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Concanavalin A receptors on the cell membrane of *Trypanosoma cruzi*[1]

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Summary

Epimastigotes and trypomastigotes of *T. cruzi* obtained from acellular culture as well as bloodstream trypomastigotes agglutinate with concanavalin A (Con A). Con A-binding sites were also localized on the cell membrane by using the Con A-horseradish peroxidase-diaminobenzidine method. Passage of epimastigotes and trypomastigotes from acellular culture through DEAE-cellulose column did not affect Con A-binding sites as detected by agglutination and electron microscopy.

Key words: Trypanosoma cruzi; epimastigotes and trypomastigotes; cell membrane; Concanavalin A receptors; electron microscopy; cytochemistry.

Introduction

Trypanosoma cruzi presents during its life cycle in the vertebrate host various developmental stages which on structural bases are identified as amastigote, epimastigote and trypomastigote forms. Only the amastigote and epimastigote forms are able to divide. The trypomastigote form plays a fundamental role in the life cycle of *T. cruzi* since it is the agent of natural infection and is able to penetrate host cells (for a review about the life cycle of *T. cruzi* see Brener, 1973).

One approach to the analysis of the complex host-parasite interactions in Chagas' disease is a comparative study of the surface properties of the invasive

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trypomastigote and noninvasive epimastigote forms. Our previous studies on the cell surface of *T. cruzi* have included the detection of carbohydrates on the cell membrane (De Souza and Meyer, 1975), estimation of the net surface charge determined by cellular electrophoretic mobility (De Souza et al., 1977) and freeze-fracture studies (Martinez-Palomo et al., 1976). In the present report we describe results obtained with the use of the plant concanavalin A (Con A) to determine the presence and distribution of specific carbohydrate-containing receptor sites on the cell surface of epi-and trypomastigote forms of *T. cruzi*. Previous studies (Alves and Colli, 1974) using Con A suggested differences in the membrane of these two forms.

