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# MORPHOLOGICAL AND MORPHOMETRIC VARIABILITY OF THREE CLONES OF *TRYPANOSOMA CRUZI* AND THE STRAIN OF ORIGIN (BOLIVIA)

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**Summary** Knowing the great diversity of medical and biological properties of *Trypanosoma cruzi*, the causal agent of Chagas' disease, we quantified the morphological parameters that typify the different forms of three clones of *T. cruzi* and their original strain, Bolivia, in comparison among themselves and with strain Bolivia, attempting to provide additional data concerning the clonal biological behaviour of this parasite. Blood forms morphology was quantified using a computarized image analysis (Videoplan/Kontron) and statistical analysis was determined using ANOVA-1 Test. Large number of quantitative differences among slender, broad, and stout forms were found. The comparison of clones I, II and III with their mother strain, leads to the emergence of significant differences in at least 12 parameters out of the 16 we studied. When clones were compared among themselves, the differences decreased. Variations of the percentages of the three kinds of clones were found along the acute infection. These data are the first step in correlating the morphological and pathogenic characteristics of the parasite.

**Key words:** *Trypanosoma cruzi*, clone, strain, polymorphism, morphometry

The protozoan parasite *Trypanosoma cruzi* causes Chagas' disease in South and Central America, an affliction that in humans is characterized by a broad spectrum of clinical symptoms. The severity and type of the disease symptoms vary in different geographical regions, possibly reflecting a heterogeneity among strains of *T. cruzi* or genetic differences in the human population. Different subpopulations of *T. cruzi* might differ in their abilities to multiply in different mammalian hosts or insect vectors. Thus, *T. cruzi* manifests a great diversity of medical and biological properties which in turn could be the origin of the clinical variability in the disease[6, 7, 9, 15, 18]. The multiclonal structure of natural populations of *T. cruzi* has been demonstrated by extensive population studies[15, 18] and might be the reason for such varied behaviour.

There have been attempts to correlate morphological and pathogenic properties[20, 8, 2, 19]. This led us to propose a morphological and morphometric study of different clonal lines of *T. cruzi* as a part of a biological comparison study, trying to provide more data to the hypothesis that the clones behave as independent genetic entities.

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## Materials and Methods

*Strain of* T. cruzi. Bolivia strain was isolated from *Triatoma vitticeps* captured in Vittichi (Bolivia) in 1971. This strain has been maintained frozen in our laboratory. The strain belongs to «major clone» 20, according to Tibayrenc and Ayala's classification[15], into which natural populations of *T. cruzi* are subdivided in clones, of which a few are able to spread unchanged over large geographical areas and long periods of time: these are the «major clones», on the basis of which the authors express

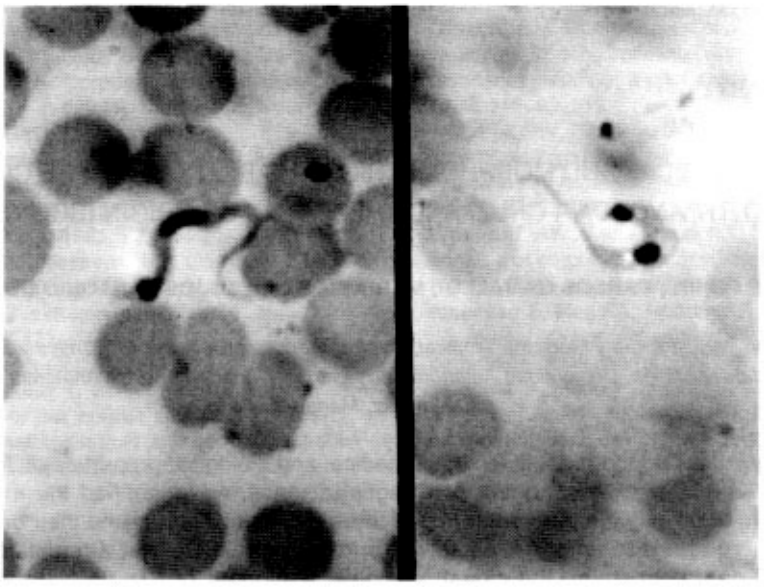

Fig. 1.— Trypanosoma cruzi *blood forms: slender form on the left side and broad form on the right side. X1000.*

the hypothesis that the major clones or genotypes, play an important role in Chagas' diseases epidemiology and pathogenicity.

