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TRYPANOSOMA CRUZI: EFFECTS OF *RHODNIUS PROLIXUS* EXTRACTS ON *IN VITRO* DEVELOPMENT

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SUMMARY

The addition of saline extracts of adult *Rhodnius prolixus* bugs to an insect-oriented culture system for *Trypanosoma cruzi* resulted in the large scale evolution of trypomastigote forms of these parasites *in vitro*. Parasite populations in these cultures reached densities of as many as 8.3×10^7 parasites per ml after 14 days incubation at 27-28°C.

INTRODUCTION

For many years research on the metabolism and immunology of *Trypanosoma cruzi* was limited because the available *in vitro* culture systems produced mainly epimastigote stages. In 1967 CASTELLANI et al. [1] reported the *in vitro* evolution of metacyclic trypomastigote forms after a series of manipulations through two types of media. WOOD & PIPKIN [3] subsequently reported that by using a system based on the insect cell culture medium of GRACE [2] (GMA), large numbers of trypomastigote stages of *T. cruzi* could be harvested. However, the insect-oriented culture required the addition of 0.5% of a specially processed hemolymph from diapausing pupae of the moth *Philosamia cynthia*. This requirement limited the availability of the insect-oriented culture system to only a few laboratories.

A search for a more available source of the insect factor(s) supplied in the moth hemolymph led to the following study.

METHODS AND MATERIALS

A myotropic strain of *T. cruzi* (Brazilian origin) was used in these experiments. The strain was maintained in Swiss albino mice by serial blood passage every 10 days.

Adult *Rhodnius prolixus*, reduviid vectors of *T. cruzi*, were obtained from a laboratory colony, and extracts (Re) were prepared in the following manner. Bugs were ground in a constantly-chilled (4°C) mortar and pestle at a ratio of one adult per ml of phosphate buffered saline, pH 6.8 (PBS). After thorough grinding, the triturate was filtered through PBS soaked, Whatman's #50 filter-paper, in a Buchner funnel. The filtered extract was collected in a chilled Erlenmeyer flask, and then heat-inactivated at 56° C for 6 minutes. The material was then transferred into centrifuge tubes and frozen at −70° C for 12 hours. Following this, the extract was quick-thawed and centrifuged at 14,000 × G for 30 minutes at 4° C. The supernatant was sterilized by filtration through a 0.22μ membrane filter and stored at −20° C.

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