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FINE STRUCTURE OF "LEPTOMONAS PESSOAI" IN THE PROMASTIGOTE STAGE

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RESUMO

A ultraestrutura da **"Leptomonas pessoai"** na fase promastigota é descrita e comparada com a de outros tripanosomatideos.

O micro organismo é envolvido por uma membrana de dupla camada, a qual reveste a bolsa flagelar e é contínua com a membrana limitante do flagelo. Uma estrutura semelhante a desmosoma promove o contacto íntimo entre o flagelo e a película. Uma única fileira de microtubulos sub-peliculares regularmente arranjados situa-se paralelamente ao eixo logitudinal da célula. Sómente uma fileira de quatro a seis microtubulos está presente sob a membrana limitante da bolsa do flagelo. O flagelo exibe, caracteristicamente, duas fibrilas centrais e nove periféricas, sendo estas duplas e constituídas por duas subfibrilas. O cinetoplasto apresenta cristas e uma densa estrutura lamelar de DNA que se apresenta frequentemente em continuidade com uma mitocondria. Um citóstoma aparece como um prolongamento da bolsa flagelar em estreita associação com o aparelho de Golgi e em forma de um funil alongado. O reticulo endoplasmático é pouco desenvolvido Ribosomas ou polisomas preenchem o citoplasma ao lado de numerosos corpos eletro-densos.

FINE STRUCTURE OF "LEPTOMONAS PESSOAI"

"Leptomonas pessoai" is an insect trypanosomatid isolated from the reduviid **Zellus leucogrammus** by Galvão et al. in 1970. Thenceforth the biology, nutrition, metabolism and immunological aspects of this organism have been extensively investigated (Souza and Roitman, 1971; Barbosa et al., 1972; Roitman et al., 1972; Souza and Barbosa, 1972; Barbosa et al., 1973; Santos et al., 1973a; 1973b). Recently Carvalho (1973) have shown that the **"Leptomonas pessoai"** is a name given to a mixed culture.

Because of advances in morphological knowledge of trypanosomatids depend mainly on electron microscopy, the ultrastructural study of **"Leptomonas pessoai"** has been undertaken and our observations on promastigote forms in two-day cultures are described in this paper.

MATERIALS AND METHODS

Two-day cultures in Warren modified medium were centrifu-

ged and the organism fixed in cold 1.4% glutaraldehyde solution in cacodylate buffer (pH 7.2) for 1 hour. The material was then post-fixed in 1% osmium tetroxide in cacodylate buffer for 45 minutes, dehydrated in ascending concentrations of ethanol followed by propylene oxide, infiltrated with propylene oxide—Epan B12 mixture and embedded in Epan 812 (Luft, 1961). At each subsequent change the tube was centrifuged, the fluid poured off, the next fluid added, and the pellet broken up very gently. Ultrathin sections were out with a diamond knife on a Porter Blum MT-1 ultramicrotome and double stained with uranyl acetate followed by lead citrate. Observations were made with a Zeiss EM 96-2 electron microscope.

OBSERVATIONS

Only promastigotes appear in the exponentially growing cultures which we studied.

Promastigotes of **"Leptomonas pessoai"** are bounded by a double osmiophilic pellicular membrane with an intermediate layer of low density (Figs. 1, 2, 6, 8, 11). The cell membrane invaginates at the anterior and of the cell to form the flagellar pocket (FP, Figs. 2, 6, 8, 9) and is continuous with the flagellar membrane. The flagellar pocket, also called reservoir, is a membrane bounded space that surrounds the proximal region of the flagellum. Zones of attachment between the pellicua and the fagellar membrane can be seen (AZ, Figs. 3, 4, 9). Such attachment zones constitute desmosome-like plaques of the "macula adherens" type.

