Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Suppl. Vol. 79 :1-5, 1984

THE CELL SURFACE OF *TRYPANOSOMA CRUZI*

WANDERLEY DE SOUZA & THAIS SOUTO-PADRÓN

The cell surface of trypanosomatids is formed by the plasma membrane and a layer of sub-pellicular microtubules which are connected to the plasma membrane. The plasma membrane is composed by proteins, lipids and carbohydrates which form the glycocalix. In this paper we will review briefly aspects related to the organization of the cell surface of *Trypanosoma cruzi.*

The glycocalix

Carbohydrate – containing sites exist on the surface of all developmental stages of *T. cruzi,* as can be seen in electron micrographs of thin section submitted to the periodic acid-thiosemicarbaride – silver proteinate technique (De Souza & Meyer, 1975). Lectins have been used to determine the nature of the sugar residues located on the surface of *T. cruzi.* With the use of the lectin WGA it was shown that only epimastigotes are agglutinated with low concentrations of the lectin while large concentrations are necessary to agglutinate trypomastigotes (Pereira et al., 1980). Several lectins were used in these studies and the results obtained by various groups show that qualitative and quantitative differences exist in the carbohydrates exposed on the cell surface of the parasite in the various stages of its life cycle (review in De Souza, 1984).

As most of the eucaryotic cells, *T. cruzi* has a net negative surface charge. Therefore, cationic particles, such as cationized ferritin, bind to the surface of the parasite (De Souza et al., 1977). A larger number of particles bind to the surface of trypomastigotes (Fig. 1) than to epimastigotes indicating a difference in the surface charge of the two forms. The surface charge of cells can be analysed in more detail by the determination of the cellular electrophoretic mobility (EPM). By this approach it was shown that differences exist in the surface charge of the various developmental stages of *T. cruzi* (De Souza et al., 1977; Souto-Padrón et al., 1984). Table I shows the mean EPM of epimastigote, amastigote and bloodstream trypomastigote forms of *T. cruzi.* As can be seen epimastigotes have the lower negative surface charge, trypomastigotes have the higher and the charge of amastigotes lies in between. It is important to point out that the mean EPM is characteristic for each developmental form, independent of the strain or the origin of the parasite. Based on the observation that treatment of the parasites with neuraminidase reduces significantly the surface charge of the cells it has been suggested that sialic acid residues contribute considerably to the surface charge of *T. cruzi.* Table II shows the effect of neuraminidase treatment on the surface charge of *T. cruzi.* The data obtained indicate that in trypomastigotes there are more sialic acid residues exposed on the surface, and susceptible to the action of neuraminidase from *Clostridium perfrigens,* than in epimastigotes. We can not exclude the possibility that sialic acid residues inacessible or insensitive to the neuraminidase used exist on the surface of *T. cruzi.*

Based on the fact that neuraminidase-treated cells recover their normal surface charge when incubated for 2-4 hs in fresh culture medium it can be concluded that the parasite synthesizes the sialic acid-containing components. The addition of inhibitors of protein synthesis (puromycin) or N-glycosylation of proteins (tunicamycin) to the culture medium where enzyme-treated cells are incubated, inhibits partially the recovery of the surface charge. However, the grade of inhibition varies between epimastigote and trypomastigote forms. Based on the data obtained it was suggested that most of the sialic acid residues located on the surface of trypomastigotes, and susceptible to neuraminidase treatment, belong to sialoglycolipids (Souto-Padrón & De Souza, in press). In epimastigotes, however, most of the sialic acid residues are associated to glycoproteins. Preliminary results obtained in epimastigotes, indicate that there are some sialic acid residues which are released by neuraminidase treatment only after previous treatment of the parasites with trypsin. The study of the surface charge of *T. cruzi* is of great interest in view of the observations reported in the last years that sialic acid residues located on the surface of trypomastigotes render the parasites resistant to complement-mediated lysis and may modulate the process of interaction of the parasite with macrophages (Kipnis et al., 1981; Araújo-Jorge & De Souza, 1984; Meirelles, Souto-Padrón & De Souza, 1984).