*Cloning procedure.* A drop of blood from a mouse's tail infected with *T. cruzi* (Bolivia strain), was put into 1 ml of sterile saline solution. A microdrop of the homogenized solution was placed on a cover slip which was then inverted over a concave slide and examined under the microscope. The drop filled a microscopic field with objective of 40X. When a single trypanosome was seen, it was washed with a saline solution, inoculated in the medium of N.N.N. culture medium[1], and incubated at 26°C. Observation began at day 10 postinoculation.

*Experimental animals.* Male mice *Mus musculus* of the Swiss strain Ico (OF1: IOPS Caw), one month old, were mainted under the following conditions: photoperiod: 12 hr light/12 hr darkness, temperature; 21°C ± 1°C, humidity: 55 ± 10%, air renewal: 10 to 15 changes/hr. Mice used are an outbred strain but reproduction is endogamic in this group due to the same initial couple.

*Morphological study.* Blood smears were made and number of slender, broad, and stout forms (Figs. 1 and 2) were noted weekly during the acute phase (63 days) on 15 mice for each of the study groups, clones I, II, III and Bolivia strain. With the data obtained, the corresponding statistical evaluations were performed.

*Morphometric study.* Smears obtained from the morphological study were used to evaluate the parameters of slender, broad, and stout forms. A total of 90 forms were evaluated, 30 for each group (slender, broad and stout) at the same day of infection in mice. Thus, sample size was of 30 elements for each measured form and for each

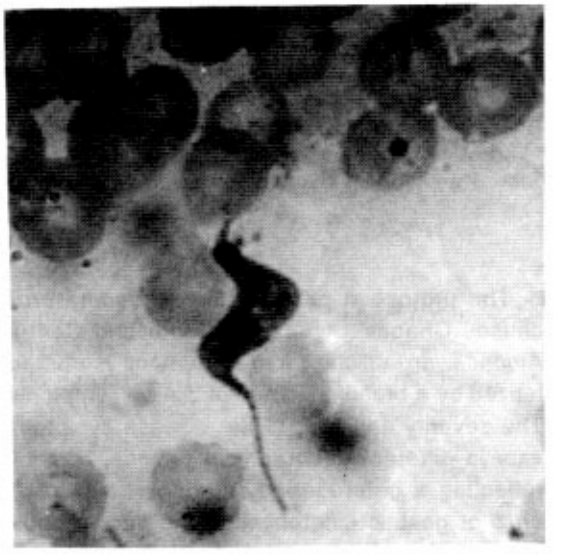

Fig. 2.— Trypanosoma cruzi *stout form. X 1000.*

parameter. A computarized image analysis (Videoplan/ Kontron) was used to quantify the following 16 parameters: total body length, including free flagellum; (L), length of free flagellum (F), cytoplasmic length (CL), distance between nucleus and posterior extremity (NP), distance between nucleus and anterior extremity (NA), distance between nucleus and kinetoplast (NK), nuclear area (NAr), kinetoplast perimeter (KPer), cytoplasmic area (CAr), cytoplasmic perimeter (CPer), kinetoplast area (KAr), kinetoplast perimeter (KPer, cellular width (W), nucleus/

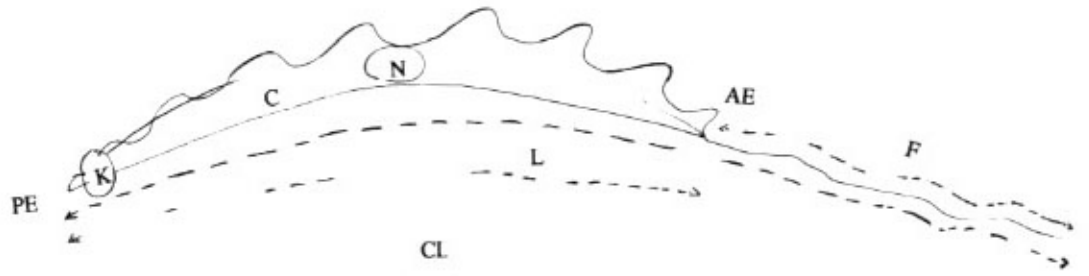


Fig. 3.— *Scheme of the main* Trypanosoma cruzi *morphological parameters: N: nucleus; C. cytoplasm; K: kinetoplast; F: flagellum; AE: anterior extremity; PE: posterior extremity; L: total body length; F: length of free flagellum; cytoplasmic length*

cytoplasm relation (N/C), nuclear index (NI = NP/NA) and flagellum/cytoplasm relation (F/C). (See fig. 3).

