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BIOLOGICAL ASPECTS OF THE DM 28C CLONE OF *TRYPANOSOMA CRUZI* AFTER METACYCLOGENESIS IN CHEMICALLY DEFINED MEDIA

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The biological characterization of the Trypanosoma cruzi *clone Dm 28c in terms of its growth in LIT medium, cell-cycle, infectivity to mice and interaction with professional and non-professional phagocytic cells shows that it behaves as a bona fide* T. cruzi *representant. The biological properties of this myotropic clone do not change according to the origin of the trypomastigote forms (i. e., from triatomines, infected mice, cell-culture or from the chemically defined TAUP and TAU3AAG media). In addition Dm 28c metacyclic trypomastigotes from TAU3AAG medium display a high infectivity level to fibroblasts and muscle cells. Experiments on binding of cationized ferritin to trypomastigotes surface show the existence of cap-like structures of ferritin in regions near the kinetoplast, however the nature and role of these anionic sites remain to be determined. The results indicate that metacyclic trypomastigotes from the Dm 28c clone obtained under chemically defined conditions reproduce the biological behaviour of* T. cruzi, *rendering this system very suitable for the study of cell-parasite interactions and for the isolation of trypanosome relevant macromolecules.*

Key words: *Trypanosoma cruzi* – metacyclogenesis – Chagas' disease – muscle cells

The life cycle of *Trypanosoma cruzi*, the ethiological agent of Chagas disease (Chagas, 1909), comprises three distinct morphological and functional types (Brener, 1973; De Souza, 1984). The transformation of epimastigotes to metacyclic trypomastigotes (metacyclogenesis process) deserves special interest since this process occurs naturally within the triatomine insect vector and involves differential gene expression (Goldenberg et al., 1984), which will ultimately enable this form to infect mammals.

Several complex *in vitro* differentiating systems have been developed allowing the transformation of epimastigotes to metacyclic trypomastigotes (Camargo, 1964; Castellani et al., 1967; Chiari, 1975; Sullivan, 1983; Contreras et al., 1985a). Recently, chemically defined in vitro differentiating conditions have been developed by us (Contreras et al., 1985b; Goldenberg et al., 1987) supporting reproducibility the metacyclogenesis of *T. cruzi*. The establishment of such in vitro differentiating media paves the way for the study of the mechanisms involved in the regulation of gene expression in *T. cruzi* as well as a model system for studying the interaction of the parasite with its invertebrate host.

However, in order to perform these studies, one must be sure that the differential gene expression observed during the metacyclogenesis process is not due to the heterogeneity of the clonal populations within a *T. cruzi* strain (Deane et al., 1984; Dvorak, 1985). This leads to the necessity of using genetic homogeneous populations (clones) for parasite differentiation studies.

Accordingly, we are presently using a clone (Dm 28c) to study *T. cruzi* differential gene expression, and therefore we present here the analysis of several biological properties of such *T. cruzi* clone.

MATERIALS AND METHODS

Parasites: T. cruzi Dm 28 strain was originally isolated from the opossum *Didelphis*

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marsupialis. The Dm 28c clone was obtained as described by Goldenberg et al. (1984). Briefly, *T. cruzi* Dm 28 trypomastigotes were diluted in culture medium to a concentration of one parasite per five microliters and used to orally infect new born mice. Three clones were obtained (Dm 28a, Dm 28b and Dm 28c) with respective prepatencies of 8, 12 and 17 days. *T. cruzi* Dm 28c clone was used, being maintained by axenic culturing in LIT medium (Camargo, 1964) and in triatomines with a passage through vertebrate and invertebrate hosts every six months.

T. cruzi Dm 28c metacyclic trypomastigotes were obtained using either the TAUP (Contreras et al., 1985b) or TAU3AAG (Goldenberg et al., 1987) chemically defined differentiating media. Briefly, epimastigotes from late exponentially growing phase in LIT medium were harvested by centrifugation at 8,500 xg and incubated for 2 h in artificial triatomine urine (TAU, 190 mM NaCl, 17 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 8 mM phosphate buffer pH 6.8) in a density of 5×10^8 cells/ml. Thereafter, the parasites were incubated in TAUP (TAU supplemented with 10 mM L-proline) or TAU3AAG (TAUP supplemented with 50 mM L-glutamate, 2 mM L-aspartate, 10 mM glucose) media to a final concentration of 5×10^6 cells/ml in a final volume of 10 ml in 25 cm^2 falcon flasks, and incubated at 28°C for 96 h.

Bloodstream trypomastigotes were obtained by differential centrifugation of the blood from swiss albino male mice, infected with axenic culture-derived metacyclic forms. Parasitemia was followed through the acute stage of murine infection as described elsewhere (Deane et al., 1984). Cell culture-derived trypo and amastigotes were obtained from the supernatant of infected *in vitro* cultured cells (LA-9 fibroblasts, primary fibroblasts, Vero cells or LLCMK2 cells). Triatomine-derived metacyclic trypomastigotes were obtained as described elsewhere (Garcia et al., 1984).

