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CYTOMETRY AND KARYOMETRY IN *Trypanosoma cruzi**

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ABSTRACT

A total of 991 *Trypanosoma cruzi* cells, from four laboratory stocks, including the three differentiation forms, had their cellular outlines, nuclei and kinetoplasts measured at 9000 x magnification. Data on the identifiable cell cycle stages were used to search for intraspecific and biological cycle heterogeneity.

Cellular areas (CA) in the interphasic differentiation forms produced ratios of 1.07 for culture epimastigotes (E), 1 for blood trypomastigotes (T), and 0.86 for tissue forms (A). Homogeneity in terms of nuclear (NA) and kinetoplast (KA) areas prevailed among the stocks, with differences of at most 6%, for modal NA of strains CL and Y. NA of T-form was larger than the basic NA of early G1 A-form. T-form kinetoplast volume was 3-fold that of A-form K-DNA nucleoids.

One of the two recently divided kinetoplasts in mitotic E-form did not correlate with CA, indicating that mitochondrial division was unequal. The KA of CL strain T-form did not correlate with NA, suggesting a mitochondrial disfunction in this thermosensitive strain.

The CL strain T-form was more heterogeneous than the Y strain for all characters, showing greater frequency of large values, even reaching the G2 levels. This heterogeneity was interpreted as functional, consequent to the thermosensitivity of the CL strain. Precocious bursting of CL strain host cells would lead to the polymorphic T-forms. Post-S phase trypomastigotes could start division soon after penetration of host cells.

INTRODUCTION

Studies of Chagas Disease are complicated by the diversity of clinical syndromes, whose prevalence also vary geographically (Rezende, 1975).

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Evidence for *Trypanosoma cruzi* intraspecific variation is available but no clear relationship has been found between the parasites' characteristics and pathogenicity to humans.

Determinations of *T. cruzi* DNA content have offered conflicting results (Riou and Pautrizell, 1969; Stohlman *et al.*, 1973; Lopetegui and Miatello, 1978; Lanar *et al.*, 1981). A limited number of isoenzyme phenotypes were characterized as zymodemes (Miles, 1979; Romanha *et al.*, 1979; Barret *et al.*, 1980). A larger number of schizodemes were delineated by restriction endonuclease cleavage of kinetoplast DNA (K-DNA) minicircles (Mattei *et al.*, 1977; Morel *et al.*, 1980). Flow microfluorometric determinations of total DNA content produced differences of up to 34% among strains and 41% among clones (Dvorak *et al.*, 1982) and static microfluorometric procedures showed that both the nuclear DNA (N-DNA) and K-DNA contents varied. Pulsed-field DNA electroforesis showed that, in contrast to *T. brucei*, mini-chromosomes are absent and the chromosomes are not very large in *T. cruzi* (Gibson and Miles, 1986; Engman *et al.*, 1987; Aymerich and Goldenberg, 1989). In *T. cruzi*, the "centromeric" bodies visualized by electron microscopy occurred in smaller numbers than those of the molecular chromosomes (Solari, 1980).

T. cruzi genomes seem to be diploid from both DNA reassociation kinetics (Lanar *et al.*, 1981; Castro *et al.*, 1981) and isoenzyme profiles (Tait, 1983), but no haploid or polyploid phases have been detected. The discovery of cells with reduced DNA contents in the mosquito phase of the life cycle of *T. brucei* (Zampetti-Bosseler *et al.*, 1986; Kooy *et al.*, 1989) could suggest that, also in *T. cruzi*, sexual processes would occur in the invertebrate cycle.

Previous morphometric studies in *T. cruzi* have not attempted to describe its cell cycle. Most of the data were collected for the purpose of morphologic characterization of isolates (Ribeiro *et al.*, 1982; Rodrigues *et al.*, 1982). Attempts at discerning the DNA contents of the organelles or of the differentiation forms proceded mostly through biochemical methods, producing average values from large samples, which do not inform correctly on the division cycle of each organelle (Riou and Gutteridge, 1978; Gutteridge and Gaborak, 1979).

We describe all three differentiation forms of *T. cruzi*. Several strains and clones were used, to illustrate the intraspecific variation of laboratory adapted isolates.

