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THE SURFACE CHARGE OF TRYPANOSOMATIDS OF THE GENUS *TRYPANOSOMA*

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Cell electrophoresis was used for determination of the electrophoretic mobility (EPM) of epimastigote and trypomastigote forms of several isolates of Trypanosoma cruzi *and some stocks of other members of the* Schizotrypanum *subgenus, such as* T. dionisii, T. vespertilionis *and* T. myoti. *The EPM of* T. brucei, T. rangeli, *and* T. conorhini *was also determined. The results obtained show that the EPM values can be useful to distinguish the parasites.*

Key words: trypanosomatids – cell surface charge – electrophoretic mobility

Studies carried out in the last years in several laboratories have shown clearly that *Trypanosoma cruzi* exists in the nature as genetically heterogeneous population which can be distinguished by various criteria such as double time in axenic and in cell culture, isoenzymes and analysis of the kinetoplast DNA (Dvorak et al., 1980; Engel et al., 1982; Dvorak, 1984; Morel, 1984). From the analysis of the results obtained using these different approaches a complex picture emerges where, in some cases, we can even discuss if a certain new isolate represents *T. cruzi* or not. More single biological approaches, such as ability to infect mice or cells in cultures is not always possible because of the small number of infective trypomastigotes obtained.

All these considerations indicate that it is important to have techniques which can identify new isolates as true *T. cruzi*. Our previous observations using cell electrophoresis have shown that each developmental stage of the two strains of *T. cruzi* analyzed (Y and CL strains) present a mean characteristic eletrophoretic mobility (EPM) (Souto-Padrón et al., 1984; Carvalho et al., 1985). In the case of epimastigotes the values obtained did not vary according to the medium where the parasites were grown (De Souza et al., 1977; Souto-Padrón et al., 1984; Souto-Padrón & De Souza, 1985). In view of these observations we decided to extend our study to several new isolates of *T. cruzi* as well as for other species of the genus *Trypanosoma*, especially those of the sub-genus *Schyzotrypanum*. The results obtained are described in this paper.

MATERIALS AND METHODS

Parasites – The following isolates of *T. cruzi* were analyzed: (a) stocks 1001, 1042 and 1048 isolated from the opossum *Didelphis albiventris;* (b) A1-DN and A1-7 clones which had been kept frozen, isolated from a chronic patient and which belong all to zymodeme A; (c) isolates 147 and 271, from chronic patients and which belong to zymodeme B; (d) isolates 181, 143, 231 and 254, from chronic patients and which belong to zymodeme C; (e) isolate 1049, from the triatomine *Panstrongylus megistus;* (f) isolates designated as Reis, Silvio and Noel, from patients with the acute phase of Chagas' disease, which belong to new zymodemes. The parasites were included in zymodemes A, B, C according to Romanha et al. (1979). All these isolates were maintained in LIT medium (Camargo, 1964). For the experiments they were collected after 5-7 days of cultivation when the population was mainly composed of epimastigotes. For other experiments the parasites were cultivated in a poor medium according to the method of Chiari (1981), where the population mainly consisted of trypomastigote and intermediate forms.

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One stock (P2) of *T. dionisii*, which was isolated from *Pipistrellus pipistrellus* in the United Kingdon (Baker, 1980), was used. It was cultivated in a monophasic liquid L4NH5 medium (Baker et al., 1976), where it grew as epimastigotes. At the stationary phase of growth trypomastigote forms were observed. They were separated using a DEAE cellulose column (Selden & Baker, 1980).

Strains 1, 3, 4 and 5 of *T. myoti*, isolated from *Myotis lucifugus* in Guelph, Canada, were analyzed (Bower & Woo, 1981a, b). They were cultivated in L4NH5 medium.