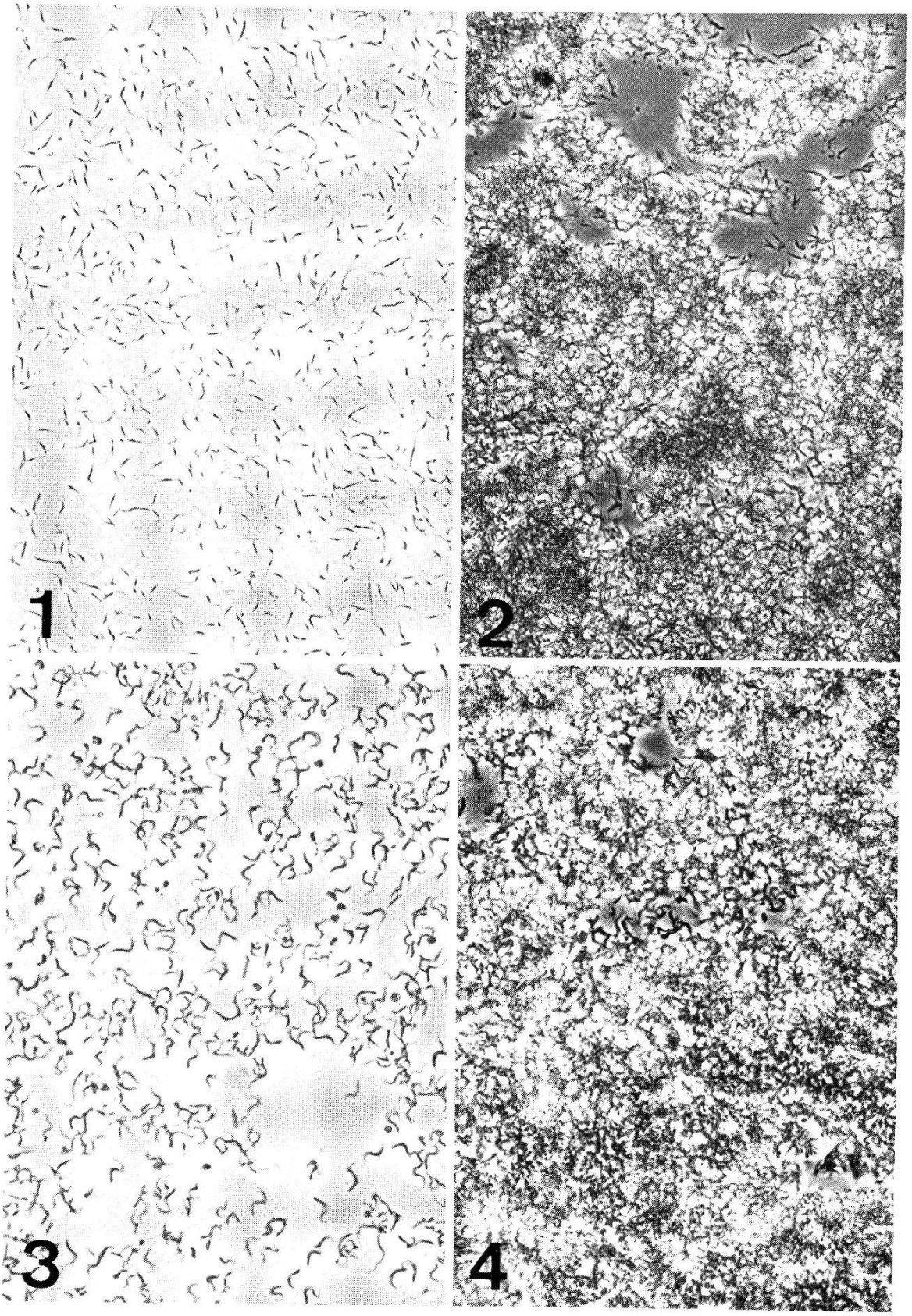
Materials and methods

The following strains of *Trypanosoma cruzi* were used: *Y*, isolated from a human patient with acute Chagas' disease (Silva and Nussenzweig, 1953); *FL*, and *CL* isolated from a naturally infected *Triatoma infestans* collected in Rio Grande do Sul, Brazil (Brener and Chiari, 1963); *Gilmar*, isolated from an acute case of Chagas' disease (Brener et al., 1976).

Trypanosoma cruzi was cultivated in LIT (Liver infusion-Tryptose) medium (Camargo, 1964) for periods varying from 3 to 8 days at 28° C. We used 3-day cultures as the source of epimastigotes and 8-day cultures as that of trypomastigotes. The cell density at time of harvesting epimastigotes and trypomastigotes was 5.10^7 and 8.10^7 cells/ml respectively. The percentage of epi- and trypomastigotes was estimated by microscopic examination. Infectivity of trypomastigotes was assayed by intraperitoneal inoculation of the parasite in mice. To separate epi- from trypomastigotes a modification of Lanham and Godfrey's DEAE-cellulose column method (Lanham and Godfrey, 1970) was employed, as described previously (Goldberg et al., 1976). Bloodstream trypomastigotes were obtained from mice infected with *FL* and *Y* strains which were bled in the 7th day of infection, with a parasitaemea of about 6.10^6 cells/ml. The blood was collected with 3.8% sodium citrate as the anticoagulant and centrifuged at 50 *g* for 15 min at 4° C. The pellet was discarded, and the supernatant fluid, which contained the trypanosomes, was collected and centrifuged at 715 *g* for 15 min at 4° C. The pellet was washed twice in cold 0.01 M phosphate-buffered 0.15 M saline (PBS), pH 7.2, and resuspended in PBS to a concentration of 2.10^7 cells/ml.

Agglutination reaction. Purified concanavalin A (Con A, grade IV) and α-methyl-D-mannoside were purchased from Sigma Chemicals. All Con A solutions were prepared freshly before each experiment. For agglutination studies the cells were collected by centrifugation at 715 *g* for 10 min at 4° C, washed twice in PBS and resuspended in PBS to a concentration of 2.10^7 (bloodstream trypomastigotes) or 2.10^8 (epi- and trypomastigote forms obtained from cultures) cells/ml. An aliquot of the cell suspension was mixed with equal volume of Con A at different concentrations and incubated at room temperature. A rapid slide agglutination test was used. It was scored qualitatively as previously described (De Souza et al., 1976). The controls were done in a system in which the specific inhibitor α-methyl-D-mannoside 0.2 M was added together with Con A.