MATERIAL AND METHODS

Strains CL and Y (the latter usually zymodeme A), regarded as polar biological variants of *T. cruzi*, have been kept in the laboratory for at least 30 years (Brener, 1977). They were maintained by serial blood innoculation in mice (each 10th or 7th day, the parasitemia peaks, respectively) or by serial seeding in LIT cultures (each 15-20 days,

28°C). Clone CL14 (zymodeme B) was obtained from Dr. Erney Camargo and the zymodeme C stock 254-R7 (considered homogeneous for various parameters) was obtained from Dr. Egler Chiari.

Tissue forms (Y strain, 7th day) were obtained from the spleen, minced and pressed through a metal sieve (Katz *et al.*, 1972) immersed in LIT medium. Culture epimastigotes (CL14) were maintained at exponential growth by 48 h serial seedings. These and zymodeme C epimastigotes were also processed for thin (0.5 μm) sectioning. Cultures were centrifuged at 2000 g for 10 min, fixed in suspension in 2.5% glutaraldehyde for 3 h, and post-fixed in 1% OsO_4 in 0.1 M phosphate buffer. Cells were embedded in 1:1 Araldite^R, sectioned by a Huxley ultramicrotome, and stained with 1% Toluidine Blue, in 1% Borax with slight flame heating. Tissue forms with cell body length/width ratios higher than 2.22 were not studied.

Glass smears (at a 45° angle) were made for all cell types, with quick cold fan drying and 10 min methanol fixation. Staining was done with three drops of Merck's 50% Giemsa in 2 ml distilled water, for about 20 min.

Cells with neat contours of the bodies and organelles were photographed. The negatives were projected onto paper and the borders of their bodies and organelles drawn with a pencil. Final magnification was 9000 x, calibrated with photographs of a micrometer slide (Jena, 1/0.01 mm).

A total of 991 cells were measured. From glass smears: 218 CL strain and 298 Y strain blood trypomastigotes (T), 179 tissue forms (A) from Y strain, and 143 epimastigotes (E) from clone CL14. From sections of E-forms: 113 were from clone CL14 and 40 from zymodeme C.

The areas of the nuclei (NA), kinetoplasts (KA) and cell bodies (CA) were measured with a planimeter. KA of T-form, being spherical, was also calculated from the average of two perpendicular diameters, taken with a micrometric lens (0.1 mm divisions). CA from E-forms were possibly underestimated when the flagellum was hidden under the cell body or its insertion was not easily defined. In this case, the end of the cell body was defined as an abrupt thinning. Cells were measured only when this was seen. In some E-forms the organelles were not measured when they were tightly juxtaposed. For the sections, NA and nuclear volumes (NV) were calculated from two perpendicular diameters. The sectioned kinetoplasts of zymodeme C were considered as disks (cut as cylinders) for KA calculations, but those of clone CL14 were irregular and measured with a planimeter. A total of 16 kinetoplasts from electron microscopic photographs of strain FL were also measured, from Maria (1975) plus others kindly supplied by her.

In pre-cytokinetic cells, with divided kinetoplasts (1N2K), nuclei (2N1K) or both (2N2K), nuclei were arbitrarily numbered (N1 and N2) and their respective closest kinetoplasts were numbered accordingly.

Definition of organelle sizes corresponding to different ploidy levels was obtained by modal analyses of frequency histograms (Schreiber, 1953) and by comparisons among histograms from cells at the identifiable cell cycle stages. Modal values were calculated from King's formula (Arkin and Colton, 1942). Basic organelle sizes (mode I) were obtained from those recently divided (early G1 phase): NAI from N1 and N2 of 2N1K and 2N2K cells, KAI from K1 and K2 of 1N2K and 2N2K cells. Doubled organelle sizes (mode II) were obtained from the sum of the two divided organelles in each cell or, whenever possible, from those just reaching the division stage (G2 phase; NAII from 1N2K cells, KAII from 2N1K cells). For CA, the doubled value could be determined, in 2N2K cells. 1N1K cells were heterogeneous but, sometimes, subpopulations were discernible.

Cells where only one organelle had divided were rare and adequate sampling of them was not always possible. Samples were used only when their coefficients of variation were below 30%.

RESULTS

Cycling forms

Mitotic activity was more intense in CL14 epimastigotes. The frequency of 1N2K cells was 1.7% in Y strain A-form and 4.2% in CL14 E-form. The 2N1K cells were not detected in Y A-form and represented 3.5% of CL14 E-form, while 2N2K cells were 16.2% and 25.2%, respectively in the above two samples.