Cell electrophoresis – The cells were collected by centrifugation (1500 g for 10 min), twice washed with 0.1 M phosphate buffer, pH 7.2 and fixed for 1 h in 2.5% glutaraldehyde in 0.1 M phosphate buffer. After fixation the cells were washed in a 0.85% sodium chloride solution with an ionic strength of 0.145 md. dm^{-3}, pH 7.2. The electrophoretic mobility of the cells was determined in a Zeiss cytopherometer with a current of 4-6 mA and a final voltage of 100 V. The cell suspension was placed into the chamber and then allowed to equilibrate for 10 min. Measurements were made at a temperature of 25 °C. When current was switched on, we measured the time necessary for one cell to travel across two vertical lines, separated by a distance of 16 μm. Then the polatiry was reversed and the time was measured again for the cell travelling in the opposite direction. About 100 cells were measured for each sample analyzed. Calibration of the equipment was made by measuring the electrophoretic mobility of fresh human erythrocytes. Statistical analysis was performed using the t- test.

RESULTS

Electrophoretic mobility (EPM) of Trypanosoma cruzi – The analysis of the several isolates of *T. cruzi* confirmed our previous observations (De Souza et al., 1977; Souto-Padrón et al., 1984) that epimastigote and trypomastigote forms of *T. cruzi* have characteristic mean EPM values. Epimastigotes from all isolates presented a mean EPM around – 0.65 $\mu m.s^{-1}.V^{-1}.cm$. When old axenic cultures were examined they presented a large number of trypomastigote and intermediate forms, so that the mean EPM were around – 1.0 $\mu.s^{-1}.V^{-1}.cm$, as can be seen in Figs 1, 2. Although all populations examined derived from the respective clone they were not homogenous in terms of surface charge, as can be seen in Figs 1, 2.