Electron microscopy. Cells were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 for 1 h at room temperature, post-fixed in 1% OsO_4 in the same buffer, dehydrated in ethanol at increasing concentrations and embedded in Epon. Ultrathin sections were cut with a LKB ultramicrotome and examined unstained or stained with lead citrate in an AEI EM6-B electron microscope. For detection of Con A-binding sites the Con A-horseradish peroxidase-diaminobenzidine technique (Bernhard and Avrameas, 1971) was used. After fixation in glutaraldehyde, the cells were pelleted and rinsed in PBS. The cells were then suspended for 30 min in a solution containing 0.01 or 0.1 mg/ml Con A in PBS. They were rinsed 3 times with PBS, resuspended for 30 min in a 50 μg/ml solution of horseradish peroxidase (Sigma, type II) in PBS, again rinsed 3 times and suspended for 30 min in a medium containing 3–3'diaminobenzidine (Sigma, tetrahydochloride) (0.5 mg/ml) in



Figs. 1–2. Epimastigotes of *T. cruzi* from LIT medium. Control (Fig. 1) and Con A agglutinated (Fig. 2) cells. ×400.

Figs. 3–4. Trypomastigotes of *T. cruzi* from LIT medium. Control (Fig. 3) and Con A agglutinated (Fig. 4) cells. ×400.

Table 1. Agglutination by concanavalin A of trypomastigotes and epimastigotes separated on DEAE-cellulose column (DE 52) and epimastigotes from LIT medium (CL strain)

Flagellates	Con A concentration (μg/ml)*					
	0	1	10	100	250	500
Trypomastigotes from column	0	0	++	++++	++++	++++
Epimastigotes from column	0	0	++	++++	++++	++++
Epimastigotes from LIT medium	0	0	++	++++	++++	++++

* In the presence of 0.2 M sucrose, identical agglutination levels were observed. α-methyl-D-mannoside (0.2 M) inhibited agglutination.

0.05 M Tris-HCl buffer, pH 7.5 and 0.01% H_2O_2. After washing with PBS the cells were post-fixed with OsO_4, dehydrated and embedded. Control specimens consisted of: a) fixed cells exposed to horseradish peroxidase and diaminobenzidine without incubation with Con A; b) fixed cells which, after reaction with Con A, were treated with diaminobenzidine without previous incubation with horseradish peroxidase; c) fixed cells which, after incubation with Con A + α-methyl-D-mannoside (0.2 M), were treated with horseradish peroxidase and diaminobenzidine.