Regularly arranged subpellicular microtubules run parallel to the longitudinal axis of the cell beneath the pellicula and form the periplast layer (PM, Figs. 2, 6, 8, 11). The invaginated cell pocket is devoid of subpellicullar membrane portion of the flagellar microtubules, with the exception of a row of four to six tubules (Figs. 3, 9, at the arrows).

The nucleus lies in the middle of the cell and is sligthly rounded to elliptical (N, Figs. 1, 2). It is surrounded by two membranes separated from each other by a space and frequently interrupted by pores (Fig. 2, at the arrows). A roundish nucleolus is in the middle portion of the nucleus and it appears as an electron-dense body. The space between nuclear membrane and nucleolus is filled with a granular material.

Immediately anterior to the nucleus is the kinetoplast (KP, Fig. 2). It appears variable in sape and has a granular matrix of density similar to the matrix of mitochondria (Figs. 2, 7, 11). Immersed in this matrix is a dense lamellated fibrous structure arranged perpendicularly to the long axis of the cell and surrounded by a homogeneous electron-translucent region (Fig. 2). In transverse sections the dense structures appear as tubular structures (Fig. 11). Continuation of the kinetoplast with a mitochondria is quite frequent in our material (Fig. 7). A double wavy membrane completely surrounds the kinetoplast and its

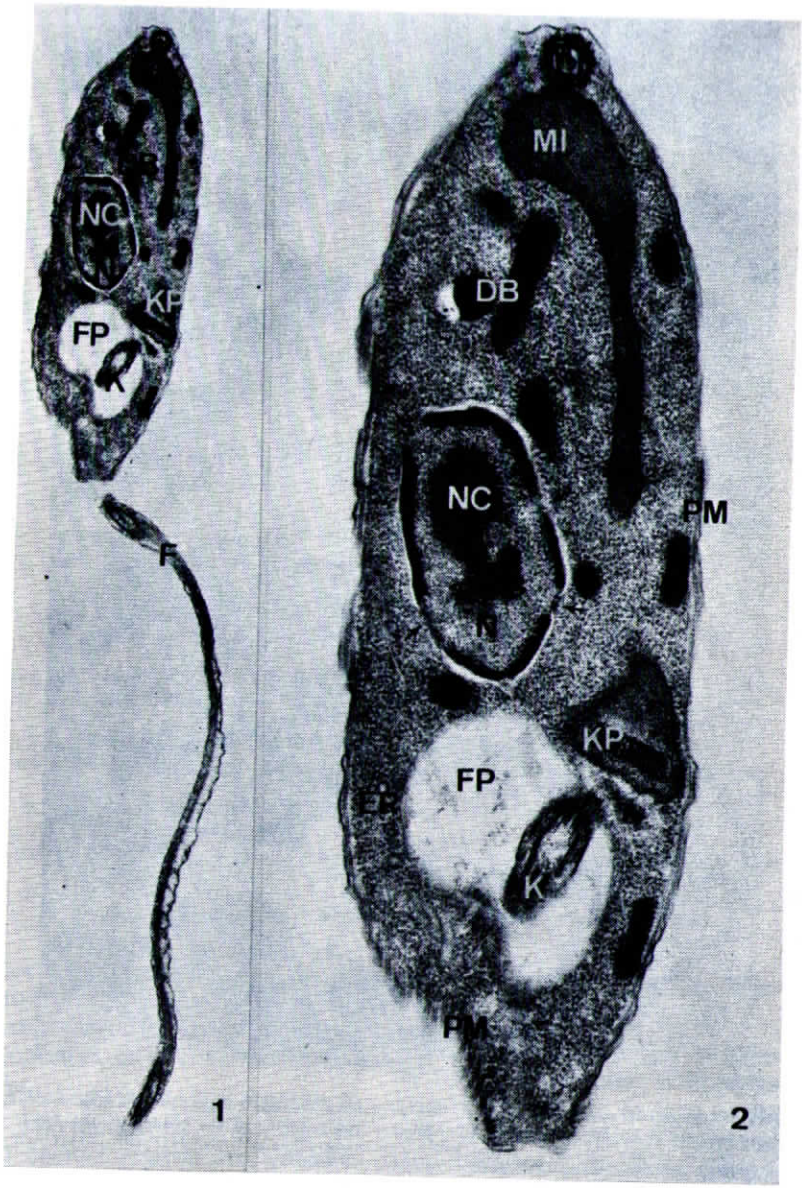

Figura 1 - General view of a promastigote forms. Note the relative position of the nucleus, kinetoplast and flagellum x 12500

