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FREEZE-FRACTURE STUDY OF *TRICHOMONAS VAGINALIS*

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The freeze-fracture technique was used to analyse the organization of the plasma membrane, as well as membranes of cytoplasmic organelles, of the pathogenic protozoan Trichomonas vaginalis. *Rosettes formed by 4 to 14 intramembranous particles were seen on the fracture faces of the membrane lining the anterior flagella as well as in fracture faces of the plasma membrane enclosing the anterior region of the protozoan and in cytoplasmic organelles. Special organization of the membrane particles were also seen in the region of association of the recurrent flagellum to the cell body.*

Key words: *Trichomonas vaginalis* – membrane structure – freeze-fracture – electron microscopy

Trichomonas vaginalis is a pathogenic protozoan of the urogenital tract of humans. Fine structural studies of members of the protozoan order Trichomonadidae have been made by transmission electron microscopy (Simpson & White, 1964; Honigberg et al., 1971), by cytochemistry (Benchimol & De Souza, 1983) and scanning electron microscopy (Warton & Honigberg, 1979). Although these techniques give important informations concerning the organization and structure of cellular components they do not permit a detailed examination of the structure and organization of the cell membranes.

In replicas of freeze-fractured membranes, the inner part of the cell membrane is exposed, allowing the examination of either the inner or the outer membrane halves. In previous papers, we have described the organization of the plasma membrane of *Tritrichomonas foetus* using the freeze-fracture technique (Benchimol et al., 1981; 1982; Benchimol & De Souza, 1984). These studies have revealed the presence of special arrays of intramembranous particles, localized in the flagellar membrane of the anterior flagella and in that portion of the flagellar membrane involved in the attachment of the recurrent flagellum to the protozoan body. Recently we used polymyxin B, a peptide antibiotic which interacts specifically with anionic phospholipids, in such freeze-fracture studies (Benchimol & De Souza, 1988). However, very few investigations have been done on *T. vaginalis*. Honigberg et al. (1984) published a study of the organization of *T. vaginalis* using freeze-fracture, in which two strains of *T. vaginalis,* one with low and one with high pathogenicity, as well as the highly pathogenic KV-1 strain of *T. foetus,* were analyzed. Some functional aspects of the various structures and differences between certain organelles revealed in the two trichomonad species by the freeze-fracture method were discussed. This study has contributed for a better understanding of the fine structure of the membranes of Trichomonadidae, however, certain aspects are still unresolved and open to further investigation.

In this report we present our observations on freeze-fracture replicas of *T. vaginalis.*

MATERIALS AND METHODS

Trichomonas vaginalis, Judith strain, was isolated from a patient attending the University Hospital, at Ilha do Fundão, Rio de Janeiro, Brazil and the cells were grown in the TYM medium (Diamond, 1957) for 24 to 48 h at 37 °C. They were collected by centrifugation at 1500 g for 10 min and fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 2 h at room temperature. After fixation,

