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RECENT CONTRIBUTIONS FOR A BETTER UNDERSTANDING OF THE *TRYPANOSOMA CRUZI*-MUSCLE CELL INTERACTION

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The process of penetration of parasites into the host cell involves an initial adhesion of the parasite to the cell surface followed by its internalization. Based on studies in which macrophages were treated with Cytochalasin B and low temperature it has been suggested that both epimastigote and trypomastigote forms of *T. cruzi* enter macrophages mainly by a process of endocytosis (Meirelles, Araújo Jorge & De Souza, 1982).

Most of the vertebrate cells tested *in vitro* can be infected by *T. cruzi*. The first studies *in vitro* of the *T. cruzi*-muscle cell interaction were done using the classic technique of hanging drop developed by Carrel, where the growth and cellular cycle of the parasite was analysed (Kofoid, Wood & Mc Neil, 1935; Meyer & Oliveira, 1948; Pereira da Silva, 1959; Hawking, 1946; Tang, 1953). Other experiments carried out with muscle cell lines gave emphasys to quantitative analysis of penetration, kinetics of interiorization, growth of trypomastigote forms, selective isolation of trypomastigotes (Dvorak & Hyde, 1973; Schmatz & Murray, 1981; Sanderson, Thomas & Twomey, 1980; Bertelli & Brener, 1980). Subsequent work examining the interaction of *T. cruzi* with different host cell has demonstrated that the parasite interacts with the surface of the host cell via some form of receptor-ligant binding. It has been postulated that adhesion and penetration phases are mediated by components of both the parasite and the host cell (Alcantara & Brener, 1978; Andrews & Colli, 1982; Piras, Piras & Henriques, 1983; Villalta & Kierszenbaum, 1983/1984; Meirelles et al., 1983; Meirelles, Souto-Padrón & De Souza, 1984).

The existence of strains of *T. cruzi* with *in vivo* tropism for different cell types was reported by early workers but in all the cases the muscle cell is the preferential host cell for the characterization of Chagas' disease. We used two groups of strains that have been previously characterized using morphological criterium, tissue tropism virulence, pathology, and immunological reactions (Andrade, 1974; Alcantara & Brener, 1978). One strain (Y) has a tropism for macrophages and two other strains (Cl and Colombiana) have a tropism for muscle cells.

The infection of muscle cells by *Trypanosoma cruzi* has been poorly studied *in vitro*. In order to investigate this interaction in more detail we have established an experimental *in vitro* system of primary culture of heart and skeletal muscle cells (Barbosa et al., 1983; Meirelles et al., 1983; Araújo Jorge et al., 1984). Myoblasts were obtained from mouse embryo and the process of differentiation *in vitro* to muscle fiber was followed.

One of the first questions that arise from our experimental work was: Does our primary cell culture from mouse embryos have the characteristic features of the muscle cells described *in vivo*?

The ultrastructural analysis of the experimental system of primary cell culture – The structural organization of the muscle cells obtained *in vitro* was analysed by transmission and scanning electron microscopy. The results obtained showed that they have the same basic structure found in muscle cells *in vivo*. We found several specialized structures such as the sarcotubular system, hypernucleation, the transverse tubular system (T-system), elongated mytochondria with dense parallel cristae, organized myofilamentar network for the skeletal muscle cell and, in the case of heart muscle cells, intercalated discs composed of interdigitating ridges between two cells and presenting the junctions responsible for cohesivinness (desmossomes and fascia adherens) and for intercellular communication (gap junction) (Barbosa et al., 1983/1984; Araújo Jorge et al., 1984; Meirelles et al., 1983) (Fig. 1).

Ultrastructural cytochemistry and the interiorization of the parasite – The mechanism by which the trypomastigote form of *T. cruzi* infects vertebrate cells is not well understood. Several investigators have examined the mode of entry of the parasite in the vertebrate cells and contradictory results have been reported (Alexander, 1975; Nogueira & Cohn, 1976; Kipnis, Galich & Dias da Silva, 1979; Alcantara & Brener, 1978; Meirelles, Araújo Jorge & De Souza, 1982; Maria, Alcantara & Brener, 1982). Based on studies of *T. cruzi*-macrophage interaction and with a few other cell lines it has been assumed that the parasite is found in a phagocytic vacuole at early times just after infection (Nogueira & Cohn, 1976; Milder, Kloetzel & Deane, 1977). Recent experiments made with non professional phagocytes like fibroblasts show that if they are previously incubated with fibronectin they can ingest 7μm diameter latex particles (Grinnel, 1984).