Figura = 2- Longitudinal section through the cell body. Nucleus (N), nucleolus (NC) kinetoplast (KP), kinetosome (K), flagellar pocket (FP), subpellicular microtubules (PM), endoplasmic reticulum (ER). Numerous electron-dense bodies (DB) scattered throughout cell as well as a large mitochondrial tube (MI). A myelin figure (MY) is present at the posterior and of the cell x 32000.

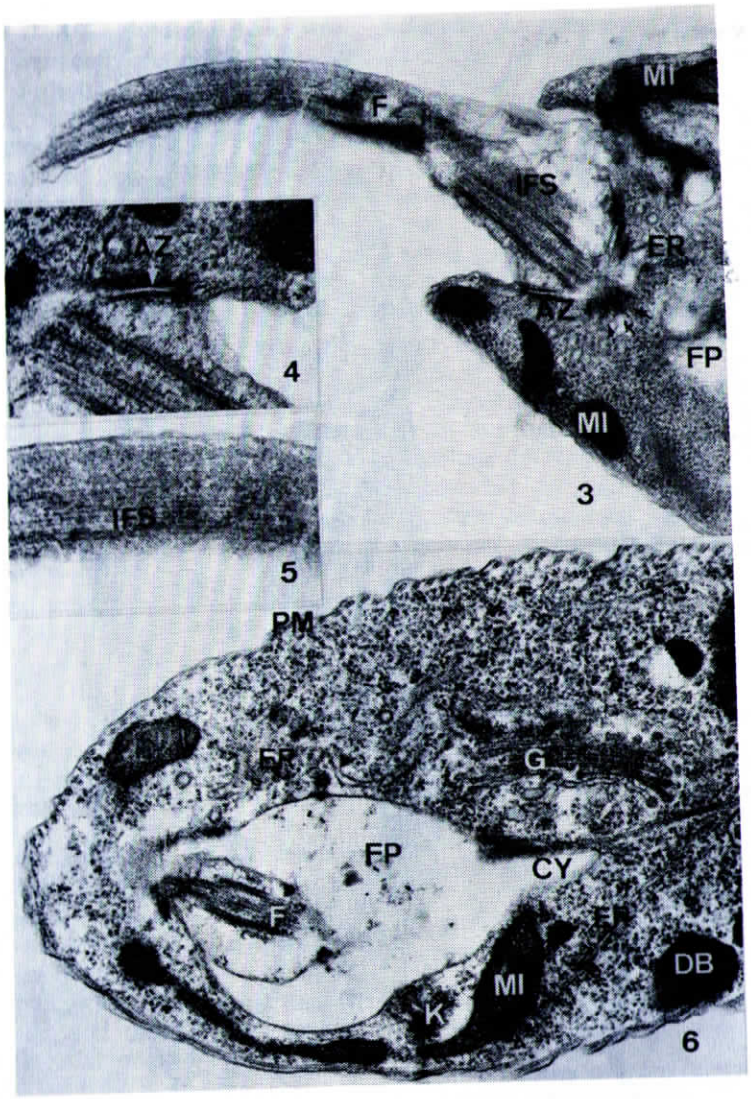

Figure 3 Attachment zones (AZ) between the pellicula and the flagellar membrane are demonstrated, Subpellicular microtubules can be seen in the border of the flagellar pocket (arrows). Endoplasmic reticulum (ER), mitochondria (MI),flagellum (F), and intra-flagellar structure (IFS). x 40000.

