## SURFACE ELECTRICAL CHARGE OF BLOODSTREAM TRYPOMASTIGOTES OF TRYPANOSOMA CRUZI STRAINS

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Bloodstream trypomastigotes of some Trypanosoma cruzi strains were processed through DEAE-cellulose columns under standardized conditions. The results obtained suggest mainly that these strains present different surface charges, that there are subpopulations of bloodstream trypomastigotes as regards electrical charges and that the broad forms are less negative than the slender ones.

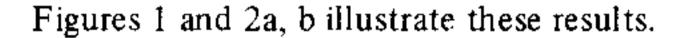
Bloodstream trypomastigotes of several strains of Trypanosoma cruzi (Y, CL, FL, Colombian, "Berenice" and "São Felipe") presented different behaviours on processing through DEAE-cellulose columns (Sousa, 1983). In the present note, some results obtained during these experiments are reported, and some deriving considerations on surface charge of this parasite are emphasized. Experiments were carried out with columns equilibrated with phosphate-saline-glucose (PSG) buffers of ionic strength (I) 0.181, pH 8.0, and stepwise elutions using PSGs of increasing ionic strengths (0.181, 0.217 and 0.253), also at pH 8.0. The infected blood for these experiments was from normal or sublethally gamma-irradiated mice. Methodological details were previously reported (Sousa, 1983). The following results are of interest:

- 1. The Y and "Berenice" strains never were eluted with PSG of I = 0.181, although the others were generally at low percentages (below 5.3%). These were only broad trypomastigotes and no contamination with blood cells or platelets in these eluates.
- 2. Running of PSG of I = 0.217 led to the highest recovery rates of the CL, FL, Colombian and "Berenice" strains, although at different and relatively characteristic values for each one (26.2 80.6%), whereas the Y and "Berenice" strains were eluted in low percentages (1.9 8.2%). This buffer mainly determined the recovery of broad trypomastigotes, although a low increase of slender forms occurred processing blood from irradiated mice. Eluted trypanosomes were free from blood cells and platelets. It is noteworthy that only the FL and "São Felipe" strains presented a striking increase in the recovery rates when collected from irradiated mice.
- 3. Running of PSG of I = 0.253 led to the highest recovery rates of the Y and "Berenice" strains (15.6 50.8%), while the others were recovered at percentages below

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those previously obtained (3.0 - 26.2%). This buffer could increase the elution of slender trypomastigotes, although for some strains (CL, FL, Colombian and "São Felipe") the increase occurred only with blood from irradiated mice. Moreover, this buffer led to the elution of some blood cells with the trypanosomes. Among the strains assayed, only the FL collected from irradiated mice was 100% recovered after passage of this buffer.