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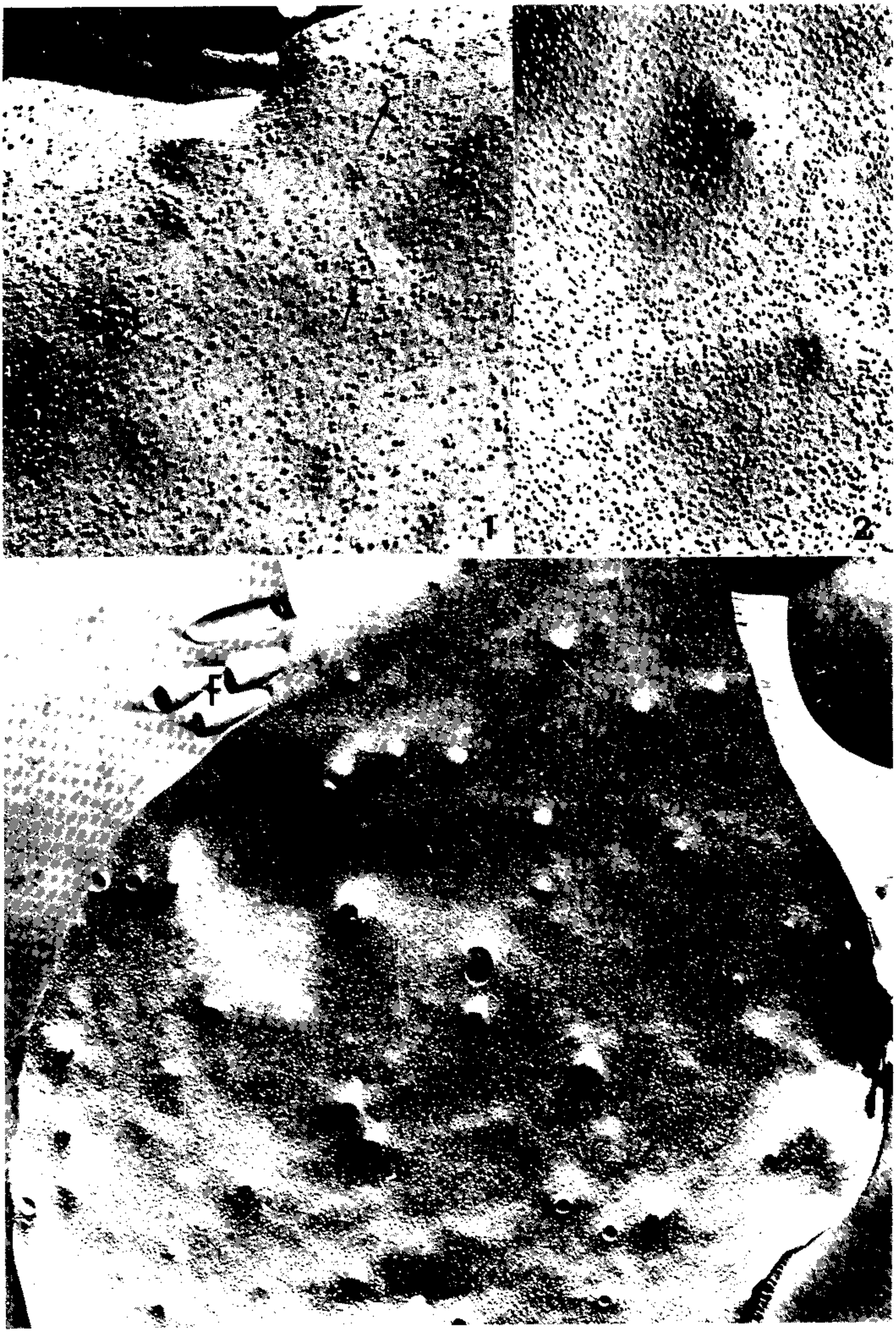
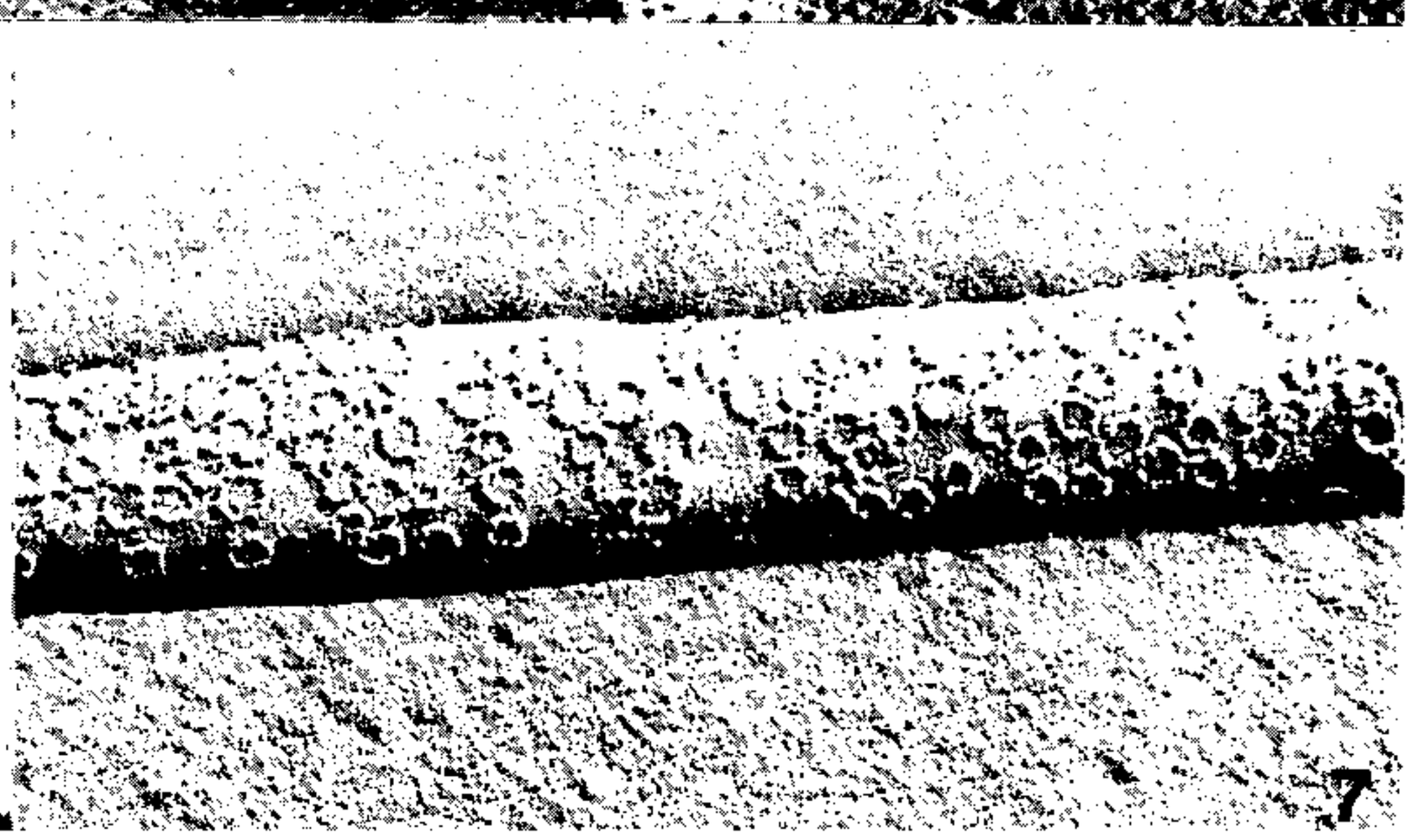


