The Challenges of Sciences. A Tribute to Carlos Chagas
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THE CONTRIBUTION OF CELL BIOLOGY FOR A BETTER UNDERSTANDING OF CHAGAS' DISEASE

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First I wish to thank the organizers of the symposium to invite me to be here today, and to congratulate the Pontifician Academy of Sciences for the initiative to organize this symposium as a tribute to the memory of its past President Carlos Chagas Filho. Since 1970, when I was 18 years old and started my scientific carreer at the Instituto de Biofísica Carlos Chagas Filho under the supervision of Hertha Meyer, I had the privilege to be in permanent contact and under the positive influence of Professor Carlos Chagas Filho. I still remember when I showed him the first micrographs where the sub-pellicular microtubules of the protozoan *Toxoplasma gondii* could be seen. He became so enthusiastic with the results and decided to present them in the French Academy of Sciences. The paper, which was my first one, was published in 1972 in the Comptes Rendues de la Academie de Sciences

Table I

TROPICAL PARASITIC DISEASES-WHO / 1999

Infected people	500.000.000
Dead per year	2.000.000
Malaria	500.000.000
Schistosomiasis	200.000.000
Filariasis	120.000.000
Amebiasis	16.000.000
Leishmaniasis	15.500.000
Chagas disease	14.000.000
Sleepness disease	3.000.000

de Paris (1). Figure 1 (see page III) illustrates one important moment of my carreer when I was introduced in 1984 as Member of the Brazilian Academy of Sciences and received the diploma from the hands of Professor Carlos Chagas Filho, at that time President of the Pontifician Academy of Sciences.

In the last 30 years I have been involved in the study of parasitic diseases from a cell biology perspective. Tropical Parasitic Diseases still constitute one of the most important public health problem in the world affecting about 500 millions of people, according to information from the WHO (Table I).

Chagas Disease, caused by the protozoan *Trypanosoma cruzi* discovered in 1909 by Carlos Chagas, father of Carlos Chagas Filho, is the most important parasitic disease in large areas of South and Central America (Fig. 2, see page III), affecting about 14 million people, with about 100 million people remaining at risk.

Important health programs have been established in the last years aiming to control transmission of the parasites from the insects to man as well as from man to man during blood transfusion as exemplefied in Figure 3 (see page IV) and Table II.

These data could led us to suggest complete elimination of new infections caused by *T. cruzi* in the next years. However, recent studies using isoenzyme and riboprinting analysis, rRNA promoter activity, sequence of mini-exon genes and microsatellite markers have provided clear evidences that *T. cruzi* is not a single species but it corresponds to two highly diver-

Table II: Human Infection by *Trypanosoma cruzi* and reduction of incidence

Southern Cone Initiative,

1983-1999

Country	Age Group (Years)	Infection in 1983	Infection in 1999	Reduction of Incidence
		(Rates x 100)	(Rates x 100)	(%)
Argentina	18	4.5	1.2	85.0
Brazil	7-14	18.5	0.17	96.0
Bolivia	1-4	33.9	ND	ND
Chile	0-10	5.4	0.14	99.0
Paraguay	18	9.3	3.9	60.0
Uruguay	6-12	2.5	0.06	99.0

gent genetic subgroups, designated as lineages 1 and 2 (2). Lineages 1 predominates in the domestic cycle while lineage 2 is mainly represented in the sylvatic cycle. Both are potentially pathogenic for man. It is important to point out that although at present there is no transmission of Chagas disease in places like the Rio de Janeiro state 100% of the small primitive monkeys known as gold lion tamarins and 84 % of the opossums found in a residual forest localized 80 km from the city of Rio de Janeiro are infected with *T. cruzi* (3). Therefore, it is important to intensify basic work on the biology of the parasite in order to develop new strategies of disease control.

From the cell biology point of view *T. cruzi* is a very interesting model to study processes such as reversible differentiation or cell transformation, mechanisms of cell invasion, etc. The central developmental stage is the trypomastigote form (Fig. 4). The basic structure of the protozoan, which is

Fig. 4 General aspect of the trypomastigote form of *T. cruzi* as seen with the high voltage electron microscope. K. Kinetoplast.

common to most of the members of the Trypanosomatidae family, is schematically shown in Figure 5. Some characteristic features deserve a few comments (4). The protozoan is an asymmetric and polarized cell where at least three surface domains exist: 1) the cell body, 2) the flagellum, and 3) the flagellar pocket, a specialized region formed due to an invagination of the plasma membrane lining the cell body, which is continuous with the flagellum. The flagellum establishes contact with the cell body through a special type of junction. The coupling of the flagellum with the cell body is so strong that when the flagellum moves the body also seems to move, giving the impression of the existence of an undulating membrane. At the base of the flagellum there is the kinetoplast, a structure which contains up to 30 % of the total cell DNA and formed by DNA arranged as cocatenated

