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CRYOPRESERVATION OF *TRYPANOSOMA RANGELI* INFECTIVE STAGES FROM EXPERIMENTALLY-INFECTED *RHODNIUS ECUADORIENSIS*

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SUMMARY

Trypanosoma rangeli infective stages, obtained from salivary glands and haemolymph of experimentally infected *Rhodnius ecuadoriensis*, were successfully preserved in liquid nitrogen. Patent infections in vertebrate hosts and subsequent normal cyclical transmission have been obtained with the flagellates preserved for 30 days. The technique may be useful for the study of *Trypanosoma rangeli* strains from vectors collected in endemic areas, preventing erratic changes which may occur in strains maintained for long periods in the laboratory.

INTRODUCTION

Preservation, at low temperature, of protozoan-parasite infective stages obtained from their vectors or within the intact insects has already been reported by different Authors: sporozoites from human malaria parasites (JEFFERY & RENDTORFF [7]), *Plasmodium gallinaceum, P. cynomolgi* (MOLINARY [9]) and *P. berghei* (BAFORD [1]); *Trypanosoma cruzi* metacyclic trypomastigotes in *Triatoma infestans* and *Rhodnius prolixus* (FLUCK [6]); metacyclic trypomastigotes from phlebotomine sandflies (MINTER & GOEDBLOED [8]). This paper reports the preservation, in liquid nitrogen, of *T. rangeli* infective stages from salivary glands and haemolymph of experimentally infected *Rhodnius ecuadoriensis*. The possibility of frozen infective stages of the parasite accomplishing their whole cycle both in vertebrate and invertebrate hosts has also been investigated.

MATERIAL AND METHODS

T. rangeli strain — isolated, in 1971, from naturally-infected *R. ecuadoriensis* collected in Cajamarca, Perú, this parasite has been cyclically transmitted to mice and guinea-pigs by bite of experimentally-infected laboratory triatomids.

R. ecuadoriensis colony — eggs of these triatomids were brought from Perú in 1971, a colony being then established in the Instituto de Endemias Rurais.

Infection of R. ecuadoriensis — clean laboratory-reared *R. ecuadoriensis* were fed on experimentally-infected mice and guinea-pigs presenting *T. rangeli* bloodstream trypomastigotes; afterwards, the insects were kept at 26°C and 70% humidity. Haemolymph invasion was determined according to D'ALESSANDRO's [4] technique and salivary gland infection detected by transmission of the parasite to normal mice through bite. In some experiments, the insects were dissected and their intestinal contents, haemolymph and salivary glands examined for flagellates.

The *R. ecuadoriensis* specimens whose salivary glands and haemolymph were removed and frozen had been infected in the 6th cyclic laboratory passage of *T. rangeli*.

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Infection of the vertebrate host — mice were infected either through the vector's bite or mechanical inoculation of metacyclic trypomastigotes by intraperitoneal route; repeated fresh blood examinations, thick smears, xenodiagnosis, haemocultures in blood-agar or "LIT" medium (CAMARGO [2]), were performed to detected *T. rangeli* infections.

Freezing methods — the insect's salivary glands were dissected out and whether whole or disrupted, suspended in a mixture of inactivated guinea-pig serum with 10% glycerol. To collect flagellates from haemolymph, the specimens were dissected and their haemocel throughly washed with the above described preservation medium. The material containing living flagellates was handled at room temperatures (23°-25°C) and transferred to polyethylene capillary tubes, which were then sealed and stored in a −73°C deep-freezer for 16-20 hours. After this period the tubes, packed in identified cardcylinders, were immediately placed in liquid nitrogen. After storage for different periods of time, the material was thawed for 2 minutes in water at 37°C and microscopically examined before inoculation.

RESULTS

Table I shows the results obtained with the inoculation into mice of infective stages of *T. rangeli* obtained from experimentally infected *R. ecuadoriensis* and preserved in liquid nitrogen. In all performed experiments the animals have been successfully infected with cryopreserved material. A fourth stage *R. ecuadoriensis* nymph which had been regularly transmitting *T. rangeli* by bite was dissected 75 days after the infective meal and its disrupted salivary glands, frozen for 30 days in liquid nitrogen, inoculated into a normal mouse. Figure 1 shows the whole sequence of cyclical transmission obtained with the frozen material.