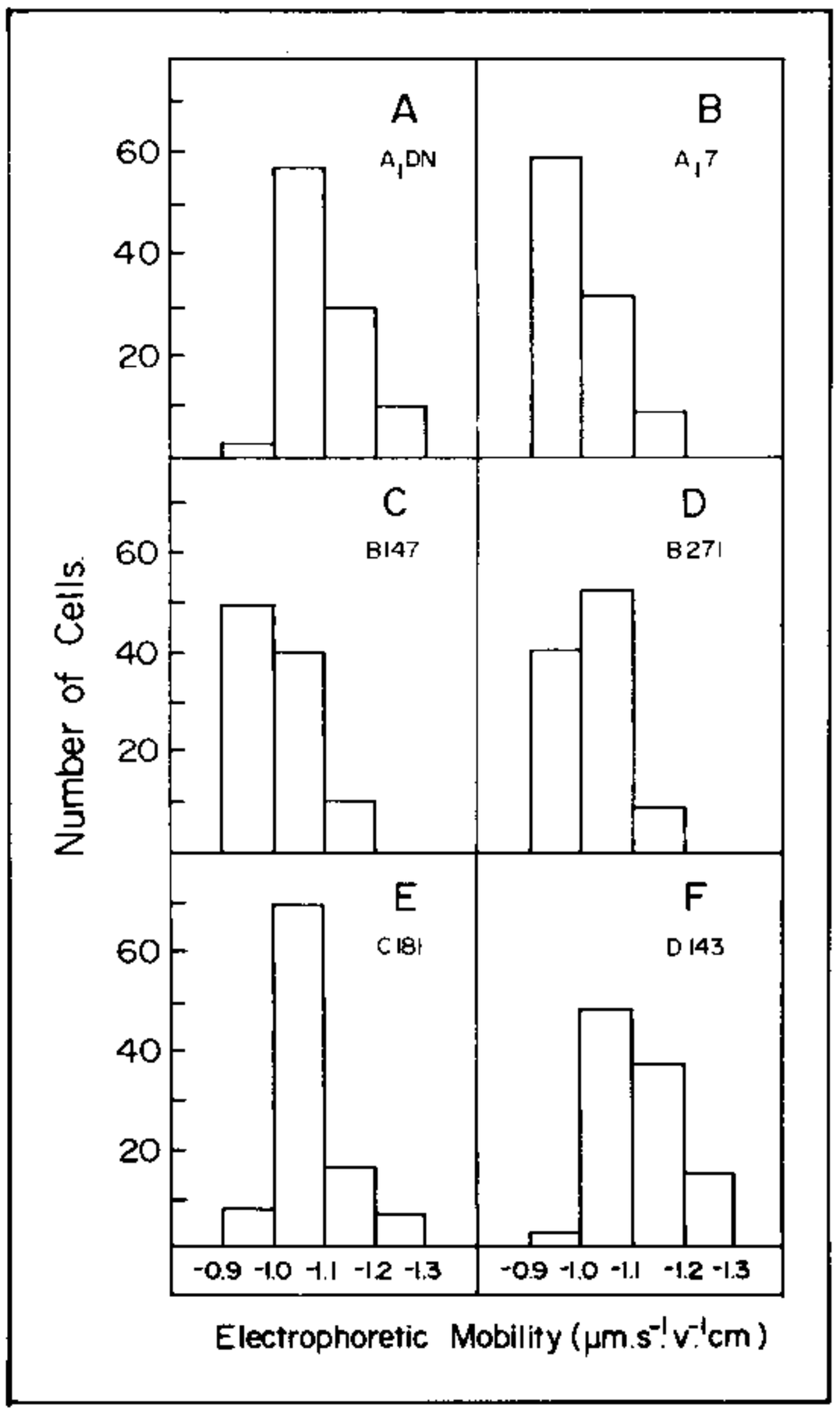

Fig. 1: distribution of the electrophoretic mobilities of populations of *Trypanosoma cruzi* isolated form patients with Chagas' disease corresponding to zymodemes A (A-B), B (C-D) and C (E-F).

The data presented in the Table show that it is possible to distinguish *T. cruzi* from other members of the sub-genus *Schizotrypanum*, such as *T. dionisii* and *T. myoti*. In the case of *T. dionisii* this difference could be observed both in epimastigote and trypomastigote forms. Trypomastigotes of *T. dionisii* have a very negative surface charge, with a mean EPM of – 1.90 $\mu m.s^{-1}.V^{-1}.cm$. It was not possible to distinguish epimastigotes of *T. cruzi*, *T. rangeli* and *T. vespertilionis*, since they present a similar mean EPM (arround – 0.60 $\mu m.s^{-1}.V^{-1}.cm$). The same was observed for epimastigotes of *T. dionisii* and *T. myoti*. It is important to point out that all stocks of *T. myoti* analyzed showed a similar mean EPM.