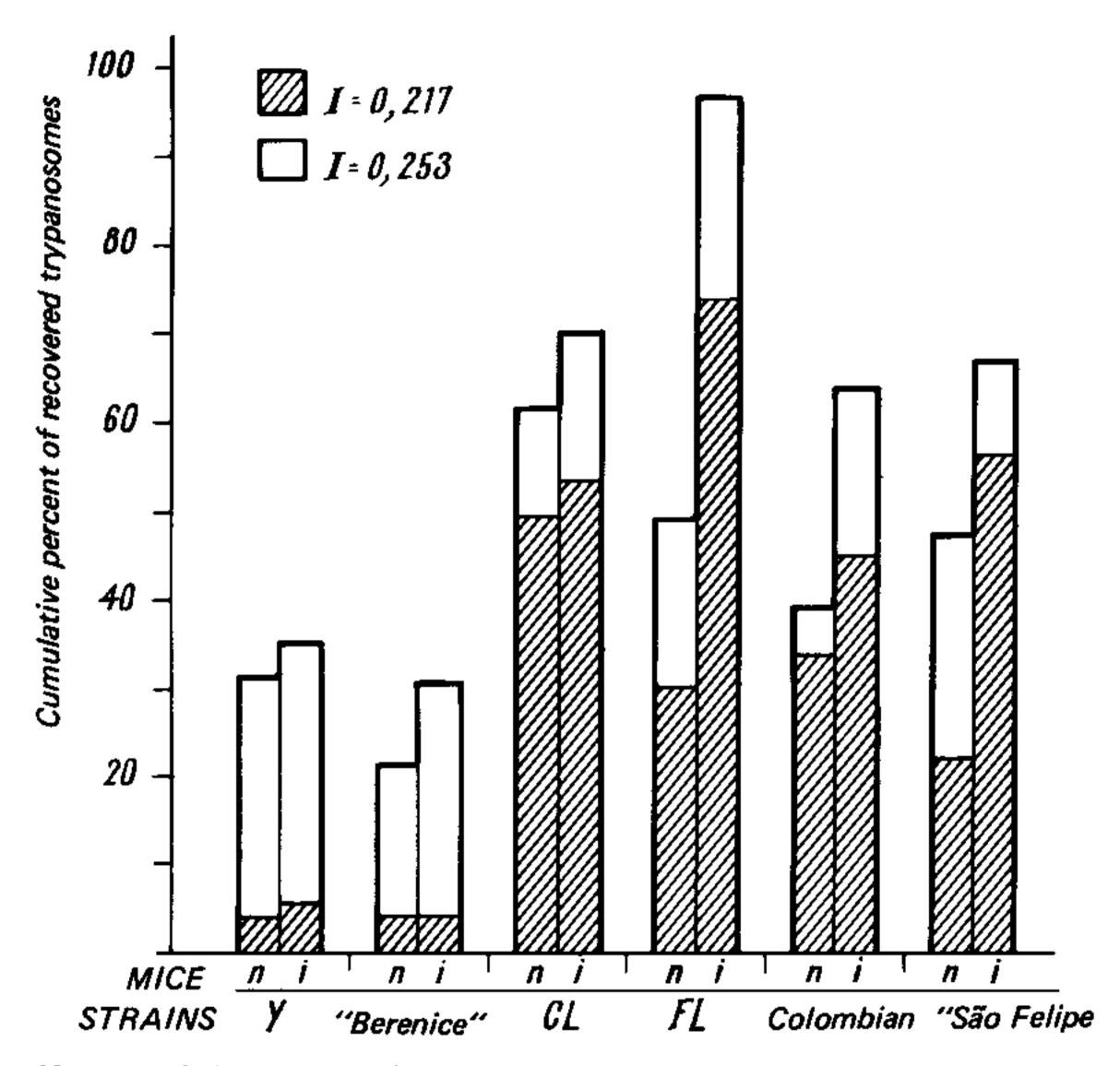


Fig 1 – Mean cumulative percents of recovered bloodstream trypomastigotes of six T. cruzi strains processed through DEAE-cellulose columns during the running of PSGs of I = 0.217 and 0.253. The infected blood was from normal (n) or irradiated (i) mice.

Lanham (1971) had already observed considerable variation in the adsorptionelution characteristics of *T. cruzi* strains processed through DEAE-cellulose; however, she did not mention the methodological conditions used, the strains assayed and their recovery rates. Since it would be expected that the degree of adsorption of cells on anion exchangers is proportional to their net surface charges (Lanham & Godfrey, 1970) and it has been demonstrated that *T. cruzi* bloodstream trypomastigotes are negatively charged (Broom, Brown & Hoare, 1936; Kreier, Al-Abbassy & Seed, 1977; Souza et al., 1977), the above mentioned results strongly suggest the following considerations.

1. The T cruzi strains present different surface charges, Y and "Berenice" probably being more negative than the others, at least when collected on the 7th day of mouse infection. However, the surface charge of some strains, as FL and "São Felipe", appeared altered when obtained from irradiated mice (Fig. 1; I = 0.217).



Fig. 2 – Bloodstream trypomastigotes of the FL strain of *T. cruzi* (from irradiated mice) recovered from a DEAE-cellulose column during the running of PSGs of I = 0.217 (Fig. 2a) and 0.253 (Fig. 2b). Note the different polymorphism pattern of the trypanosomes in each eluate.

- 2. There is surface charge heterogeneity among the bloodstream trypomastigotes of the strains assayed, since subpopulations of them were recovered during the running of buffers of increasing ionic strengths. Trypanosomes eluted with each buffer possibly present similar surface charges. In addition, as only the FL strain from irradiated mice was completely recovered under the experimental conditions used, it is possible that other parasite subpopulations presenting higher surface charges had remained adsorbed on the columns.
- 3. Broad trypomastigotes are less negative than the slender forms, since they are recovered at lower ionic strengths, and the surface charge of the latter can be modified by the host immune response, hence their elution was favoured collecting blood from irradiated mice (Fig. 2a, b).
- 4. There is a certain correlation between the predominant morphological type of a strain and its surface charge, since *T. cruzi* strains presenting mainly broad forms, such as CL, FL, Colombian and "São Felipe", were more easily recovered than those with predominantly slender forms, such as Y and "Berenice" (Fig. 1).

