Rev. Soc. Mex. Hist. Nat., 47:19-23 (1997).

Biological Properties of Metatrypomastigotes of *Trypanosoma cruzi* from the Anal Glands of Urban *Didelphis marsupialis*.*

Propiedades Biológicas de Metatripomastigotes de Trypanosoma cruzi de Glándulas Anales de Didelphis marsupialis Urbanos.

S. Urdaneta-Morales, I. Nironi and L. Herrera**

ABSTRACT

Metacyclic trypomastigotes of *Trypanosoma cruzi* from the anal glands of naturally infected urban *Didelphis marsupialis* and cultures derived from these were inoculated i.p., orally, and into the eyes of juvenile opossums. The parasite could develop in blood, tissues, and anal glands. The blood forms of these infections colonized *Rhodnius* (Hemiptera, Reduviidae, Triatominae) employed for xenodiagnosis. NMRI mice were infected by the same routes by metacyclics from the same sources. All mice died with parasitemias up to 10^6 /ml blood, while all opossums survived with parasitemias no higher than 10^3/ml. Opossums showed limited cardio - and myotropism, while mice were extensively parasitized, in addition, in the smooth muscle of the intestines and lung, and in the liver. The continuing presence of *T. cruzi* in the anal glands close to the excretory orifices would suggest transmission of the disease by contamination.

Keywords: *Trypanosoma cruzi*, metatrypomastigotes, *Didelphis marsupialis*, opossums, anal glands, urban American trypanosomiasis, Venezuela

RESUMEN

Metatripomastigotes de *Trypanosoma cruzi* obtenidos de glándulas anales de rabipelados (zarigueyas) *Didelphis marsupialis* (Marsupialia, Didelphidae) urbanos naturalmente infectados y de sus cultivos inoculados i.p., oral y ocularmente en rabipelados juveniles se desarrollaron en la sangre, tejidos y glándulas anales. Flagelados hemáticos y glandulares infectaron, respectivamente a *Rhodnius* (Hemiptera, Triatominae) así como a rabipelados y ratones NMRI. Resultados similares se obtuvieron al inocular por las mismas vías a ratones con metacíclicos de ambas fuentes. Todos los ratones murieron con parasitemias de 10^6 parásitos/ ml de sangre; los marsupiales sobrevivieron con máximos de 10^3/ ml de sangre. Limitado mio y cardiotropismo en los rabipelados contrastó con extensa invasión de estos tejidos y de músculo liso de los intestinos y pulmón así como del hígado de los ratones. La continua presencia de metacíclicos en glándulas anales de los marsupiales urbanos experimentales y la cercanía anatómica de estas glándulas con el recto y los órganos urogenitales son discutidos en relación con la importancia epidemiológica de estos marsupiales.

Palabras claves: *Trypanosoma cruzi*, metatripomastigotes, *Didelphis marsupialis*, rabipelados (zarigueyas), glándulas anales, Tripanosomiasis americana urbana, Venezuela.

*** En honor del Dr. Eucario Lopez-Ochoterena: distinguido protozoologo mexicano.**

**** Seccion de Parasitologia, Instituto de Zoologia Tropical, Facultad de Ciencias, Universidad Central de Venezuela.Apartado 47058. Caracas 1041-A, Venezuela.**
Corresponding author - FAX No. 58.2.7535897

Introduction

The metacyclic trypomastigote infective stage of *Trypanosoma cruzi*, pathogen of Chagas' disease, normally develops in the lumen of the Malpighian tubules and gut of the bloodsucking insect vectors (Hemiptera,Reduviidae, Triatominae) (Garcia & Azambuja , 1991). Transmission of the disease is usually by contamination with the bug's excreta (Dias, 1979).

Also, the whole cycle of development of the parasite can occur within the anal odor glands of the opossum *Didelphis marsupialis* (Marsupialia, Didelphidae) (Deane *et al.*, 1984). These workers showed that, in experimental infections of opossums, there developed multiplicative epimastigotes and sphaeromastigotes, and metacyclic trypomastigotes in the fluid content of the glands; the morphology of these was like that in insect feces.