The Y strain A-form showed two main cell types: spherical (S; 62%) and fusiform (F; 20%). A free flagellum could be seen in 28% of the cells, mostly (65% of the flagellated ones) in the fusiform cells. Although this cell type was apparently slightly larger than the spherical ones, there were no significant differences among the parameters ($\overline{X} \pm SD$, in μm^2; CA: S = 19.09 ± 0.94, F = 20.00 ± 1.28; NA: S = 4.12 ± 0.22, F = 4.39 ± 0.24; KA: S = 1.68 ± 0.10, F = 1.79 ± 0.15).

The basic (mode I) NA was defined in the 2N2K A-form (Figure 1A, Table IB). The main modes were larger in the 1N1K A-form and the largest in the 1N1K or 2N2K E-form (Figure 1E, Table IB). The doubled (mode II) NA was estimated by adding the values for the two nuclei of the 2N2K A-form (Table IB), which coincided with the secondary modes observed in the 1N1K A-form (Figure 1A) and E-form (Figure 1E).

The basic (mode I) KA was lower in the E-form (Figure 2E) than in the A-form (Figure 2A, Table IB) 2N2K cells. The main KA modes were only slightly larger in the 1N1K cells of both A- and E-forms. The doubled (mode II) KA was estimated by adding the values for the two kinetoplasts of the 2N2K cells (Table IB) which coincided with the secondary mode observed in the 1N1K E-form (Figure 2E). The E-forms of

Table I - Comparison among stocks and the three differentiation forms of *Trypanosoma cruzi*.
Data are (*A*) the average ± SD of cell body (CA), nuclear (NA) and kinetoplast (KA) areas, and (*B*) the modal values calculated from histograms. Only the data for interphase (1N1K) cells, from smears, are presented. Some modal values for sectioned E-forms are shown in (*C*), which allowed volume calculations (NV, KV). Ratios among stocks and differentiation forms are also shown. Data are in μm^2 (areas) or μm^3 (volumes). * = not available.

Differentiation forms	Source	CA	NA	KA
A - Trypomastigote (T)	Y	21.06 ± 3.77	3.26 ± 0.66	0.89 ± 0.20
	CL	21.83 ± 5.53	3.31 ± 0.75	1.02 ± 0.30
	CL/Y	1.04	1.02	1.15
Tissue (A)	Y	19.16 ± 4.51	4.18 ± 0.98	1.72 ± 0.51
	A/T	0.91	1.28	1.93
Epimastigote (E)	CL_{14}	23.79 ± 5.96	4.04 ± 1.22	1.39 ± 0.44
	CL_{14}E/CLT	1.09	1.22	1.36
B - Trypomastigote (T)	Y	20.97	3.09	0.95
	CL	22.80	3.30	0.95
	CL/Y	1.09	1.07	1.00
Tissue (A)	Y	17.11	3.41	1.51
	Modes I-II	* - 26.89	2.91-(5.82-6.10)	1.47-2.91
	A/T	0.82	1.10	1.59
Epimastigote (E)	CL_{14}	24.10	3.59	1.11
	Modes I-II	* - 34.44	3.60-(6.04-6.53)	(1.10-1.15)-(2.42-2.50)
	CL_{14}E/CLT	1.06	1.09	1.17

Differentiation forms	Source	NA	NV	KA	KV
C - Epimastigote (E)	CL_{14}	3.41	5.04	0.58	*
	Zymodeme C	3.47	4.98	0.55	1.21
	C/CL_{14}	1.02	0.99	0.95	*

