Characterization of a *Trypanosoma rangeli* Strain of Colombian Origin

C Zuñiga, T Palau[*], P Penin[**], C Gamallo[***], JA de Diego[/+]**

Departamento de Medicina Experimental, Facultad de Medicina, Campus Occidente, Universidad de Chile, Santiago, Chile [*]Instituto Nacional de la Salud, Colciencias, Bogotá, Colombia [**]Unidad de Parasitología y Medicina Tropical, Departamento de Medicina Preventiva y Salud Pública, Facultad de Medicina, Universidad Autónoma de Madrid, c/ Arzobispo Morcillo 4, 28029 Madrid, España [***]Departamento de Anatomía Patológica, Hospital La Paz, Madrid, España

A Colombian strain of Trypanosoma rangeli *was characterized by analyzing its behaviour in different axenic and cellular culture, its infection rate and the histopathological lesions produced in experimental animals. Although slight inflammatory infiltrations were shown in different histopathological sections, no pseudocysts could be observed. Grace's insect medium is better than liver infusion tryptose or artificial triatomine urine supplemented with proline when studying* T. rangeli *metacyclogenesis, with a peak of 32% trypomastigotes. High infection rates were found in VERO and J774 cells. Because of its 100% infectivity rates and adequacy of parasitemia levels, C23 strain is a suitable model of* T. rangeli *biology study.*

Key words: *Trypanosoma rangeli* - histopathological study - culture - infection rate - Colombia

Trypanosoma rangeli is a trypanosome species that infects man in Central and South America where it can be found in mixed infections together with *T. cruzi* in both invertebrate and vertebrate hosts. While *T. cruzi* causes Chagas' disease, *T. rangeli* is considered nonpathogenic to man (D'Alessandro 1976, De Souza 1984). Mixed infections represent a serious problem to the differential diagnosis as it shares common antigenic determinants with *T. cruzi* (Guhl & Marinkelle 1982). Both parasites have a similar geographical distribution, the same vertebrate hosts, and, in some regions, identical insect vectors (D'Alessandro 1976, Guhl et al. 1985).

Study of parasitism of this flagellate in mammalian hosts has been hampered by the difficulty of obtaining bloodstream forms in the laboratory animals with very low parasitemias and short duration in experimental animals (Urdaneta-Morales & Tejero 1986). Another important fact is the scarcity of studies about the influence of several factors in the infection such as parasite strain, inoculum dose, hosts age and sex, and immune response related to the host. This kind of studies as well as those related to the mixed infections with *T. rangeli* and *T. cruzi* in experimental animals requires *T. rangeli* strains with high infectious capacity able to produce high and prolonged parasitemia levels which permits its evolution study during the infection.

The present paper describes the characterization of a Colombian strain of *T. rangeli* in several aspects such are: biological behaviour in acellular media, *in vitro* infections of cell line cultures and analysis of the mice infection with reference to the parasitemia evolution and the histopathological study in different tissues.

MATERIALS AND METHODS

Trypanosomes - Strain C23 of *T. rangeli* was used. It was isolated from a primate *Aotus trivirgatus* in 1982 in San Marcos, Sucre, Colombia and identified by polymerase chain reaction (PCR) (Campbell et al. 1993).

Experimental animals - Groups of 10 male mice, one month aged, of the strain Swiss ICO NMRI (IOPS) were used to study the parasitemia evolution and the histopathological analysis.

Parasitemia study - A group of 10 mice was inoculated intraperitoneally with 2.5 x 10^5 bloodstream trypomastigotes of *T. rangeli* from mice whose infection was previously stabilized. Parasitemias were determined daily until they were negative. For this study, blood of each mice were collected in a microhaematocrit tube (60 µl) by the method described by Arias and Ferro (1988).

Histopathological study - A group of 10 mice infected with 2.5 x 10^5 parasites was sacrified at

[+]Corresponding author. Fax: +34-1-397.5353. E-mail: JADEDIEGO@mvax.fmed.uam.es
Dr Zuñiga and Dr Palau benefited of BIBA Fellow, FIS and ICI Fellow, Madrid, Spain, respectively.
Received 9 September 1996
Accepted 21 March 1997

days 8, 10, 13, 20, 28 and 30 postinfection (p.i.). For each interval a study of heart, brain, skeletal muscle, liver, spleen and colon was made. Sections were fixed in formalin and embedded in paraffin and 8 µm thick sections were stained with haematoxylin and eosyin. The severity of inflammatory infiltration or pseudocysts presence was graded on a 3-point scale: + indicates less of 25% of affected area; ++ between 25 and 50% of affected area; and +++: more than 50% of affected area.