Figs 1-3: views of fracture faces of the membrane which encloses the cell body on *Trichomonas vaginalis.* At the anterior region an array of particles forming rosettes is evident (arrows in Fig. 1). Areas of ondulation are also seen (asterisc in Fig. 2). A large number of endocytic sites is evident on the E fracture face (Fig. 3). F, flagellum. Fig. 1: X 45,000. Fig. 2: X 35,000. Fig. 3: X 13,000.



Figs 4-7: different views of the fracture faces of the membrane which encloses the anterior flagella. Some flagella have few rosettes (arrows in Figs 4-5). Others, however, have many rosettes (Fig. 7). A linear array of particles is seen at the base of the flagellum (arrow in Fig. 6). Figs. 4-5: X 30,000. Fig. 6: X 39,000. Fig. 7: X 35,000.

the cells were washed twice in cacodylate buffer and then exposed to ascending concentrations of glycerol in cacodylate buffer until, after 30 min, a final concentration of 30% (v/v) glycerol was attained. The cells remained in 30% glycerol in cacodylate buffer for 3 to 20 h. Specimens were mounted on Balzers support disks and rapidly frozen in the liquid phase of Freon 22 cooled by liquid nitrogen. Freeze-fracturing was carried out at -115 °C in a Balzers apparatus equipped with a turbomolecular pump. The specimens were shadowed with platinum/carbon at 2.10^{-6} Torr immediately after fracturing. Replicas were recovered in distilled water, cleaned with sulphuric acid and sodium hypochlorite, mounted on 200-mesh grids, and examined in a Jeol 100-CX electron microscope.

RESULTS

Cells studied by freeze-fracture replication may be cleaved through the hydrophobic region of the plasma membrane, exposing large areas of the inner or the outer membrane halves, or through the cytoplasm exposing membranes of cellular components and other nonmembranous structures (Pinto da Silva & Branton, 1970).