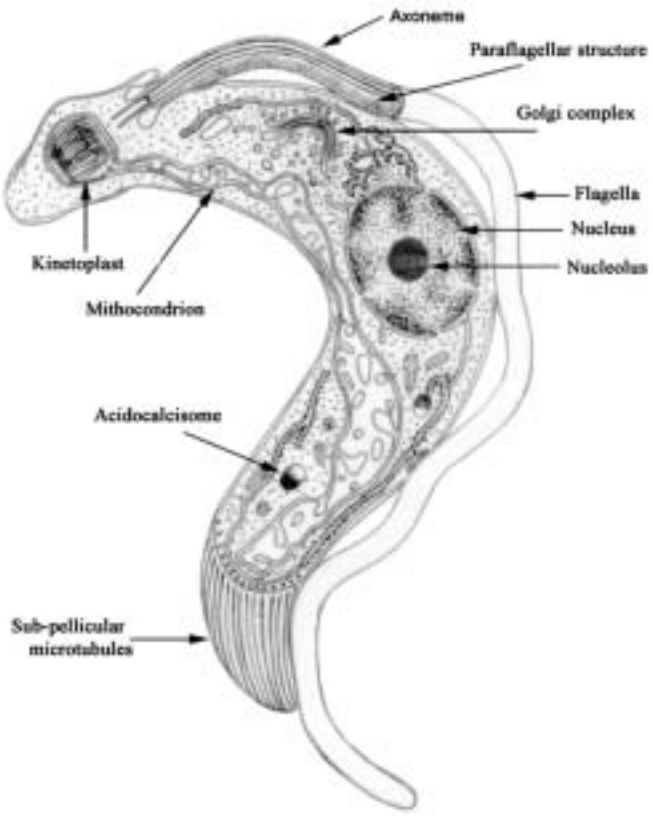

Fig. 5 Schematic view of the trypomastigote form of *T. cruzi*

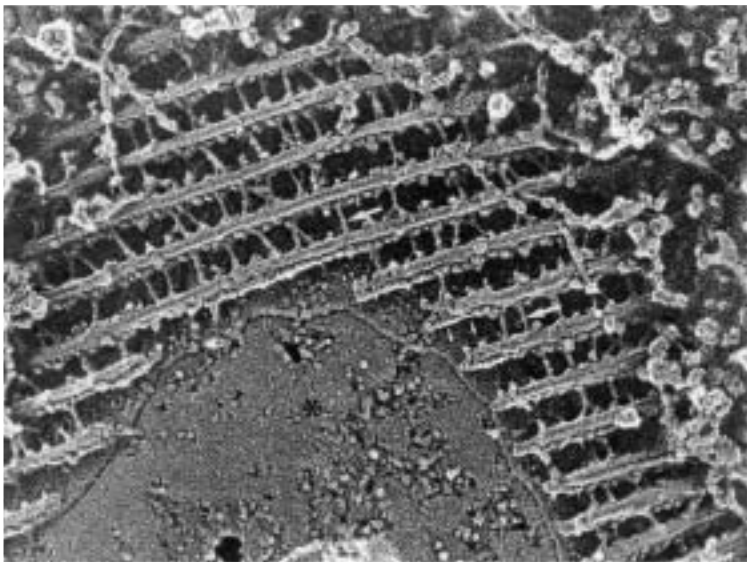

Fig. 6 Deep-etching view of the association of sub-pellicular microtubules to each other (white arrow) and the plasma membrane (asterisk).

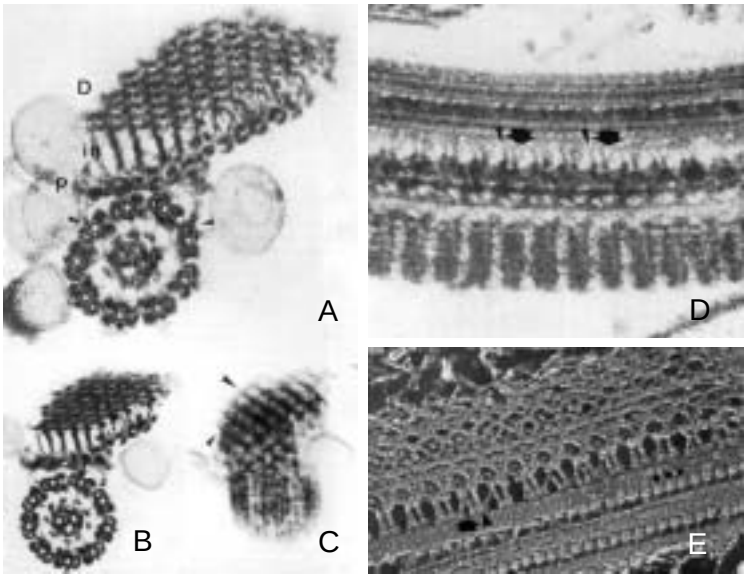

Fig. 7 General view of the flagellar axoneme and its association with a network of filaments which make the paraflagellar structure.