Freeze-fracture

The examination of thin sections of *T. cruzi* does not reveal differences in the structure of the membrane which encloses the cell body, the flagellar pocket region and the flagellum. With the freeze-fracture technique the inner part of the cell membrane is exposed allowing the examination of either the inner or the outer membrane halves. Both fracture faces (P, protoplasmic and E, extracellular) show a large number of structures which have been designated as intramembranous particles. Since the fracture plane

---

This work has been supported by UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (FINEP) and Conselho de Ensino para Graduados da UFRJ (CEPG).

Instituto de Biofísica, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941 Rio de Janeiro, RJ, Brazil.

TABLE I

Mean cellular electrophoretic mobility of *Trypanosoma cruzi**

Developmental stage	Source	Strain	EPM ($-\mu m.s^{-1}.V^{-1}cm$)	
			Mean	S.D.
Epimastigote	M 16 Medium	Y	0.65	0.11
Epimastigote	M 16 Medium	CL	0.74	0.11
Epimastigote	Warren Medium	Y	0.62	0.09
Amastigote	Spleen	Y	0.86	0.20
Amastigote	J 774 G 8	Y	0.85	0.02
Trypomastigote	M 16 Medium	Y	1.20	0.20
Trypomastigote	M 16 Medium	CL	1.10	0.15
Trypomastigote	tissue culture	Y	1.04	0.11
Trypomastigote	tissue culture	CL	1.04	0.05
Trypomastigote	bloodstream	Y	1.15	0.17
Trypomastigote	bloodstream	CL	1.10	0.07

*For details see Souto-Padrón et al., 1984; Carvalho, Souto-Padrón & De Souza, 1984.

TABLE II

Effect of neuraminidase treatment on the surface charge of *Trypanosoma cruzi**

Developmental stage	EPM ($-\mu m.s^{-1}.V^{-1}.cm$)		% of decrease
	control	enzymetreated	
Epimastigote	0.62	0.54	13
Amastigote	0.85	0.55	36
Trypomastigote	1.15	0.55	53

*For details see Souto-Padrón et al., 1984; Carvalho, Souto-Padrón & De Souza, 1984.

TABLE III

Density of intramembranous particles on the fracture faces of the plasma membrane of *Trypanosoma cruzi**

Development stage	Number of particles per square micrometer	
	Face P	Face E
Epimastigote	1.836	1.450
Amastigote	303	541
Trypomastigote	122	125

*For details see De Souza et al., 1978; Carvalho, Souto-Padrón & De Souza, 1984.

passes through the center of the lipid bilayer of the membrane, the intramembranous particles represent integral proteins located in the hydrophobic region of the membrane structure.

With the use of the freeze-fracture technique, it was observed that the density of the intramembranous particles varied according to the developmental stage of the parasite (De Souza, Martinez-Palomo & Gonzales-Robes, 1978; Carvalho, Souto-Padrón & De Souza, 1984). As shown in Table III epimastigotes have the higher density of particles. These data suggest that quantitative differences exist in the integral proteins of the three developmental stages of *T. cruzi*. It was also observed that the density of membrane particles is much higher in the membrane which lines the cell body than in that which lines the flagellum (Fig. 2). Areas where the membrane particles showed an organized array were seen in the membrane which lines (a) the region of the cystostome found in epimastigotes and amastigotes, (b) in the region of attachment of the flagellum to the cell body, and (c) in the membrane which lines the base of the flagellum (De Souza, Martinez-Palomo & Gonzales-Robes, 1978).