Examination of freeze-fracture replicas showed large surfaces of the inner or outer membrane halves (Figs 1-5). Observations of the fracture faces of the *T. vaginalis* plasma membrane revealed a marked heterogeneity in the size and distribution of membrane particles. A great number of areas of pinocytosis were seen throughout the whole plasma membrane (Fig. 3). In most cases the intramembranous particles were randomly distributed. However, at the anterior region of the protozoan, close to the point of emergence of the flagella, some particles were organized forming rosettes (Fig. 1). Areas of ondulation of the surface were also seen (Fig. 2). Determination of the density of intramembranous particles showed the presence of 396 ± 85 and 293 ± 83 particles/μm^2 of the protoplasmic and extracellular faces, respectively.

The structure of the flagellar membrane was different from that of the membrane of the cell body (Figs 4-7). A remarkable feature of the flagellar membrane was the presence of clusters of 4 to 14 intramembrane particles forming rosettes (Figs 4, 5, 7). The diameter of each rosette was approximately 90 ± 27 nm. The number of rosettes per flagellum varied. In some replicas we found 60 rosettes/μm^2 flagellar membrane and in others only 2 rosettes/μm^2. Comparison of the rosettes revealed differences in their number and distribution in the different anterior flagella. No rosettes were ever observed on the P or E faces of the membrane of the recurrent flagellum in all replicas examined during the present study (Figs 8, 11). At the base of the flagella linear array of particles, perpendicular to the major flagellar axis, was seen (Fig. 6).

A specialized region of the cell surface of *T. vaginalis* is the undulating membrane. At this region the recurrent flagellum is attached to a cleft of the cell surface (Figs 8-13). In some replicas it was possible to see the fracture plane jumping from the flagellar membrane to the membrane of the cell body or vice versa. The area of attachment of the recurrent flagellum to the cytoplasmic fold of the undulating membrane was typically marked by four parallel rows of intramembranous particles located on the P face of the flagellar membrane (Fig. 11). The membrane which encloses the protozoan body at the region of contact with the recurrent flagellum showed significant modifications in the pattern of distribution of membrane particles (Figs 8-13). One portion of this area contained very few membrane particles (Figs 8, 12). In another region a large number of particles were organized as parallel rows.

In many cases, the fracture plane deviated from the plasma membrane and entered the cytoplasm, exposing faces of the membrane of intracellular organelles (Figs 14-19). In some cases, double membrane bounded-organelles were seen – they may correspond to hydrogenosomes (Figs 14-15). In some of them cap-like protrusions of their surfaces were found (Fig. 15). There were differences in number and distribution of intramembranous particles in both membranes – the outer membrane had less IMP and when they could be seen they were clustered in a corner of the membrane.

In trichomonads, the Golgi complex corresponds to the parabasal body, which has a close spatial relationship with the parabasal filaments, periodic structures easily recognized, always near to the stacks of the Golgi complex. The micrographs showed an average of eight stacks of closely apposed flattened cisternae and vesicles, still attached to or free of the cisternae (Fig. 17). We could see 2 or 3 dictiosomes in the same cell and the vesicles were found budding from any part of the stacks (Fig. 14).

The nucleus of *T. vaginalis* was seen as an elongated structure showing nuclear pores (Figs 14-16, 18). The pores varied in number and position within the nucleus. Usually, they were randomly distributed (Figs 14-16), but occasionally a concentration of them in one part of the nucleus was observed (Fig. 18).

The mean diameter and mean number/μm^2 of the nuclear pores were 102 ± 21 and 11 ± 5, respectively. Sometimes we found 2 nuclei in the same cell.