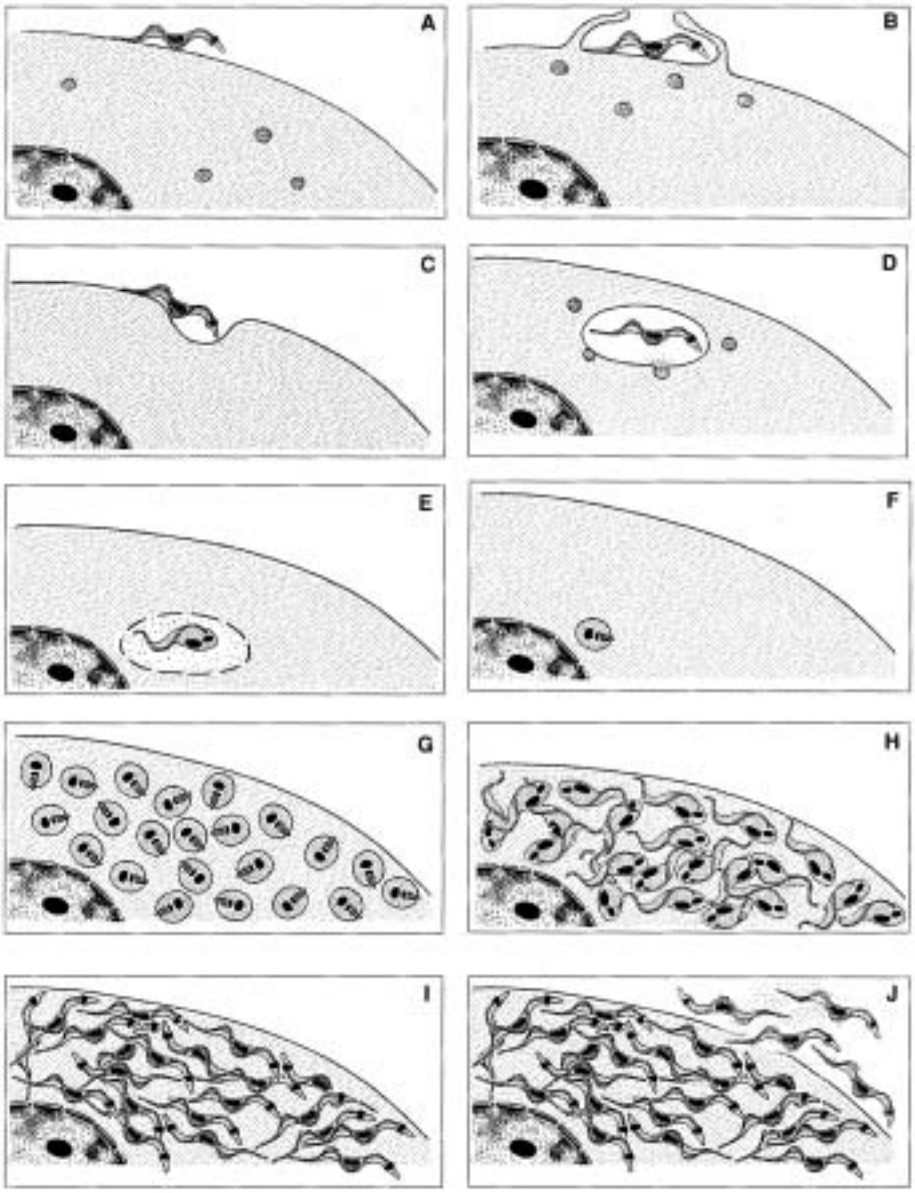

Fig. 9 Schematic view of the process of interaction of *T. cruzi* with host cells.

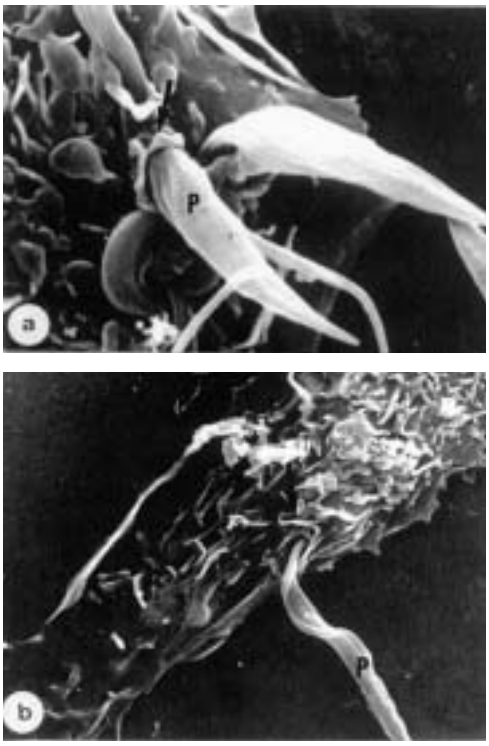

Fig. 10 Scanning electron microscopy of the process of interaction of *T. cruzi* with macrophages.

minicircles and maxicircles located within the single mitochondrion. Below the plasma membrane there is a layer of microtubules, known as sub-pellicular microtubules, connected to each other and to the plasma membrane via small bridges (Fig. 6). Two special organelles are observed in all trypanosomatids: (a) the glycosome, a special type of peroxisome, which contains the glycolytic enzymes, which has been characterized in detail by Fred Opperdoes and co-workers in Belgium (5), and (b) the acidocalcisome, which is an acidic organelle involved in the uptake of calcium, thus helping in the regulation of the intracellular concentration of this ion (Review in 6). The flagellum of the trypanosomatids exhibits, in addition to the axoneme, a complex array of filaments that form the paraxial structure (Fig. 7) whose function is still not completely defined. Recent studies implicate its role in the flagellar movement. The paraxial structure is formed by a complex

array of 25 and 70 nm thick filaments made of two major proteins of 69 and 80 kDa (7-8).

The life cycle of *Trypanosoma cruzi* starts with the ingestion of trypomastigote forms, found in the blood of the vertebrate host, during biting by insects of the Reduviidae family (Fig. 8, see page IV). Following blood meal the insect dramatically increase its size and weight. In the stomach the long trypomastigote forms transform into rounded, flagellated forms known as spheromastigotes (Reviews in 9-10). These forms migrate to the intestine and transform into short and long epimastigote forms. Subsequently, intense division of the epimastigote forms takes place in the intestine and later on they transform into infective trypomastigote forms, also known as metacyclic forms, which are released through the feces and urine of the insect during its bloodmeal in vertebrates, including the man. The parasites deposited on the