In vitro cellular infection: Primary culture cells from heart tissue of mouse embryos yielded the heart muscle cells (HMC) and fibroblasts (Fib) used, obtained as previously described (Meirelles et al., 1986). Cells were maintained in Dulbecco's modified Eagle medium (DME) supplemented with 10% horse serum, 10% fetal calf serum (FCS), 2% chicken embryo extract, 1% L-glutamine, 1000 U/ml penicillin and 50 μg/ml streptomycin. HMC and Fib were cultivated for 3-7 days prior the interaction assay, with daily changes of the medium. Macrophages (MO) were obtained from normal mouse by peritoneal wash and cultivated in DME-FCS 10% for 24 h before the interaction assay. For quantitative evaluation of parasite-cell interaction, the cells were spread over round glass coverslips in 24-wells culture plates (Nunclon). The interaction experiments were performed at 37°C in a 5% CO_2 humidified atmosphere, in DME without serum for 1-2 h. The cultures were then washed and fixed in Bouin and stained with Giemsa. The percentage of infected cells and the total number of parasites per 100 cells were evaluated in each slide from a total sample of 200 cells/slide, counting triplicates in each experiment. In some assays, an hypotonic shock (30 seconds with 1:5 water diluted phosphate-buffered saline) was applied to the cultures before fixation, resulting in the lysis of adherent parasites and allowing the quantification of interiorized parasites (Andrews & Colli, 1982). For ultrastructural analysis the cells were spread over culture milk flasks and infected with parasites for different periods of time before fixation in glutaraldehyde.

Morphological analysis: The morphology of the parasites and their intracellular fate in infected mammalian cell cultures were studied in Giemsa-stained preparations using a Zeiss photomicroscope.

Histopathological studies: Three infected mice, casually chosen from a lot of 10 animals inocculated with metacyclic trypomastigote forms from the urine of *R. prolixus*, were necropsiated. The organs were fixed in 10% formol and included in paraffin. Thick sections (5 μm) were stained with haematoxilin-eosin and observed in a Zeiss photomicroscope.

Ultrastructural analysis: The isolated parasites or the infected cultures were washed and fixed in 2.5% glutaraldehyde in 0.1 M cacodilate buffer pH 7.2. The infected cell cultures were scraped off with a rubber-policeman and further processed in suspension like the parasites, with sequential series of centrifugations and resuspensions. The fixed parasites or infected cells were washed and post-fixed in 1% OsO_4 in cacodylate buffer, dehydrated in acetone and embedded in Epon. Ultrathin sections were contrasted with uranyl acetate

and lead citrate and observed in a Zeiss EM 10 electron microscope at 50 KV. For anionic sites detection the parasites were treated with 1 mg/ml cationized ferritin after glutaraldehyde fixation (Danon et al., 1972).

RESULTS

Parasitological and morphological aspects of the Dm 28c clone: The growth curve of *T. cruzi* Dm 28c in LIT medium at 28°C showed that the stationary phase is attained at the 8th day (results not shown). *T. cruzi* Dm 28c displayed three distinct morphological types depending upon the localization of the kinetoplast and the presence of the flagellum. The replicative stage in axenic culture was a typical epimastigote form (Fig. 1), similar to those which replicate within the invertebrate host. Trypomastigotes were observed in the supernatant and within *in vitro* cultured cells infected with *T. cruzi,* and circulating in the blood stream of infected animals (Fig. 2). In this case the parasites displayed a typical stout form (Fig. 2). We also observed trypomastigotes in the excreta of infected triatomines (Fig. 3) and in the chemically defined differentiating media developed by us (Fig. 4). The non-flagellated amastigote forms were observed within infected mammalian cells cultured *in vitro* (Fig. 5) or in different tissues of infected animals, namely heart muscle cells (Fig. 6) and spleen cells (Fig. 7). Ultrastructural analysis of these different forms confirmed their characteristics of epimastigotes, trypomastigotes and amastigotes, as reviewed by De Souza (1984).