*Statistic study.* Data were processed in a Macintosh SE/30 using the StatView SE program. We first looked for means and standard deviations for each parameter within each blood group. Then, significant differences on measured morphometric parameters were searched for between the same forms of different groups. An Anova-1 variance analysis was conducted. Significance level has been taken as 95% ($p < 0,05$).

## Results

*Blood study*

Evolution of blood pleomorphism: In studying the morphological forms of *T. cruzi*, Bolivia strain, stout forms are seen to predominate from day 14 postinoculation when they first appear. Slender forms were practically insignificant at less than 5% and disappear at day 56 (Fig. 4). Clone I showed all the three forms initially without great percentage differences, although as the infection progressed, stout forms increased until they reached values of over 50% (Fig. 5). In clone II stout forms predominated from the first day and disappeared at day 42 postinfection (Fig. 6). In the last group studied, clone III, stout forms continued to predominate except on the last day when they fall to 15%-20%, while the slenders increase to 32% simultaneously (Fig. 7).

*Morphometric study.*

Tables 1, 2, 3 and 4 show the mean and standard deviation values of Bolivia strain and clones I, II and III. Significant differences obtained by the variance analysis are related in Tables 5, 6 and 7.

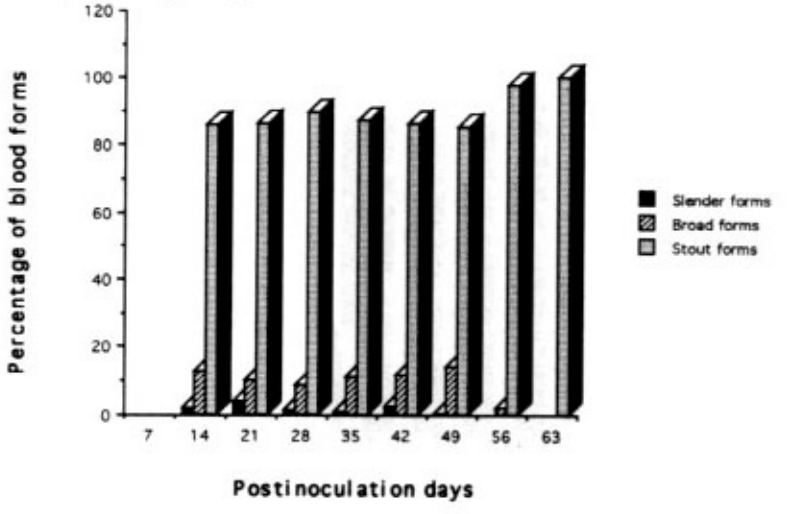


Fig. 4.—*Percentage evolution of different blood forms in the acute phase of infection by* Trypanosoma cruzi *(Bolivia Strain).*

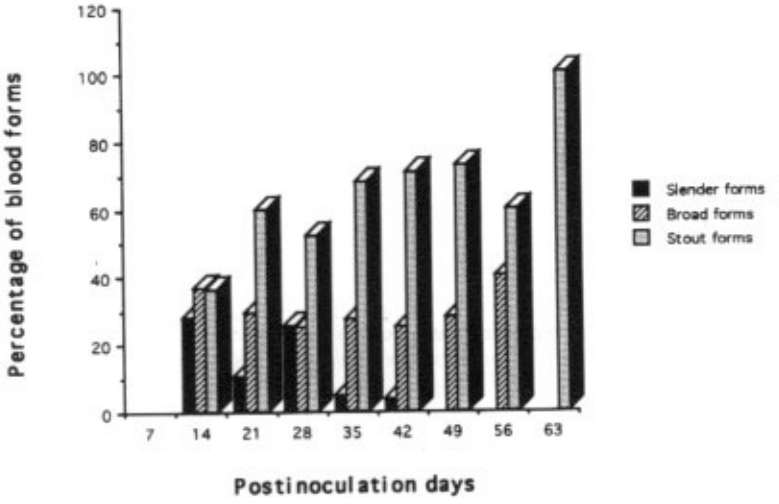


Fig. 5.— *Percentage evolution of different blood forms in the acute phase of infection by* Trypanosoma cruzi *(Clone I).*