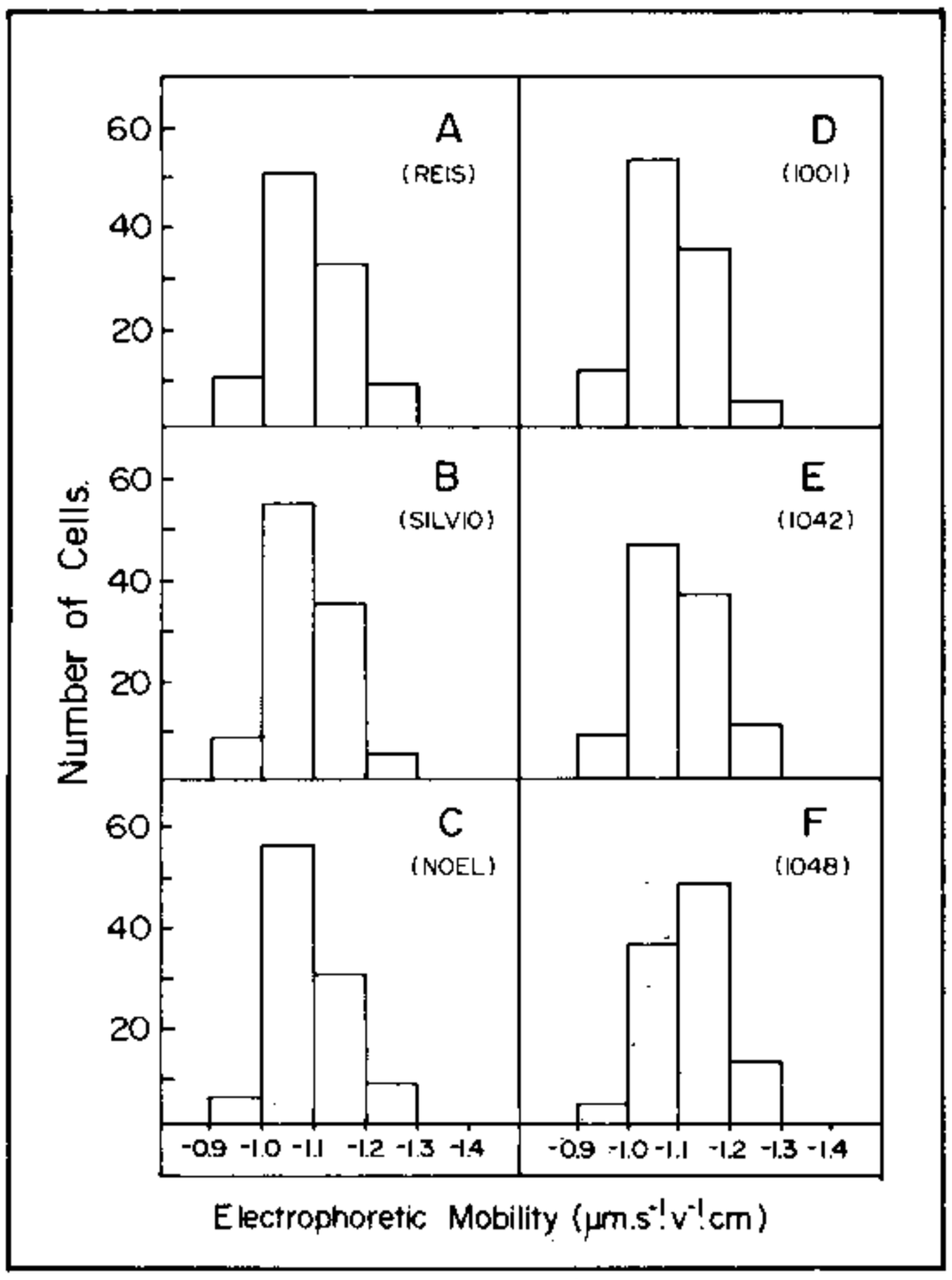

Fig. 2: distribution of the electrophoretic mobilities of populations of *Trypanosoma cruzi* (axenic medium containing intermediate and trypomastigote forms) isolated from patients in the acute phase of Chagas' disease (A, B, C) and from opossum (D, E, F).

TABLE

Mean electrophoretic mobility (EPM) of different species of the genus *Trypanosoma*[a]

Species	Strain	Developmental form	Mean EPM ($\mu m.s^{-1}.V^{-1}.cm$)
T. brucei		epi	− 0.83 ± 0.03
T. brucei		trypo (b)	− 0.68 ± 0.02
T. cruzi	Y	epi	− 0.65 ± 0.02
T. cruzi	Y	trypo (c)	− 1.15 ± 0.17
T. rangeli		epi	− 0.62 ± 0.02
T. vespertilionis		epi	− 0.61 ± 0.04
T. conorhini		epi	− 1.73 ± 0.10
T. dionisii		trypo (d)	− 1.90 ± 0.22
T. dionisii		epi	− 1.05 ± 0.20
T. myoti	1	epi	− 1.03 ± 0.06
T. myoti	3	epi	− 1.03 ± 0.06
T. myoti	4	epi	− 1.06 ± 0.06
T. myoti	5	epi	− 1.14 ± 0.07

a: in all cases cells were fixed in glutaraldehyde, washed and ressuspended in a 0.85% sodium chloride solution.
b: bloodstream trypomastigote isolated using DEAE cellulose column.
c: bloodstream trypomastigote isolated by differential centrifugation.
d: trypomastigotes obtained in axenic culture medium and isolated using DEAE cellulose column.