Study of the metacyclogenesis - Transformation studies in three acellular media: liver infusion tryptose (LIT) (Camargo 1964), Grace (Grace 1962, Sullivan 1982) and artificial triatomine urine supplemented with proline (TAUP) (Contreras et al. 1988) were made. Parasites were preincubated for 2 hr in artificial triatomine urine (TAU) (190 m*M* NaCl, 17 m*M* KCl, 2 m*M* $MgCl_2$, 2 m*M* $CaCl_2$, 0.035% NaHCO3, 8 m*M* phosphate buffer pH 6.0) prior the study in TAUP medium. An initial population of 10^4 parasites was incubated at 26ºC in an acellular culture of a total volume of 10 ml into sterile plastic tissue culture flasks of 25 cm^2 surface area (Costar® N. 3055, Cambridge). Evolution of parasite transformation was made each five days by examining a drop of medium fixed in methanol and stained in Giemsa stain. Metacyclic forms were counted in a total of 200 parasites.

Study of in vitro *infectivity* - A VERO cell line and a murine reticulum sarcoma line (J774) were cultivated originally at 37ºC in Dulbecco modified medium, pH 7.2, established by Dulbecco and Freeman (1959) supplemented with 10% of heat-inactivated fetal calf serum. The second cell line having a similar behaviour to the macrophage culture cells line (Ralph et al. 1975).

Cells were previously counted and centrifuged and adhesion took place onto a 12 mm diameter cover-slides placed into tissue culture flasks (25 cm^2 growth area) containing 5 ml of medium which were incubated at 37ºC for 24 hr waiting for cell adhesion. Parasites were collected from an axenic culture in a metacyclic known percentage, leading a 10 parasite/cell relation. Cells were exposed for 24 hr to parasites and incubated at 37ºC. At the end of the exposure period, non interiorized parasites were removed by repeated washings. Cultures were observed daily the first 3 days. Days 4, 6 and 8, we took out cover-slides that were washed with PBS, stained in Giemsa stain and put onto slides.

Infection index was evaluated by counting the infected cell number and intracellular forms randomly chosen in 200 cells.

All assays were made twice.

In order to rule out any possibility of *T. cruzi* contamination of the studied samples, PCR test was carried out once more at the end of the experiments.

RESULTS

Data concerning the development of the parasite in mice, one month old, infected with 2.5 x 10^5 parasites of the C23 strain of *T. rangeli* are shown in Fig. 1. Prepatent period was of 4 days and the peak of parasitemia reached 10^6 parasites/ml at day 14 p.i. No bloodstream parasites were seen after day 30 p.i. Although every inoculated mouse was infected, no mouse infected with *T. rangeli* died.

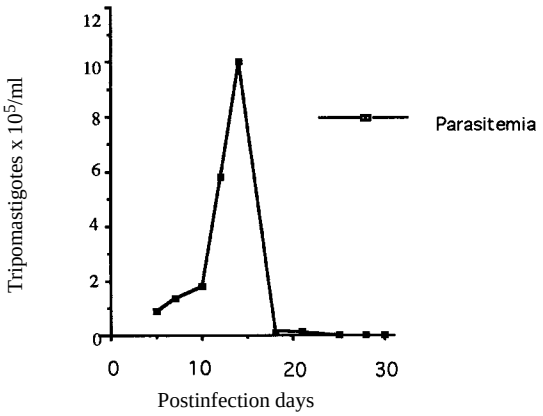

Fig. 1: *Trypanosoma rangeli*. Parasitemia evolution in mice, one month aged, infected with 25 x 10^4 trypomastigotes.

The histopathological study disclosed slight inflammatory infiltration foci in brain, skeletal muscle and liver sections. No one of the studied tissues showed pseudocysts (Table I).