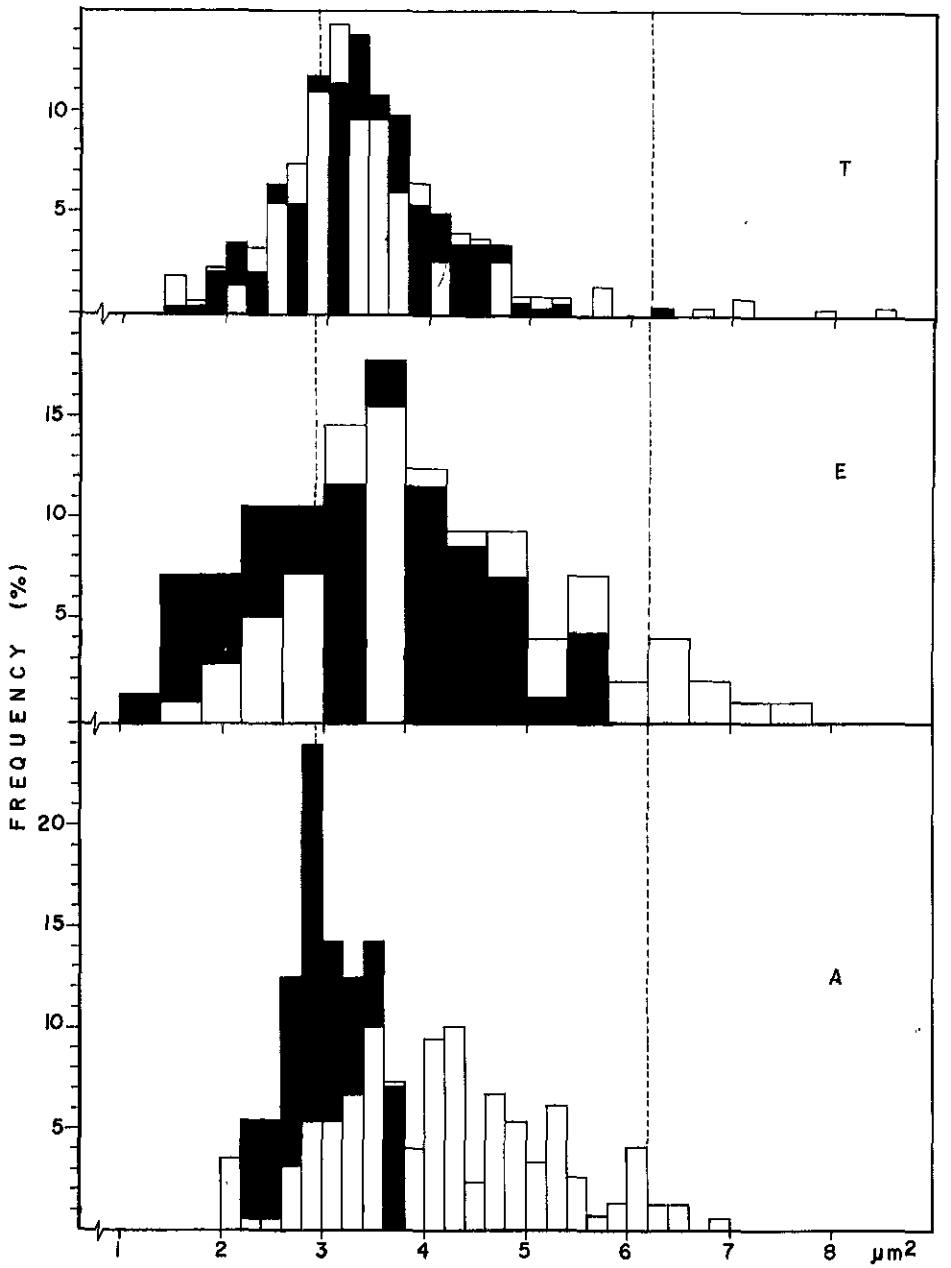

Figure 1 - Nuclear areas in the differentiation forms of *Trypanosoma cruzi*. T - Blood trypomastigotes of strains Y (■) and CL (□), mononuclear and monokinetoplastic (1N1K). E - Culture epimastigotes of clone CL_{14}, 1N1K (□) and 2N2K (■). A - Tissue forms of strain Y, 1N1K (□) and 2N2K (■). All data are from glass smears. Data for the two nuclei of 2N2K cells were pooled together. The vertical dashed lines mark the basic (2.91 μm^2) and the doubled (between 5.8-6.5 μm^2) modes.