In our current study of urban reservoirs of *T. cruzi* in the valley of Caracas, Venezuela (Herrera & Urdaneta-Morales, 1992) we have infected opossums and mice with metacyclics from anal glands, and from cultures of these forms. These all proved virulent when inoculated i.p., s.c., orally, or into the eye. Previous investigations (Urdaneta-Morales & Nironi, 1996) showed marked cardio- and myotropism with intracellular parasites in experimental infections with glandular material from naturally infected opossums.

Material and Methods

The capture of opossums, their examinations, and culture of parasites from anal glands have been previously described (Urdaneta-Morales & Nironi, 1996).

Experimental infections.

Glandular material from a naturally infected adult opossum was inoculated i.p. or s.c. into 6 juvenile opossums (av wt 350 g) and orally into an adult opossum (2.5 kg wt). All inocula were of 200 metacyclics/g body weight (Jansen *et al.*, 1991), quantified in fresh material according to Brener (1962). All opossums, including the mother of the juveniles, were negative for *T. cruzi* by blood examination and xenodiagnosis.

Three lots of 3 juvenile opossums (av wt 350 g) were inoculated s.c., orally, and into the eye with the same doses of metacyclics harvested from LIT cultures derived from the glandular material.

For controls of each experiment, lots of 3 NMRI mice (av wt 7 g) were inoculated in the same manner. Mice were examined daily to measure the average parasitemia, and opossums were examined every other day, from the third day PI until the parasitemia disappeared or the animal died. Anal glands of the opossums were examined weekly, and material from these was inoculated s.c. into mice, which were examined as above.

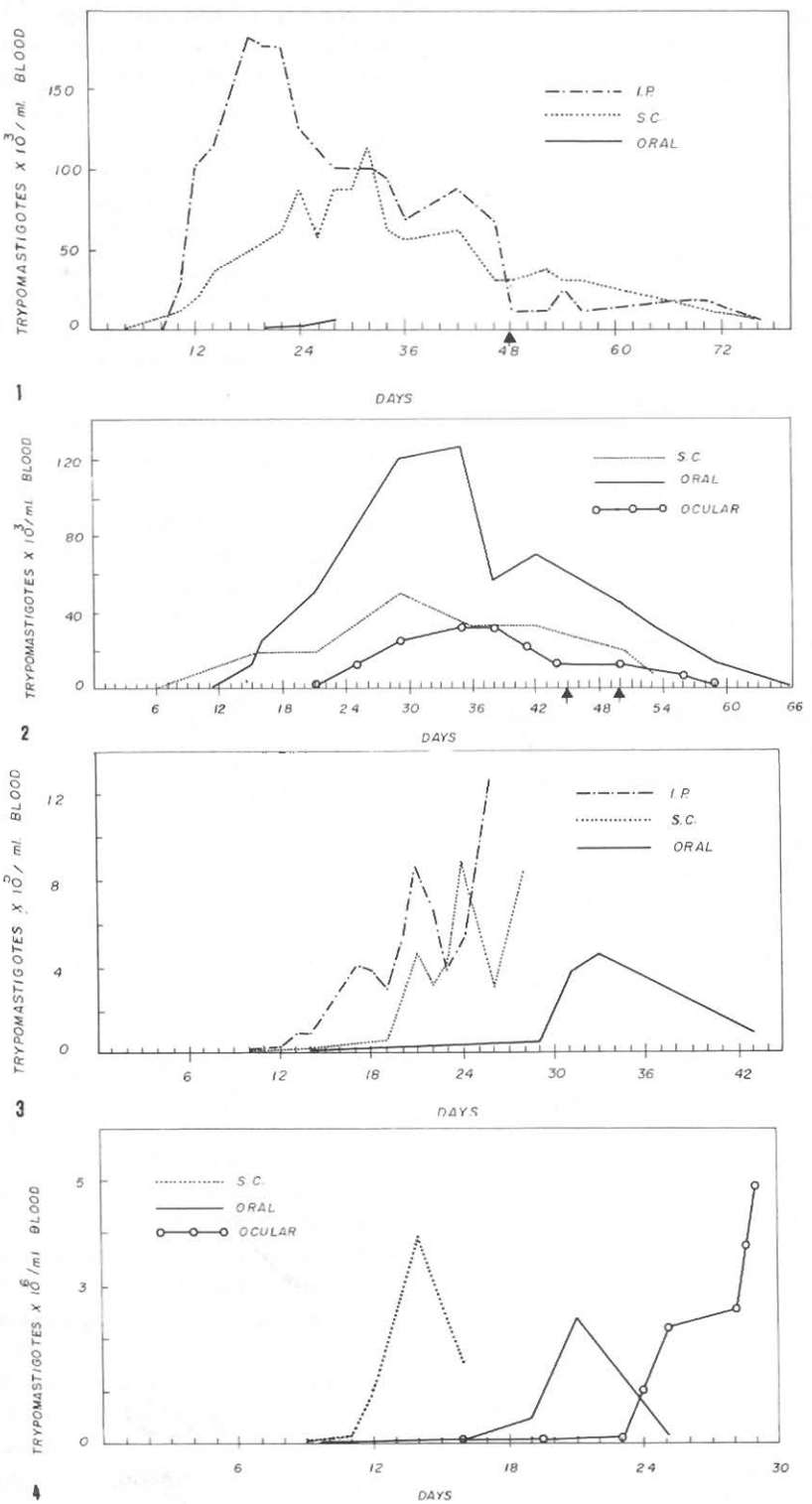
Opossums and mice were sacrificed by anesthetic overdose and cervical dislocation, respectively. Tissue samples were taken from heart, skeletal muscle, liver, spleen, small and large intestine, diaphragm, lung, and anal glands. These were fixed in 10% formalin, imbedded in paraffin, cut into 5μ sections, and stained with hematoxylin-eosin. Presence of parasites was microscopically (1000 X) checked by two independent observers.