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Fig. 1: skeletal muscle cell: general aspect of a myotube of embryo mouse culture showing some specialized structures such as hypernucleation (N), myofilaments arrangements (arrows) and several mytochondria (arrow heads). x 10.000. Fig. 2: intercalated disc membrane of heart muscle cells showing a reaction product indicative of the presence of Adenilyl Cyclase (arrows), mytochondria (M) and zig-zag polisomes (arrow head). x 48.750. Fig. 3: a typical trypomastigote form within an endocytic vacuole of a heart muscle cell. Arrow heads showing the membrane of parasite; arrows showing the membrane of endocytic vacuole. (K) Kinetoplast. x 105.000.

Aiming to verify the process of interiorization of *T. cruzi* by a nonphagocytic cell we infected primary culture of heart muscle cells with bloodstream forms of *T. cruzi*. We found, independently of the strain and the parasite-cell ratio used, that the infection is very low at the first hours (Barbosa et al., 1984). After 20 hours of interaction the bloodstream forms of *T. cruzi* are found within an endocytic vacuole located in the cytoplasm of the heart muscle cell. This observation suggests that the infection of heart muscle cells occurs by a process of endocytosis (Meirelles et al., 1984; Meirelles et al., 1984 – manuscript in preparation).

Working with ultrastructural cytochemistry we localized two enzyme markers at the surface of heart muscle cells: the basic Ca^{2+} - Mg^{2+} - ATPase and Adenilyl Cyclase. We found the basic ATPase in the membrane lining the whole surface of the cell but it was not found in the regions of contacts of the cell to each other. For the enzyme Adenilyl Cyclase a very strong reaction was found at the surface membrane and at the intercalated disc but there was no reaction, in the regions of desmosomes, fascia adherents and gap junctions. Typical trypomastigote forms were found within cytoplasmic vacuoles of heart muscle cells which had been maintained for 20-24 hs in the presence of the parasite. No reaction product, indicative of the presence of the two enzymes, was seen in the membrane of the vacuole (Figs. 2 and 3). This observation