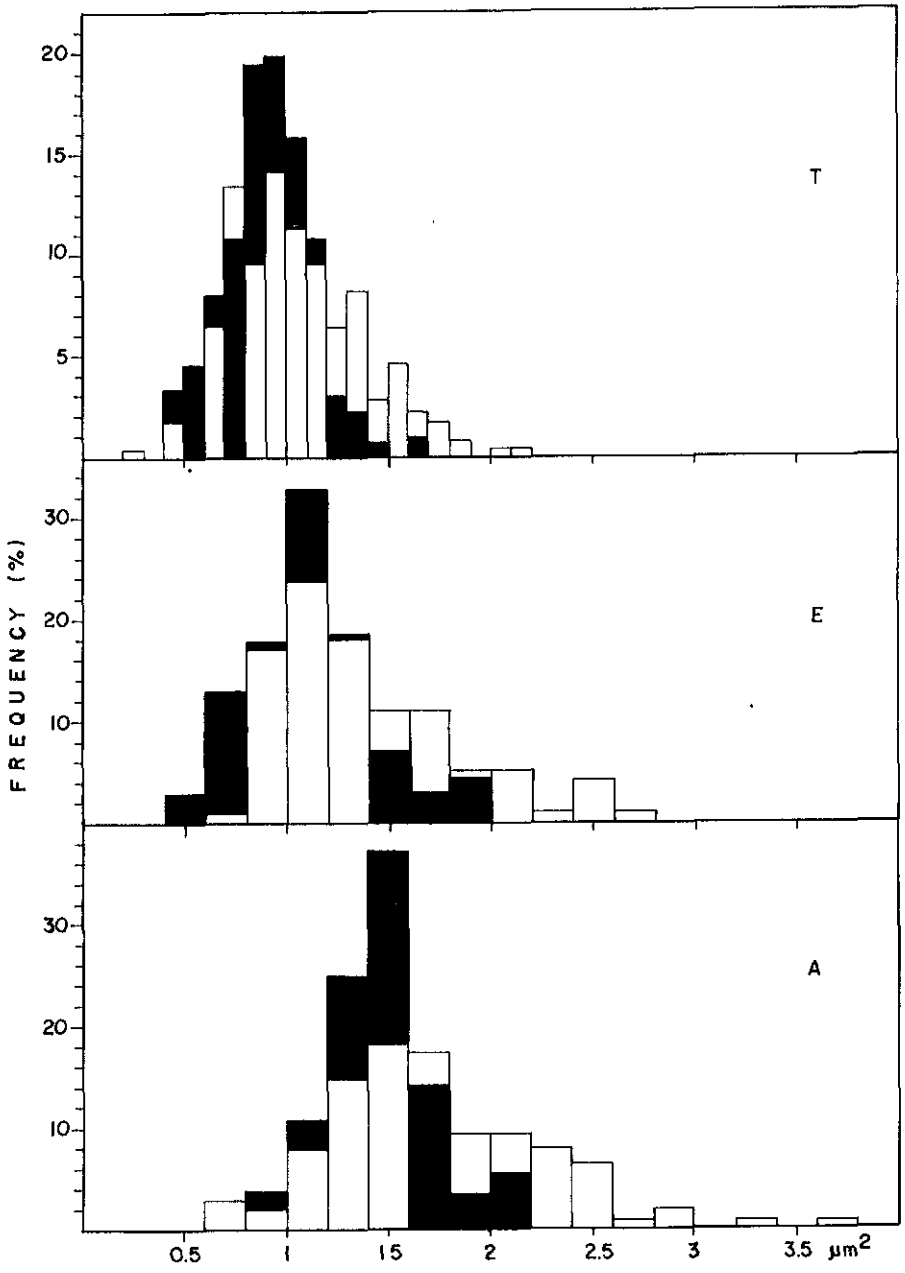

Figure 2 - Kinetoplast areas in the differentiation forms of *Trypanosoma cruzi*. T - Blood trypomastigotes of strains Y (■) and CL (□), mononuclear and monokinetoplastic (1N1K). E - Culture epimastigotes of clone CL14, 1N1K (□) and 2N2K (■). A - Tissue forms of strain Y, 1N1K (□) and 2N2K (■). All data are from glass smears. Data for the two nuclei of 2N2K cells were pooled together.

zymodeme C and clone CL14, when compared in sections, showed differences of only 2% for NA and 5% for KA (Table IC).

The doubled (mode II) CA was obtained in the 2N2K cells (Table IB), being larger in the E-form than in the A-form. While in the 1N1K tissue forms all parameters produced asymmetric histograms, skewed towards the higher values, only the KA values showed this asymmetry in the E-form.

Blood trypomastigotes

The three parameters were larger in CL than in Y strain (Figures 1T and 2T, Table IA and IB). The greatest differences were a 10% larger CA mode, a 6% larger NA mode, and a 15% larger KA average (only this last was significant). For all parameters, the CL strain gave histograms with greater heterogeneity than the Y strain, the classes with larger values being more abundant. NA was the most homogeneous; CA and KA showed significant asymmetry values, but the KA modes were identical.