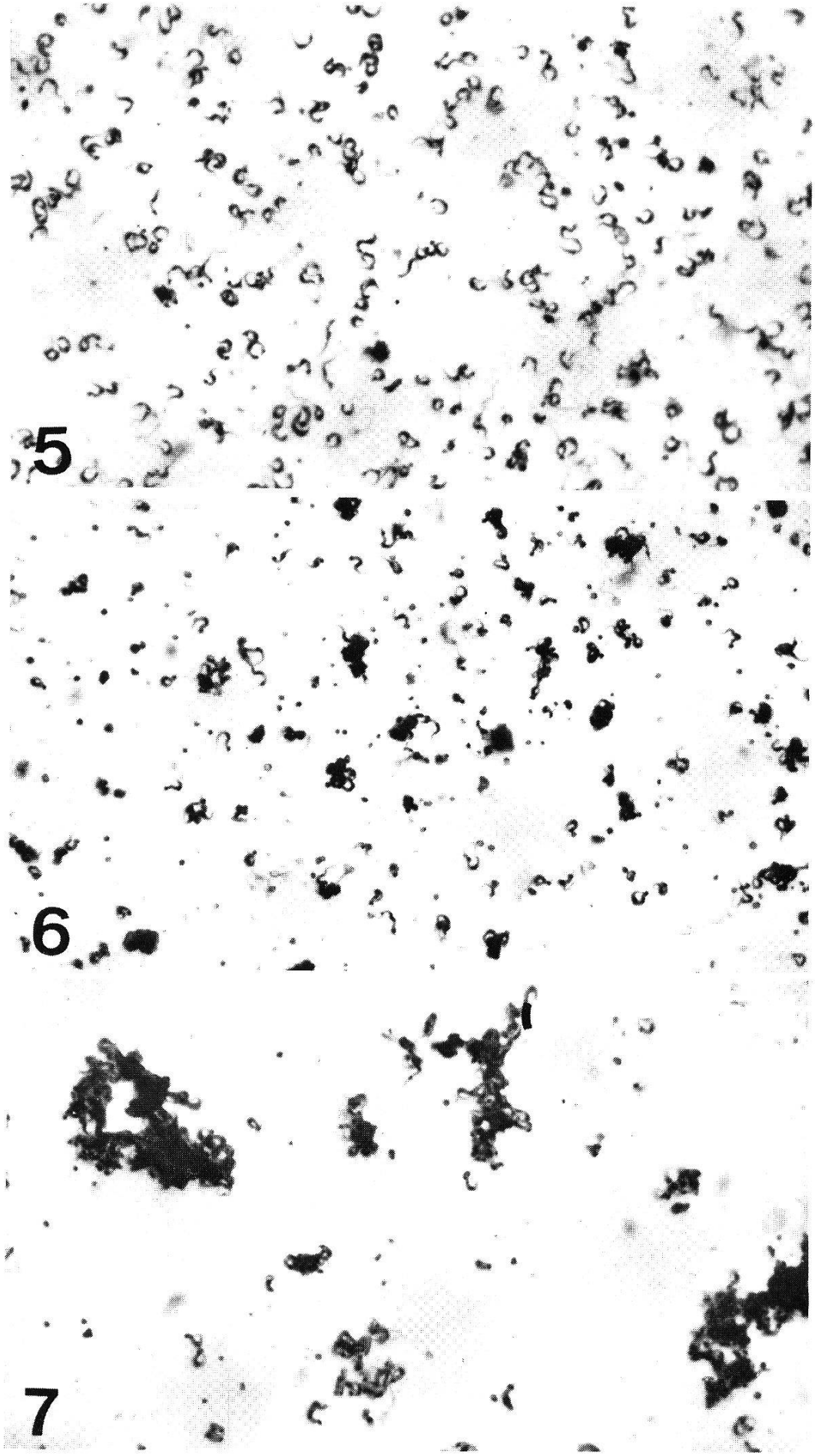
Results

Agglutination. Normal epimastigote, epi- and trypomastigote forms obtained by means of DEAE-cellulose column as well as bloodstream trypomastigotes were agglutinated by various concentrations of Con A used (Table 1, Figs. 1–7). The intensity of the agglutination was the same to all forms used independent of the strain and was influenced by the concentration of Con A used. In the absence of Con A as well as in the presence of the specific inhibitor α-methyl-D-mannoside spontaneous agglutination did not occur. Sucrose (0.2 M) did not inhibit agglutination. Since it is difficult to obtain large quantities of bloodstream trypomastigotes, concentrations of $2 \cdot 10^7$ cells/ml were used. For epi- and trypomastigote forms isolated from cultures, concentrations of $2 \cdot 10^8$ cells/ml were used. In both cases we could see that practically all cells were agglutinated.

In order to see if passage of the cells through DEAE-cellulose column (a procedure necessary to isolate trypomastigotes from LIT medium) interferes with agglutination of trypomastigote forms we used stationary phase populations containing epi- and trypomastigote forms which were not passed through the column. We observed that the two forms were agglutinated.

Cytochemistry. By using the Con A-horseradish peroxidase-diaminobenzidine technique a positive reaction was observed as an electron-dense deposit on the external face of the cell membrane of all forms of *T. cruzi* used (Figs. 8, 10).