Comparative study of metacyclogenesis of the C23 strain in the three culture media was done in Fig. 2. The best data of transformation were ob-

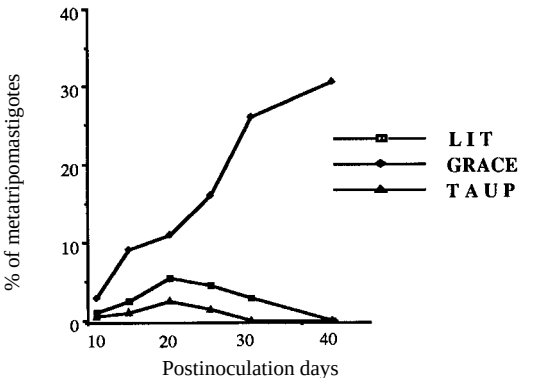

Fig. 2: comparative study of the metacyclogenesis of *Trypanosoma rangeli* in three axenic media; LIT: liver infusion tryptose; TAUP: artificial triatomine urine supplemented with proline.

TABLE I

Trypanosoma rangeli (C23 strain). Histopathological study in mice

Postinoculation days		8	10	13	20	28	30
Parasitemia [a]		134	180	1000	13	1	-
Heart	Inflammation	-	-	-	-	-	-
	pseudocysts	-	-	-	-	-	-
Brain	Inflammation	-	-	-	-	+	-
	pseudocysts	-	-	-	-	-	-
Skeletal muscle	Inflamation	-	-	-	-	+	-
	pseudocysts	-	-	-	-	-	-
Spleen	Inflammation	-	-	-	-	-	-
	pseudocysts	-	-	-	-	-	-
Liver	Inflammation	+	-	-	-	-	-
	pseudocysts	-	-	-	-	-	-
Colon	Inflammation	-	-	-	-	-	-
	pseudocysts	-	-	-	-	-	-

a: parasitemia measured in parasites x 10^3/ml; + < 25 % ; ++ between 25 % and 50% ; +++ > 50% of affected tissue

TABLE II

Vero and J774 cells infected with *Trypanosoma rangeli* (C23 strain)

Postinfection days	% infected cells	% cells with amastigotes	No. amastigotes x infected cells[a]	% cells with trypomastigotes	No. trypomastigotes x infected cells[a]
VERO cells					
4	46	36	10	19	13
6	50	36	87	20	106
8	62	58	43	6	75
J774 cells					
4	47	53	24	1	33
6	47	39	31	7	53
8	56	50	23	5	22

a: mean values of infected cells

served in Grace's insect medium with a peak of 32% at day 40 p.i. When LIT and TAUP media were used the initial parasite development had a progressive decline since day 20 p.i. in both cases, sharing peaks of 15% and 6%, respectively.

Results of *in vitro* infectivity after 4 hr of incubation showed a large number of parasites adherent to cells, few of them appearing inside. At day 4 p.i. 46% of VERO cells and 47% of J774 cells showed amastigote and/or trypomastigotes forms enclosed within them. After 6 days p.i. 50% of VERO cells and 47% of J774 cells were infected, but intracellular parasites were at this time increased. Day 8 p.i. seems to show the beginning of a second cycle of infection with a large number of cells containing amastigote forms but less quantity being infected with trypomastigote forms (Table II). Figs 3 to 7 showed VERO and J774 cells infected by *T. rangeli* at different days p.i.

Infection with bloodstream parasites shared no significant rate of infection.

The absence of contamination of the *T. rangeli* utilized in this study was confirmed by PCR.

Fig. 3: VERO cells infected with *Trypanosoma rangeli* at day 4 postinfection. Giemsa X 400.