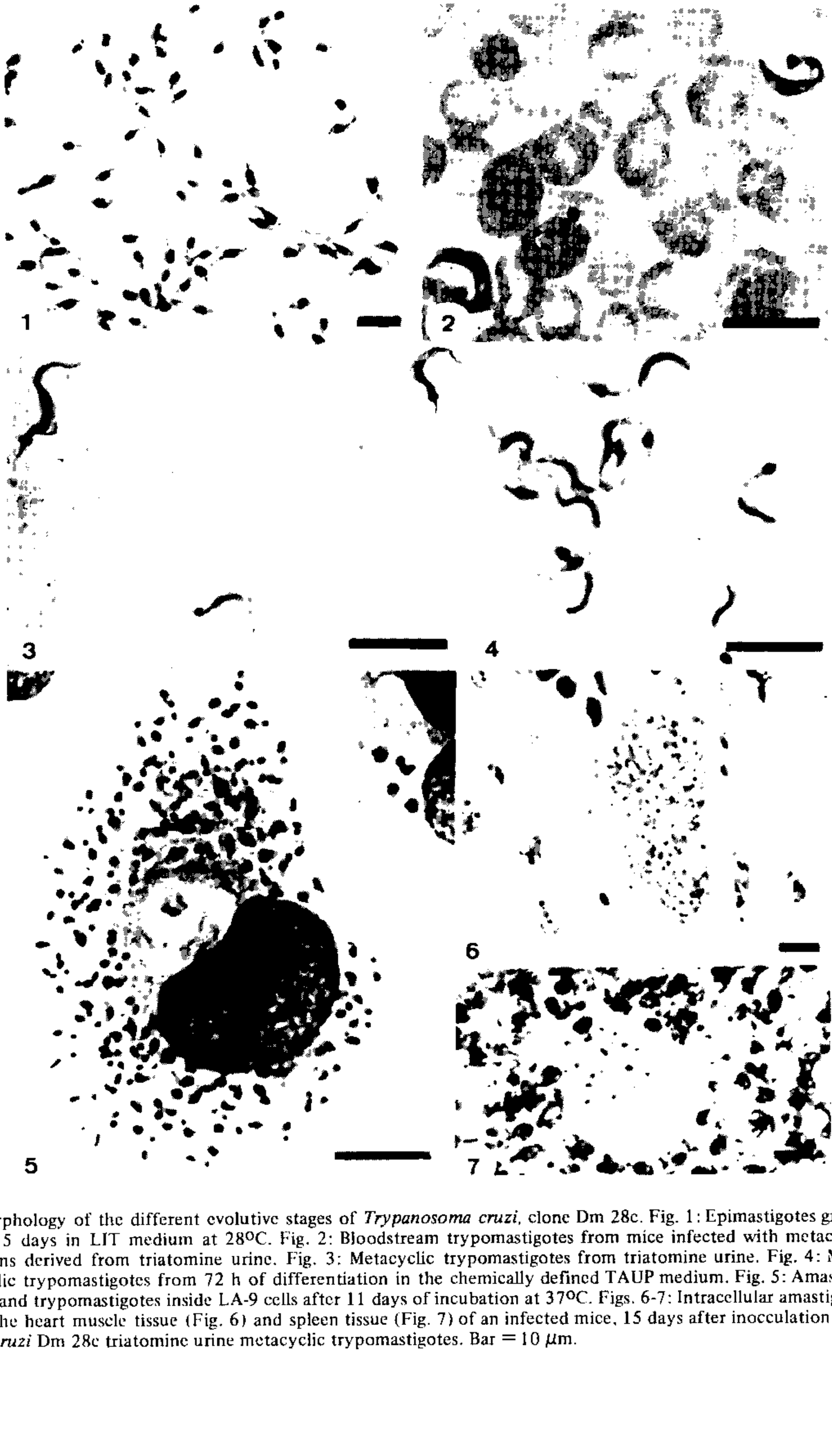
The characterization of the surface anionic sites of trypomastigote forms showed a coat of cationized ferritin particles (Figs. 8-12) in parasites derived from cell culture supernatants (Figs. 8-10), from the chemically-defined TAU3AAG medium (Figs. 11-12) and from the blood of infected mice (not shown). In the case of cell culture derived trypomastigotes, the cationized ferritin is disposed in a thick coat formed by 5-7 superimposed layers of particles in most of surface extension areas, with the thinnest regions having at least three layers (Figs. 8-10). Cap-like structures of ferritin could be seen in regions of the plasma membrane near the kinetoplast of parasites derived from cell culture supernatants and from TAU3AAG medium (Figs. 8-10, 12) or the flagellum (Fig. 11), with 18-25 layers of particles.

In vivo infectivity of the Dm 28c clone: In order to investigate whether *T. cruzi* Dm 28c trypomastigotes display the biological properties of such forms, we have infected mice with blood trypomastigotes, triatomine urine metacyclic trypomastigotes and metacyclic trypomastigotes from the chemically defined TAUP medium. The results are presented in Table I and show that 100% of the mice became infected regardless of the origin of the trypomastigotes, although the animals infected with *in vitro* differentiated metacyclic trypomastigotes presented an increased prepatency period. However, the parasitaemia curves of the animals infected with different Dm 28c metacyclic trypomastigotes were essentially the same peaking at the 17th day (Fig. 13).

