

TRITOMINE'S EMBRYO EXTRACTS PROMOTE GROWTH OF CULTURE FORMS OF *TRYPANOSOMA CRUZI*

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Among 59 studied chronic chagasic patients, in four of them it was only possible to isolate *Trypanosoma cruzi* using haemoculture, but not xenodiagnosis¹. This failure in isolating *T. cruzi* using xenodiagnosis performed with three different triatomine species, represents an opportunity to evaluate the interaction between *T. cruzi* and its infection within the triatomine vector. Based on observation that a triatomine embryo-cell-line allows the differentiation of the parasite to metacyclic forms³ and the induction of parasite growth and differentiation in cultures, when supplemented with triatomines' intestinal extracts² we argue that some of the questions related to triatomine's susceptibility and infection could be answered by studying the influence of the vector's organ extracts on parasite growth in culture media.

The present study is being conducted to analyze the role of different organ homogenates, from embryos to adult stages of triatomines, on the growth and differentiation of two *T. cruzi* strains (Herm and Fran) isolated only by hemoculture from two chagasic patients and frozen immediately after isolation. The isolates were used after five passages in LIT media.

Suspensions of four and three days old embryos, of *Dipetalogaster maximus* and *Rhodnius prolixus* respectively, were obtained from ground eggs in PBS (pH 7.2). The extracts were filtered either with 0.22 or 0.45µm Millipore filters, in order to observe possible differences in the flagellates growth due to the

presence of protein molecules of different size, and stored in -20°C before being added to LIT culture medium. The protein concentration of the extracts was measured after Lowry et al⁴ and adjusted to 160µg/ml in the culture (final concentration). The initial inoculum was of 3 x 10⁶ flagellates in the logarithmic growth for the various samples. The cultures were maintained at 27°C. Eleven days after addition of the extracts to the cultures, the number of flagellates was determined using a blood cell counter from triplicate cultures.

The results have shown that both embryo extract preparations (*R. prolixus* and *D. maximus*) were able to promote an exacerbation on the growth of the two *T. cruzi* strains achieving numbers of flagellates eight times higher than the one observed in the control cultures of the parasite (Table 1).

Table 1 - Number of flagellates/ml ($\times 10^6$) of two *T. cruzi* strains (Herm and Fran) after 11 days in LIT culture medium with *R. prolixus* (Rh) or *D. maximus* (Dm) embryo extracts filtered through two different membrane pores (0.22 and 0.45µm).

Strains	Control	Rh (0.22)	Rh (0.45)	Dm (0.22)	Dm (0.45)
Herm	4.7	20.1	15.7	17.6	24.3
Fran	2.5	15.3	13.4	18.8	20.2

No significant differences were observed in the percent of metacyclic trypomastigotes in the cultures.

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