Results

Both glandular material and cultures derived from it infected all animals. Parasitemias developed early in the young opossums inoculated i.p., s.c., or orally. Ocular inoculation in the young and oral in the adult delayed parasitemia (Figs. 1 & 2). Unfortunately, the adult died of other causes shortly after parasitemia was detected. The curves of parasitemia were similar in form for all methods of inoculation in the opossums, but none died.

Glandular and cultured metacyclics were equally virulent when inoculated i.p., s.c., and orally; lowest parasitemias were observed from cultured metacyclics inoculate s.c. and into the eye.

Control mice inoculated with glandular metacyclics (Fig. 3) showed parasitemias with longer prepatent periods and higher levels. The animals showed acute phases, dying with erection of hair, inhibition of movement, and copious urination. The control mice inoculated with cultured metacyclics showed



Parasitemias produced in opossums (figs. 1&2) and in mice (figs. 3 & 4) by inoculation of metatrypomastigotes of *Trypanosoma cruzi* obtained from anal glands of naturally infected urban *Didelphis marsupialis*, and from cultures of these forms, respectively. The arrows mark the days postinoculation in which the colonization of the anal glands of the experimentally infected opossums was detected.

infections which varied in prepatent period and time of peak parasitemia, according to route of inoculation (Fig. 4). The animals showed high levels of bloodstream forms, and all died. *Rhodnius* employed for xenodiagnosis produced metacyclics in feces, equally, virulent for mice. The final examinations, 6 months PI, showed blood parasites in the opossums. Those inoculated i.p. with glandular metacyclics, and orally and into the eye with cultured forms, had colonized anal glands with up to 17 x 10^4 metacyclics/ml at this time.

Tissue invasion in all experimental opossums and mice was primarily in cardiac and skeletal muscle, with amastigote nests in these tissues. Also, in mice inoculated i.p., there was invasion of smooth muscle of small and large intestine and lung. The liver was also invaded.