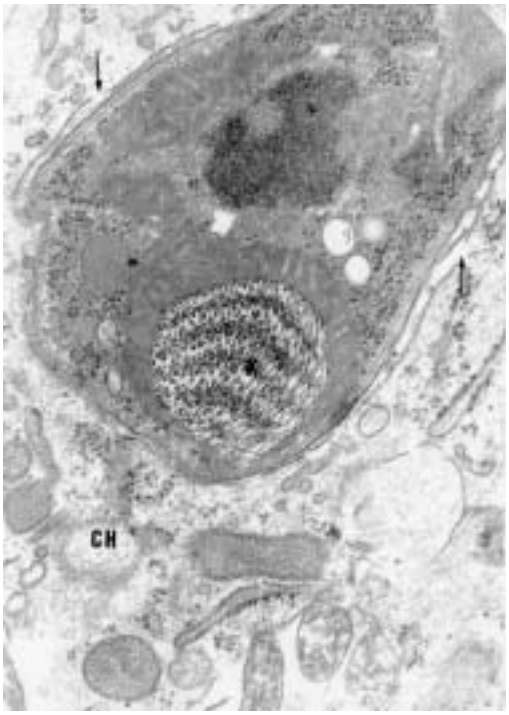

Fig. 11. Transmission electron microscopy of a thin section showing a trypomastigote form of *T. cruzi* within a parasitophorous vacuole whose membrane is in process of desintegration (arrows).

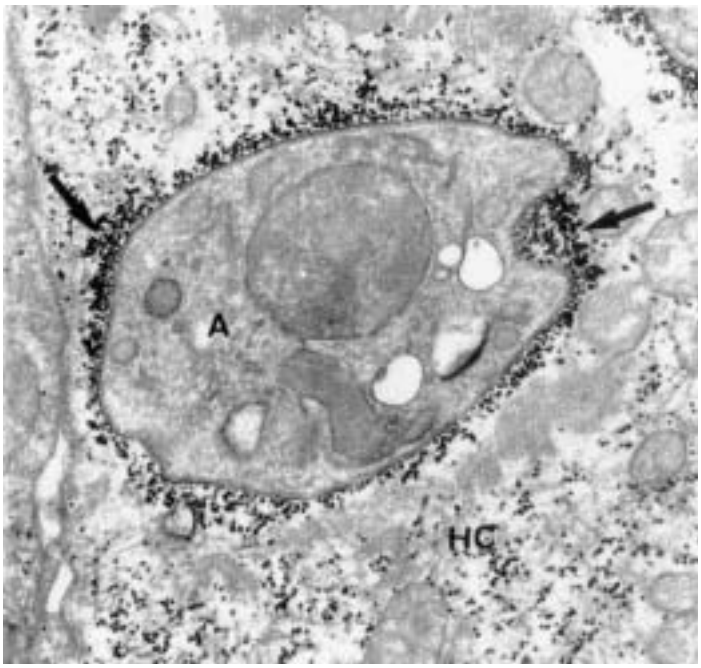

Fig. 12. Amastigote form of *T. cruzi* (A) in direct contact with components of the host cell cytoplasm (HC). The arrows indicate the presence of glycogen particles.

skin of the vertebrate host may have access to the host tissues if small skin lesions exist or are formed during insect biting, and then enter in contact with the surface of host cells (Fig. 9) such as macrophages, muscle cells, neurons and fibroblasts. Such contact involves a process of cell-to-cell recognition event. Studies carried out in the last years have shown that sialic acid-containing macromolecules found on the surface of the host cells are involved in the recognition process (11). In addition, a large number of parasite surface molecules play a fundamental role. Among them I would like to emphasize the importance of a family of proteins known as trans-sialidase which has a dual function since they simultaneously express neuraminidase and sialyl transferase activities (Reviews in 12-14). Using not yet clarified mechanisms these molecules may interfere with the level of sialylation of surface-exposed molecules found both on the parasite and on the host cell. Once attached to host cell surface the infective trypomastigote form is either ingested through

a typical phagocytic process (Fig. 10) or induces an endocytic activity of the host cell in a process that involves calcium release, protein phosphorylation, and lysosomal migration (Reviews in 15-17). Both processes correspond to an endocytic event with formation of a parasitophorous vacuole (Fig. 11). Next, the parasite changes its form to a rounded one (amastigote), with the concomitant disruption of the membrane lining the vacuole (Fig. 11) due to release of enzymes. Then the parasite enters in direct contact with the cytoplasmic structures of the host cell (Fig. 12) interacting with cytoskeletal elements such as microtubules and microfilaments (Fig. 13, see page V). After about 35 hours the amastigote forms start to divide, with a generation time of

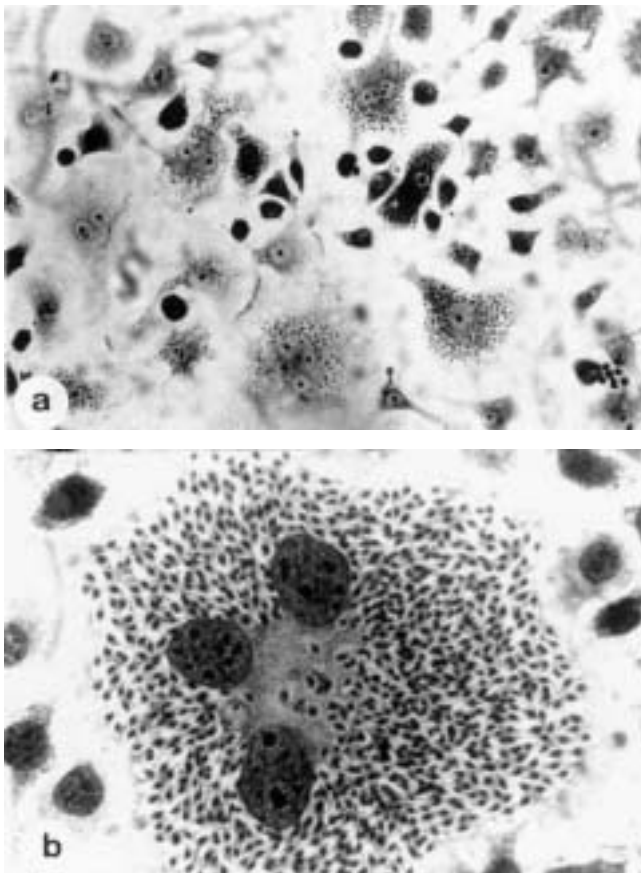

Fig. 14. Light microscopy of cells infected with *T. cruzi*.