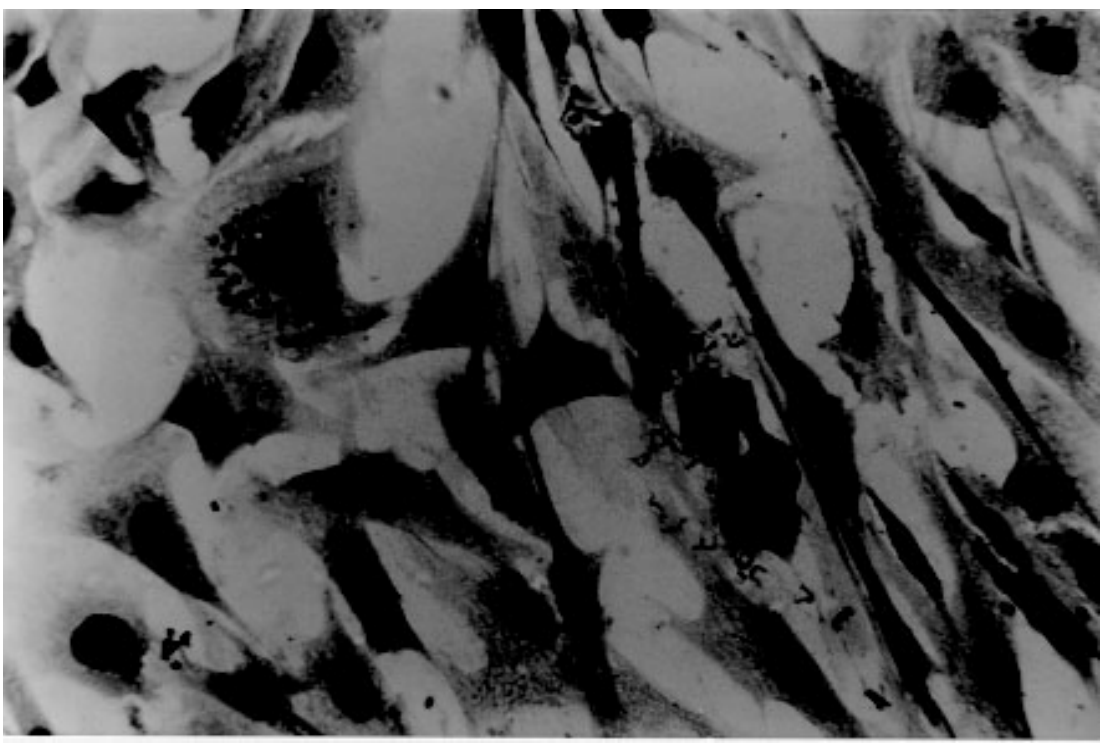

Fig. 4: VERO cells infected with *Trypanosoma rangeli* at day 6 postinfection. Giemsa X 400.