Figure 4 Flagellar attachment zone is morphologically similar to the desmosomes of vertebrate cells x 80000.

Figure 5. Longitudinal section through the intra-flagellar structure (IFS).It is composed of filaments, giving this structure a honey-comb-like appearance x 95000.

Figure 6. Longitudinal section of the anterior region of the cell demonstrating the Golgi apparatus (G), composed of sac-like membranes and vesicles, in close association with the cytostome (CY). The endoplasmic reticulum (ER) is represented by smoothsurfaced membranes. Kinetosome (K), flagellar pocket (FP), subpellicular microtubules (PM), mitochondria (MI) x 40000.

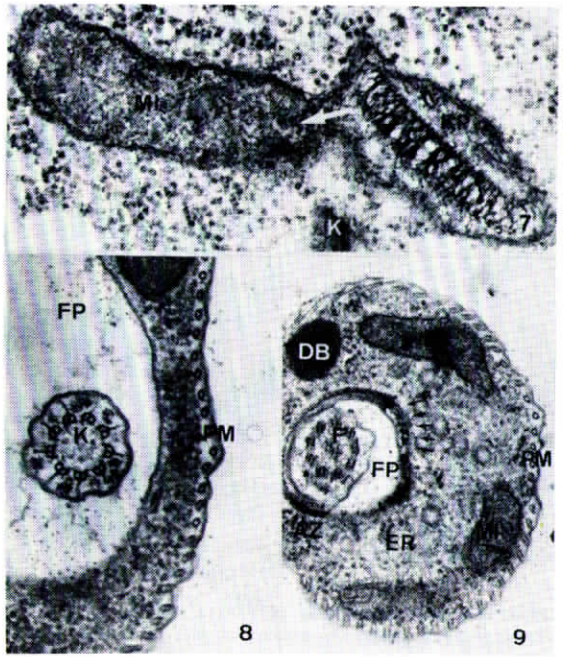

Figure 7 - Details from a kinetoplast (KP) Arrow indicates a continuation of this organelle with a mitochondria. It contains the dense lamellated fibrous structure embedded in the mitochondrial matrix. Kinetosome (K) is observed in close association to the kinetoplast x 80000.

Figure 8. Cross section through the distal portion of the kinetosome (K) in the flagellar pocket (FP). The doublet pattern of fibrils is shown. A single row of subpellicular microtubules (PM) is seen beneath the cell membrane x 75000.

Figure 9 - Cross section through apical region of the cell showing a semi-circular attachment zone (AZ). Arrow heads indicate five microtubules liying below the membrane of the flagellar pocket (FP). Subpellicular microtubules (PM), endoplasmic reticulum (ER), mitochondria (MI) x 70000.

mitochondrial extensions. Cristae mitochondriales have also been found within the limits of the kinetoplast.

The kinetosome lies anterior to the kinetoplast (K, Fig. 2) and two distinct regions are identifiable. In cross section the proximal portion has nine peripheral groups of three fibrils. The distal portion has nine groups of two fibrils with a septa arising from each one of these nine doublets and projecting towards the kinetosome membrane (Fig. 8).

The flagellum, which originates in the kinetosome, is approximately three times as long as the cell. It has two central fibrils and nine peripheral doublets composed of two subfibrils A and B (F, Fig. 10). Subfibril A has a circular profile, while subfibril B appears crescentic and sharing a part of the wall of subfibril A. Appendages of the subfibrils A