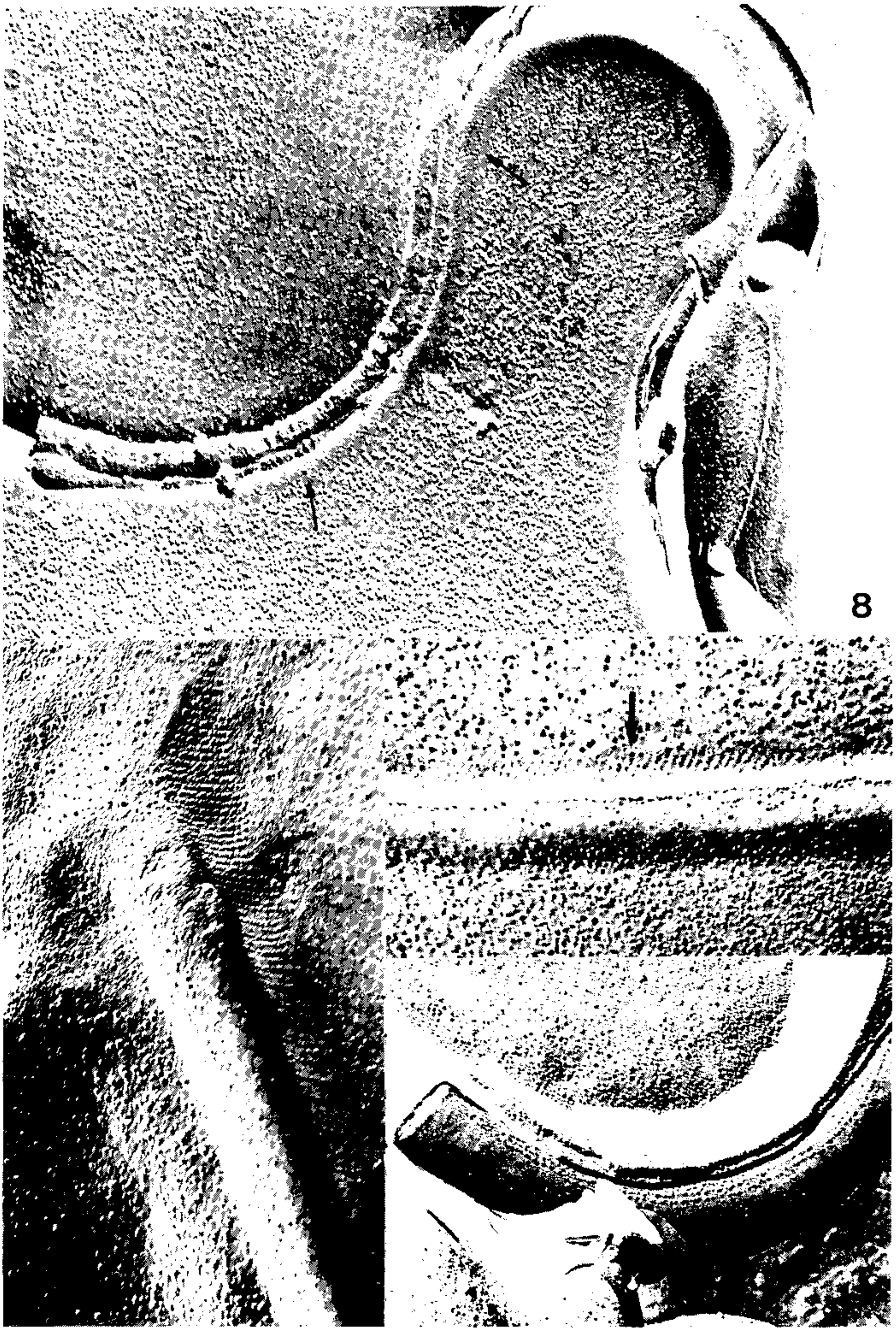