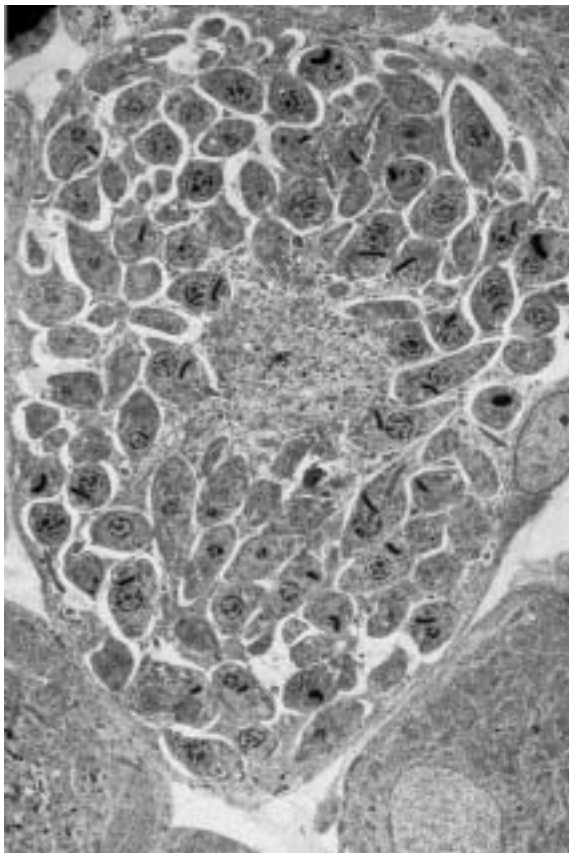

Fig. 15. General aspect of a neuron infected in vitro with *Trypanosoma cruzi*. Most of the cytoplasm of the cell is occupied by intracellular parasites. (Courtesy of H. Meyer.)

about 14 hours. After about 5 days the host cell is completely filled with amastigote forms (Figs. 14-15), which then starts a gradual process of transformation into trypomastigotes. Due to the intense movement of the intracellular parasites there is a rupture of the host cell and release of hundreds of trypomastigote forms into the intercellular space, from where they infect neighboring cells or reach the bloodstream and infect other tissues such as heart, skeletal muscle and the nervous system.

From the cell biology point of view *T. cruzi* is also very interesting. The analysis of the mechanisms used by the parasite to ingest macromolecules

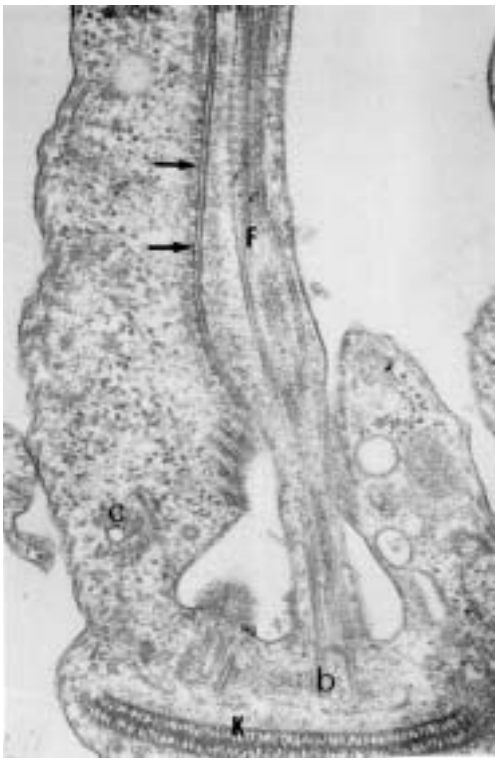

Fig. 16. The section of an epimastigote form of *T. cruzi* showing the kinetoplast (K), two basal bodies(b), and the region of the attachment of the flagellum (F) to the cell body (arrows). An oblique section through the cystostome (C) is also seen.

from the medium and how it regulates the biosynthesis of sterols have provided information which is opening possibilities to develop new alternatives for the chemotherapy of Chagas disease. Biochemical studies have shown that *T. cruzi* is not able to synthesize cholesterol (18). However, it incorporates this important molecule as part of the LDL complex through a highly polarized endocytic process which takes place at the cytostome (19), a highly organized region of the protozoan surface (20) (Figs. 16-18). In the absence of LDL in the medium the parasites synthesize ergosterol which is subsequently incorporated into the cell membranes. This observation opened the possibility to use inhibitors of the ergosterol biosynthesis, largely employed against fungi, to kill *T. cruzi*. In collaboration with Julio

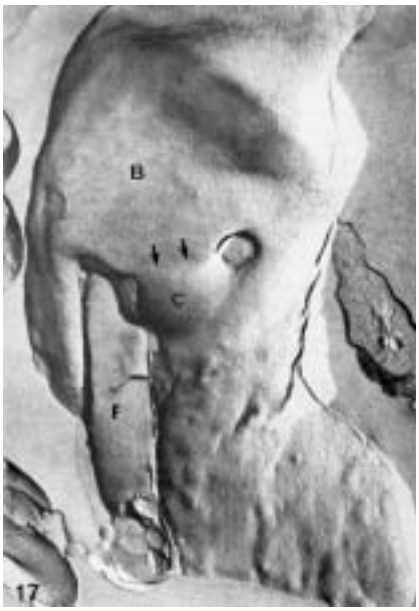

Fig. 17. Freeze-fracture view of the membrane lining the cell body (B), the flagellum (F) and the cytostome (C) of *T. cruzi*.

