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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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Cultures of *T. cruzi* were initiated by inoculating one drop of cardiac blood from an infected mouse into 10 ml of GMA plus 10% fetal calf serum (FCS). The blood and media were thoroughly mixed and 5 ml aliquots introduced into each of two 30 ml plastic tissue culture flasks. When dividing epimastigotes were plentiful in the primary cultures, usually after seven days of incubation at 27°C, they were subcultured.

The following combinations of media were employed:

(1) GMA + 10% FCS, (2) GMA + 10% FCS + 2% Re, (3) GMA + 10% FCS + 1% Re, (4) GMA + 10% FCS + 0.5% Re, (5) GMA + 10% FCS + 0.25% Re, (6) GMA + 10% FCS + 0.1% Re, and (7) GMA + 10% FCS + 0.5% moth hemolymph (WOOD & PIPKIN [3]). For these studies, thirty thousand parasites from primary cultures were added to 10 ml of medium and each medium was dispensed in five ml aliquots in 30 ml plastic tissue culture flasks. All cultures were incubated at 27-28°C.

Cultures were examined daily using an inverted phase-contrast microscope; parasite numbers were established in a hemocytometer. To determine the percent composition of trypomastigotes in a given culture, one drop of media plus parasites was placed on a microscope slide, fixed with 0.2% formalin, dried, stained, and the forms present recorded.

RESULTS

Parasite populations in each of the five Re-containing culture media reached maximum population densities, comparable with those of the moth hemolymph control, on the 13-14th day of incubation (Table I).

TABLE I

Maximum *T. cruzi* population densities in insect-oriented media after 14 days incubation

Media	Maximum population densities	Percent trypomastigotes
(1) GMA (1) + 10% FCS (2)	2.5×10^6	3%
(2) GMA + 10% FCS + 2.0% Re (3)	7.0×10^7	64%
(3) GMA + 10% FCS + 1.0% Re	6.8×10^7	52%
(4) GMA + 10% FCS + 0.5% Re	7.1×10^7	66%
(5) GMA + 10% FCS + 0.25% Re	8.3×10^7	85%
(6) GMA + 10% FCS + 0.1% Re	4.7×10^7	35%
(7) GMA + 10% FCS + 0.5% hemolymph (4)	5.6×10^6	75%

(1) Grace's Medium for *Antheria* (GRACE, 1962)
(2) Fetal Calf Serum
(3) Extract of *Rhodnius prolixus* adult bugs
(4) Hemolymph from the moth *Phylosamia cynthia* prepared according to GRACE, 1962.

Dividing epimastigote stages were the only morphological forms observed in all cultures for the first five days. On the 6th day a few trypomastigotes less than 1% of the total population were observed in the cultures with one or more percent Re. By the tenth day, cultures containing 0.5% or more Re averaged 40% trypomastigotes. The media containing 0.25% and 0.1% Re had less than 3% trypomastigotes at this time. By the 14th

day 85% of the forms present in the cultures with 0.25% Re were trypomastigotes (Fig. 1). These results compared favorably with the 75% trypomastigote populations observed in preparations to which moth hemolymph had been added. The cultures with the two highest concentrations of Re (2.0% and 1.0% respectively) never exhibited more than 64% trypomastigotes. On the other hand, the cultures containing 0.1% Re never differentiated to more than 35% trypomastigotes.

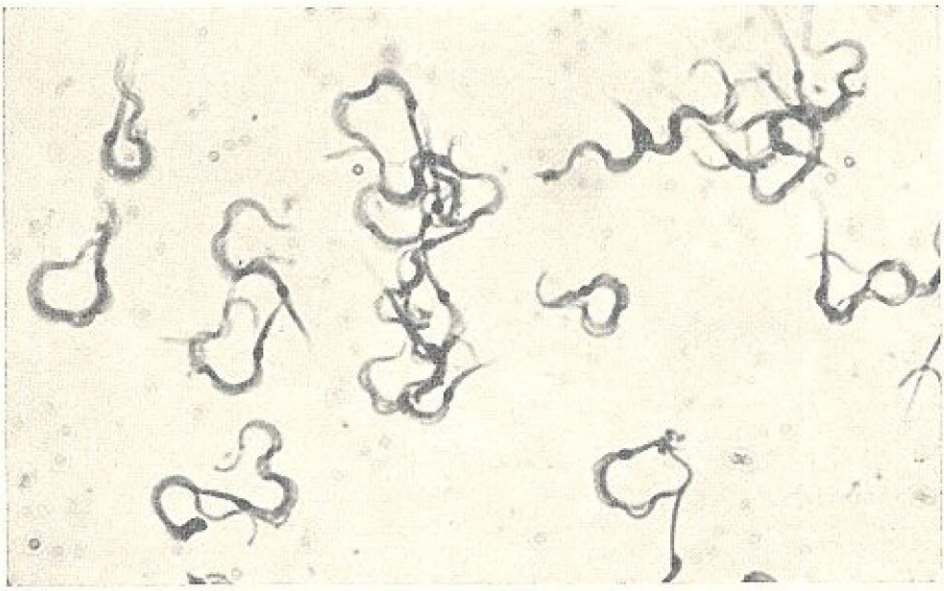

Fig. 1 — Trypomastigotes of *Trypanosoma cruzi* from *in vitro* culture in a medium consisting of GMA + 10% FCS + 0.25% Re, after 14 days of incubation at 27-28°C.

DISCUSSION

The fact that extracts of *Rhodnius* vectors can fulfill the requirements of moth hemolymph in the culture system of WOOD & PIPKIN [3] makes this method available to many laboratories that are working on Chagas' disease. The advantages of this culture system are many. It is a clear medium in which the development of the parasites can be readily followed and it yields reproducible results. With this medium large quantities of trypomastigotes can be obtained for use in metabolic, drug susceptibility, and immunologic studies. The use of trypomastigotes in immunological investigations is thought to be of particular importance since it is these stages that are most often experienced by the mammalian host's immune systems.

RESUMO

Trypanosoma cruzi: *Efeitos de extratos de* Rhodnius prolixus *sobre seu desenvolvimento* in vitro

Mediante a adição de extratos salinos das formas adultas do triatomíneo *Rhodnius prolixus* a um novo meio de inseto-culturas para o *Trypanosoma cruzi*, foi possível obter uma pronunciada evolução das formas de tripomastigotas metacíclicos destes parasitos *in vitro*. As populações desses parasitos nos sistemas de culturas acima citados alcançaram densidades de até 8.3×10^7 parasitos por ml após 14 dias de incubação à temperatura de 27-28°C.

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