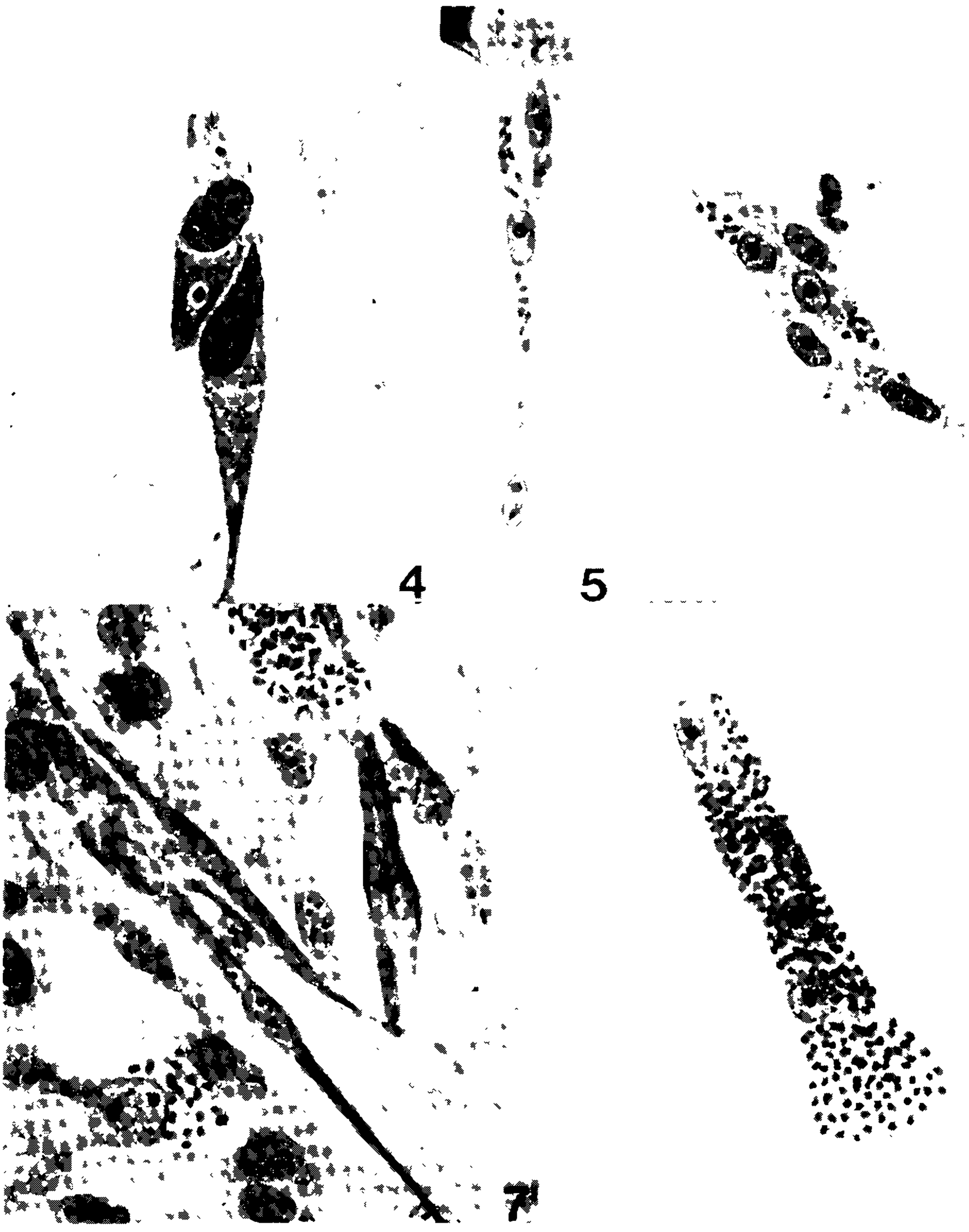

Fig. 4: alignment of two myoblasts infected with Cl strain of *T. cruzi* after 36 hs of infection. x 1.200. Fig. 5: alignment of three myoblasts infected with Colombiana strain of *T. cruzi* after 72 hs of infection without signs of fusion. x 900. Fig. 6: alignment of infected myoblasts with Y strain with 72 hs of interaction showing the begining of fusion. x 900. Fig. 7: general aspect of a culture infected with Cl strain with 5 days of interaction showing differentiated myotubes without parasites or recently infected. The fibroblasts (F) present in the culture are full of parasites. x 900. Fig. 8: a five day old culture myotube infected with Y strain presenting a normal pattern of infection of *T. cruzi*. x 900.

suggests that mechanisms exist which control the components of the plasma membrane of the host cell which are interiorized together with the parasite as already shown for macrophage-*T. cruzi* interaction (Meirelles & De Souza, 1983; Meirelles et al., 1983; Meirelles, Souto-Padrón & De Souza, 1984).

The skeletal muscle differentiation and the process of *T. cruzi*-muscle cells interaction – We have studied the behaviour of the Y, Cl and Colombiana strains of *T. cruzi* in skeletal muscle cells (Araújo Jorge et al., 1984).

The development of the skeletal myogenic cultures of mouse embryos occurs in three main phases:

a) intense proliferation and alignment of myoblasts;

b) fusion of myoblasts, which lasts for 2 or 3 days, to form large multinucleated myotubes;

c) the completion of fusion and the formation of a network of multinucleated fibers against a background of fibroblastic and myoblastic cells.

The observation of the myoblasts infected on the second and third day of culture have shown, for all the strains tested an independently of the medium used (with or without EGTA) that the cells have 2 or 3 parasites inside the cytoplasm during the first 24 hours of interaction and 5 to 8 parasites between 26-48 hours. By the third day of infection the fusion starts and in the experiments with the Y strain of *T. cruzi* we could follow the fusion of infected and non infected myoblasts. By the fourth and fifth days we observed the presence of numerous myotubes full of parasites showing the same aspect of the infected fibroblast also present in the culture (Araújo Jorge et al., 1984 – manuscript in preparation). With the cultures infected with the Cl and Colombiana strains we found a persistence of myoblasts infected that lasted for the fourth and fifth days in culture. The presence of myotubes in these cultures were characterized for being completely free of parasites or containing 2 or 3 parasites recently penetrated (Figs. 4-8).

