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Research Note

Effect of Low pH on Transformation of *Trypanosoma cruzi* Trypomastigote to Amastigote

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Trypanosoma cruzi has four developmental stages; the epimastigote and metacyclic trypomastigote in insects, and the amastigote and trypomastigote in mammals. Although the natural process of transformation from trypomastigote to amastigote occurs intracellularly (Brener, 1973), it was shown (Piras *et al.*, 1982: Andrews *et al.*, 1987) that extracellular trypomastigotes, which had emerged from host cells and were kept in tissue culture medium at 37°C, transformed within 24–48 hours into round forms which resembled intracellular amastigotes. However, Kanbara and Nakabayahsi (1983a) observed that about 60% of bloodstream trypomastigotes failed to transform in mouse macrophage culture medium for at least one week, nor was transformation or phagocytosis observed even when the trypomastigotes were transferred daily to fresh macrophage cultures. Considering the intracellular site of transformation, it was presumed that there existed either factors of stimulation within the host cell or suppressive factors in the bloodstream. The present study examined a) the effects of a decreasing pH as a transforming factor because of the lowered pH the trypomastigotes encounter within the phagosomes of phagocytic cells, and b) the ability of serum to suppress transformation.

Firstly, trypomastigotes from the high-virulent clone (H-23) and the low-virulent clone (L-38), derived from the Tulahuen strain (Kanbara *et al.*, 1987), were developed in fibroblast cultures derived from ICR newborn mouse skins. The parasites were separated from other cell components by passage through a CM-cellulose column (Kanbara and Nakabayashi, 1983b). About 2×10^6 trypomastigotes/ml were incubated, at 37°C, in 199 medium containing 1% bovine albumin (199-Albumin), at pH 5.0, 6.0, and 7.0. As controls, equivalent numbers of parasites were incubated in 0.85% saline containing 0.2% glucose (SG), at pH 5.0, 6.0, and 7.0. The pH of the incubation medium was adjusted by 10 mM citrate-phosphate buffer, under a pH meter. Morphological changes of the trypomastigotes were monitored by hourly sampling and Giemsa staining (Fig. 1, 2). Both the high- and low-virulent trypomastigotes transformed to amastigotes more efficiently at a low pH than at neutral. Low-virulent trypomastigotes, after 7 hours of incubation in 199-Albumin, showed 100% transformation at low pH, and 80% transformation at neutral pH. In contrast, although over 95% of high-virulent trypomastigotes transformed at pH 5.0, only 30 and 20% transformed at pH 6.0 and 7.0, respectively. In SG medium, high-virulent trypomastigotes were almost completely agglutinated and disintegrated after 7 hours at pH 5.0, and to a lesser degree at neutral pH. Low-virulent trypomastigotes were not damaged in spite of

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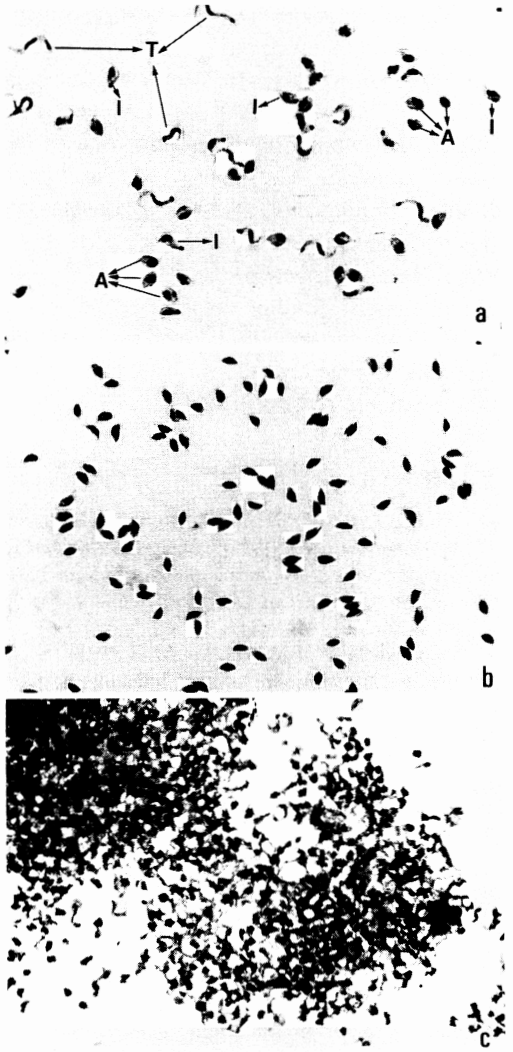

Fig. 1 Transformation of trypomastigotes after 7 hours of incubation. Trypomastigote (T); intermediate forms (I); amastigote (A). a, Clone H-23 trypomastigotes in 199-Albumin, pH 5.0. b, L-38 trypomastigotes in 199-Albumin, pH 5.0. Almost complete transformation to amastigotes. c, H-23 trypomastigotes in SG, pH 5.0; clumping in large aggregates and disintegration.

