INVITED REVIEW

The Hydrogenosome

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Abstract

The hydrogenosome is an organelle described in trichomonads, parasitic protists where most studies were carried out and also in organisms phylogenetically distant such as rumen ciliates, some free-living ciliates and chytrid fungi (Chytridiomycota). The organisms presenting hydrogenosomes live in anaerobic habitats and they all lack mitochondria.

The hydrogenosome contains enzymes that participate in the metabolism of pyruvate and is the site of formation of ATP and molecular hydrogen. The origin of the hydrogenosome brought a great debate, since like mitochondria, it has a double membrane envelope, divides autonomously by fission, imports proteins post-translationally, and produces ATP. However, it differs from mitochondria in that it seems to lack a genome, a respiratory chain, cytochromes, the F_0F_1 system, the tricarboxylic acid cycle, oxidative phosphorylation, and cardiolipin. The hydrogenosome uses pyruvate:ferredoxin oxidoreductase, a counterpart to the mitochondrial pyruvate dehydrogenase complex and presents hydrogenase, an enzyme typically restricted to anaerobes.

Hypotheses have been proposed for the origin of the trichomonad hydrogenosome: an independent endosymbiosis of an anerobic eubacterium with a eukaryotic host or the conversion of an established mitochondrion adapted to an anaerobic lifestyle. The common origin hypothesis for mitochondria and hydrogenosome was refined to state that both organelles evolved from a common progenitor structure present in eukaryotes before the advent of true mitochondria or hydrogenosome.

Hydrogenosome is a spherical or slightly elongated structure (when in process of division), 0.5-1.0 μm diameter, usually associated with cytoskeletal structures such as the axostyle and costa in trichomonads. The matrix of hydrogenosome is homogeneously granular. Crista-like invaginations of inner-membrane were described in hydrogenosome of some rumen ciliates. The matrix also presents calcium deposits. The hydrogenosome of trichomonads contains a flattened, membrane-bounded vesicle completely dissimilar from the hydrogenosomal matrix. This vesicle contains high levels of Mg^{++}, Ca^{++} and P and possibly functions in intracellular calcium regulation. Its membrane presents N-acetyl-glucosamine-containing glycoconjugates.

Close proximity, and even continuity, between endoplasmic reticulum and hydrogenosome was observed.

Hydrogenosomal proteins are synthesized on free polyribosomes, protein import occurs post-translationally, is dependent on the presence of a cleavable N-terminal presequence, cytosolic protein (s) and energy in the form of temperature, ATP and a transmembrane electrochemical potential, similar to that of mitochondria and chloroplasts.

Introduction

During the evolutionary process eukaryotic microorganisms presenting special organelles appeared. One example is the glycosome, a particular type of peroxisome found in protozoa of the kinetoplastida order, which contains most of the glycolytic enzymes (Opperdoes, 1984; Attias and De Souza, 1995). A second example is the hydrogenosome initially found in protozoa of the Trichomonadida Order and which contains enzymes that participate in the metabolism of pyruvate formed during glycolysis and is the site of formation of molecular hydrogen and ATP (Müller, 1993). The