However, since *T. cruzi* strains present different polymorphism patterns, which can be modified under certain conditions, as by the host species (Sogayar, 1978), its immunological state (Pizzi, 1953) and the infection phase (Silva, 1959; Brener, 1965; Sogayar, 1978), conclusive data on surface charges of bloodstream trypomastigotes represent a complex problem, so that the above considerations must be limited by the experimental conditions used.

Souza et al. (1977) reported that T. cruzi bloodstream trypomastigotes present higher negative charge than the metacyclics based on data of electrophoretic mobilities;

however, these authors were expecting that both stages had similar charges. Pinho & Castro (1982) processing acellular cultures of T. cruzi through DEAE-cellulose columns under the basic conditions of the present work promptly recovered isolated metacyclics at I = 0.181, that is an unfavourable ionic strength to elute blood trypomastigotes of this parasite, as previously mentioned. Moreover, the slender blood forms, which resemble the metacyclics, are eluted at higher ionic strength (Fig. 2b). These data corroborate the previous observations that bloodstream trypomastigotes of T. cruzi have higher negative charges than the metacyclics.

## **RESUMO**

Tripomastigotas sanguíneos de algumas cepas de Trypanosoma cruzi foram processadas em colunas de DEAE-celulose sob condições padronizadas. Os resultados obtidos sugerem principalmente que estas cepas possuem cargas superficiais diferentes, que em relação a este aspecto existem subpopulações de tripomastigotas e que as formas largas são menos negativas do que as finas.

## REFERENCES

- BRENER, Z., 1965. Comparative studies of different strains of Trypanosoma cruzi. Ann. Trop. Med. Parasitol., 59:19-26.
- BROOM, J.C.; BROWN, H.C. & HOARE, C.A., 1936. Studies in microcataphoresis. II The electric charge of haemoflagellates. *Trans. R. Soc. Trop. Med. Hyg.*, 30:87-100.
- KREIER, J.P.; AL-ABBASSY, S.N. & SEED, T.M., 1977. Trypanosoma cruzi. Surface charge characteristics of cultured epimastigotes, trypomastigotes and amastigotes. Rev. Inst. Med. trop. São Paulo, 19:10-20.
- LANHAM, S.M., 1971. The separation of stercorarian trypanosomes from infected blood using DEAE-cellulose. Trans. R. Soc. Trop. Med. Hyg., 65:248-249.
- LANHAM, S.M. & GODFREY, D.G., 1970. Isolation of Salivarian Trypanosomes from Man and Other Mammals using DEAE-cellulose. Exp. Parasitol., 28:521-534.
- PINHO, R.T. & CASTRO, B.G., 1982. Purification of biologically and antigenically unaltered metacyclic trypomastigotes of *Trypanosoma cruzi* on DEAE-cellulose columns. Presented at the IX Annual Meeting on Basic Research on Chagas' Disease, pp. 132, Caxambu, MG, Brazil.
- PIZZI, T., 1953. Sobre el problema de las formas delgadas de Trypanosoma cruzi. Bol. Chil. Parasitol., 8:26-30.
- SILVA, L.H.P., 1959. Observações sobre o ciclo evolutivo do Trypanosoma cruzi. Rev. Inst. Med. trop. São Paulo, 1:99-118.
- SOGAYAR, R., 1978. Infecção experimental de ratos albinos Wistar com diferentes cepas de *Trypa-nosoma cruzi* Chagas, 1909. Thesis, Minas Gerais Federal University, Belo Horizonte, Brazil, 100 pp.
- SOUSA, M.A., 1983. A simple method to purify biologically and antigenically preserved bloodstream trypomastigotes of *Trypanosoma cruzi* using DEAE-cellulose columns. *Mem. Inst. Oswaldo Cruz*, 78:317-333.
- SOUZA, W.; ARGUELLO, C.; MARTINEZ-PALOMO, A.; TRISSL, D.; GONZÁLEZ-ROBLES, A. & CHIARI, E., 1977. Surface charge of *Trypanosoma cruzi*. Binding of cationized ferritin and measurements of cellular electrophoretic mobility. *J. Protozool.*, 24:411-415.