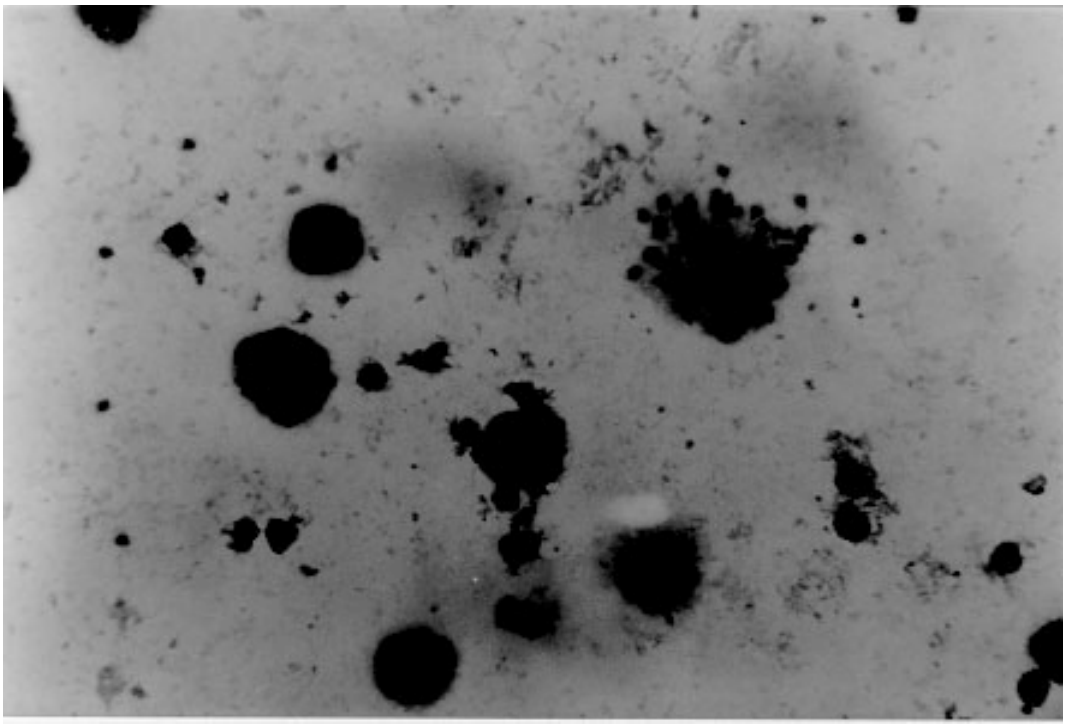

Fig. 5: J774 cells infected with *Trypanosoma rangeli* at day 4 postinfection. Giemsa X 400.

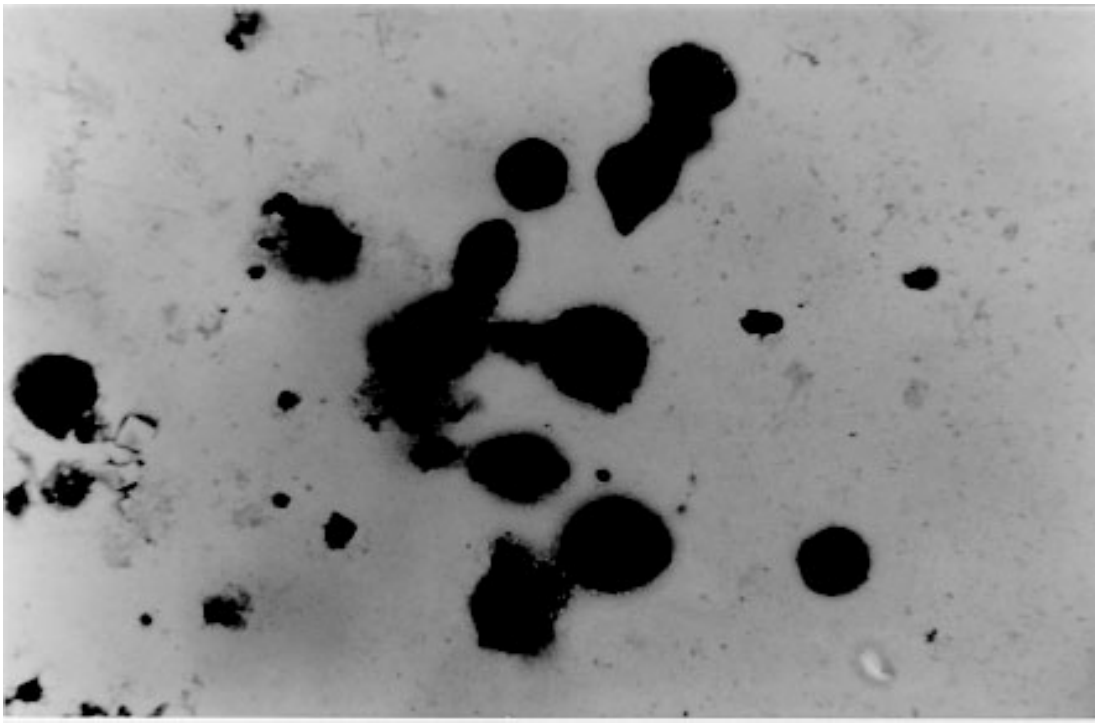

Fig. 6: J774 cells infected with *Trypanosoma rangeli* at day 6 postinfection. Giemsa X 400.