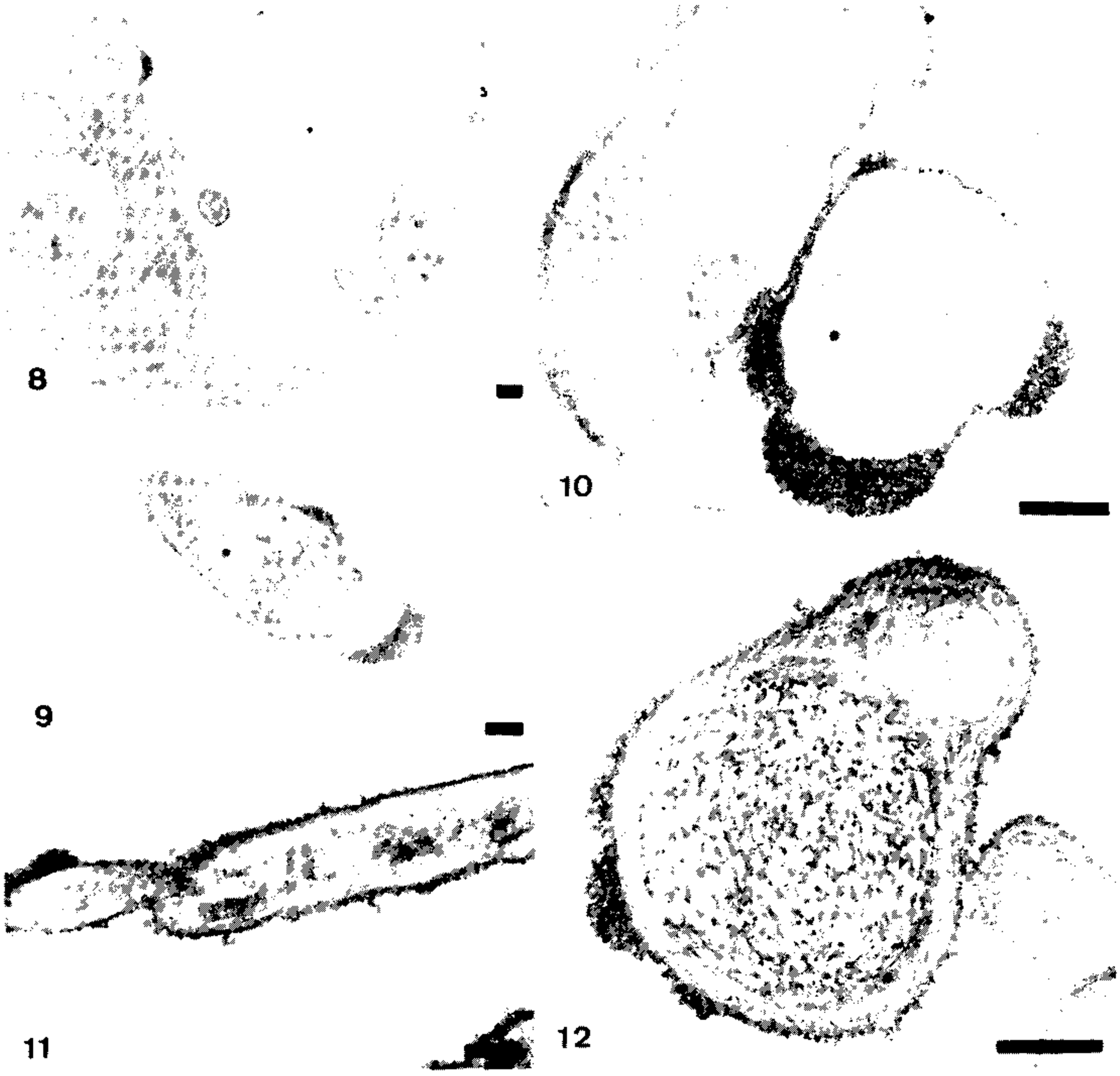
The study of the histotropism of the clone Dm 28c showed that out of 16 organs analysed, 8 were infected with parasites (Table II). The analysis of the infected organs indicates that the Dm 28c clone is myotropic according to the criteria of Melo & Brener, 1978.

A last point to confirm the *T. cruzi* nature of the Dm 28c clone is its ability to confer to infected mice the resistance to reinfection by a well-characterized *T. cruzi* strain. The results presented in Table III show that indeed animals infected with *T. cruzi* Dm 28c are resistant to reinfection with *T. cruzi* Y strain.

In vitro infectivity of the Dm 28c clone: We have also investigated the ability of *T. cruzi* Dm 28c metacyclic trypomastigotes to infect *in vitro* mice peritoneal macrophages and primary cultures of mice fibroblasts or heart and skeletal muscle cells. We could verify that the Dm 28c clone displayed high infectivity levels to both professional and facultative phagocytic cells, even with interaction periods as short as 1 or 2 h (Table IV). After one hour of parasite-cell incubation, 20 to 40% of the macrophages, 20 to 30% of the fibroblasts and 10 to 15% of the myocardial cells showed associated (adherent and/or interiorized) parasites. In the second hour more parasites were found associated to the cells and could be studied at the ultrastructural level. Regions of parasite – muscle cell apposition were seen by both the parasite body and flagellum (results not shown). In some cases, an area of stronger electron density could be seen in the adherence zone. Bloodstream trypomastigotes from the Dm 28c clone seem to associate slightly more than TAU3AAG derived metacyclic trypomastigotes.



Morphology of the different evolutive stages of *Trypanosoma cruzi*, clone Dm 28c. Fig. 1: Epimastigotes grown for 5 days in LIT medium at 28°C. Fig. 2: Bloodstream trypomastigotes from mice infected with metacyclic forms derived from triatomine urine. Fig. 3: Metacyclic trypomastigotes from triatomine urine. Fig. 4: Metacyclic trypomastigotes from 72 h of differentiation in the chemically defined TAUP medium. Fig. 5: Amastigotes and trypomastigotes inside LA-9 cells after 11 days of incubation at 37°C. Figs. 6-7: Intracellular amastigotes in the heart muscle tissue (Fig. 6) and spleen tissue (Fig. 7) of an infected mice, 15 days after inocculation with *T. cruzi* Dm 28c triatomine urine metacyclic trypomastigotes. Bar = 10 μm.



Surface anionic sites localization by cationized ferritin particles binding in cell culture derived trypomastigotes (Figs. 8-10 unstained sections) and TAUP derived trypomastigotes (Figs. 11-12 uranil plus lead stained sections) of *T. cruzi* Dm 28c. Caps of ferritin particles are seen in surface regions near the kinetoplast (Figs. 8-10, 12) and the flagellum (Fig. 11). Bars = 0.2 μm.

