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SHORT COMMUNICATION

SEPARATION WITH CM-CELLULOSE OF THE TRYPOMASTIGOTE FORM OF *TRYPANOSOMA CRUZI* FROM THE FORMS GROWN IN FIBROBLAST CELL CULTURES

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Lanham (1968) reported studies on the separation of trypanosomes from the blood of infected rats and mice using anion exchangers; among various species of trypanosomes tested, only *Trypanosoma cruzi* could not be separated from infected mouse blood. On the basis of Lanham's method Al-Abbassy, Seed and Kreier (1972) isolated the trypomastigote form of *T. cruzi* from a mixture of the trypomastigote and epimastigote forms grown in HEp-2 cell cultures using a DEAE-cellulose (anion exchanger) column. Since the trypomastigote forms from infected mouse blood were adsorbed on DEAE-cellulose, whereas the trypomastigote forms produced in HEp-2 cell cultures were not adsorbed at the same pH and ionic strength, the question arises as to whether these forms have different surface charges. Howells and Chiari (1973) separated the trypomastigote form of *T. cruzi* from the white blood cells in the peritoneal fluid of infected mice using Cellex-SE cation exchange resin. Kanbara, Enriquez and Inoki (1974) and Kanbara (1975) reported antigenic differences in the surface coating substances of epimastigote, amastigote and trypomastigote forms. In this work, we used anion (DEAE-cellulose) and cation (CM-cellulose) exchangers for separating the three separate forms of *T. cruzi* from a mixture. Tulahuen strain of *T. cruzi* was obtained from the National Institute of Health, U.S.A. through Keio University, Japan and maintained in modified NNN diphasic medium and in an established fibroblast cell culture from a Balb-C mouse. Epimastigotes were collected from tube cultures and mixed with trypomastigotes and amastigotes grown in the fibroblast cell cultures (Fig. 1). The mixture was centrifuged at 3,000 r.p.m. for 10 min and suspended in

FIGURE 1 Original mixture composed of 73% of epimastigote (E), 13% amastigote (A) and 14% trypomastigote (T) forms.

10 ml of the initial elution buffer. Phosphate-saline-glucose buffer (PSG), pH 8.0, pH 7.0 and pH 6.0, consisting of 300 ml of 0.2 M phosphate buffer of the respective pH values, 300 ml of 0.85% (W/V) NaCl and 400 ml of 2.5% (W/V) glucose, were prepared according to Lanham's description, except for the proportions of monobasic and bibasic sodium phosphate used. DEAE-cellulose and CM-cellulose were activated and equilibrated with the respective initial elution buffers. Columns (2×5 cm) of DEAE-cellulose and CM-cellulose were prepared in glass syringes plugged with a wad of glass wool. The parasite mixture was layered on the surface of a column and the first buffer was added after the sample had entered the adsorbent.

Stepwise elution from the DEAE-cellulose column was done by decreasing the pH of the buffer (PSG) and conversely stepwise elution from CM-cellulose was done by increasing the the pH. Two hundred ml of PSG of the respective pH was passed through the column and the elute was collected in a flask and centrifuged at 3,000 r.p.m. for 10 min. The supernatant was discarded and the precipitate was resuspended in 1 ml of saline, stained with Giemsa and examined under a microscope. All forms of *T. cruzi* were adsorbed to DEAE-cellulose except for a small number of epimastigotes eluted in the last fraction of PSG, pH 6.0. According to Al-Abbassy et al. (1972), both the trypomastigotes and epimastigotes cultured in HEp-2 cells were eluted from DEAE-cellulose with PSG, pH 8.0, but trypomastigotes passed through faster than epimastigotes. Our results were the opposite of theirs but agreed with those of Lanham (1968); we found that trypomastigotes produced in fibroblast cell cultures could not be separated by DEAE-cellulose as well as trypomastigotes in infected mouse blood. The trypomastigotes were eluted from CM-cellulose with PSG of all pH values tested and the last fractions eluted with buffer of pH 8.0 were contaminated with a few epimastigotes (Figs. 2, 3). The amastigotes could not be eluted from either DEAE- or CM- cellulose under these conditions. Therefore, practically, trypomastigotes could be isolated from CM-cellulose with PSG, pH 8.0 from the forms grown in cell cultures which didn't contain epimastigotes and 20 to 30% of recovery was obtained.

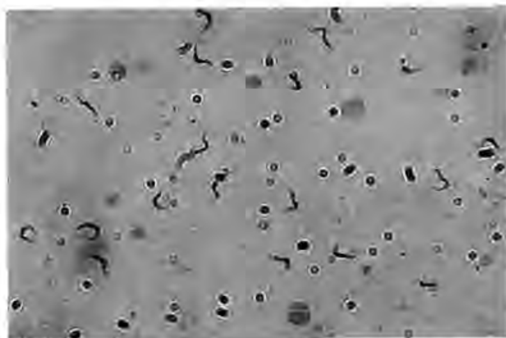

FIGURE 2 Only trypomastigotes were eluted from CM-cellulose with PSG, pH 6.0.

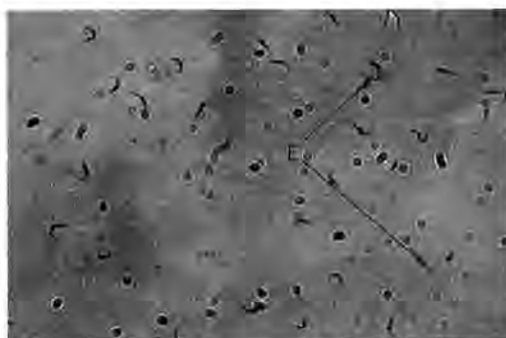

FIGURE 3 About 10% of epimastigotes was eluted with trypomastigotes from CM-cellulose with PSG, pH 8.0.

These results may show that trypomastigotes are less positively charged than epimastigotes and amastigotes, but it is uncertain whether separation of trypanosomes on an ionexchanger is wholly dependent on differences in their surface charges.

It is known that a molecule of protein having the same net surface charge density as another (and hence a similar electrophoretic mobility) but exceeding the latter in size, requires stronger conditions than the latter for elution

because it is capable of forming more bonds with the adsorbent. Moreover, the surface charge is only the average of many different charges on the surface. The surface of a cell is composed of many materials with various charged sites and thus the elution pattern of trypanosomes is probably very complicated. Our results show that the trypomastigote form has different surface character from other forms and thus can be separated from the latter on CM-cellulose.

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