Discussion

The anal glands of the marsupials *D. marsupialis*, *D. albiventris*, and *Lutreolina crassicaudata* can be infected naturally or experimentally with *T. cruzi* (Deane *et al.*, 1984; Steindel & Pinto, 1988; Steindel *et al.*, 1988; Fernandez *et al.*, 1989; Urdaneta-Morales & Nironi, 1996). These infections show a double cycle of development, in the blood and in the anal glands, suggesting an ancient relationship between the parasite and its vertebrate host (Lenzi *et al.*, 1984).

In our experiments, glandular metacyclics from naturally infected *D. marsupialis* induced the double infection of *T. cruzi* when inoculated into young opossums i.p., orally, or into the eye. The blood flagellates of these animals infected *Rhodnius* and the glandular flagellates infected other opossums and mice.

Strains of *T. cruzi* isolated from *D. marsupialis* have generally shown low virulance in their proper reservoir hosts or in experimental animals, with transitory parasitemias usually detectable only indirectly and low tissue parasitism (Deane *et al.*, 1984; Bice & Zeledon, 1970; Barr *et al.*, 1991). In contrast, our experimental infections of mice with glandular metacyclics or cultures of these induced high parasitemias, with death of all animals, together with the intense tissue parasitism reported in a previous paper (Urdaneta-Morales & Nironi, 1996).

The content of the anal glands of opossums is forcibly expelled in territorial marking and in response to threat (Deane *et al.*, 1986). Also, the proximity of the anal glands to the end of the alimentary tract and to the urogenital organs would readily allow the contamination of feces and urine with glandular metacyclics (Lenzi *et al.*, 1984; Steindel & Pinto, 1988). These forms, in contact with buccal or conjuntival mucosa', readily induce *T. cruzi* infections. Thus, the contamination of foodstuffs eaten raw by the ejecta of opossums could lead to infections by *T. cruzi*, experimentally proven by Jansen & Deane (1985). This could easily explain outbreaks of Chagas' disease in areas where triatomines are essentially isolated from human beings (references in Lenzi *et al.*, 1984). The epidemiological potential for the valley of Caracas, where opossums naturally infected abound in contact with a large human population of marginal sanitation, needs no emphasis.

In our experimental opossums, glandular infections were detected when the initially high parasitemia had begun to decline, in agreement with other investigators (Lenzi *et al.*, 1984; Deane *et al.*, 1984; Jansen *et al.*, 1991), who postulated a correlation between the two parasitoses. According to Jansen (1988), invasion of the glands depends on a certain level of systemic parasitosis, prior to its decline. Thus, Lenzi *et al.* (1984) have suggested that the anal glands of the marsupials provide a habitat for the flagellate that protects it from the defense mechanisms of the animal.

Those animals with intense glandular infections showed high titers of circulatory anti- *T. cruzi* antibodies and were negative in parasitological examinations for long periods (Jansen, 1988). Our naturally and experimentally infected opossums continued to show glandular infections for up to 6 months post-infection, while parasitemias were apparently absent or only detectable by xenodiagnosis. This would explain the intermittent appearance of parasitemia in *D. marsupialis*, unusual for *T. cruzi* (Deane *et al.*, 1984; Lenzi *et al.*, 1984); the prolonged systemic infections characteristic for opossums (Jansen, 1988) would provide a guaranteed source of infection for the vector.

The presence of pseudocysts in the deep layers of the walls of the anal glands (Urdaneta-Morales & Nironi, 1996) would facilitate the passage of the

parasites to the lumen of the anal glands by rupture of these pseudocysts themselves. A similar mechanism for transmission of *T. cruzi* by urine in mice has been proposed by Herrera & Urdaneta-Morales (1992) upon finding parasitization of the urogenital organs in experimental mice.