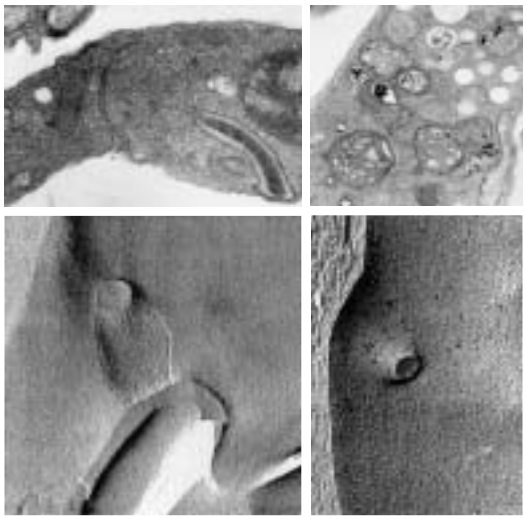

Fig. 18. Different views of the endocytic system of the epimastigote form of *T. cruzi*. A and B are thin sections of cells incubated in the presence of gold-labeled transferrin C and D show the cystostome.

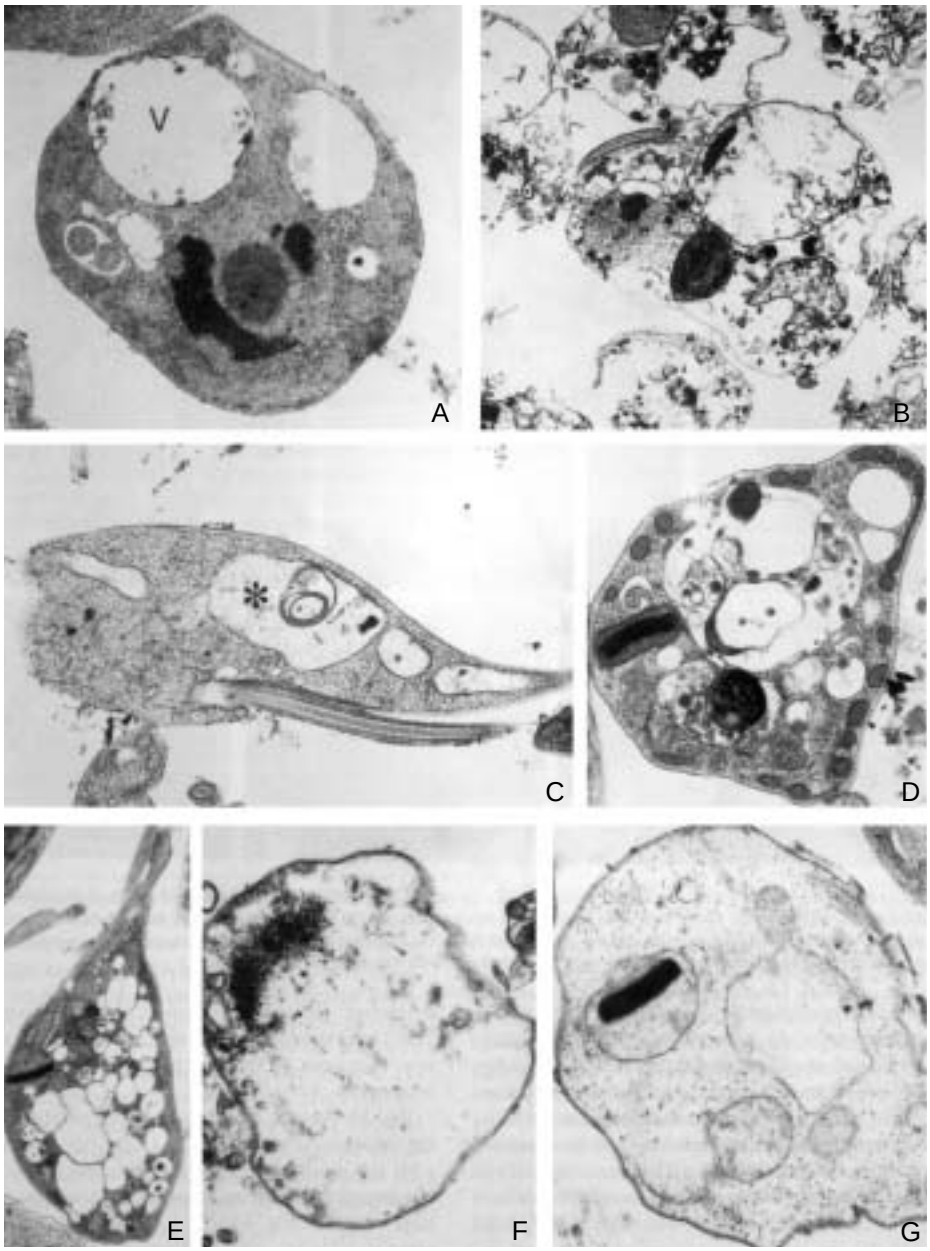

Fig. 19.Different views of *T. cruzi* incubated in the presence of an inhibitor of the biosynthesis of ergosterol. The parasite is completely desintegrated.

Urbina and colleagues in Venezuela we have tested several of such compounds (21-22). The results we have obtained confirmed this assumption and show that some drugs are highly active, efficiently killing the parasite (Fig. 19) and are now in clinical trials.

I would like to conclude saying that much need to be done on the study of basic aspects of the cell biology of *T. cruzi* in order to identify new parasite targets for more specific chemotherapy and for the development of a vaccine.