The observation of 8 and 10 days old cultures have shown that the myotubes formed were infected with all the three strains of *T. cruzi.* This infection, which corresponds to the parasite second cell cycle, indicates that once formed the myotubes is well infected by different strain of the parasite.

Our results point that once inside the myoblast the two myotropic strains of *T. cruzi* tested are able to change in some way important steps necessary to the completion of fusion.

Further investigations are necessary to determine the nature of the changes induced by infection of myoblasts with myotropic strains which led to a blockage of the process of cell fusion.

REFERENCES

ALCANTARA, A. & BRENER, Z., 1978. The *in vitro* interaction of *Trypanosoma cruzi* bloodstream forms and mouse peritoneal macrophages. *Acta Tropica, 35* :209-219.

ALEXANDER, J., 1975. Effect of the antiphagocitic agent Cytochalasin B on macrophage invasion of *Leishmania mexicana* promastigotes and *Trypanosoma cruzi* epimastigotes. *J. Protozool., 32* :237-270.

ANDRADE, S.G., 1974. Caracterização de cepas do *Trypanosoma cruzi* isoladas do Recôncavo Baiano. *Rev. Patol., 3* :65-121.

ANDREWS, N.N. & COLI, W., 1982. Adhesion and interiorization of *Trypanosoma cruzi* in mammalian cells. *J. Protozool., 29* :264-269.

ARAÚJO JORGE, T.C.; BARBOSA, H.S.; DE SOUZA, W. & MEIRELLES, M.N.L., 1984. Skeletal muscle differentiation myogenesis is differently affected by myotropic and non myotropic strains of *Trypanosoma cruzi.* Manuscript in preparation.

ARAÚJO JORGE, T.C.; MIRANDA, C.F.; BARBOSA, H.S.; DE SOUZA, W. & MEIRELLES, M.N.L., 1984. Cepas miotrópicas do *T. cruzi* inibem a miogênese de células esqueléticas *in vitro.* Resumo BI-47. XI Reunião Anual de Pesquisa Básica em Doença de Chagas. Caxambu, MG.

BARBOSA, H.S.; ARAÚJO JORGE, T.C.; MEIRELLES, M.N.L. & DE SOUZA, W., 1983. Desenvolvimento de um modelo experimental *in vitro* para o estudo da interação *Trypanosoma cruzi*-célula muscular. Resumos BI-08. X Reunião Anual de Pesquisa Básica em Doença de Chagas. Caxambu, MG.

BARBOSA, H.S.; ARAÚJO JORGE, T.C.; DE SOUZA, W. & MEIRELLES, M.N.L., 1984. Análise ultraestrutural de um sistema *in vitro* da célula muscular cardíaca durante a interação com o *T. cruzi.* Resumos BI-49. XI Reunião Anual de Pesquisa Básica em Doença de Chagas. Caxambu, MG.

BERTELLI, M.S. & BRENER, Z., 1980. Infection of tissue culture cells with bloodstream trypomastigotes of *Trypanosoma cruzi. J. Parasit., 66* :992-997.

DVORAK, J.A. & HYDE, T.D., 1973. *Trypanosoma cruzi:* interaction with vertebrate cells *in vitro.* I – Individual interaction at the cellular and subcellular levels. *Exp. Parasit., 34* :268-283.

GRINNEL, F., 1984. Fibroblast spreading and phagocytosis similar cells response to different sized substrats. *J. Cell Physiol., 119* :58-64.

HAWKING, F., 1946. Growth of Protozoa in tissue culture. *Trans. Roy. Soc. Trop. Med. Hyg., 40* :345-349.

KIPNIS, T.L.; GALICH, V.L.C. & DIAS DA SILVA, W., 1979. Active entry of bloodstream forms of *Trypanosoma cruzi* this macrophage. *Parasit., 78* :89-98.

KOFOID, C.A.; WOOD, F.D. & Mc NEIL, E., 1935. The cycle of *Trypanosoma cruzi* in tissue culture of embryonic heart muscle cell. *Unit. Calif. Publ. in Zool., 41* :23-24.

MARIA, T. de A.; ALCANTARA, A. & BRENER, Z., 1982. Ultrastructural studies on the *in vitro* interaction of *Trypanosoma cruzi* bloodstream forms and mouse peritoneal macrophages. *Acta Trop., 39* :99-109.