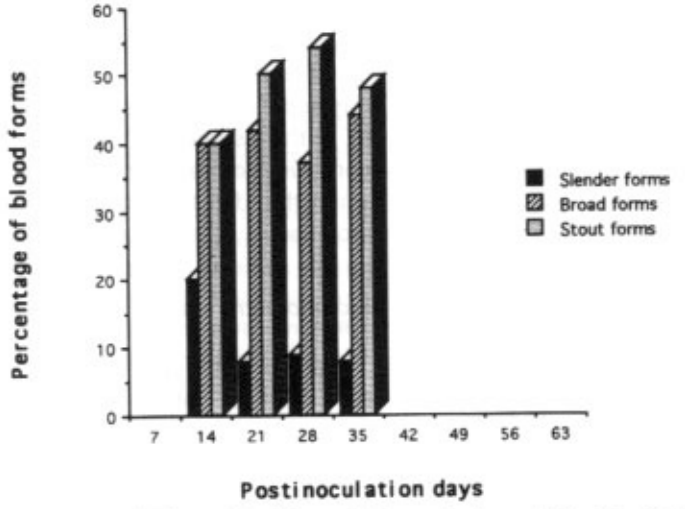


Fig. 6.— *Percentage evolution of different blood forms in the acute phase of infection by* Trypanosoma cruzi *(Clone II)*

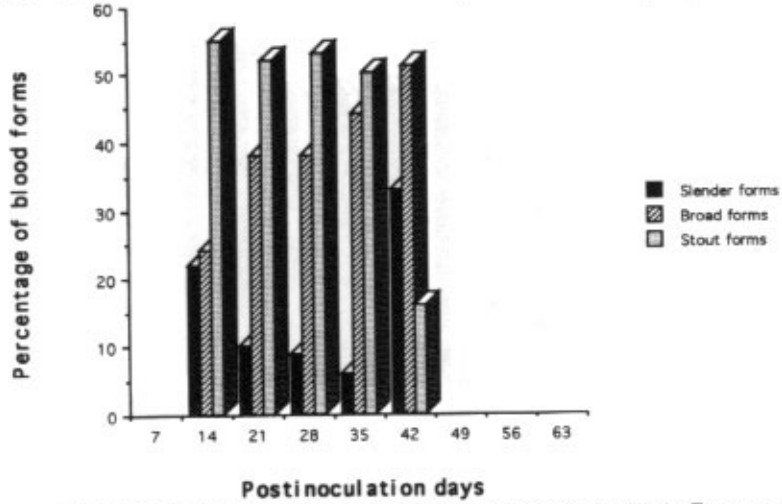


Fig. 7.— *Percentage evolution of different blood forms in the acute phase of infection by* Trypanosoma cruzi *(Clone III).*

TABLE 1.— *Blood forms morphometric data (in μm) of T. cruzi Bolivia strain*

| Studied Parameters | Slender | | Broad | | Stout | |
|---|---|---|---|---|---|---|
| | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. |
| L | 25.63 | 2.87 | 28.57 | 3.83 | 33.44 | 3.21 |
| F | 6.07 | 1.71 | 7.69 | 2.63 | 8.89 | 1.27 |
| NP | 0.95 | 2.21 | 11.67 | 1.72 | 13.70 | 1.95 |
| NA | 8.58 | 2.10 | 9.52 | 1.66 | 10.41 | 1.91 |
| NAr | 2.75 | 0.65 | 3.30 | 0.83 | 3.66 | 0.82 |
| NPer | 8.44 | 1.48 | 7.23 | 1.19 | 7.58 | 1.08 |
| CAr | 16.58 | 3.20 | 26.78 | 4.46 | 46.32 | 11.02 |
| CPer | 34.42 | 5.44 | 37.08 | 2.48 | 38.20 | 3.33 |
| KAr | 1.20 | 0.28 | 1.77 | 0.26 | 1.81 | 0.34 |
| KPer | 4.10 | 0.52 | 4.85 | 0.37 | 4.93 | 0.48 |
| W | 1.29 | 0.29 | 2.40 | 0.34 | 5.00 | 1.73 |
| NK | 9.86 | 1.86 | 9.61 | 1.83 | 12.46 | 1.77 |
| N/C | 0.16 | 0.03 | 0.12 | 0.03 | 0.08 | 0.02 |
| IN | 1.34 | 0.41 | 1.26 | 0.29 | 1.36 | 0.34 |
| CL | 19.55 | 2.66 | 20.87 | 2.74 | 24.54 | 2.87 |
| F/C | 0.31 | 0.10 | 0.37 | 0.13 | 0.36 | 0.06 |