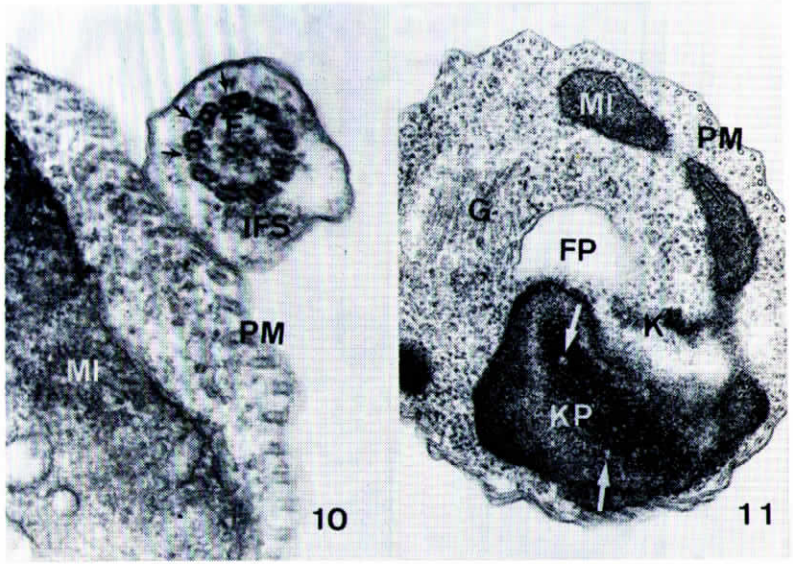

Figure 10 . Cross section through the pellicula and the flagellum exhibits the characteristic 9+2 arrangement of the fibrillar elements. Arm-like appendages are indicate by arrows x 100000.

Figure 11. Transverse section of the kinetoplast. The dense lamellated fibrous structures appear as tubular structures (arrows). Golgi apparatus (G), flagellar pocket (FP); subpellicular microtubules (PM), mitochondria (MI), kinetosome (K). x 40000.

oppose the subfibrils B of adjacent doublets. Running alongside the fibrilar elements of the flagellum there is a moderately dense matrix, which constitute the intra-flagellar structure (IFS, Figs. 3, 5, 10). It is composed of filaments, giving this structure a honey-comb-like appearance.

Mitochondria appear as tubular structures with a granular matrix and limited by two membranes, a relatively smooth outer one and a inner one having few sparce cristae (MI, Figs. 2-3, 6, 10, 11). A single large mitochondrial tube extends along the cell, more or less parallel to and underneath the pellicula, from the posterior face of the kinetoplast to the posterior and of the organism (Fig. 2).

The Golgi apparatus lies in the region of the flagellar pocket (G, Figs. 6, 11). It comprises several parallel cisternae and many Golgi vesicles budding off laterally. In close association with the Golgi elements is the cytostome, with a shape of an elongated funnel, being as a prolongation of the flagellar pocket (CY, Fig. 6). A few microtubules are seen under the limiting membrane of the cytostome.

The poorly developed endoplasmic reticulum is chiefly represented by smooth membranes situated in the vicinity of the flagellar pocket. Rough-surfaced membranes are unusual. Dense granules, isolated or in clumps, that correspond to ribosomes or polysomes, fill the cytoplasm.

Numerous electron—dense bodies are distributed throughout the cytoplasm (DB, Figs. 2, 6, 9). They present a flocculent to fine-granular matrix, but sometimes it appears as myelin figures (MY, Fig. 2).

DISCUSSION

The fine structure of **"Leptomonas pessoai"** in the promastigote stage does not differ significantly from other trypanosomatids and only few points need comment.

The arrangement of the subpellicular microtubules and of the mastigont system agree with what has been described for other trypanosomatids. However the fine—filamentous intertubular connections described in trypanosomes (Steiger, 1973) were not observed. The single row of four to six microtubules beneath the limiting membrane of the flagellar pocket is of interest. Similar microtubules have been reported in a wide variety of free living and parasitic organisms (Schulz and MacClure, 1961; Vickerman, 1962; Anderson and Ellis, 1965; Burton, 1966; Kusel et al., 1967; Paulin, 1969; Taylor and Godfrey, 1969; Paulin and McGhee, 1971; Steiger, 1973). Their function is unknown, but Angelopoulos (1970) has suggested that they may have a role in attaching the flagellum to the pellicular microtubules.