Figs. 5–7. Bloodstream trypomastigotes of *T. cruzi*. Control (Fig. 5), Con A 10 μg/ml (Fig. 6) and Con A 100 μg/ml (Fig. 7). $\times 400$.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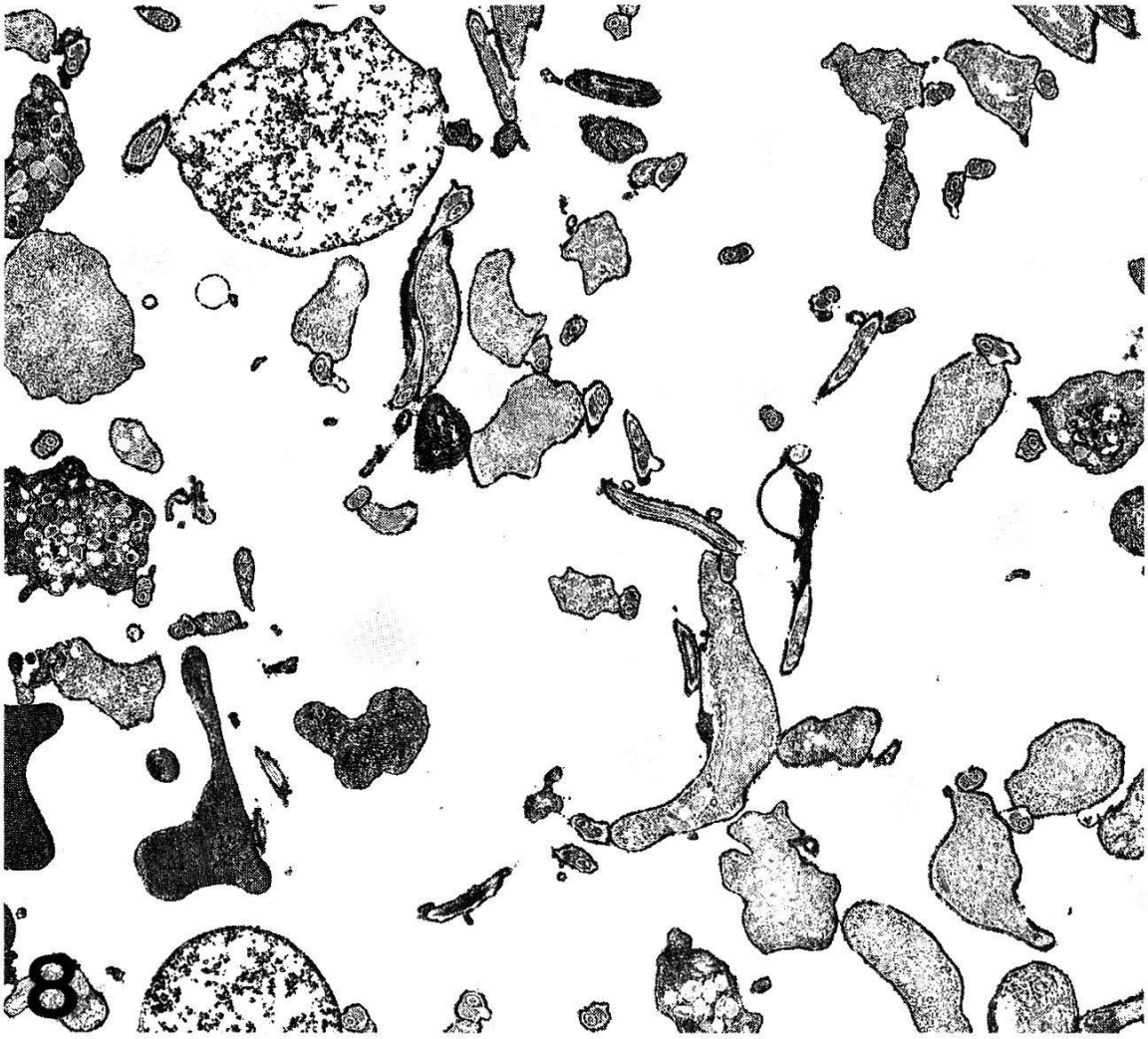
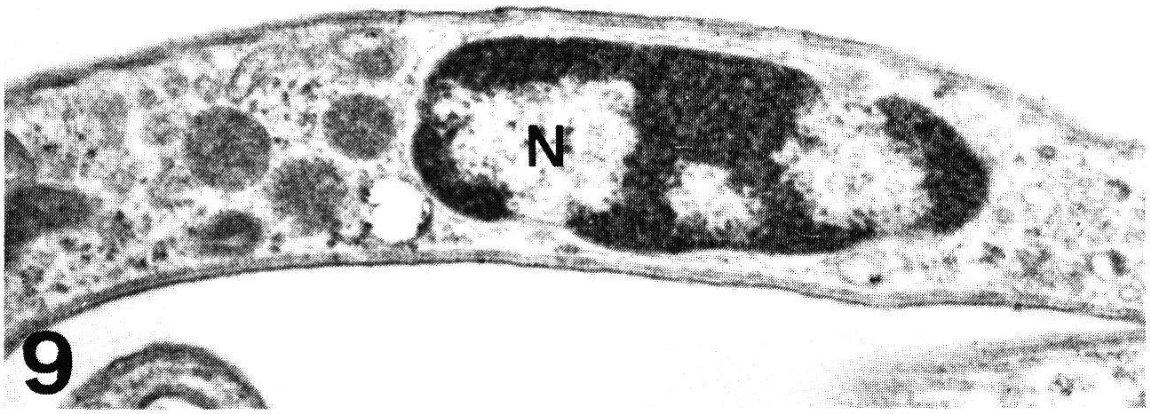