TABLE 2.— *Blood forms morphometric data (in μm) of T. cruzi Clone I*

| Studied Parameters | Slender | | Broad | | Stout | |
|---|---|---|---|---|---|---|
| | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. |
| L | 9.08 | 1.15 | 12.34 | 1.41 | 14.70 | 1.41 |
| F | 3.17 | 0.57 | 4.99 | 0.83 | 5.65 | 0.64 |
| NP | 2.61 | 0.33 | 3.46 | 0.57 | 4.39 | 0.69 |
| NA | 1.37 | 0.51 | 2.23 | 0.54 | 2.75 | 0.66 |
| NAr | 0.96 | 0.25 | 1.03 | 0.27 | 1.51 | 0.34 |
| NPer | 4.45 | 0.72 | 3.97 | 0.59 | 4.65 | 0.58 |
| CAr | 3.41 | 0.85 | 6.09 | 1.42 | 9.96 | 1.80 |
| CPer | 12.34 | 1.35 | 15.73 | 1.70 | 8.25 | 1.84 |
| KAr | 0.44 | 0.15 | 0.48 | 0.21 | 0.54 | 0.19 |
| KPer | 2.52 | 0.39 | 2.59 | 0.55 | 2.71 | 0.49 |
| W | 0.82 | 0.26 | 1.08 | 0.24 | 1.55 | 0.36 |
| NK | 1.73 | 0.30 | 2.61 | 0.55 | 3.30 | 0.66 |
| N/C | 0.28 | 0.09 | 0.20 | 0.17 | 0.14 | 0.02 |
| IN | 2.20 | 0.87 | 1.64 | 0.52 | 1.80 | 0.59 |
| CL | 5.93 | 0.83 | 7.34 | 0.98 | 8.94 | 1.20 |
| F/C | 0.53 | 0.10 | 0.68 | 0.12 | 0.63 | 0.10 |

TABLE 3.— *Blood forms morphometric data (in µm) of T. cruzi Clone II*

| Studied Parameters | Slender | | Broad | | Stout | |
|---|---|---|---|---|---|---|
| | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. |
| L | 10.17 | 1.09 | 13.51 | 1.12 | 16.09 | 1.42 |
| F | 3.86 | 0.77 | 5.29 | 0.83 | 6.44 | 0.76 |
| NP | 2.88 | 0.52 | 4.09 | 0.59 | 4.70 | 0.78 |
| NA | 1.39 | 0.39 | 2.31 | 0.52 | 3.01 | 0.81 |
| NAr | 0.92 | 0.25 | 1.11 | 0.29 | 1.53 | 0.38 |
| NPer | 4.54 | 0.70 | 4.20 | 0.80 | 4.67 | 0.63 |
| CAr | 3.37 | 0.76 | 6.07 | 1.36 | 9.42 | 3.00 |
| CPer | 12.59 | 1.11 | 16.21 | 1.68 | 18.28 | 1.93 |
| KAr | 0.40 | 0.09 | 0.47 | 0.12 | 0.54 | 0.20 |
| KPer | 2.39 | 0.39 | 2.51 | 0.34 | 2.68 | 0.45 |
| W | 0.79 | 0.19 | 1.08 | 0.24 | 0.43 | 0.39 |
| NK | 1.99 | 0.42 | 3.00 | 0.48 | 0.51 | 0.51 |
| N/C | 0.27 | 0.08 | 0.18 | 0.06 | 0.16 | 0.03 |
| IN | 2.22 | 0.65 | 1.87 | 0.53 | 1.68 | 0.51 |
| CL | 6.30 | 0.62 | 8.21 | 0.96 | 9.63 | 1.13 |
| F/C | 0.61 | 0.12 | 0.65 | 0.14 | 0.67 | 0.11 |