TABLE I

Results of inoculation into mice of cryopreserved infective stages of *T. rangeli* from experimentally infected *R. ecuadoriensis*

Triatomids used	Method used to detected infection in triatomids (day of infection)	Preserved material (days)	Result of inoculation into mice	Observations
Fourth stage nymph	Transmission by bite (78th day)	Whole salivary glands (15 days)	Infection detected by hemoculture	
Adult	Transmission by bite (65th day)	Whole salivary glands (30 days)	Infection detected by xenodiagnosis	Two nymphs infected after feeding
Adult	Transmission by bite (78th day)	Disrupted salivary glands (15 days)	Infection detected by hemoculture	
Fourth stage	Haemolymph examination (65th day)	Haemolymph (30 days)	Infection detected by fresh blood examination	5 out of 10 nymphs became infected after feeding on the mice

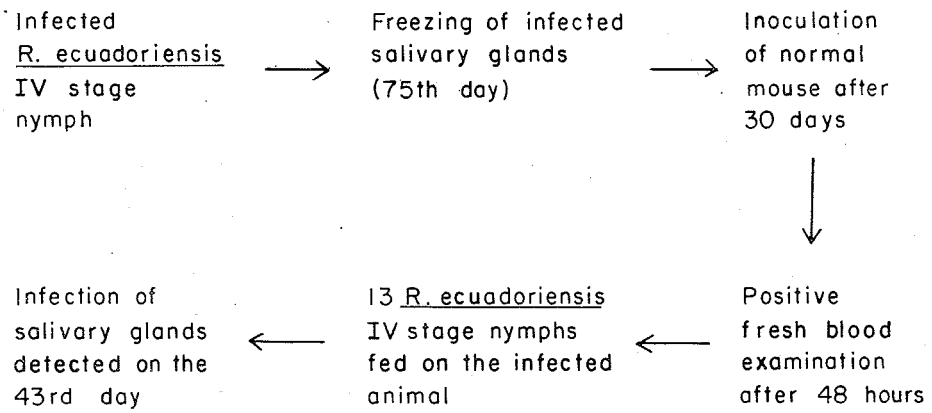

Fig. 1 — Sequence of cyclic transmission obtained after inoculation of cryopreserved *T. rangeli* infective stages obtained from *R. ecuadoriensis*.

DISCUSSION

The number of *T. rangeli* bloodstream trypomastigotes is usually very low and/or fluctuating, and the maintenance of the parasite in the laboratory is basically dependent upon cyclical transmission between vertebrate hosts and susceptible triatomines which may propagate it by bite. This routine procedure may be faced with a problem when large numbers of strains are to be collected and studied in the laboratory. D'ALESSANDRO [5], for instance, has recently emphasized the need of studying large numbers of *T. rangeli* strains from different areas in order to define this parasite either as a species complex or a biological complex of variant strains. For this purpose infective stages from naturally-infected vectors, collected in endemic areas, could be stored at low temperatures and then gradually investigated through comparative studies. This research sequence may provide a good picture of *T. rangeli* natural behaviour especially with regard to the important aspect of vector-parasite relationship. Furthermore, there may be avoided changes already undergone by the different strains during long-term maintenance in the laboratory, such as the progressive increase of infected salivary glands displayed by a strain kept for years through cyclical passages (TOBIE [10]).

T. rangeli culture forms are easily kept in the laboratory and when inoculated in the haemocel of susceptible triatomines they may develop into an "anterior-station" parasitism, with invasion of salivary glands and transmission by bite (ZELEDON [11]). Loss of infectivity to triatomines after prolonged cultivation has, however, been reported (D'ALESSANDRO [5]), which makes culturing unsuitable for regular infection of invertebrate hosts for *T. cruzi* comparative studies.