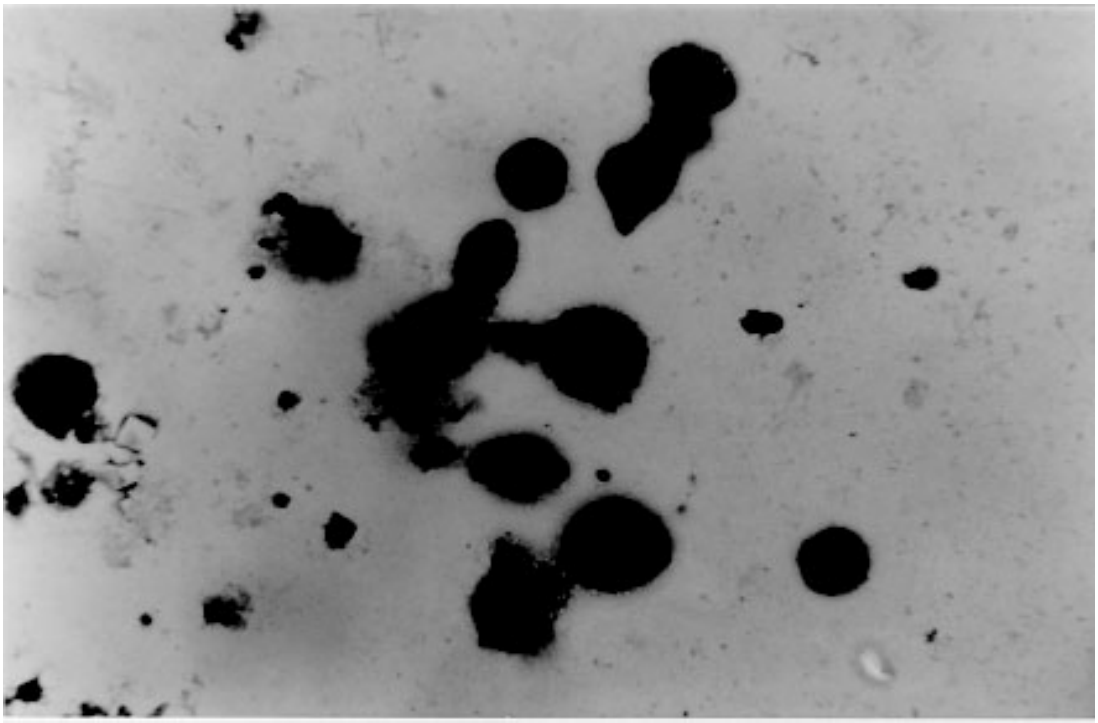

Fig. 7: J774 cells infected with *Trypanosoma rangeli* at day 8 postinfection. Giemsa X 200.

DISCUSSION

Important factors are decisive to get high levels of parasitemia in the experimental infection with *T. rangeli* other than the used strain, such as the number of trypomastigote forms contained in the inoculum. When difficulties to obtain a large number of bloodstream parasites were found, it could be interesting to have a culture medium with a good transformation rate from epimastigote to trypomastigote forms. The Grace medium showed to be the best of the studied media to obtain metatrypomastigotes (32%) in contrast with the LIT medium which shared a transformation similar with those reported for other *T. rangeli* strains (Urdaneta-Morales & Tejero 1985). TAUP medium was not suitable to *T. rangeli* growth and transformation differing from most strains of *T. cruzi* (Homsy et al. 1989).

Metacyclic forms of C23 strain obtained from Grace culture showed to have a high infectious capacity in VERO cells and J774 cells after 4 days of incubation of parasites and cell lines together. When *T. cruzi* has been observed during the *in vitro* host-paratise interaction, varied results have been obtained: metacyclic forms of the Dm 28c clone, obtained after 72-96 hr of differentiation in an axenic medium are able to associate to 30-60% of heart muscle or fibroblastic cells within 2 hr of parasite contact and 30-50% of the associated parasites are already interiorized (Contreras et al. 1988). Indeed, authors working with bloodstream trypomasytigotes and non-professional phagocytic cells like VERO or BEMS cells (Bertelli & Brener 1980), fibroblasts (Kongtong & Inoki 1975), primary heart or skeletal muscle cells (Araujo-Jorge et al. 1986, Meirelles et al. 1986) pointed out to the need of at least 9-24 hr of parasite contact to obtain indexes varying between 1 and 9% of infected cells, depending on the parasite strain used. Comparative studies between *T. rangeli* and *T. cruzi* strains are controversial due to the heterogeneity of clonal populations within a *T. cruzi* strain (Deane et al. 1984, Dvorak 1984, Tibayrenc & Ayala 1988, 1991, De Diego et al. 1991, Penin et al. 1996).