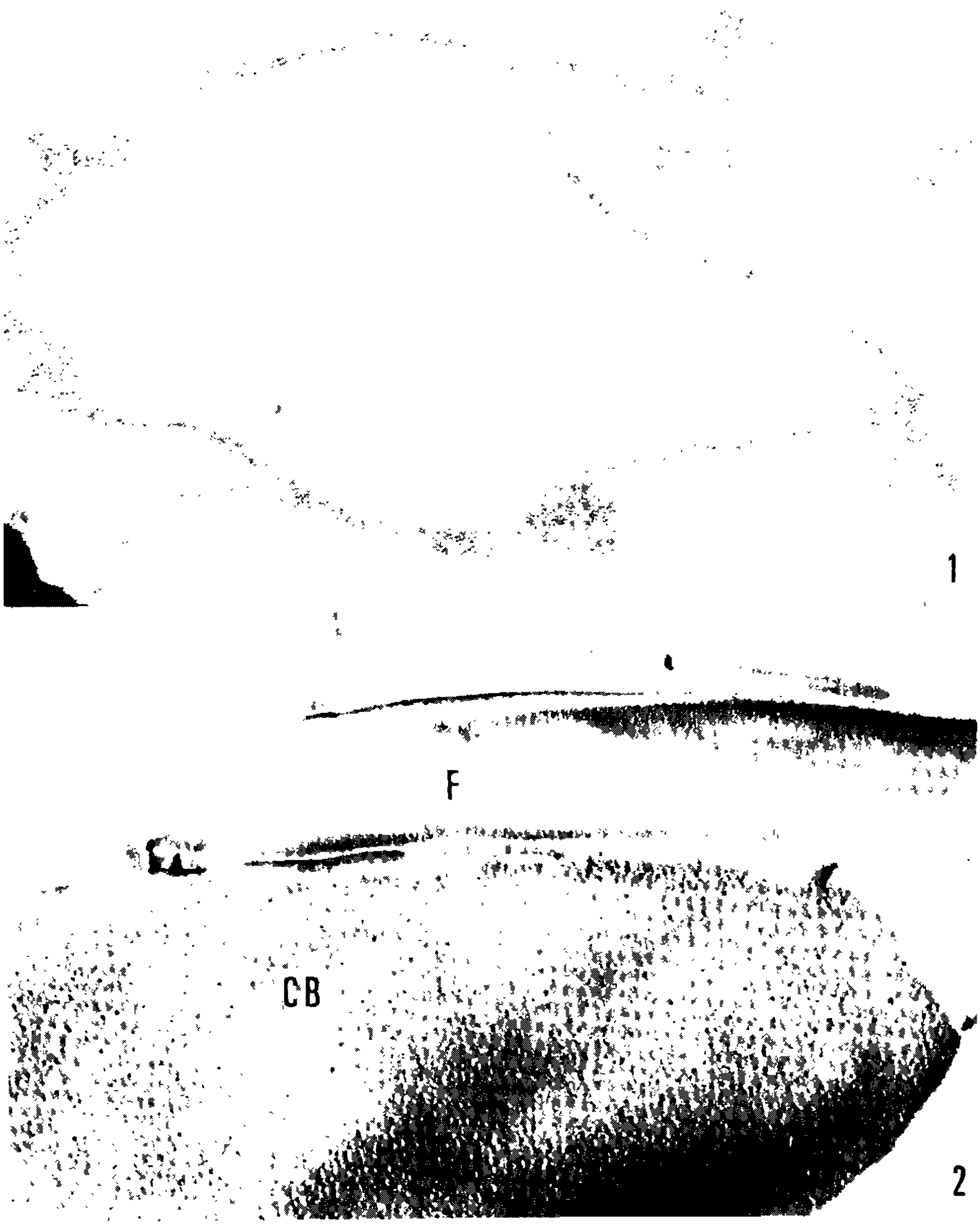

Fig. 1: thin section of a trypomastigote form of *T. cruzi* showing the presence of cationized ferritin particles on the cell surface. X 40.000. Fig. 2: freeze-fracture of a form in transition from the epimastigote to the trypomastigote form. A large number of intramembranous particles are seen in the membrane which lines the cell body (CB). Few particles are seen in the membrane which lines the flagellum (F). X 55.000.

The polyene antibiotic filipin was used for the visualization of hydroxy-sterols (usually cholesterol or ergosterol) in the plasma membrane of epimastigotes of *T. cruzi* (Souto-Padrón & De Souza, 1983). Filipin-sterol complexes (Fig. 3), which appear as protuberances with a mean diameter of 31 nm, were seen throughout the plasma membrane of the protozoan. In some cells the protuberances were linearly arranged forming parallel bands. The flagellar membrane showed a larger number of protuberances than the membrane lining the cell body.