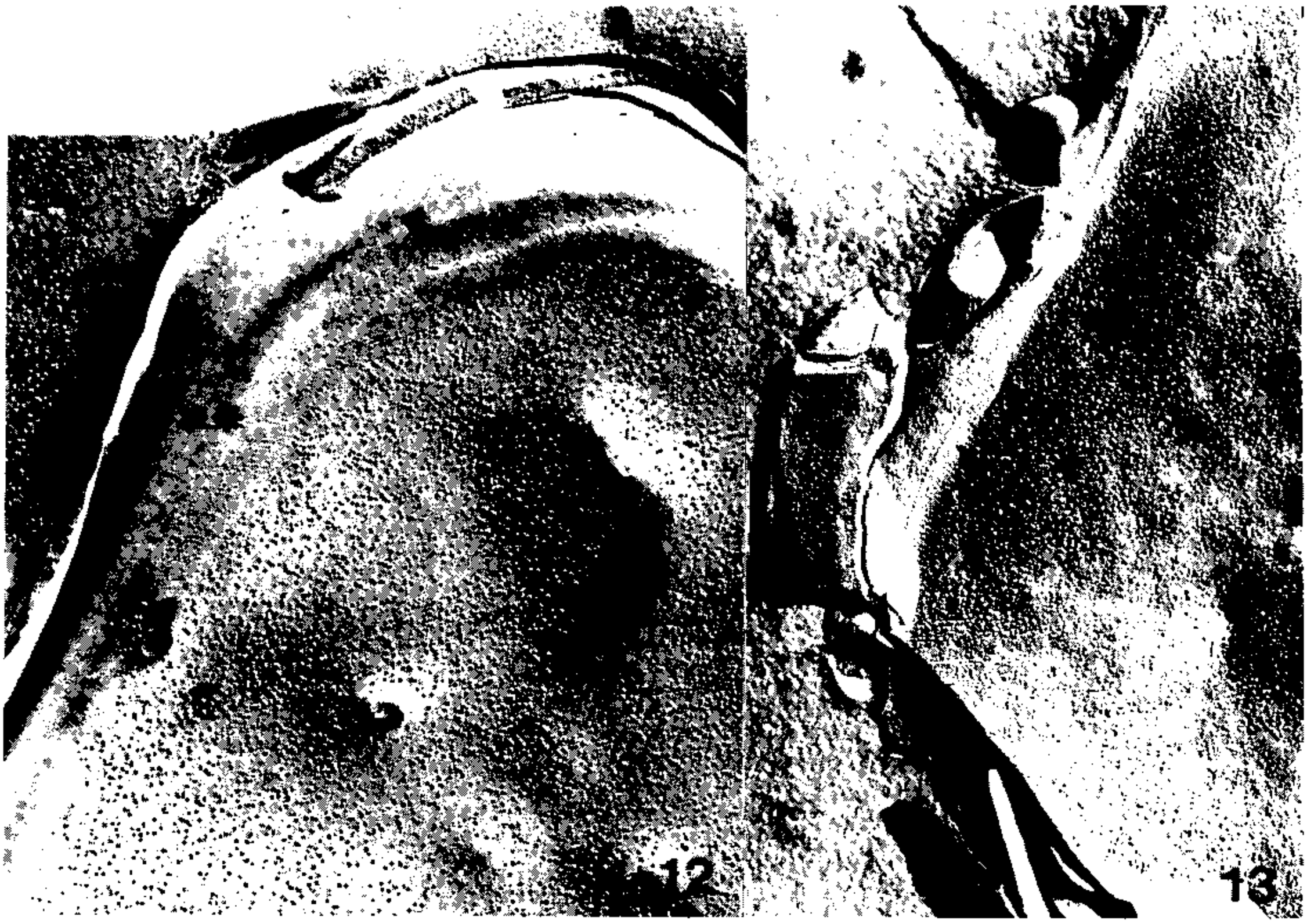