Correlation coefficients

Correlations between pairs of the three parameters, in the T-form, demonstrated some differences between strains: the CA x NA correlation was slightly higher for Y strain, CA x KA was higher for CL strain, and NA x KA was significant only for Y strain (Table IIA). In Y strain 1N1K tissue forms, the correlations were all significant (Table IIA), while in 2N2K cells (Table IIB) the only significant correlation was between NA1 and NA2, among 10 tests.

In clone CL14 E-form (1N1K cells), all correlations were significant (Table IIA). In 2N2K cells, a striking difference between the two early G1 kinetoplasts was seen (Table IIB): KA1 showed the highest r value, with CA, but KA2 was the only non-significant correlation obtained, with the same CA. Among the nine significant correlations, the lowest also involved KA2, with its correspondent NA2.

Kinetoplast shape, structure and size

Detailed measurements were conducted on smeared and on sectioned cells, to elucidate the contribution of the nucleoid (K-DNA) and of the electron-translucent area around it, limited by the mitochondrial membrane, to the kinetoplast shape and size in the different cell types (Table III).

In electron micrographs of sectioned A-form, the width of the electron translucent area around the nucleoid was about 0.29 μm (Table IIIA,B). Both sectioning methods produced approximately equivalent results. The widths of the kinetoplasts were

Table II - Correlation coefficients between nuclear, kinetoplast and cell body areas in the differentiation forms of *Trypanosoma cruzi*.

Cellular (CA), nuclear (NA) and kinetoplastic (KA) areas; significant correlations ($P < 0.05$) are indicated by *. In B, tissue form, only the three topmost r values are shown.

A - Interphase cells (mononucleated and monokinetoplastic)

Differentiation forms	Strain	CA x NA	CA x KA	NA x KA
Blood trypomastigote	Y	0.58*	0.22*	0.29*
	CL	0.50*	0.35*	0.07
Culture epimastigote	CL_{14}	0.78*	0.58*	0.75*
Tissue forms	Y	0.72*	0.68*	0.66*

B - Mitotic cells (binucleated and bikinetoplastic)

Differentiation forms	Strain	NA_1	NA_2	KA_1	KA_2
Culture epimastigote	CL_{14}				
	CA	0.68*	0.68*	0.89*	0.31
	NA_1	-	0.88*	0.63*	0.69*
	NA_2	-	-	0.60*	0.52*
	KA_1	-	-	-	0.80*
Tissue form	Y				
	NA_1	-	0.51*	0.31	-
	KA_1	-	-	-	0.28

similar in electron micrographs from A-form and in sectioned toluidine-stained culture E-form (Table IIIB,F). The diameters of Giemsa-stained kinetoplasts in smeared 1N1K A-forms were 15% larger than those of the electron micrographs (Table IIIB,C). Where the electron translucent area around the nucleoid was absent (T-form), the diameters were similar for the nucleoid in electron micrographs and for the organelle in Giemsa smears (Table IIIA,G).

Table III - Kinetoplast dimensions in the differentiation of *Trypanosoma cruzi*.
Average ± SD, data in μm or μm^3 (volumes). A, B: FL strain; C, D, E, G: Y strain; F: zymodeme C; n = 16 in all samples. Kinetoplast heights were not measured in C and D, where they were seen as disks flattened on the glass surface. Nucleoid heights were not measured in E and F, where the basic dyes stained the whole organelle. In spherical organelles (G), the diameter was the only dimension measured. Volumes were calculated from the dimensions shown in the corresponding lines or (*) using the height of the nucleoid in A.

Differentiation forms	Treatment	Diameter	Height	Volume
Tissue	Electron microscopy			
	A - Nucleoid	1.144 ± 0.379	0.179 ± 0.070	0.199 ± 0.148
	B - Total	1.446 ± 0.406	0.459 ± 0.089	0.822 ± 0.498
	Giemsa smears			
	C - Circular, 1N1K	1.665 ± 0.293		* 0.402 ± 0.085
	D - Circular, 2N2K	1.475 ± 0.176		* 0.329 ± 0.085
	E - Kidney-shaped, 2N2K		0.935 ± 0.184	2.461 ± 1.138
		1.878 ± 0.358		
				* 0.514 ± 0.180
Epimastigote	Sections, Toluidine Blue		0.439 ± 0.184	1.275 ± 0.407
	F - 1N1K	1.896 ± 0.283		
				* 0.515 ± 0.137
Trypomastigote	Giemsa smears			
	G - 1N1K	1.046 ± 0.121		0.639 ± 0.226