In spite of *T. rangeli* intracellular multiplication could remain in the speculative field, some authors have been able to find parasite tissular forms in vertebrate hosts (Urdaneta & Tejero 1986). Moreover, the viability of *T. rangeli* intracellular parasites has been suggested to be able to survive intracellularly over extended periods (Osorio et al. 1995).

Few inflammatory infiltrates were found in the histopathological sections, suggesting as Osorio et al. (1995) that *T. rangeli* infection did not elicit a strong host response, in agreement with the low antigenicity of natural infections by *T. rangeli* (D'Alessandro-Bacigalupo & Saravia 1992) and the low and brief immune responses observed in experimentally infected animals (Añez et al. 1985, Urdaneta-Morales & Tejero 1986).

The scarcity of intracellular forms and the possibility that parasites could be localized in lymphoreticular tissues have been argued as causes of failure to detect amastigote forms in tissular sections of the experimentally infected animals (Deane 1969, Osorio et al. 1995) as shown in our data. However, some lymphoreticular tissues have been also studied by us, such are lymph nodes. Special attention was also made to the visceral capillaries with negative results.

Although it is well known that *T. rangeli* is difficult to establish in experimental animals (Hoare 1972) as shown by characteristically low and inconstant parasitemias, C23 strain of *T. rangeli* have been shown to produce high and prolonged levels of parasitemia, high infection rates in VERO and in J774 cells without serious pathological tissular findings which makes it a good model to study *T. rangeli* in a murine model due to its easy maintenance by mice passages. This strain could also be an interesting model to study the mixed infection by *T. rangeli* and *T. cruzi* for high, sustained and without histopathological damage.

ACKNOWLEDGEMENTS

To M Ayala and S Nicholls, Instituto Nacional de Salud, Bogotá, Colombia for providing the C23 strain of *T. rangeli.*

REFERENCES

Añez N, Velandia J, Rodriguez AM 1985. Estudios sobre *Trypanosoma rangeli* Tejera 1920. VIII. Respuestas a las reinfecciones en dos mamíferos. *Mem Inst Oswaldo Cruz 80*: 149-153.

Araujo-Jorge TC, Barbosa HS, Moreira AL, de Souza W, Meirelles MNL 1986. The interaction of myotropic and macrophagotropic strains of *Trypanosoma cruzi* with myoblasts and fibers of skeletal muscle. *Z Parasitenkd 72*: 577-584.

Arias AR, Ferro E 1988. Quantification of *Trypanosoma cruzi* parasitaemia by direct micromethod. *Trans R Soc Trop Med Hyg 82*: 248.

Bertelli MST, Brener Z 1980. Infection of tissue culture cells with bloodstream trypomastigotes of *Trypanosoma cruzi*. *J Parasitol 66*: 992-997.

Camargo EP 1964. Growth and differentiation in *Trypanosoma cruzi*. I. Origin of metacyclic trypanosomes in liquid media. *Rev Inst Med Trop São Paulo 6*: 93-100.

Campbell D, Gonzalez C, Jaramillo C, Montilla M, Rojas W, Labrada L, Lopez W, Mejia D, Osorio Y, Santrich C 1993. Resúmen del taller sobre el uso de la reacción en cadena de la polimerasa (PCR) para distinguir entre *Trypanosoma cruzi* y *Trypanosoma rangeli*. *Biomédica* (Colombia) *2*: 94-101.

Contreras V, Araujo-Jorge T, Bonaldo M, Thomaz N, Barbosa H, Merielles M, Goldenberg S 1988. Biological aspects of the DM 28c clones of *Trypanosoma cruzi* after metacyclogenesis in chemically defined media. *Mem Inst Oswaldo Cruz 83*: 123-133.

D'Alessandro A 1976. The biology of *Trypanosoma rangeli* Tejera 1920, p. 327-403. In WHR Lumsden, DA Evans (eds), *Biology of the Kinetoplastida*, vol. 1. Academic Press, London, New York, San Francisco.

D'Alessandro-Bacigalupo D, Saravia NG 1992. *Trypanosoma rangeli*, p. 1-54. In JP Kreier, JR Baker (eds), *Parasitic protozoa*, Vol. 2. Academic Press, New York.