MEIRELLES, M.N.L.; ARAÚJO JORGE, T.C.; BARBOSA, H.S. & DE SOUZA, W., 1983. Observações ultraestruturais e citoquímicas preliminares da interação *Trypanosoma cruzi*-célula muscular. Resumos BI-35. X. Reunião Anual de Pesquisa Básica em Doença de Chagas. Caxambu, MG.

MEIRELLES, M.N.L.; ARAÚJO JORGE, T.C. & DE SOUZA, W., 1982. Interaction of *Trypanosoma cruzi* with macrophages *in vitro* dissociation of the attatchment and internalization phases by low temperatures and Cytochalasin B. *Z. Parasitenkd, 68* :7-14.

MEIRELLES, M.N.L.; ARAÚJO JORGE, T.C.; DE SOUZA, W. & BARBOSA, H.S., 1984. Presença de vacúolo fagocítico em célula muscular cardíaca recém penetrada pelo *T. cruzi.* Resumos BI-48. XI Reunião Anual de Pesquisa Básica em Doença de Chagas. Caxambu, MG.

MEIRELLES, M.N.L.; ARAÚJO JORGE, T.C.; DE SOUZA, W. & BARBOSA, H.S., 1984. The localization of trypomastigote form of *T. cruzi* within an endocytic vacuole in heart muscle cells revealed by ultrastructural and enzyme cytochemistry analysis. Manuscript in preparation.

MEIRELLES, M.N.L. & DE SOUZA, W., 1983. Interaction of lysosomes with endocytic vacuoles in macrophages simultaneously infected with *Trypanosoma cruzi* and *Toxoplasma gondii. J. Submicrosc. Cytol., 15* (4) :889-896.

MEIRELLES, M.N.L.; MARTINES PALOMO, A.; SOUTO-PADRÓN, T. & DE SOUZA, W., 1983. Participation of Concanavalin A binding sites in the interaction between *Trypanosoma cruzi* and macrophages. *J. Cell Sci., 62* :287-291.

MEIRELLES, M.N.L.; SOUTO-PADRÓN, T. & DE SOUZA, W., 1984. Participation of cell surface anionic sites in the interaction between *T. cruzi* and macrophages. *J. Submicrosc. Cytol., 16* :533-545.

MEYER, H. & XAVIER DE OLIVEIRA, M., 1948. Cultivation of *Trypanosoma cruzi* in tissue culture: a four year of study. *Parasitology, 39* :91-94.

MILDER, R.T.; KLOETZEL, J. & DEANE, M.P., 1977. Observation of the interaction of peritoneal macrophages with *Trypanosoma cruzi.* II – Intracellular fate of bloodstream forms. *Rev. Inst. Med. trop. São Paulo, 19* :313-322.

NOGUEIRA, N. & COHN, Z., 1976. *Trypanosoma cruzi:* mechanism of entry and intracellular fate in mammalian cell. *Exp. Med., 143* :1402-1420.

PEREIRA DA SILVA, L.H., 1959. Observações sobre o ciclo evolutivo do *Trypanosoma cruzi. Rev. Inst. Med. trop. São Paulo, 1* :99-118.

PIRAS, R.; PIRAS, M.M. & HENRIQUES, D., 1983. *Trypanosoma cruzi*-fibroblastic cell interactions necessary for cellular invasion. Cytopathology of Parasitic Disease Pitman Boon London (Ciba Foundation Symposium 99), pg. 31-51.

SANDERSON, C.J.; THOMAS, J.A. & TWOMEY, C.E., 1980. The growth of *Trypanosoma cruzi* in human diploid cells for the production of trypomastigotes. *Parasit., 80* :153-162.

SCHMATZ, D.M. & MURRAY, P.K., 1981. Selective isolation of pure trypomastigote from cultural muscle cell. *J. Parasitol., 67* :517-521.

TANG, C.C., 1953. Cultivation of *Trypanosoma cruzi* in tissue culture. *Chinese Med., 71* :115-125.

VILLALTA, F. & KIERSZENBAUM, F., 1983. Role of cell surface mannose residues in host cell invasion by *Trypanosoma cruzi. Biochem. Biophys. Acta, 736* :39-44.

VILLALTA, F. & KIERSZENBAUM, F., 1984. Host cell invasion by *Trypanosoma cruzi:* role of cell surface galactose residues. *Biochem. Biophys. Res. Commun., 119* :228-235.