Viable preservation, at low temperatures, of *T. rangeli* forms from infected triatomines has now been demonstrated by the recovery of motile flagellates which can infect vertebrate host after −196°C freezing for at least 30 days in liquid nitrogen. Those frozen forms were able to induce patent infection in mice and subsequently kept on developing in a high percentage of triatomines, where they also displayed their normal distribution in the digestive tract, haemolymph and salivary glands. The characteristics of infection in the vertebrate host (low parasitemia, 24-48 prepatent period) and in the vectors (rate of infectivity; sequence of intestine, haemolymph and salivary glands invasion; transmission by bite) observed with the frozen parasites were rather similar to those seen during the regular cyclical transmission of this Peruvian *T. rangeli* strain (CUBA [3]).

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RESUMO

Criopreservação de estágios infectantes do Trypanosoma rangeli *obtidos em* Rhodnius ecuadoriensis *experimentalmente infectados*

Glândulas salivares e hemolinfa de *Rhodnius ecuadoriensis* experimentalmente infectados com uma cepa peruana de *Trypanosoma rangeli* foram submetidos a criopreservação em nitrogênio líquido durante 30 dias. Infecções patentes no hospedeiro vertebrado, bem como subseqüente infecção de triatomíneos e transmissão por picada a animais normais foram obtidas após inoculação do material preservado por diferentes períodos de tempo. Os Autores sugerem que a criopreservação dos estágios infectantes do *T. rangeli* possa ser útil para o estudo de diferentes amostras do parasita encontradas em triatomíneos naturalmente infectados assim como para a prevenção de eventuais alterações decorrentes da prolongada manutenção do parasita em condições de laboratório.

REFERENCES

1. BAFORD, J. — The effects of low temperature preservation on the viability of the sporozoites of *Plasmodium berghei*. *Ann. Trop. Med. Parasitol.* **62**:301-304, 1968.

2. CAMARGO, E.P. — Growth and differentiation in *Trypanosoma cruzi*. I. Origen of metacyclic trypanosomes in liquid media. *Rev. Inst. Med. trop. São Paulo* **6**:93-100, 1964.

3. CUBA, C. — *Evolução de uma cepa peruana de* Trypanosoma rangeli *em* Rhodnius ecuadoriensis *e Panstrongylus herreri*. Thesis. University of Minas Gerais, 84 pp., 1973.

4. D'ALESSANDRO, A. — Technique of haemolymph examination to detect *Trypanosoma rangeli* in triatomid bugs. Rio de Janeiro, *Proc. VII Inter. Congr. Trop. Med. Malaria* **2**:247, 1973.

5. D'ALESSANDRO, A. — New experimental vectors of Colombian *Trypanosoma rangeli*. *J. Med. Entol.* **9**:187-195, 1972.

6. FLÜCK, V. — Tiefkühl Konservierung von *Trypanosoma cruzi* in einem der natürlichen Überträger (*Triatoma infestans*). *Acta Trop.* **19**:355-357, 1962.

7. JEFFERY, G.M. & REDTORFF, R.C. — Preservation of viable human malaria sporozoites by low-temperature freezing. *Exptl. Parasit.* **4**:445-454, 1955.

8. MINTER, D.M. & GOELBLOED, E. — The preservation in liquid nitrogen of tsetse flies and phlebotomine sandflies naturally infected with trypanosomatid flagellates. *Trans. Roy. Soc. Trop. Med. Hyg.* **65**:175-181, 1971.

9. MOLINARY, V. — The action of low temperatures on Plasmodia. *J. Trop. Med. Hyg.* **64**:225-230, 1961.

10. TOBIE, J. — Increased infectivity of a cyclical maintained strain of *Trypanosoma rangeli* to *Rhodnius prolixus* and mode of transmission by invertebrate host. *J. Parasitol.* **50**:493-598, 1964.

11. ZELEDÓN, R. — *Trypanosoma rangeli* en glandulas salivares de *Rhodnius pallescens* de Panamá. *Rev. Biol. Trop.* **13**:157-159, 1965.

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