TABLE I

Infectivity of the trypomastigote forms of *Trypanosoma cruzi* Dm 28c clone to normal mice

Parasite origin	Inoculum p/g tissue**	Prepatent period (days) x ± sd***	Infected/ inoculated	Dead/ inoculated	Survival (days) x ± sd
Metacyclic – *R. prolixus*	1,000	6 ± 1	7/7	3/7	16 ± 60****
Bloodstream trypomastigotes	870	6 ± 1	6/6	0/6	60
Metacyclic Taus*****	1,000	8 ± 0	6/6	2/6	18 ± 60
Metacyclic TAUP	1,000	8 ± 0	5/5	1/5	18 ± 60

*Swiss male mice 21 days old; ** p/g = Parasite per gram of mice weight; *** x ± sd = Mean value ± standard deviation; **** mice were followed by 60 days; ***** TAUS = Artificial triatomine urine supplemented with 10% New Born calf serum (Contreras et al., 1985a).

TABLE II

Histotropism of *Trypanosoma cruzi* Dm 28c to mice*

Organ	Presence of amastigote nest	Organ	Presence of amastigote nest
Brain	–	Spleen	+
Cerebellum	–	Bladder	+
Lymphatic ganglia	–	Heart	+
Liver	–	Small intestine	+
Gallbladder	–	Alsophagus	+
Pancreas	–	Longue	+
Kidney	–	Subcutaneous tissue	+
Colon	–	Skeletal muscle	+

* 21 days old swiss male mice were inocculated with 1000 triatomine derived metacyclic trypomastigotes per gram of body weight and sacrified 15 days after infection. Randon fields of the organ sections were examined for three inocculated animals.

TABLE III

Trypanosoma cruzi Dm 28c induced resistance to reinfection in mice*

Origin of parasites	Pre-infection with Dm 28c	Inocculum p/g tissue**	Prepatent period (days) x ± sd***	Infected/ inocculated	Dead/ inocculated	Survival (days) x ± sd***
Y Bloodstream trypomastigotes	–	2900	4 ± 0	8/8	7/8	12 ± 60****
Y Bloodstream trypomastigotes	+	2900	–	0/15	0/15	60

* Swiss male mice older than 90 days, in a chronic phase after a pre-inocculum with *T. cruzi* Dm 28c; ** p/g = parasites per gram of body weight; *** x ± sd = mean ± standard deviation; **** mice were followed by 60 days.

TABLE IV

Infection levels of professional or facultative phagocytes by *Trypanosoma cruzi* Dm 28c

Host cell	Dm 28c	Interaction time	Percentage of cells with associated parasites*
Macrophages	Mt**	1 h	20-40
Fibroblasts	Mt	1 h	20-30
	Mt	2 h	40-60
	Bt***	1 h	40-60
Heart muscle cells	Mt	1 h	10-15
	Mt	1 h	25-50
	Bt	2 h	25-40
	Bt	2 h	30-50
Skeletal muscle cells	Mt	2 h	45-70

* Maximum variation in triplicates from three experiments; ** Mt = Metacyclic trypomastigotes from 96 h of differentiation in TAU3AAG; *** Bt = Bloodstream trypomastigotes.