A minority of A-forms showed kidney-shaped kinetoplasts. They were more densily stained and presented some discrepantly large dimensions (Table IIIE) but did not have larger areas (30.1% of the circular ones had areas larger than 2.13 μm^2, as opposed to 15.9% in the kidney-shaped). They were present also in 2N2K cells (in these, the kidney-shaped kinetoplasts had areas of 1.45 ± 0.12 μm^2, while the circular and the intermediate ones had 1.43 ± 0.12 and 1.47 ± 0.10 μm^2, respectively).

Kinetoplast volume in T-form was approximately 3X that of A-form nucleoids (Table IIIA,G). Considering a constant height (Table IIIA, the nucleoid height), volumes

became a function of the diameters and grew in approximate ratios of 1 (early G1 phase 2N2K A-form, Table IIID), 1.2 (advanced G1 + S phase 1N1K A-form, Table IIIC), and 1.6 (advanced G1 + S phase 1N1K E-form, Table IIIF). The latter ratio was also obtained considering the volumes measured in the sectioned E-form and the A-form whole organelles (Table IIIA,F).

Comparison among the differentiation forms

The differentiation forms, in smears, were compared, according to the three area measurements in 1N1K cells, by analysis of variance and the confidence intervals of the averages. The average and modal values are also shown in Table IA and IB, respectively. Sectioned epimastigotes from two sources were also compared (Table IC).

NAs were larger in the reproductive forms than in T-form $\{(A,E) > T; P < 0.01\}$. Both A- and E-forms showed approximately 10% higher modes. The skewing was more intense towards the higher classes, resulting in 22-28% larger averages than those of T-form. A-forms had 9-19% smaller body areas, and E-form 5-9% larger ones, than those of T-form ($P < 0.001$ for all three contrasts). KA were $A > E > T$ ($P < 0.001$).

DISCUSSION

The high magnification optical micrometric method resolved even kinetoplast subclasses. It was expected, therefore, that variations in nuclear sizes would be easily detectable. Comparison of the modal organelle areas in the cell cycle stages enabled the definition of the basic (early G1) and doubled (late G2) sizes.

Taking the early G1 NA of A-form as a reference, it was shown that the nuclei of the T-form were larger. These are non-reproductive cells and their chromatin staining with Giemsa is more purple and homogeneous than that of other stages. It has been suggested (Crane and Dvorak, 1980) that the T-form should be classified as the G0 stage. Their larger sizes and the higher frequency of doubled sizes in CL strain, together with the findings that they are highly active in some aspects of the metabolism (Guimarães and Gutteridge, 1987; Stohlman *et al.*, 1973) indicated that they should be regarded as a non-cycling but highly active specialized cell type. If the main conotation of the G0 denomination was for comparison with the cycling cells of the same differentiation form, the proposed denomination would not be necessary.

Our data on the log-phase E-forms indicated that they were more active than the A-form. The recently divided nuclei of pre-cytokinetic cells were as large as those in the advanced interphase, suggesting that the G1 stage was short, S phase starting soon after karyokinesis. The same was shown for kinetoplast sizes. The recently divided organelles,

in A-form, showed smaller modes than those of advanced interphase cells but, in E-form, both showed similar values.

Consistent with biochemical data (Riou and Gutteridge, 1978), kinetoplasts of T-form had three times the volume of A-form K-DNA nucleoids. The different structure (and dynamics) of kinetoplasts in the reproductive forms, as disks with large electron-translucent spaces which are stained by basic dyes, resulted in their larger areas, especially in the flattened A-form organelles. The very large kidney-shaped kinetoplasts observed in A-form were interpreted as artifacts, due to their folding when settled perpendicularly to the glass surface.

Two pairs of protozoan stocks were compared. The differences found were at most 6%, for the modal NA of the T-form of CL and Y strains, and 5%, for the modal KA of clone CL_{14} and the zymodeme C stock. It is possible that the intraspecific homogeneity observed was due to the stocks having been maintained under constant laboratory conditions for a long time. The extensive heterogeneity of DNA contents detected by Dvorak *et al.* (1982), needs to be further checked in natural populations, as greater heterogeneity was found among recently established laboratory clones.