Our electron microscopic observations in promastigote forms of **"Leptomonas pessoai"** show that its flagellum is physically attached to the cell surface. Such an attachment zone is morphologically similar to the desmosome of vertebrate epithelial cells when viewed in cross sections. They are desmosome-like plaques of the "macula aherens" type (Fawcett, 1966). These adhesion zones were previously described in **Trypanosoma lewisi** (Anderson and Ellis, 1965), **T. gambiense** (Boisson et al., 1965) and **T. rhodiense** (Vickerman, 1969b). The presence of these attachment zones in members of the genus **Leptomonas** has not been demonstrated before.

The central part of the kinetoplast is made up of tubular fibrous structures. These tubules are mostly parallelly arranged, but sometimes they interlace. According to several authors (Cosgrove and Anderson, 1954; Steinert, 1960; Schulz and MacClure, 1961; Anderson and Ellis, 1965; Wollace and Hertig, 1968; Delain and Riou, 1969; Sanabria, 1970; Ozeki et al., 1971) they are DNA threads composed by DNA molecules forming a continuous spiral in shape of a figure eight. This dense band of kinetoplastic DNA is not unlike other trypanosomatids studied. Mitochondrial cristae were also observed within the limits of the kinetoplast. The majority of investigators postulate that the kinetoplast is responsible for the mitochondrial activity of the try-

panosomatid cell, being actively involved in the formation of mitochondria under the control of DNA.

A cytostomal system has been observed in **Trypanosoma mega** (Steinert and Novikoff, 1960), **T. conorrhini** and **T. cruzi** (Milder and Deane, 1969; Sanabria, 1970) and **T. raiae** (Preston, 1969), but not in culture forms of **T. brucei,** in the course of its developmental cycle in the blood stream and vector (Steiger, 1973), and in culture forms of **T. vivax, T. congolense** and **T. equinum** (Sanabria, 1970). To our knowledge the presence of cytostome in promastigote forms has not been demonstrated. In this paper we present evidence for the existence of a cytostome in promastigotes of **"Leptomonas pessoai"**. The function of the subpellicular microtubules related to the cytostome is obscure, but probable they allow movements that open and close the entrance of the organelle.

The electron-dense bodies correspond to the peroxisome-like organelles of **Trypanosoma congolense** (Vickerman, 1969a) and **T. cruzi** (Maria et al., 1972). According to Vickerman (1969a) they are extramitochondrial sites of enizymatic activity. A high peroxidatic activity was reported from culture epimastigotes of **Trypanosoma cruzi** (Kallinikova, 1968). The presence of myelin figures suggests that they may contain a phospholipid material.

SUMMARY

FINE ESTRUCTURE OF "LEPTOMONAS PESSOAI" IN THE PROMASTIGOTE STAGE

The fine structure of **"Leptomonas pessoai"** in the promastigote stage is described and compared to that of other trypanosomatids. The organism is bounded by a double-layered unit membrane which lines the flagellar pocket and is continuous with the limiting membrane of the flagellum. A desmosome-like structure maintains continual cell membrane contact with the flagellum and the pellicula. A single row of regularly arranged subpellicular microtubules runs parallel to the longitudinal axis of the cell. Only a row of four to six microtubules are present under the limiting membrane of the flagellar pocket. The flagellum exhibit the characteristic two central and nine peripheral fibril doublets composed of two subfibrils; it projects from its origin near the anteriorly placed kinetoplast and kinetosome. The kinetoplast contains cristae in addition to a dense lamellated DNA structure and often is continuous with a mitochondria. A cytostome, with a shape of an elongated funnel, appears as a prolongation of the flagellar pocket, in close association with the Golgi elements. The endoplasmic reticulum is poorly developed. Ribosomes or polysomes fill the cytoplasm. Numerous electron-dense bodies are found distributed throughout the cytoplasm.

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