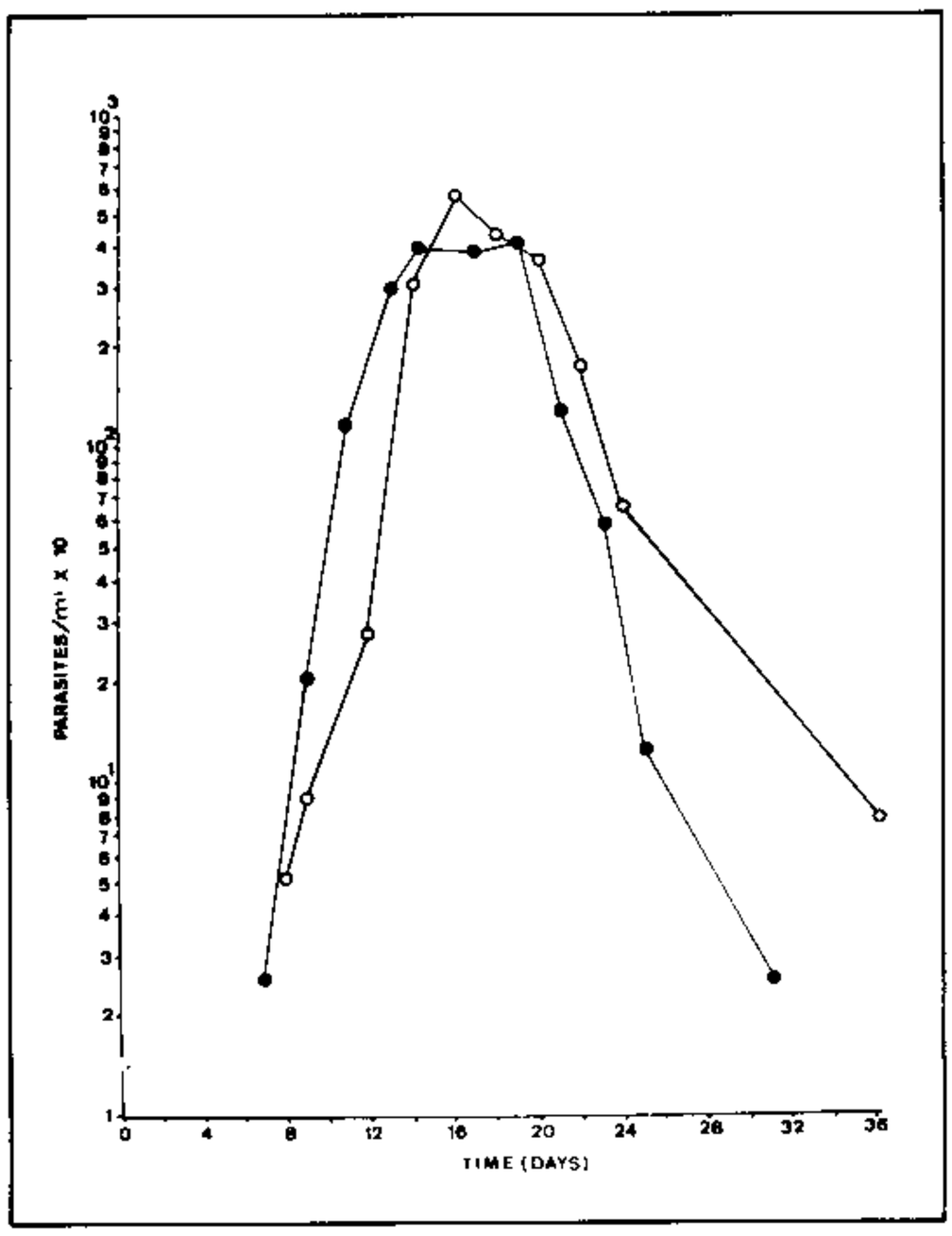


Fig. 13: Evolution of the parasitaemia in mice infected with triatomine derived metacyclic trypomastigotes (black circles) or TAUP medium metacyclic trypomastigotes (white circles) of *T. cruzi* Dm 28c.

In order to investigate the parasite-cell interaction, we submited adherent parasites to an osmotic lysis. The results presented in Table V show that after 2 h of parasite-cell contact, 32 to 54% of the cells interiorized the associated trypomastigotes, while in 46 to 68% of the cells the parasites were susceptible to the hipotonic shock.

The intracellular fate of metacyclic trypomastigotes from the Dm 28c clone was observed after 2,48 and 96 hours of parasite infection (Figs. 14-18), following the general cycle already described for *T. cruzi* (Dvorak & Hyde, 1973). Adherent epimastigotes and trypomastigotes forms and interiorized trypomastigotes can be seen after 2 h of parasite-cell contact (Fig. 14). By this time bloodstream-derived trypomastigotes have a rounded shape (not shown) while axenic-culture-derived metacyclic trypomastigotes remained in a slender form for longer periods of time. Amastigotes are easily recognized 24 h after infection and dividing amastigotes could be seen after 48 h (Fig. 15) while transition forms and differentiated trypomastigotes were observed after 96 h (Figs. 16-18).