DISCUSSION

Biological and biochemical studies show that *T. cruzi* is a heterogeneous group of parasites with significant differences in their behaviour both *in vitro* and *in vivo*. Such differences are in some cases so great that for some isolates the designation of the parasites as *T. cruzi*-like has been used. Modern biochemical methods such as the analysis of isoenzymes (Zimodemes) and the kinetoplast DNA (Schizodemes) have provided important informations on this subject (reviews in Brener, 1973; Dvorak, 1984; Morel, 1984).

We have shown previously that the mean electrophoretic mobility of epimastigote and trypomastigote forms of the Y and CL strains of *T. cruzi* was basically the same, independent on the origin of the parasites (Souto-Padrón et al., 1984). The present study extend those observations to a large number of clones of parasites isolated from patients in the acute phase of Chagas' disease, from the invertebrate host or from wild animals, belonging to the various classes of zymodemes or shizodemes. In all cases a characteristic mean EPM for epimastigote and trypomastigote forms was found.

Our observations show that using cell electrophoresis under standard conditions (well defined pH and ionic strength of the solution) it is possible to distinguish epimastigotes of *T. cruzi* from similar forms of *R. dionisii* and *T. myoti*. This observation is of interest in view of the fact that these parasites were isolated from bats and showed to be able to infect vertebrate cells where they reproduce as amastigotes, with an intracellular behaviour characteristic of members of the *Schizotrypanum* subgenus (Baker et al., 1972). However, it was not possible to distinguish epimastigotes of *T. cruzi* from those of *T. vespertilionis*, which also belongs to the *Schizotrypanum* sub-genus. Previous immunological studies have shown that there are several common antigens in these trypanosomes. However, characteristic antigens for *T. myoti*, *T. vespertilionis* and *T. dionisii* were identified for each one (Bower & Woo, 1982).

Using cell electrophoresis, it was possible to distinguish epimastigotes of *T. cruzi* from those of *T. brucei* and *T. conorhini* but not from those of *T. rangeli*. The impossibility to distinguish *T. cruzi* from *T. rangeli* is an important

drawback of the method since these two trypanosomes can be found in some countries in the same species of triatomines. However, they can be distinguished using other methods such as sialic acid content (Schottelius, 1984) and the presence of neuraminidase in the supernatant of the cultures (Schottelius, 1987).

We analyzed the surface charge of trypomastigotes obtained from three members of the *Trypanosoma* genus, *T. cruzi*, *T. dionisii*, and *T. brucei*. They present different mean EPM values and could be easily distinguished from each other. It is important to point out that trypomastigotes of *T. dionisii* presented the greater negative surface charge, with a mean EPM of $-1.90\ \mu m.s^{-1}.V^{-1}.cm$. The mean EPM values we determined for trypomastigotes of *T. brucei* differed from that previously determined by Holingshead et al. (1963) although the value found for epimastigotes was similar.

Trypanosoma dionisii and *T. myoti* have been isolated from bats of the old and the new world, respectively. Although they can be differentiated by analysis of their polypeptide profiles (Taylor et al., 1982) epimastigotes from these two species present the same mean EPM. It would be interesting to measure the EPM of trypomastigotes of *T. myoti* in order to determine if they also present the same mean EPM. It is important to point out that all the four different isolates of *T. myoti* we analyzed presented similar mean EPM values.

In conclusion, our observations on various members of the *Trypanosoma* genus obtained under different conditions, fixed and stored for different periods of times in a glutaraldehyde solution indicate that cell electrophoresis, under well controlled conditions, is a useful method for the analysis of these parasites. Our observations with other trypanosomatids (Esteves et al., 1988; Soares et al., 1988) enable us to extend this conclusion to other genera of the Trypanosomatidae family.

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