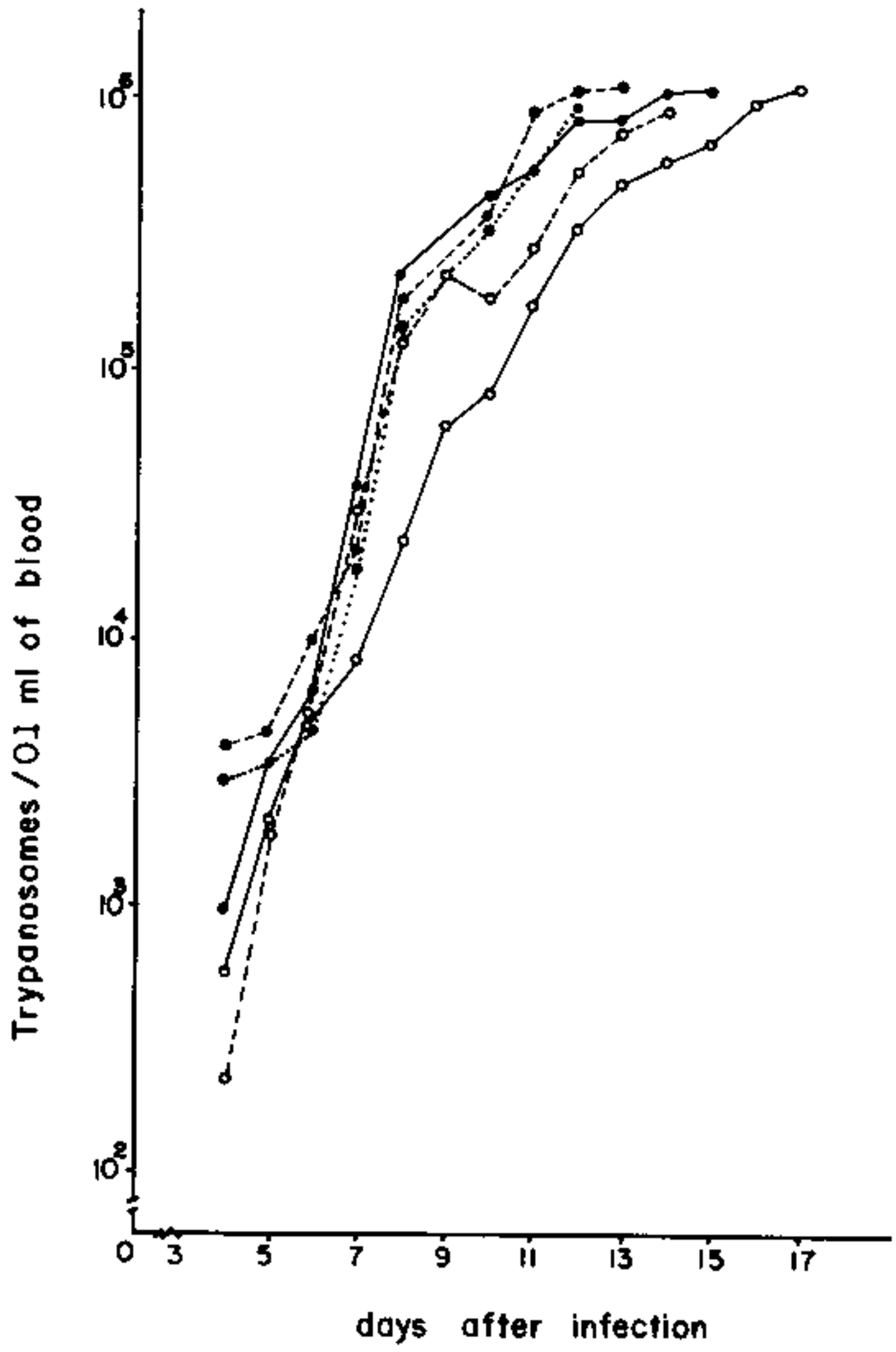

Fig. 3: parasitemia of *Trypanosoma cruzi* strain 147 at different passages in C3H mice.
Passages: 3rd (o —— o), 11th (• —— •), 21st (o – – – o), 32nd (• – – – •) and 41st (• . . . •).