Acknowledgements

The authors wish to thank Mr. Ian McLure for the English translation and aid in preparing the manuscript. Funds for the study were obtained from the Consejo Nacional de Investigaciones Cientificas y Tecnologicas (CONICIT-Venezuela) grant No. SI-2444, and from the Consejo de Desarrollo Científico y Humanistico de la Universidad Central de Venezuela, grant No. 03-31-3702-96.

Literature cited

Barr, S.C., C.C. Brown, V.A. Dnnis and T.R. Klei, 1991. The Lesions and Prevalence of *Trypanosoma cruzi* in Opossums and Armadillos from Southern Louisiana. *J. Parasitol., 77*: 624-627.

Bice, D.E. and R. Zeledon, 1970. Comparison of infectivity of strains of *Trypanosoma cruzi* (Chagas, 1909). *J. Parasitol., 56*: 663-670.

Brener, Z., 1962. Therapeutic activity and criterion of cure on mice experimentally infected with *Trypanosoma cruzi. Rev. Inst. Med. Trop. Sao Paulo, 4*: 389-396.

Deane, M.P., H.L. Lenzi and A.M. Jansen, 1984. *Trypanosoma cruzi* vertebrate and invertebrate cycles in the same mammal host, the opossum *Didelphis marsupialis. Mem. Inst. Oswaldo Cruz., 79*: 513-515.

Deane, M.P., H.L. Lenzi and A.M. Jansen, 1986. Double development cycle of *Trypanosoma cruzi* in the opossum. *Parasitology Today, 2*: 146-147.

Días, J.C., 1979. Mecanismos de transmissao. In: Z. Brener and Z. Andrade (Eds.) *Trypanosoma cruzi e doenca de Chagas*. P. 152-154, Guanabara Koogan, Brazil.

Fernández, A.J., L. Diotaiuti, J.C. Días, A.J. Romanha and E. Chiari, 1989. Infeccao natural das glandulas anais de gambas (*Didelphis albiventris*) pelo *Trypanosoma cruzi* no Municipio de Bambui, M.G.. *Mem. Inst. Oswaldo Cruz, 84*: 87-93.

García, E.S. and P. Azambuja, 1991. Development and interactions of *Trypanosoma cruzi* within the insect vector. *Parasitology Today, 7*: 240-244.

Herrera, L. and S. Urdaneta-Morales, 1992. *Didelphis marsupialis*: a primary reservoir of *Trypanosoma cruzi* in urban areas of Caracas, Venezuela. *Ann. Trop. Med. Parasitol., 86*: 607-612.

Jansen, A.M., 1988. Aspects of successful host-parasite association: opossum and *Trypanosoma cruzi. Mem. Inst. Oswaldő Cruz, 83*: (Supp. I) 491-496.

Jansen, A.M. and M.P. Deane, 1985. *Trypanosoma cruzi* infection of mice by ingestion of food contaminated with material of the anal glands of the opossum *Didelphis marsupialis. XII Reuniao Anual sobre Pesquisa Basica em Doenca de Chagas, Caxambu, Minas Gerais*, P. 39, BI-09.

Jansen, A.M., L. Leon, G.M,. Machado, M.H. da Silva, S. Souza-Leao and Deane, 1991. *Trypanosoma cruzi* in the opossum *Didelphis marsupialis*: Parasitological and serological follow-up of the acute infection. *Exp. Parasitol., 73*: 249-259.

Lenzi, H.L., A.M. Jansen and M.P. Deane, 1984. The recent discovery of what might be a primordial escape mechanism for *Trypanosoma cruzi. Mem. Inst. Oswaldo Cruz, 79*: 13-18.

Steidel, M. and C.J. Carvalho-Pinto, 1988. *Trypanosoma cruzi* developmental in the anal glands of experimentally infected *Lutreolina crassicaudata* (Marsupialia, Didelphidae). *Mem. Inst. Oswaldo Cruz, 83*: 397.

Urdaneta-Morales, S. and I. Nironi, 1996. *Trypanosoma cruzi* in the anal glands of urban opossums. *Mem. Inst. Oswaldo Cruz, 91*: 399-403.