Fig. 8-11: different views of the region of association of the recurrent flagellum to the protozoan body. The characteristic array of particles of the membrane enclosing the cell body is seen in several positions (arrows in Figs 8-10). The rows of particles located on the P face of flagellar membrane and longitudinally oriented in relation to the major flagellar axis are also evident (arrowheads in Fig. 11). Fig. 8: X 23,000. Figs. 9-10: X 60,000. Fig. 11: X 35,000.



Figs 12-13: additional views of the region of association of the recurrent flagellum with the protozoan cell body. Very few membrane particles are seen in part of the attachment region (asterisc in Fig. 12). The characteristic organization of particles is indicated by the arrow in Fig. 13. x 20,000.

In some experiments, we obtained micrographs showing in some organelles (Fig. 19) special arrangements of membrane particles such as rosettes seen in the anterior flagella.

Profiles of the endoplasmic reticulum was seen surrounding the nucleus, appearing as flattened sacculae. The costae and the axostyle were found, albeit infrequently.

In some areas it was possible to see many membrane bounded-organelles, probably lysossomes, endocytic vesicles, phagosomes, etc. Lipid-like bodies were also found (Figs 14, 16).

DISCUSSION

The development of the freeze-fracture technique opened the possibility to analyse at high resolution the distribution of membrane integral proteins which form structures recognized in replicas as intramembranous particles. The application of this technique to some pathogenic protozoa has provided valuable informations, revealing the existence of interesting specialized membrane domains such as (a) the cytostome of epimastigote and amastigote forms of *Trypanosoma cruzi* (Martinez-Palomo et al., 1976; De Souza et al., 1978; Carvalho et al., 1985), (b) the region of attachment of the flagellum to the cell body of trypanosomatids (Reviews in De Souza, 1984; 1989), and (c) the rows of membrane particles seen in the inner membrane of the pellicle of trophozoites of sporozoa (Dubremetz & Torpier, 1978; Cintra & De Souza, 1985).

Previous studies have shown the presence of interesting membrane specializations in *T. foetus* and *T. vaginalis* (Benchimol et al., 1981; 1982). Our present observations add further informations on the membrane organization of *T. vaginalis*. We will discuss only some points which in our opinion are the more interesting.

Figs 14-16: visualization of cytoplasmic structures in freeze-fracture replicas. The random distribution of nuclear pores is evident. Several cisternae of the Golgi complex (G), hydrogenosomes showing a cap-like structure (star in Fig. 15) and cytoplasmic structures surrounded by two membranes are indicated (asterisks in Figs 14, 16). N, nucleus. X 12,000.

Fig. 17: fracture face view showing the nucleus (N), and the various cisternae and vesicles forming the Golgi complex (G). X 20,000. Fig. 18: fracture face showing a concentration of nuclear pores (arrow) at certain regions of the nucleus (N). X 15,000. Fig. 19: a membrane-bounded cytoplasmic structure showing particles arranged as rosettes (arrow). X 24,000.

General views with low magnification of the inner portions of the membrane which encloses the cell body, especially the E fracture face, revealed that *T. vaginalis* presents a large number of protusions characteristic of pinocytic areas, compared with *T. foetus* (for comparison see Fig. 3 in the present study and Fig. 1 in Benchimol et al., 1982). This observation indicates that under the conditions used in both studies, the endocytic activity is higher in *T. vaginalis* than in *T. foetus*. This observation is in agreement with quantitative data related with the endocytic activity of these protozoa (Benchimol et al., 1990).

A second point which deserved some consideration is the organization of the membrane which encloses the anterior flagella. We reported initially the presence of intramembranous particles organized in rosettes on both fracture faces of the flagellar membrane of *T. foetus* (Benchimol et al., 1981;1982). This observation was then confirmed by other authors (Bardele, 1981; Honigberg et al., 1984) and observed also in *T. vaginalis*. Our present observations confirm these studies and add two new informations: (a) the density of rosettes is not the same in all anterior flagella or in the flagella of different cells. In some cases few rosettes are seen; in others, however, the density is so high (see Fig. 7) that there is only relatively small area of the flagellum which does not present rosettes. It is important to point out that almost all particles seen on both fracture faces of the membrane of the anterior flagella are associated with the rosettes; (b) some rosettes, similar to those seen in the flagellar membrane, were seen in the membrane which encloses some cytoplasmic vesicles and in the portion of the membrane which encloses the cell body located close to the region where the anterior flagella emerges. These observations suggest the possibility that the rosettes are assembled in the cytoplasm and then incorporated to the plasma membrane and subsequently concentrated in the membrane lining the anterior flagella. If this idea is correct *T. vaginalis* may constitute an excellent model for the analysis of the process of synthesis and posterior sorting of membrane proteins which are part of specific membrane domains.

A third point which we would like to discuss is related to the region of adhesion of the recurrent flagellum to the cell body. Our previous observation in *T. foetus* (Benchimol et al., 1982) showed the presence of 4-6 ribbons of membrane particles longitudinally oriented in relation to the main flagellar axis. A similar organization of membrane particles was seen in that portion of the membrane of the recurrent flagellum which faces the cell body of *T. vaginalis*. However, only 4 ribbons of closely apposed particles were seen instead of 6, as observed in *T. foetus* (Benchimol et al., 1982). In relation to the membrane lining the cell body at the region of flagellum-cell body attachment, our observations show that there are few randomly distributed particles at this region. Indeed, most of the particles are organized. Although such organization was previously observed by Honigberg et al. (1984) in *T. vaginalis* it was not so clearly visualized in *T. foetus*. Further studies are necessary in order to obtain a clear picture of the three-dimensional organization of this membrane junction, which is significantly different from other junction types.

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