The results of isoenzyme and k-DNA patterns of each strain are shown elsewhere (Carneiro et al., 1990).

DISCUSSION

The experimental development of *T. cruzi* strains from different zymodemes in C3H mice showed marked differences. The infectivity of culture forms using standard inoculum and inbred C3H mice, recognized as susceptible to *T. cruzi* infection (Pizzi et al., 1954; Watkins, 1966; Trischman et al., 1977) showed that nine strains produced patent and four subpatent parasitemia. Difficulties to adapt *T. cruzi* strains in mice have also been described by other authors, who demonstrated the absence of patent parasitemia in mice with strains isolated from sylvatic reservoirs and chronic chagasic patients (Deane et al., 1963; Schlemper Jr, 1982).

Comparing infectivity of the strains with their zymodeme, it was possible to separate the strains from zymodeme C from the others. Strains from zymodemes A, B and D presented patent parasitemia, whereas strains from zymodeme C presented subpatent parasitemia. Surprisingly, the 222 strain changed parasitemia from subpatent to patent after eight alternate passages. Changes in the biological behavior of *T. cruzi* strains, when maintained by serial passages in mice, were also described by other authors and the virulence increasing has been the aspect most often observed (Haushcka, 1949; Brener & Chiari, 1963; Brener et al., 1974; Schlemper Jr, 1982). The importance of laboratory maintenance conditions as a factor in selection of *T. cruzi* strains in mice submitted to double infection was demonstrated by Deane et al. (1984). Studies on cloned populations have confirmed that *T. cruzi* strains consist of distinct subpopulations, which are either expressed or not depending on the maintenance conditions (Morel et al., 1980; Araujo & Chiari, 1988; Dvorak et al., 1989). Our results suggest that the alternate passages of the 222 strain, selected a parasite subpopulation more adapted to the development in mice, though it has not happened with the other three strains under identical conditions. The change in parasitemia of 222 strain was followed by changes in zymodeme and schizodeme (Carneiro et al., 1990).

The two parasitemia curve profiles observed corresponded to types III and II described by Andrade (1979). Two strains of high virulence (144 and 147), seven of medium and four of low virulence were found. The virulence distribution among the strains confirmed previous studies where the majority of the strains isolated comprised those with medium and low virulence (Andrade, 1974; Schlemper Jr et al., 1983; Lana & Chiari, 1986). In confirmation of other authors observations we emphasize the need of studies on low virulence strains. Due to the difficulty in experimental maintenance those strains have been less studied or even abandoned. The use of strains with high virulence may therefore have distorted concepts of experimental Chagas' disease.

The polymorphism of blood trypomastigotes showed a predominance of broad forms during the infection. Only two strains presented slender forms at the beginning of the infection and very broad forms thereafter. There was a preferential tropism to heart and skeletal muscle tissues. The histiotropism of broad and very broad forms confirmed previous studies (Bice & Zeledon, 1970; Tay et al., 1973; Andrade, 1974; Melo & Brener, 1978; Schlemper Jr et al., 1983).

Our results show that strains from zymodeme A were more adapted to mice than the others. This group was homogeneous and the parameters evaluated were kept constant throughout the 18 months of maintenance in mice. This stability was not observed in strains from other zymodemes (Carneiro et al., 1990). The zymodeme B strains divided in two schizodeme groups presented distinct behabior in mice. Only the strains 144 and 147 from the same schizodeme presented high virulence with 100% animal mortality, suggesting a correlation between schizodeme and high virulence (Gonçalves et al., 1984).

Our results differ, however, from some published data, which suggested a correlation between isoenzyme patterns and biological behavior in strains isolated from the same geographic region (Andrade, 1985; Miles et al., 1977, 1981; Andrade et al., 1983). The biological characterization confirmed previous isoenzyme and k-DNA patterns heterogeneity observed for the *T. cruzi* strains isolated from chronic chagasic patients from Bambuí county, Minas Gerais State.

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