TABLE 4.— *Blood forms morphometric data (in µm) of T. cruzi Clone III*

| Studied Parameters | Slender | | Broad | | Stout | |
|---|---|---|---|---|---|---|
| | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. |
| L | 10.08 | 1.46 | 12.71 | 1.30 | 15.45 | 1.51 |
| F | 3.94 | 0.87 | 5.38 | 0.67 | 6.47 | 0.91 |
| NP | 2.84 | 0.62 | 3.75 | 0.60 | 4.51 | 0.75 |
| NA | 1.43 | 0.49 | 2.07 | 0.54 | 2.56 | 0.61 |
| NAr | 0.86 | 0.28 | 0.97 | 0.29 | 1.35 | 0.40 |
| NPer | 4.24 | 0.72 | 3.92 | 0.55 | 4.37 | 0.69 |
| CAr | 3.71 | 1.95 | 6.39 | 1.89 | 8.57 | 2.17 |
| CPer | 13.44 | 2.03 | 16.14 | 1.56 | 17.88 | 1.81 |
| KAr | 0.42 | 0.14 | 0.51 | 0.32 | 0.50 | 0.12 |
| KPer | 2.36 | 0.45 | 2.57 | 0.56 | 2.56 | 0.32 |
| W | 0.75 | 0.22 | 1.15 | 0.37 | 1.28 | 0.27 |
| NK | 2.13 | 0.59 | 2.84 | 0.58 | 3.34 | 0.53 |
| N/C | 0.25 | 0.09 | 0.15 | 0.05 | 0.15 | 0.05 |
| IN | 2.26 | 0.89 | 1.94 | 0.62 | 1.95 | 0.85 |
| CL | 6.13 | 0.89 | 7.32 | 1.14 | 8.74 | 1.56 |
| F/C | 0.64 | 0.13 | 0.75 | 0.17 | 0.73 | 0.14 |

TABLE 5.— *Slender Forms: Analysis of the Variance of the Comparative Study among Clones I, II and III of* Trypanosoma cruzi *and the Strain of Origin (Bolivia)*

| Parameters | Bolivia *Vs* Clone I | Bolivia *Vs* Clone II | Bolivia *Vs* Clone III | Clone I *Vs* Clone II | Clone I *Vs* Clone III | Clone II *Vs* Clone III |
|---|---|---|---|---|---|---|
| L | 0.00* | 0.00* | 0.00* | 0.00* | 0.00* | 0.78 |
| F | 0.00* | 0.00* | 0.00* | 0.00* | 0.00* | 0.69 |
| NP | 0.13 | 0.60 | 0.80 | 0.01* | 0.07 | 0.76 |
| NA | 0.00* | 0.00* | 0.00* | 0.88 | 0.54 | 0.73 |
| NAr | 0.27 | 0.03* | 0.00* | 0.49 | 0.10 | 0.00* |
| NPer | 0.02* | 0.00* | 0.15 | 0.58 | 0.18 | 0.06 |
| CAr | 0.00* | 0.00* | 0.00* | 0.81 | 0.37 | 0.31 |
| CPer | 0.00* | 0.00* | 0.00* | 0.41 | 0.01* | 0.03* |
| KAr | 0.19 | 0.00* | 0.02* | 0.06 | 0.49 | 0.38 |
| KPer | 0.08 | 0.00* | 0.00* | 0.11 | 0.10 | 0.71 |
| W | 0.00* | 0.00* | 0.00* | 0.59 | 0.11 | 0.45 |
| NK | 0.00* | 0.19 | 0.49 | 0.00* | 0.00* | 0.24 |
| N/C | 0.03* | 0.02* | 0.01* | 0.60 | 0.07 | 0.12 |
| IN | 0.00* | 0.00* | 0.00* | 0.93 | 0.72 | 0.81 |
| LC | 0.00* | 0.00* | 0.00* | 0.02* | 0.34 | 0.35 |
| F/C | 0.05* | 0.62 | 0.14 | 0.00* | 0.00* | 0.30 |

* significant differences (level of significance $p \leq 0.05$)

TABLE 6.— *Broad Forms: Analysis of the Variance of the Comparative Study among Clones I, II and III of* Trypanosoma cruzi *and the Strain of Origin (Bolivia)*

| Parameters | Bolivia *Vs* Clone I | Bolivia *Vs* Clone II | Bolivia *Vs* Clone III | Clone I *Vs* Clone II | Clone I *Vs* Clone III | Clone II *Vs* Clone III |
|---|---|---|---|---|---|---|
| L | 0.00* | 0.00* | 0.00* | 0.00* | 0.24 | 0.00* |
| F | 0.00* | 0.00* | 0.00* | 0.11 | 0.10 | 0.51 |
| NP | 0.88 | 0.00* | 0.13 | 0.00* | 0.04* | 0.00* |
| NA | 0.00* | 0.00* | 0.00* | 0.46 | 0.22 | 0.06 |
| NAr | 0.79 | 0.27 | 0.24 | 0.25 | 0.43 | 0.02* |
| NPer | 0.31 | 0.02* | 0.36 | 0.17 | 0.75 | 0.10 |
| CAr | 0.00* | 0.00* | 0.00* | 0.95 | 0.44 | 0.34 |
| CPer | 0.00* | 0.00* | 0.00* | 0.23 | 0.34 | 0.85 |
| KAr | 0.00* | 0.00* | 0.11 | 0.89 | 0.58 | 0.52 |
| KPer | 0.00* | 0.00* | 0.00* | 0.48 | 0.91 | 0.54 |
| W | 0.00* | 0.00* | 0.00* | 0.96 | 0.30 | 0.28 |
| NK | 0.00* | 0.00* | 0.00* | 0.00* | 0.08 | 0.15 |
| N/C | 0.00* | 0.00* | 0.00* | 0.46 | 0.08 | 0.02* |
| IN | 0.11 | 0.00* | 0.00* | 0.05* | 0.02* | 0.58 |
| LC | 0.00* | 0.00* | 0.00* | 0.00* | 0.91 | 0.00* |
| F/C | 0.00* | 0.01* | 0.00* | 0.31 | 0.04* | 0.00* |