REFERENCES

1. De Souza, W. *C.R. Acad. Sci.* 275:2899-2902, 1972
2. Zingales, B. *Mem. Inst. Oswaldo Cruz 95 (suppl)*: 10-12, 2000
3. Fernandes, O., Mangia, R.H., Lisboa, C.A., Pinho A.P., Morel, C.M, and Zingales, B. *Parasitology* 118: 161-166, 1999
4. De Souza, W. *Int. Rev. Cytol.* 86: 197-283, 1984
5. Opperdoes, F.R. *Ann. Rev. Microbiol.* 41: 127-151, 1987
6. Docampo, R., and Moreno, S.N. *Parasitol Today* 15: 443-448, 1999
7. Farina, M., Attias, M., Souto-Padras, T., and De Souza, W. *J. Protozool.* 33: 552-558, 1986
8. Gull, K. *Annu. Rev. Microbiol.* 53: 629-653, 1999
9. Garcia, E.S., and Azambuja, P. *Parasitol. Today* 7: 240-244, 1991
10. Kolien, A.H., and Schaub, G.A. *Parasitol. Today* 16: 381-387, 2000
11. Ciavaglia, M.C., Carvalho, T.V., and De Souza, W. *Biochem. Biophy. Res. Comm.* 193: 718-722, 1993
12. Schenckman, S., Einchinger, D., Pereira, M.E.A. and Nussenzweig, V. *Ann. Rev. Microbiol.* 48: 499-523
13. Pereira, M.E.A., Zhang, K., Gong, Y., Herrera, E.M., and Ming, M. *Infect. Imm.* 64: 3884-3892, 1996
14. Schenckman, S., Ferguson, M.A.J., Heék, N., Cardoso de Almeida, M.L., Mortara, R.A., and Yoshida, N. *Mol. Biochem. Parasitol* 59: 253-304, 1993
15. Docampo, R., and Moreno, S. *Parasitol Today* 12: 61-65, 1996
16. Vieira, M., Carvalho, T.V. and De Souza, W. *Biochem. Biophys. Res. Comm.* 203: 967-971, 1994
17. Andrews, N.W. *Trends Cell Biol.* 5: 133-137, 1995
18. Urbina, J. *Parasitology* 114: 591-599, 1997
19. Soares, M.J. and De Souza, W. *Parasitol Res.* 77: 461-468, 1991
20. De Souza, W. *Prog. Protistol.* 3: 87-184, 1989.

Fig. 1.

Fig. 2.

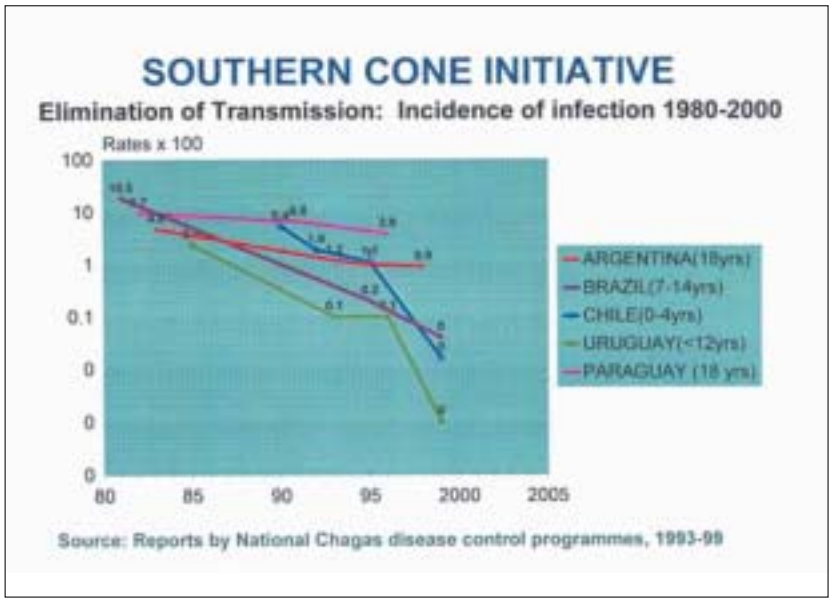

Fig. 3.

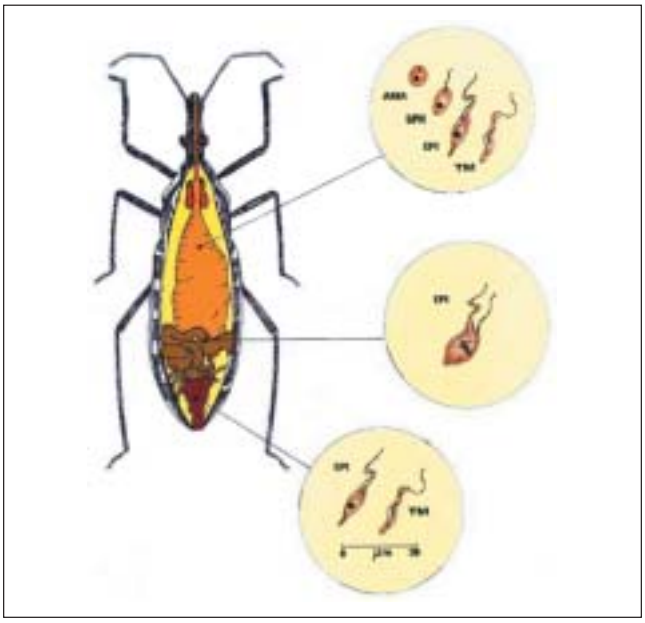

Fig. 8. Schematic view of the life cycle of *T. cruzi* in the insect vector.