Our *in situ* methods might be criticized as smearing could produce deformations in internal structures, but the correlations obtained among all parameters argue to the contrary. The 1N1K A-form, which was the bulk of the population and the one used for the comparisons, produced as high correlation coefficients as the other cell types.

Very interesting was the detection of asynchrony between one of the AK and AC or AN in the pre-cytokinetic E-form, while the other showed the highest of all correlations with the same AC or its corresponding nucleus. The data indicated that the partition of the mitochondrion was unequal; one of the daughter organelles would carry most of the activity, so its kinetoplast remained, correlating well with body size, while the other was rudimentary, to the regenerated later, after cytokinesis. The data also suggest that some kind of physical link, not yet perceived, remained, most probably between the most active sister mitochondrion and its corresponding sister nucleus. Otherwise, the organelles would have acquired scrambled positions in the preparations, leading to averaged correlations.

CL and Y strain T-forms were distinguished mainly by the former presenting greater frequencies of higher values of cellular and organelles areas. The modal KA in both strains were identical and the significance of the slightly different modal NA and CA is not yet testable. While some genetic or populational heterogeneity might have survived the prolonged maintenance of CL strain in the laboratory, there were indications that the heterogeneity would be just physiological. One striking characteristic of CL strain is its temperature-sensitivity for intracellular growth and differentiation (Bogliolo *et al.*, 1980). At 37°C, the A-forms reproduce less and showed only residual differentiation of T-forms. In spite of the slower growth dynamics, Bogliolo *et al.* (1980) found that the

host cell nuclei were intensely affected by the parasitism. The data indicated that host cell bursting would be precocious in CL strain parasitism, relative to the growth and differentiation rates of the parasites. These would be liberated mostly as immature forms, the differentiation into T-forms to be accomplished predominantly in the extracellular environment. This would produce the more heterogeneous trypomastigote phenotypes, with abundance of broad forms.

The greater frequency of G2 stage T-form in CL strain is consistent with the interpretation above. Host cell bursting, occurring at a stage when intracellular parasites were still proliferating, would catch some of them just before organelle division. These cells would differentiate into the T-form as such, without karyokinesis and kinetoplastokinesis. This would facilitate the progress of the next intracellular parasitism cycle. After having differentiated into A-form, they could directly enter mitosis without having to traverse an intracellular S phase. Accumulation of purine pools by trypomastigotes (Guimarães and Gutteridge, 1987) is another indicator of this virulence strategy, especially usefull for the CL strain.

It is also suggested that a mitochondrial dysfunction may contribute to the peculiar CL strain behavior. The lack of correlation between T-form KA and NA is interpreted as a disruption between kinetoplast growth and mitochondriogenesis, the latter being a strong determinant of cell size.

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RESUMO

Os corpos celulares, núcleos e cinetoplastos foram medidos em 991 células de *Trypanosoma cruzi*, ampliadas 9000x, em quatro estoques laboratoriais e nas três formas de diferenciação. Os dados sobre os estágios do ciclo celular identificáveis foram usados para a procura de heterogeneidade intraespecífica e no ciclo biológico.

As proporções entre as áreas celulares (AC) das formas de diferenciação interfásicas foram 1,07 em epimastigotas de cultura (E), 1 em tripomastigotas sanguícolas (T) e 0,86 em formas tissulares (A). A homogeneidade entre estoques foi elevada quanto às áreas nucleares (AN) e cinetoplásticas (AK); a diferença máxima foi de 6% quanto à AN modal das cepas CL e Y. A AN da forma T foi maior que a AN básica da forma A em G1 precoce. O volume dos cinetoplastos da forma T foi três vezes o dos nucleóides da forma A.

Um dos dois cinetoplastos recém-divididos da forma E mitótica não se correlacionou com AC, indicando que a divisão mitocondrial é desigual. A AK da forma T da cepa CL não se correlacionou com AN, sugerindo uma disfunção mitocondrial nesta cepa termosensível.

A forma T da cepa CL foi mais heterogênea para todos caracteres, do que a cepa Y, com maior freqüência dos valores maiores, atingindo até os níveis G2. Esta heterogeneidade foi interpretada como funcional, conseqüente à termosensibilidade da cepa CL. Ruptura precoce das células hospedeiras da cepa CL produziria as formas T polimórficas. Tripomastigotas no estágio G2 poderiam iniciar a divisão logo após a penetração nas células hospedeiras.

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