DISCUSSION

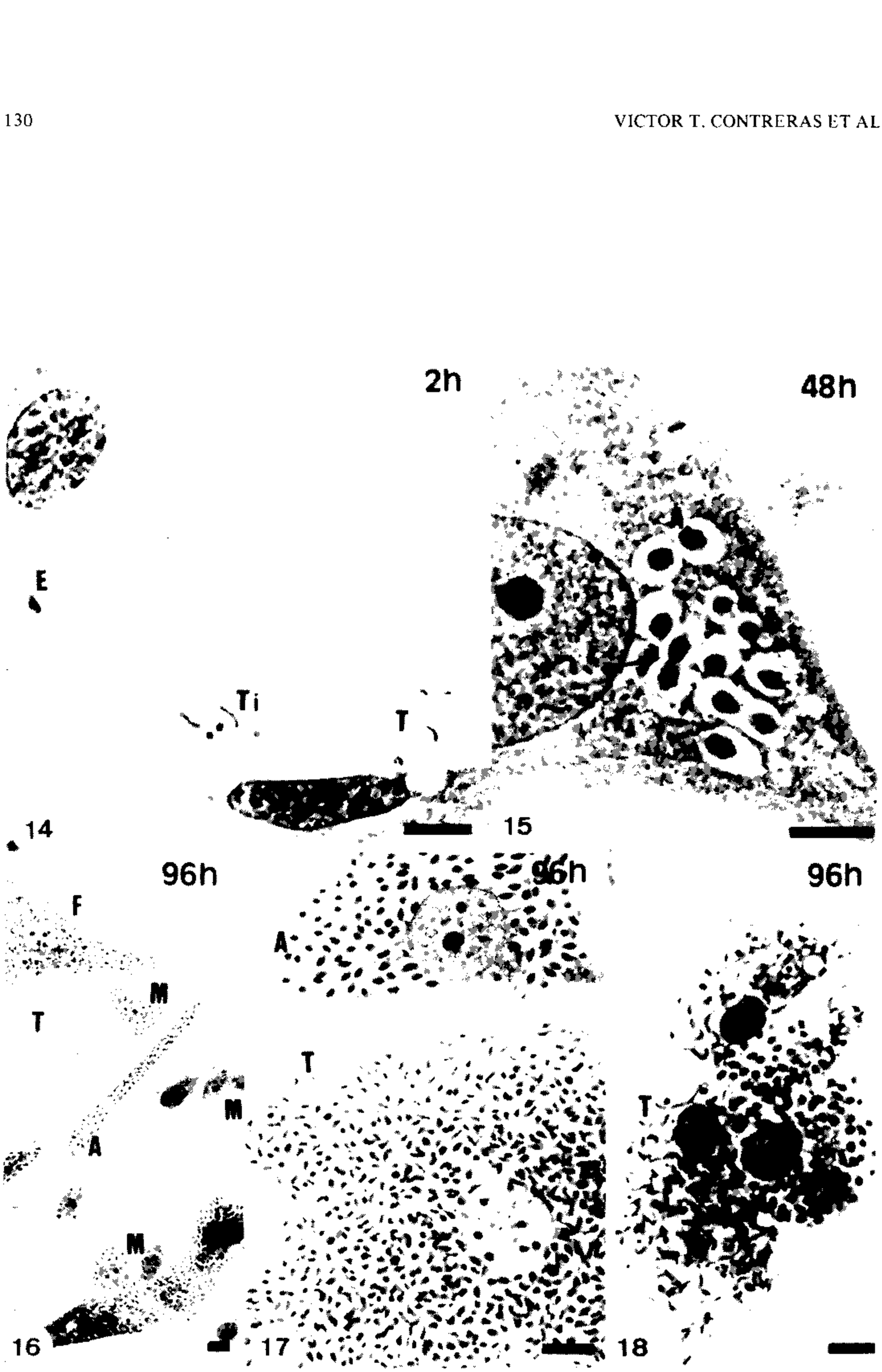
The metacyclogenesis of *T. cruzi* can be simulated *in vitro* (Camargo, 1964; Chiari, 1975; Sullivan, 1983; Contreras et al., 1985a). The experimental system of *T. cruzi* metacyclogenesis under chemically defined conditions developed in our laboratory (Contreras et al., 1985b; Goldenberg et al., 1987) circunvented the two main problems posed by *in vitro* differentiating conditions: chemical definition of the axenic culture media to induce the differentiation and genetic homogeneity of populations. Indeed, high yields of metacyclic trypomastigotes can be obtained with this methodology for different *T. cruzi* strains with excelent experimental reproducibility (Contreras et al., 1985b). We have previously charac-

TABLE V

Adhesion and interiorization levels of *Trypanosoma cruzi* Dm 28c in heart muscle cells and fibroblast after two hours of parasite-cell contact

Host cell	Exp. nº	Percentage of cells with parasites**		
		adherent***	interiorized***	associated
Heart muscle cells	1	34 (54%)****	29 (46%)	63 (100%)
	2	20 (62%)	12 (38%)	32 (100%)
	3	24 (61%)	15 (39%)	39 (100%)
Fibroblasts	1	22 (68%)	10 (32%)	32 (100%)
	2	20 (46%)	23 (54%)	43 (100%)
	3	16 (50%)	16 (50%)	32 (100%)

* *T. cruzi* Dm 28c metacyclic trypomastigotes after 72 h of differentiation in TAU3AAG; ** Mean value of triplicates in each experiment; *** The interiorized parasites were quantified after a 30 sec hypotonic shock (1 PBS: 5 H_2O). The adherent trypomastigotes were estimated by the difference between the total number of cells with associated parasites and with interiorized parasites in pair slides in a same experiment; **** The number inside parentheses indicates the relative percentage to the total number of cells with associated parasites.



Morphology of the intracellular stages of the Dm 28c clone of *Trypanosoma cruzi* in fibrolasts (F) or in heart muscle cells (M). Fig. 14: Epimastigotes (E) and trypomastigote adhesion (T) or interiorization (Ti) in heart muscle cells 2 hours after infection *in vitro*. Fig. 15: Amastigote forms and amastigote division (arrow) in fibroblasts 48 hours after infection. Figs. 16 to 18: Amastigotes and trypomastigotes that can be seen inside the cells 96 hours after the infection. In some cases free trypomastigotes recently liberated from dammaged cells are seen (Fig. 16). Bars = 20 μm.

terized the metacyclic trypomastigote forms of Dm 28c clone in respect to some trypomastigote stage specific biological properties such as resistance to complement lysis, resistance to macrophage digestion and infectivity to animals (Contreras et al., 1985b). The appearance of a single pattern of synthesized polypeptides, that accounts for the most relevant biological properties of the infective forms of *T. cruzi*, facilitates the identification of the genes involved in the process of parasite infection, opening new perspectives on therapeutic studies on Chagas' Disease (Contreras et al., 1985b).