De Diego JA, Penin P, del Rey J, Mayer R, Gamallo C 1991. A comparative pathological study of three strains of *Trypanosoma cruzi* in an experimental model. *Histol Histopathol 6*: 199-206.

De Souza W 1984. Cell biology of *Trypanosoma cruzi*. *Int Rev Cytol 86*: 197-283.

Deane MP 1969. On the life cycle of trypanosomes of the lewisi group and their relationships to other mammalian trypanosomes. *Rev Inst Med Trop São Paulo 11*: 34-43.

Deane MP, Jansen AM, Mangia RHR, Gonçalves AM, Morel CM 1984. Are our laboratory strains representative samples of *Trypanosoma cruzi* population that circulate in nature? *Mem Inst Oswaldo Cruz 79*: 19-24.

Dulbecco R, Freeman G 1959. Plaque production by the Polyoma virus. *Virology 8*: 396-397.

Dvorak JA 1984. The natural heterogeneity of *Trypanosoma cruzi*. Biological and medical implications. *J Cel Biol 24:* 357-360.

Grace TDC 1962. Establishment of four strain of cells from insect tissue grown *in vitro*. *Nature 195*: 788-789.

Guhl F, Marinkelle C 1982. Antibodies against *Trypanosoma cruzi* in mice infected with *Trypanosoma rangeli*. *Ann Trop Med Parasit 76*: 361.

Guhl F, Hudson L, Marinkelle C, Morgan S, Jaramillo C 1985. Antibody response to experimental *Trypanosoma rangeli* infection and its implications for immunodiagnosis of South American trypanosomiasis. *Acta Trop 42*: 311-318.

Hoare CA 1972. *Herpetosoma* from man and others mammals: *Trypanosoma (Herpetosoma) rangeli*, Tejera 1920, p. 288-313. In *The trypanosomes of mammals: a zoological monograph.* Blackwell Scientific Publications, Oxford.

Homsy J, Granger B, Krassner S 1989. Some factors inducing formation of metacyclic stages of *Trypanosoma cruzi*. *J Protozool 2*: 150-153.

Kongtong P, Inoki S 1975. Scanning electron microscopic studies on penetration of *Trypanosoma cruzi* into fibroblastic cells. *Jap J Parasitol 24*: 284-293.

Meirelles MNL, Araujo-Jorge TC, Miranda CF, de Souza

W, Barbosa HS 1986. Interaction of *Trypanosoma cruzi* with heart muscle cells: ultrastructural and cytochemical analysis of endocytic vacuole formation and effect upon myogenesis. *Eur J Cell Biol 41*: 198-206.

Osorio Y, Travi BL, Palma GI, Saravia NG 1995. Infectivity of *Trypanosoma rangeli* in a promonocytic mammalian cell line. *J Parasitol 81*: 678-693.

Penin P, Gamallo C, de Diego JA 1996. Biological comparison between three clones of *Trypanosoma cruzi* and the strain of origin (Bolivia) with reference to clonal evolution studies. *Mem Inst Oswaldo Cruz 91*: 285-291.

Ralph P, Prichard J, Cohn M 1975. Reticullum cell sarcoma: an effector cell in antibody-dependent cell-mediated immunity. *J Immunol 2*: 898-905.

Sullivan JJ 1982. Metacyclogenesis of *Trypanosoma cruzi in vitro:* A simplified procedure. *Trans R Soc Trop Med Hyg 3*: 300-303.

Tibayrenc M, Ayala FJ 1988. Isoenzyme variability in *Trypanosoma cruzi*, the agent of Chagas' disease. Genetical, taxonomical and epidemiological significance. *Evolution 42*: 277-292.

Tibayrenc M, Ayala FJ 1991. Towards a population genetic of microorganisms: the clonal theory of parasitic protozoa. *Parasitol Today 7*: 228-232.

Urdaneta-Morales S, Tejero F 1985. *Trypanosoma (Herpetosoma) rangeli* Tejera 1920: Mouse model for high sustained parasitaemia. *J Parasitol 4*: 409-414.

Urdaneta-Morales S, Tejero F 1986. *Trypanosoma (Herpetosoma) rangeli* Tejera 1920: Intracellular amastigote stages of reproduction in white mice. *Rev Inst Med Trop São Paulo 28*: 166-169.