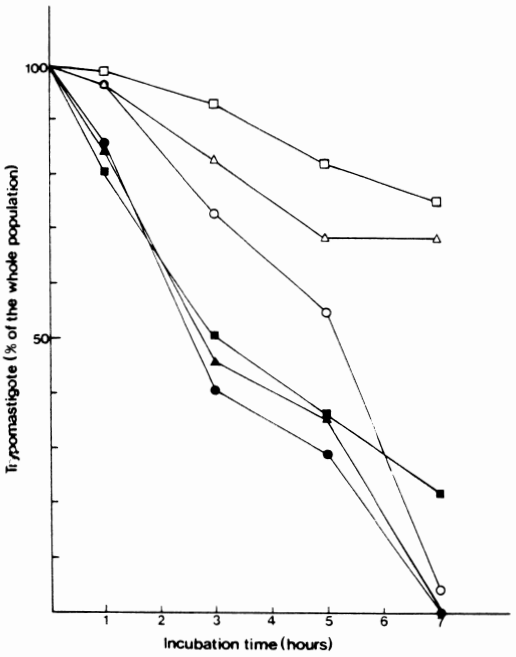

Fig. 2 Hourly changes in the percentage of trypomastigotes to the whole population after inoculation of clones H-23 and L-38 into media (199-Albumin) of different pH.

H-23 in pH 7.0 (□), H-23 in pH 6.0 (△), H-23 in ph 5.0 (○), L-38 in pH 7.0 (■), L-38 in pH 6.0 (▲), L-38 in pH 5.0 (●).

marked retardation of transformation (data not shown). The disintegration seemed to be caused by the release of chemical substances from the trypomastigotes during transformation, and probably corresponded to the hemolysins secreted from trypomastigotes and amastigotes at low pH (Andrew and Whitlow, 1989). The interesting point is that these secretory products were produced predominantly by the high-virulent trypomastigotes, but neutralized by the bovine albumin. Secondly, the effect of newborn bovine serum was examined. Trypomastigotes were incubated in medium 199, adjusted to pH 7.4 by 25 mM HEPES and 0.2% $NaHCO_3$, and supplemented by volume, with 0, 5, 10 and 20% newborn bovine serum. After every 24 hours of incubation, samples were taken and the media changed. Trypomastigotes from clone H-23 were deformed and had died within 24 hours in media lacking serum, whereas more than 80% remained viable in media containing 5–20% serum (Fig. 3). On the other hand, trypomastigotes from clone L-38 tended to transform more rapidly, and, interestingly, almost all transformed within

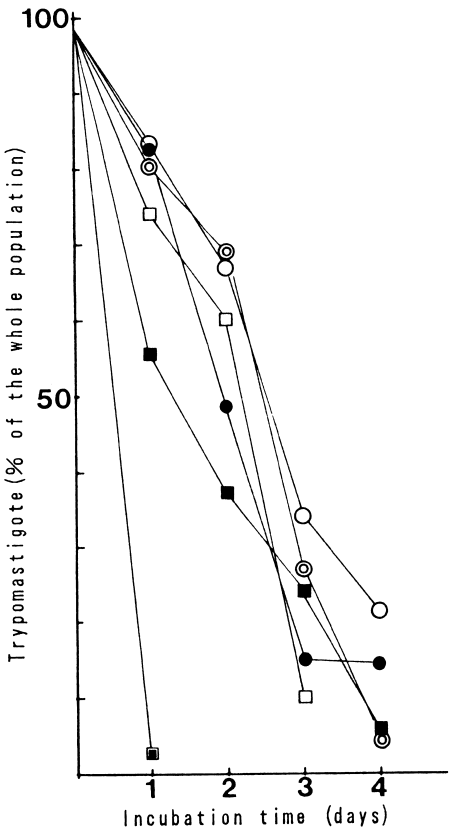

Fig. 3 Daily changes in the percentage of trypomastigote to the whole population after incubation in medium 199 supplemented with 0–20% newborn bovine serum, pH7.4.

H-23 in 5% serum (●), H-23 in 10% serum (○), H-23 in 20% serum (◎), L-38 in 0% serum (▣), L-38 in 5% serum (■), L-38 in 20% serum (□).

24 hours in media without serum but did not undergo disintegration. These results showed that a neutral pH plus the presence of serum were essential to maintain the trypomastigote form, although lytic factors were gradually released even at neutral pH. In other experiments, medium 199, at pH 7.4, was supplemented either with 10% newborn bovine serum or with 1% bovine albumin. The ability of the bovine albumin to maintain the trypomastigote stage was shown to be less than that of the newborn bovine serum (data not shown).

The present finding describe a convenient experimental system in which to study the mechanisms governing the transformation from trypomastigote to amastigote.

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