* significant differences (level of significance $p \leq 0.05$)

TABLE 7.— *Stout Forms: Analysis of the Variance of the Comparative Study among Clones I, II and III of* Trypanosoma cruzi *and the Strain of Origin (Bolivia)*

| Parameters | Bolivia *Vs* Clone I | Bolivia *Vs* Clone II | Bolivia *Vs* Clone III | Clone I *Vs* Clone II | Clone I *Vs* Clone III | Clone II *Vs* Clone III |
|---|---|---|---|---|---|---|
| L | 0.00* | 0.07 | 0.00* | 0.00* | 0.01* | 0.06 |
| F | 0.00* | 0.01* | 0.01* | 0.00* | 0.00* | 0.89 |
| NP | 0.00* | 0.00* | 0.00* | 0.07 | 0.47 | 0.28 |
| NA | 0.00* | 0.00* | 0.00* | 0.14 | 0.24 | 0.01* |
| NA | 0.34 | 0.23 | 0.34 | 0.83 | 0.07 | 0.04* |
| NPer | 0.03* | 0.01* | 0.80 | 0.84 | 0.07 | 0.03* |
| CAr | 0.00* | 0.00* | 0.00* | 0.35 | 0.00* | 0.18 |
| CPer | 0.00* | 0.00* | 0.00* | 0.94 | 0.43 | 0.37 |
| KAr | 0.00* | 0.00* | 0.00* | 0.93 | 0.24 | 0.30 |
| KPer | 0.00* | 0.00* | 0.00* | 0.084 | 0.12 | 0.26 |
| W | 0.00* | 0.00* | 0.00* | 0.14 | 0.00* | 0.09 |
| NK | 0.00* | 0.03* | 0.00* | 0.14 | 0.76 | 0.17 |
| N/C | 0.00* | 0.00* | 0.00* | 0.01* | 0.44 | 0.45 |
| IN | 0.00* | 0.02* | 0.00* | 0.33 | 0.37 | 0.06 |
| LC | 0.00* | 0.01* | 0.00* | 0.00* | 0.43* | 0.00* |
| F/C | 0.05* | 0.00* | 0.00* | 0.11 | 0.00* | 0.03* |

* significant differences (level of significance $p \leq 0.05$)

The slender forms (Table 5) in Bolivia strain were longer, having both a greater distance between the nucleus and anterior extremity, and a greater nuclear area. Kinetoplast areas, however, remained with similar values in all clones. The nucleo-cytoplasm relation was greater in Bolivia strain while its nuclear index was smaller. In this study the slender forms showed an anterior location of the nucleus in every case. There were big differences between Bolivia strain and each of the clones, but among the clones themselves these distances decreased considerably, appearing only between clones I and II for the distance between the nucleus and the anterior extremity, and the nucleus and kinetoplast.

The broad forms (Table 6) when Bolivia strain and clone I were compared, showed significant differences in all parameters except in the distance between the nucleus and the anterior and posterior of the parasite, the perimeter of the nucleus, and the nuclear index. Between Bolivia strain and clone III parameters without differences are the nuclear area, the perimeter and kinetoplast area. Differences among the clones were much smaller, especially those between clone I and the two other clones.

In the stout forms (Table 7) Bolivia strain continued to be longer, although differences are less well defined. In fact, there was no difference between the Bolivia strain and clone II nor between clone II and clone III.

Neither were there any differences in the nuclear area, except between clone II and III, nor in the kinetoplastic area between the clones themselves, although there were some differences between the clones and Bolivia strain.

We can conclude that Bolivia strain parameters showed significant differences in almost all of the 16 morphological parameters studied, when compared with each of the clones in most of them, but among the clones themselves these distances decrease considerably. There were more differences as forms evolve to stout ones. We emphasize that in every case, differences between mother strain and each of its clones were greater than among clones themselves.