Membrane – microtubule association

Since the first observations made in thin sections of tryponosomatids it was observed that there is a system of microtubules which is associated with the plasma membrane (review in De Souza, 1984). With the improvement of the techniques for processing of biological samples for electron microscopy it was observed that filamentous structures connect the sub-pellicular microtubules with each other and with the plasma membrane. These connections are seen in great detail when replicas of quick-frozen, freeze-fracture, deeply-etched, and rotary-replicated cells (Fig. 4) are examined by transmission electron microscopy (Souto-Padrón et al., 1984).

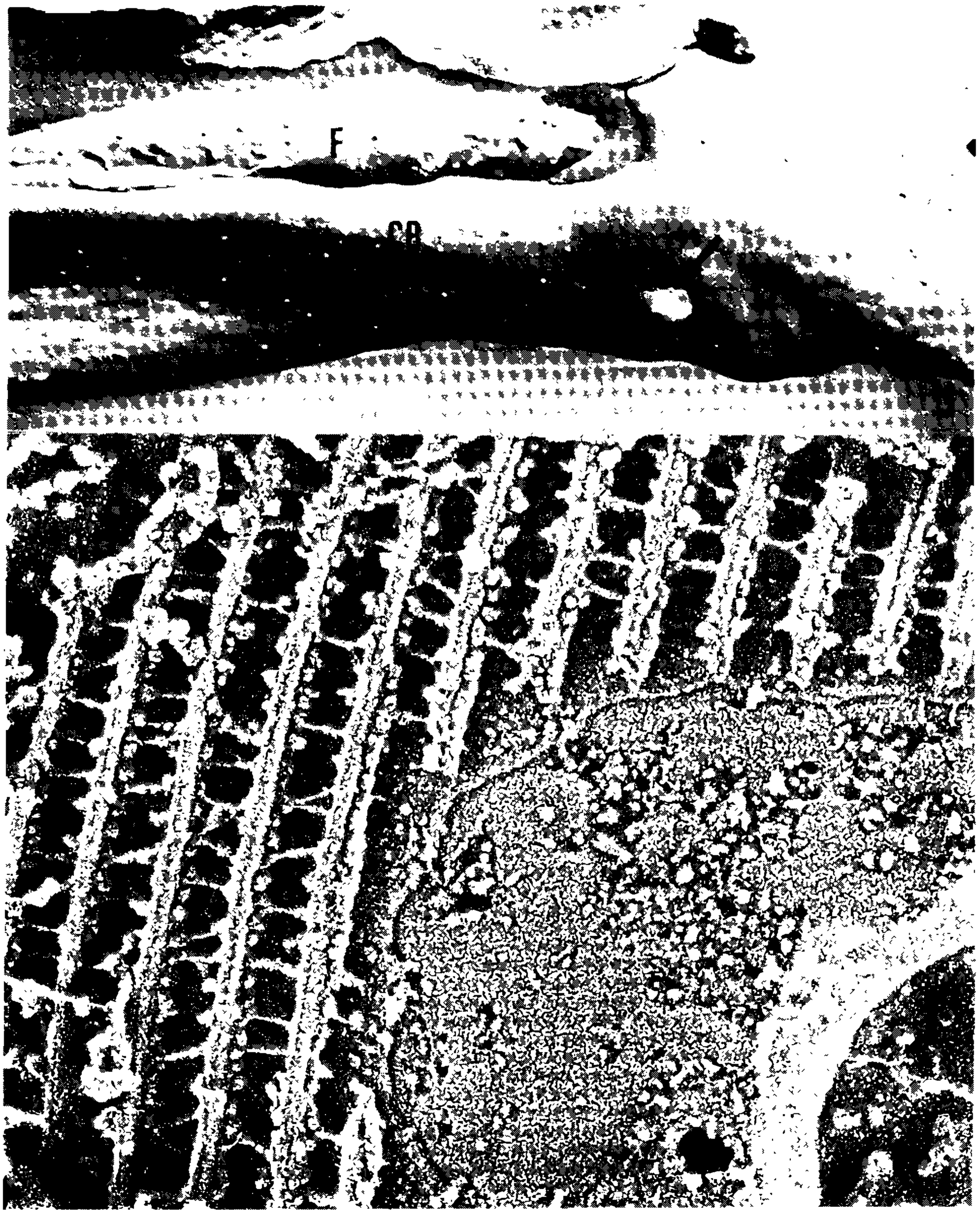

Fig. 3: freeze-fracture of an epimastigote form treated with filipin. Protuberances, indicative of the presence of filipin-sterol complexes, are seen in the membrane lining the cell body (CB) and the flagellum (F). The cystostome (arrow) can be seen. X 22.000. Fig. 4: image of a parasite treated with Triton X-100 before glutaraldehyde fixation. The cell was quick-frozen, deep-etched and rotary replicated. Filamentous structures can be seen connecting the sub-pellicular microtubules. X 150.000.

For some members of the Trypanosomatidae family it has been shown that even after disruption of the parasites and purification of a membrane fraction, the microtubules remain associated to the plasma membrane (review in De Souza, 1984). In the case of *T. cruzi* it has not been possible yet to obtain a fraction containing microtubules associated to the plasma membrane.

ACKNOWLEDGEMENTS

The authors thank Dr. H. Meyer for reading the manuscript.

REFERENCES

ARAÚJO-JORGE, T.C. & DE SOUZA, W., 1984. Effect of carbohydrates, enzymes and periodate in the process of endocytosis of *Trypanosoma cruzi* by macrophages. *Acta. Trop., 41* :17-28.

CARVALHO, T.U.; SOUTO-PADRÓN, T. & DE SOUZA, W., 1984. *Trypanosoma cruzi:* Surface charge and freeze-fracture of amastigotes. *Exp. Parasitol.* In press.