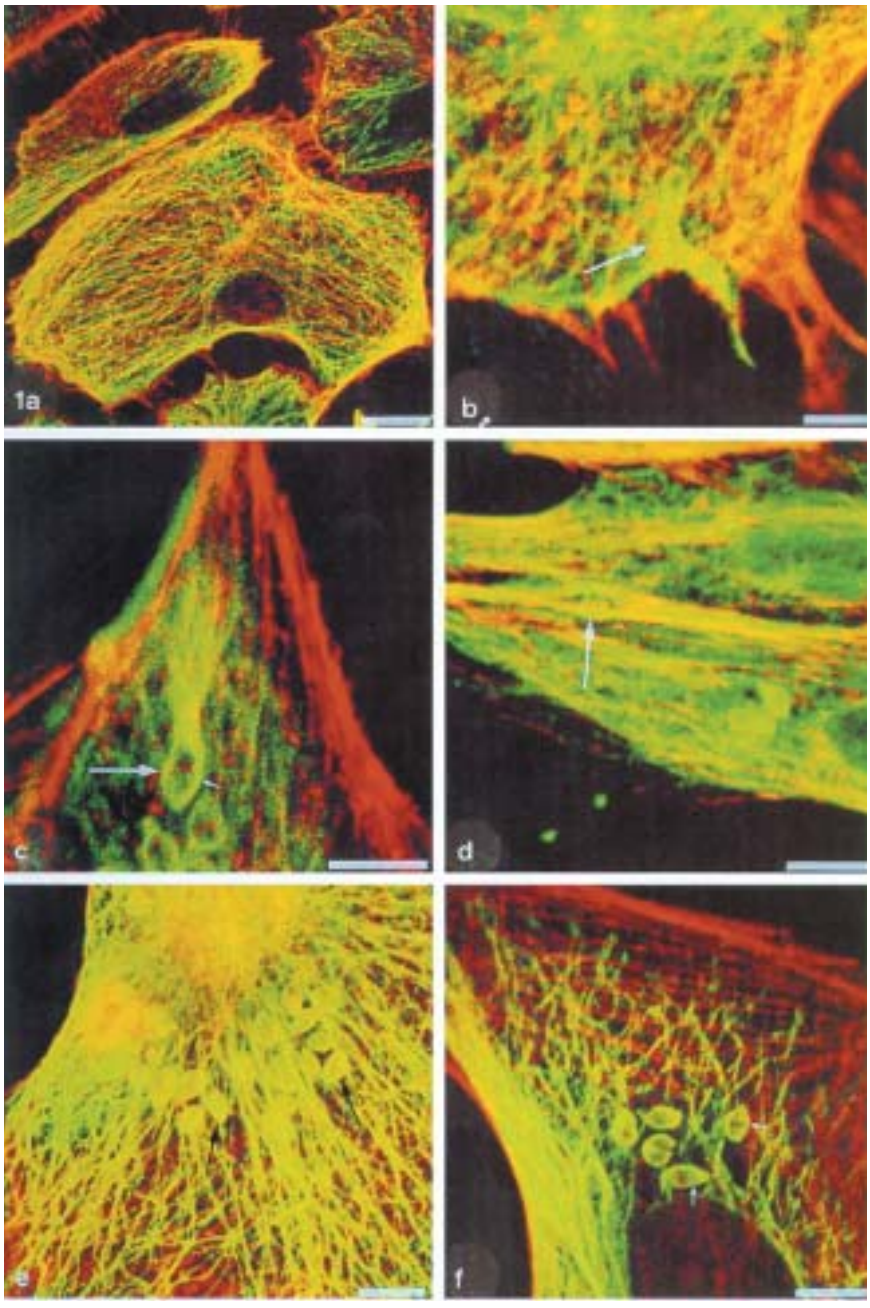

Fig. 13. Confocal laser scanning microscopy of cells infected with *T. cruzi*.

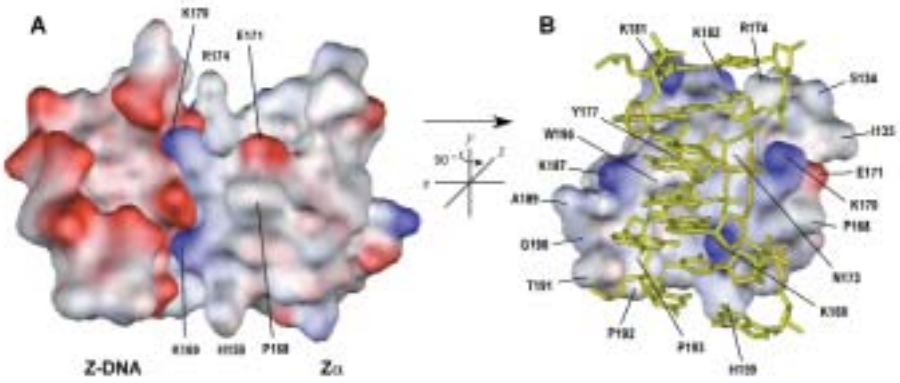

Figure 7. Electrostactic complementarity of the Zα·Z-DNA complex. (A) Residues 134 to 198 (numbering from human ADAR1) of one Zα protein and the 6 bp duplex d(TCGCGCG) are shown represented as solvent-exposed van der Waals surfaces with coloring indicating electrostatic potentials (red indicates negative potential, and blue represents positive potential). The complementarity in shape and electrostatic potential is striking. (B) The same complex as in A, rotated by ~90 °, with the DNA duplex displayed as a stick-model allowing direct viewing of the Zα interaction surface. Amino acid residues on the surface are labeled.