Fig. 8. Bloodstream trypomastigotes of *T. cruzi* submitted to the Con A-horseradish peroxidase-diaminobenzidine method. The Con A receptors are distributed in the surface of the parasite. ×4,000.

The intensity of the reaction was the same on the membrane covering the body of the parasite and the flagellum. Generally the reaction zone presented an average thickness of 25 nm. The reaction was observed with Con A at 10 and 100 μg/ml. As previously described (Martinez-Palomo et al., 1976), in epimastigotes the reaction was more intense in the cytostome. Passage of the cells through DEAE-cellulose column did not alter the distribution of Con A-binding sites as detected by electron microscopy. The reaction was evident in sections which were not stained with uranyl acetate or lead citrate. However, in order to have sections with good contrast they were briefly stained with lead

Fig. 9. Control preparation. The cell was processed by the Con A-horseradish peroxidase method, but the reaction was inhibited by the addition of α-methyl-D-mannoside. N = nucleus. ×35,000.

Fig. 10. Bloodstream trypomastigotes submitted to the Con A-horseradish peroxidase method. Reaction is seen, in the region of adhesion of the flagellum to the cell body (arrow). FP = flagellar pocket; K = Kinetoplast. ×22,500.



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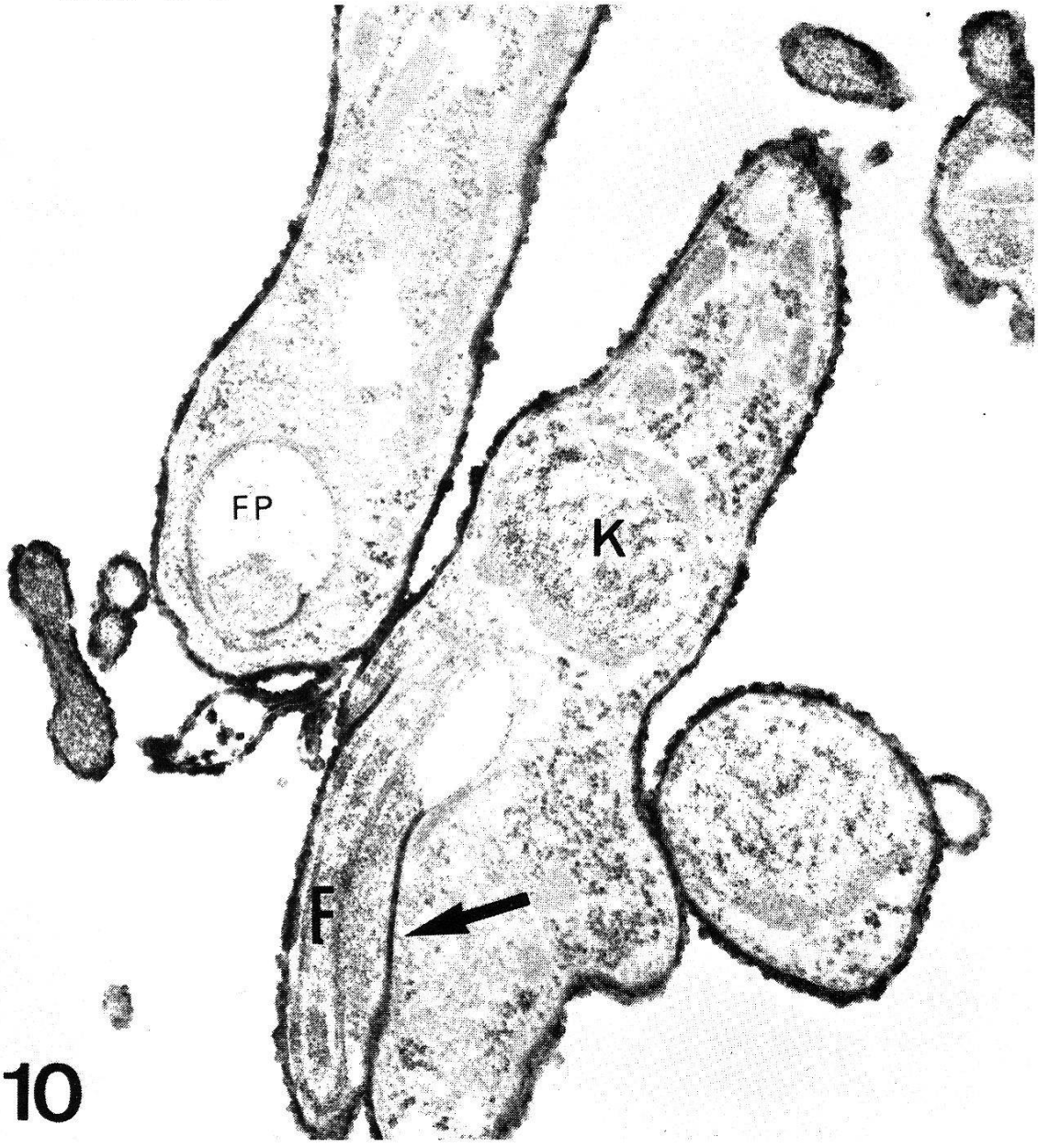