In the present article we characterized the Dm 28c clone in respect to other biological parameters with special attention to its interaction with non-professional phagocytic cells and to the course of experimental acute stage of infection. The results indicate that the Dm 28c clone behaves as a bona fide *T. cruzi* representant. The doubling time of the Dm 28c clone is of 35 hours and the morphology of the different forms at the various developmental stages also follows the known morphological characteristics of these parasites (reviewed by De Souza, 1984). The anionic sites distribution on the metacyclic forms induced in TAU3AAG medium correspond to the patterns described for trypomastigotes (Souto-Padron & De souza, 1986).

Since metacyclic trypomastigotes from TAU3AAG and TAUP media display cell electrophoretic mobility values characteristic for this developmental stage (Bonaldo et al., 1987), we tested the binding of cationized ferritin to the surface of the parasite. Surprisingly, we noticed that ferritin particles were distributed in some membrane areas, very often with a spatial relationship with the kinetoplast, forming cap-like structures. This finding is relatively frequent in the ultrathin sections and it is present in Dm 28c trypomastigotes irrespective of their origin, although cell-culture derived trypomastigotes exhibited it more sharply. As yet, we do not know by shure what these caps represent and why anionic sites are specially concentrated in these regions. We are presently investigating the nature and potential role of these anionic sites.

Metacyclic trypomastigote forms of the Dm 28c clone infected 100% of inocculated mice (Contreras et al., 1985b and present results) with a clear myotropism. The induction of resistance to re-infection after survival from an acute phase caused by a low inoccula (Brener, 1980) implies that specific immune responses are built up in the infected mice. The intracellular development of Dm 28c metacyclic trypomastigotes follows the typical time course of division and differentiation reported for *T. cruzi* (Dvorak & Hyde, 1973) but its total cycle time (96 h) is slightly shorter than that observed in most of the clones studied by Engel et al. (1985).

A surprising feature was observed during the *in vitro* host-parasite interaction assays: the high infectivity levels of Dm 28c metacyclic trypomastigotes to non-professional phagocytic cells at short periods of time. Indeed, authors working with bloodstream trypomastigotes and non-professional phagocytic cells like Vero or BESM cells (Bertelli & Brener, 1980), fibroblasts (Kongtong & Inoki, 1975), primary heart or skeletal muscle cells (Meirelles et al., 1986; Araujo-Jorge et al., 1986) pointed out to the need of at least 9-24 h of parasite-cell contact to obtain indexes varying between 1% and 9% of infected cells, depending on the parasite strain used. In our own experience, even when the highly infective Colombiana strain is used, levels of 25-30% of muscle cells infected with blocdstream trypomastigotes can be obtained only after 18-24 h of parasite-cell contact (Meirelles et al., 1986). The metacyclic forms of the Dm 28c clone, obtained after 72-96 h of differentiation in TAU3AAG medium, are able to associate to 30-60% of heart muscle or fibroblastic cells within 2 h of parasite cell contact and 30-50% of the associated parasites are already interiorized. This *in vitro* behaviour of Dm 28c metacyclic forms on cell-interaction assays facilitates morphological studies on *T. cruzi* adhesion and invasion into non-professional phagocytic cells (Meirelles et al., 1987) and allows the study of the importance of different glycoproteic antigenic sites on the surface of the parasite for the recognition of the host cells, specially heart muscle cells.

In conclusion, the present results indicate that metacyclic trypomastigotes from the cloned Dm 28c strain obtained under chemically defined conditions reproduce the biological behaviour of *T. cruzi* myotropic strains, displaying high infectivity levels to primary cultured macrophages, fibroblasts and muscle cells. This system provides a useful tool in studies of parasite surface ligands components that might

be involved in the cell invasion process. In addition, due to the simple experimental procedures allowing the obtention of high yields of bona fide metacyclic trypomastigotes, this system should allow the identification and isolation of *T. cruzi* biologically relevant macromolecules.

RESUMO

Aspectos biológicos do clone Dm 28c de *Trypanosoma cruzi* após metaciclogênese em meio quimicamente definido – A caracterização biológica do clone Dm 28c de *Trypanosoma cruzi* em termos do seu crescimento em meio LIT, ciclo celular, infectividade para camundongos e interação com células fagocíticas profissionais e não-profissionais, mostra que o mesmo comporta-se como um fiel representante da espécie *T. cruzi.* As propriedades biológicas deste clone miotrópico não mudam de acordo com a proveniência das formas tripomastigotas (i. e., de triatomíneos, de camundongos infectados, de cultura celular ou dos meios quimicamente definidos TAUP e TAU3AAG). Ainda mais, formas tripomastigotas metacíclicas do clone Dm 28c derivadas do meio TAU3AAG apresentam um alto grau de infectividade para fibroblastos e células de músculo. Experimentos de ligação de ferritina cationizada à superfície de tripomastigotas, mostram a existência de acúmulos ("caps") de ferritina em regiões próximas ao cinetoplasto, todavia a natureza e o papel destes sítios aniônicos resta a ser determinado. Os resultados indicam que tripomastigotas metacíclicos do clone Dm 28c, obtidos em condições quimicamente definidas, reproduzem o comportamento biológico de *T. cruzi,* tornando este sistema bastante apropriado para o estudo da interação célula-parasito e para o isolamento de macromoléculas relevantes.

Palavras-chave: *Trypanosoma cruzi* – metaciclogênese – doença de Chagas – células de músculo

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