DE SOUZA, W., 1984. Cell biology of *Trypanosoma cruzi. Int. Rev. Cytol., 86* :197-283.

DE SOUZA, W.; ARGUELLO, C.; MARTINEZ-PALOMO, A.; TRISSL, D.; GONZALES-ROBES, A. & CHIARI, E., 1977. Surface charge of *Trypanosoma cruzi:* binding of cationized ferritin and measurement of cellular electrophoretic mobility. *J. Protozool., 29* :411-415.

DE SOUZA, W.; MARTINEZ-PALOMO, A. & GONZALES-ROBES, A., 1978. The cell surface of *Trypanosoma cruzi:* Cytochemistry and freeze-fracture. *J. Cell. Sci., 33* :285-299.

DE SOUZA, W. & MEYER, H., 1975. An electron microscopic and cytochemical study of the cell coat of *Trypanosoma cruzi* in tissue cultures. *Z. Parasitenkd., 46* :179-187.

KIPNIS, T.L.; DAVID, J.R.; ALPER, C.A.; SHER, A. & DA SILVA, W.D., 1981. Enzymatic treatment transforms trypomastigotes of *Trypanosoma cruzi* into activators of alternative complement pathway and potentiates their uptake by macrophages. *Proc. Natl. Acad. Sci. USA, 78* :602-605.

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

PEREIRA, M.E.A.; LOURES, M.A.; VILLALTA, F. & ANDRADE, A.F.B., 1980. Lectin receptors as markers for *Trypanosoma cruzi. J. Exp. Med., 152* :1375-1392.

SOUTO-PADRÓN, T.; CARVALHO, T.U.; CHIARI, E. & DE SOUZA, W., 1984. Further studies on the cell surface charge of *Trypanosoma cruzi. Acta. Trop., 41* :215-225.

SOUTO-PADRÓN, T. & DE SOUZA, W., 1983. Freeze-fracture localization of filipin-cholesterol complexes in the plasma membrane of *Trypanosoma cruzi. J. Parasitol., 69* :129-137.

SOUTO-PADRÓN, T. & DE SOUZA, W., 1985. Sialoglycoproteins and sialoglycolipids contribute to the negative surface charge of epimastigote and trypomastigote forms of *Trypanosoma cruzi. Biochem. Biophys. Acta.* In press.

SOUTO-PADRÓN, T.; DE SOUZA, W. & HEUSER, J.E., 1984. Quick-freeze, deep-etch, rotary replication of *Trypanosoma cruzi* and *Herpetomonas megaseliae. J. Cell Sci., 69* :167-178.