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citrate. In all controls used no reaction was observed in any region of the cell membrane (Fig. 9).

Discussion

Plant lectins which bind to specific carbohydrate residues are useful probes for assessing some of the properties of cell surface components such as the localization and organization of carbohydrate-containing receptor sites (Nicolson, 1974). Among lectins Con A is frequently used, being specific for receptor sites containing α-D-mannopyranose-like residues. Previous studies using cytochemical methods associated with electron microscopy show that in *T. cruzi* carbohydrates are localized on the cell membrane and in membranes of some cytoplasmic vesicles (De Souza and Meyer, 1975). Recently a lipopeptidophosphoglycan was isolated from epimastigotes of *T. cruzi*. Based on the fact that low concentrations of this substance inhibit Con A-induced agglutination of *T. cruzi* together with the cytochemical evidence that carbohydrates are mostly located on the cell surface of *T. cruzi* (De Souza and Meyer, 1975), it was suggested that the lipopeptidophosphoglycan is located on the cell membrane (Lederkremer et al., 1976).

It was reported that epimastigotes of *T. cruzi* agglutinated in the presence of Con A whereas trypomastigotes did not (Alves and Colli, 1974). Since this result could indicate differences in surface properties of the two forms, which might facilitate their separation by affinity chromatography, we decided to investigate the problem. Our first results obtained by using the Con A-horseradish peroxidase method show the presence of Con A-binding sites on the cell surface of all stages of *T. cruzi* examined. We also observed agglutination of trypomastigotes from cultures or isolated from the blood of infected mice.

Absence of reaction product in the flagellar pocket has been described in some trypanosomatids when cytochemical methods are applied suggesting that in some cells the flagellar pocket is closed or occluded by the emerging flagellum (Dwyer et al., 1974). In *T. cruzi* the reaction product (Con A-peroxidase) was absent from the flagellar pocket only in trypomastigote forms. The same result is observed by using cationized ferritin particles to detect anionic sites (De Souza et al., 1977). However, ferritin particles were not seen in the region of adhesion of the flagellum to the cell body while Con A-peroxidase was.

As shown in the present report, passage of *T. cruzi* through DEAE-cellulose column did not affect the interaction of Con A with Con A-binding sites localized on the cell surface. Previous work showed that passage of *T. cruzi* through DEAE-cellulose interferes with the net surface charge as measured by electrophoretic mobility (De Souza et al., 1977), the binding of cationized ferritin particles (De Souza et al., 1977) and the transport of lysine and arginine (Goldberg et al., 1976). It was suggested that during the passage of trypomastigotes through the DEAE-cellulose column some of the negative groups are

detached from the cell surface. Since this treatment did not affect Con A-binding sites we can conclude that these sites are strongly attached to the cell membrane of *T. cruzi*.

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