## Discussion

A striking feature of *T. cruzi* is its extreme heterogeneity with respect to biochemical, medical and other biological properties[4, 5, 6, 9, 10, 11, 16, 17]. The multiclonal structure of natural populations of *T. cruzi* has been shown by genetic studies of populations[15, 18] the main question being whether the high clonal diversity might be responsible for all or part of this variability[13, 14]. The distinctive characteristics of clonal lines are potentially important to the knowledge of the human disease.

The general objective of this work has been to study the hypothetical correlation between natural clones and their genetic variability. Specifically, a comparative morphological and morphometric study has been done among three clones of Bolivia strain. These clones have been compared among themselves and each of them with the mother strain, observing if they behave as independent genetic entities.

First, in our paper, a morphological study was done. Blood pleomorphism of Bolivia strain and the three clones had an absolute predominance of stout forms during the acute phase, which conforms to the characteristics of Andrade's group II[2]. Previous classifications by Brener and Chiari[3] refering only to blood pleomorphism did not find these characteristics. However, Ribeiro et al.[12] corroborates the predominance of stout forms in the Bolivia strain in infections in Swiss mice. We can thus establish that not only *Trypanosoma cruzi* but also its clones fit within the framework of Andrade's Group II with relatively slow multiplication, medium and variable virulence and with a predominance of stout forms.

The NI has been found to vary in nature from 0.9 to 1.9 according to the position of the nucleus, either median or in the anterior part of the body. Our data showed that in slender forms the nucleus layed in the anterior part of the body in the Bolivia strain as well as in the three clones. It has been stated before[8] that there is a connexion between the anterior situation of the nucleus of *T. cruzi* and its infective capacity. It is generally thought that the slender trypanosomes are the young forms recently emerged from the pseudocyst, whereas the stout ones are the adult forms with slow movements and lesser ineffective capacity.

Some of the most important differences were those related to the kinetoplast, which have shown statistical differences when comparing each blood form between the clones and its original strain. Vickerman[19] stated that the greatest size of the kinetoplast may be due to its polyenergetical character. Wallace[20] has emphasized the importance of this structure in the survival of *T. cruzi*.

We can conclude that in the study of blood pleomorphism Bolivia strain and its three clonal lines show a similar behaviour in the evolution of its forms.

From the morphometrical standpoint, the present results lead to these initial conclusions: a) our strain of *T. cruzi* is composed by different clone lines; b) the comparative study between clones and the original stock showed significant differences in almost all the 16 morphometric parameters, being greater the differences as they evolve to stout forms; c) clones isolated from Bolivia strain showed less than 6 statistical differences when comparing morphometric parameters. We consider that this fact could be due to the clonal selection that occurs when strains or isolates remain in a particular environment.

The extent of morphometric significant differences point to the great heterogeneity of *T. cruzi*, more studies being necessary to determine the relationship with clinical variability.


## Resumen

*Variabilidad morfológica y morfométrica de tres clones de* Trypanosoma cruzi *y su cepa de origen (Bolivia)*

Dada la gran diversidad de propiedades médicas y biológicas de *Trypanosoma cruzi*, agente productor de la Enfermedad de Chagas, hemos cuantificado los parámetros morfológicos que tipifican las diferentes formas de tres clones de *T. cruzi* así como de su cepa de origen, Bolivia, estableciendo comparaciones entre ellos y con la cepa Bolivia, con el objeto de aportar datos adicionales acerca del comportamiento biológico clonal de este parásito. En el estudio morfológico de las formas sanguíneas se utilizó el sistema VIDEOPLAN. Se aplicó un análisis de la varianza (Anova-1) para la explotación estadística, pudiéndose constatar un gran número de

diferencias significativas en la comparación de formas slender, broad y stout, cuando los clones son comparados entre sí, así como cuando lo son con la cepa original. La comparación de cada uno de los clones I, II y III con su cepa original mostró diferencias significativas en al menos 12 de los 16 parámetros estudiados. Cuando los clones fueron comparados entre sí, el número de diferencias significativas decreció. Se observaron variaciones en el porcentaje de los tres tipos de formas sanguíneas a lo largo de la fase aguda de infección. Estos datos suponen un primer paso en la correlación de características morfológicas y patogénicas del parásito.

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*La lengua, que es siempre y últimamente la lengua materna, no se aprende en gramáticas y diccionarios, sino en el decir de la gente*

Ortega y Gasset (1883-1955)

*El hombre y la gente*