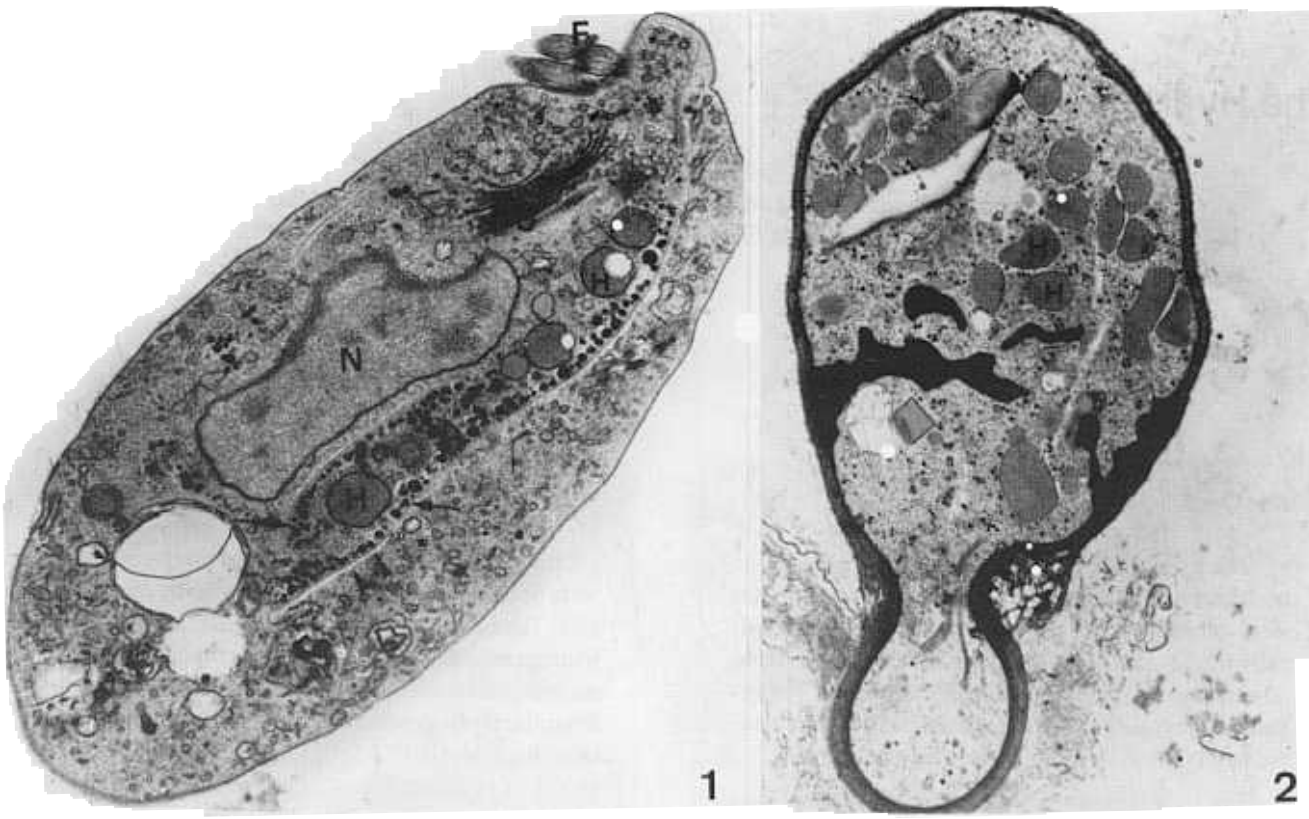


Fig 1: *General aspect of Tritrichomonas foetus seen in the transmission electron microscopy in a routine thin section. The hydrogenosomes follow the axostyle (arrowheads). The proximity of glycogen granules and hydrogenosomes is shown by arrows. The following structures are visible: the anterior nucleus (N), numerous spherical, electron-dense hydrogenosomes (H), the Golgi complex (G) and flagella (F). X 11,000. (from Benchimol* et al., *1996 a).*

Fig. 2: *General view of the fungus Neocallimastix frontalis in a routine thin section showing the overall distribution of hydrogenosomes in the rhizoid. The hydrogenosomes (H) are seen as spherical or elongated structures and are more concentrated at the anterior region of the cell. X 7,000 (from Benchimol* et al., *1997).*

hydrogenosome was discovered in 1973 by Lindmark and Müller in the protist *Trichomonas vaginalis*. It was named hydrogenosome by its property of molecular hydrogen production. Hydrogenosomes have been found in a number of unicellular organisms that inhabit anoxic or low O_2 environments.

Hydrogenosomes had been recognized by light microscopists for a long time as paraxostylar and paracostal granules, by its close proximity with the axostyle (bundle of microtubules) (Fig. 1, 6-7) and costa (Figs. 6-7)- a periodic proteinaceous structure present in the trichomonads. Only biochemical studies revealed their functional significance, which demonstrated the molecular hydrogen production as a metabolic end product (Lindmark and Müller, 1973). This organelle presents an unusual function: under anaerobic conditions it oxidizes pyruvate to malate producing molecular hydrogen (Müller, 1993).

More recently hydrogenosomes were found in several anaerobic species, such as other protists of the Trichomonadida Order (Müller, 1993), in rumen ciliates (Yarlett *et al.*, 1981, 1983, 1984; Snyers *et al.*, 1982; Paul *et al.*, 1980), some free-living ciliates (van Bruggen *et al.*, 1984; Finlay and Fenchel, 1989) and chytrid fungi (Chytridiomycota) (Fig. 2) (Yarlett *et al.*, 1986; Marvin-Sikkema *et al.*, 1992). Until today, no hydrogenosome has yet been found in multicellular animals or plants. The most extensive studies on this organelle have been carried out in trichomonads species. The broad systematic distribution of hydrogenosome in different Phyla raised questions concerning its evolutionary origin.

The hydrogenosome is spherical or slightly elongated granules 0.5 μm to 1.0 μm in diameter (Figs. 3-5) containing enzymes that participate in the metabolism of pyruvate (Müller, 1993). This process is accompanied by synthesis of ATP at the substrate level and the transfer of electrons either to protons or molecular oxygen (Müller, 1973). Nowadays it is an organelle of evolutionary interest due to its similarities (double membrane, certain enzymes, ATP production) and differences (no genetic material, presence of pyruvate: ferredoxin oxidoreductase and hydrogenase) with mitochondria.

Ultrastructure

Since the first morphological observations in trichomonads it has been shown that the hydrogenosome is mainly located close to the costa and the axostyle (Figs. 1, 6-7) and it was named paracostal or/and paraxostylar granules. This localization can be well appreciated in views of the whole protozoan by high voltage electron microscopy (Benchimol and De Souza, 1987) (Figs. 6-7). However, hydrogenosomes can be seen in almost all regions of the cell, even in the more posterior tip of the protozoan. This localization is probably due to the participation of components of the cytoskeleton, which may connect the hydrogenosome to each other (Benchimol *et al.*, 1996a). Morphometrical analysis showed that it represents 4% of the total cell volume of *T. foetus* (Benchimol *et al.*, 1996a), and 6% in *T. vaginalis* (Nielsen and Diemer, 1976).

Different size and shape of hydrogenosomes are found when trichomonads are incubated in presence of drugs such as hydroxyurea (Figs. 39-40), metronidazol, and cytochalasin (unpublished observations). They are frequently seen as enlarged structures presenting internal membranes.

A large number of hydrogenosomes present a protusion towards the cytoplasm, that could be seen by differential contrast light microscopy, in thin sections and freeze-fracture replicas (Figs 12, 27, 29). In the protist *Monocercomonas sp* the hydrogenosom seems to be elongated structures reaching the length of several micrometers (Fig. 5) (Diniz and Benchimol, 1998). The examination of thin sections, especially in serial sections used for 3D reconstruction, showed the presence of intraorganellar invaginations and vesicles (Figs. 8, 11, 15). These structures resemble mitochondrial cristae and their number varies from one hydrogenosome to the other. Its functional role is unclear (Benchimol *et al.*, 1996a).

Hydrogenosome Envelope:

When excellent preservation of the membranes structure is achieved it is clear that two closely apposed unit membranes (Benchimol and De Souza, 1983) envelop the

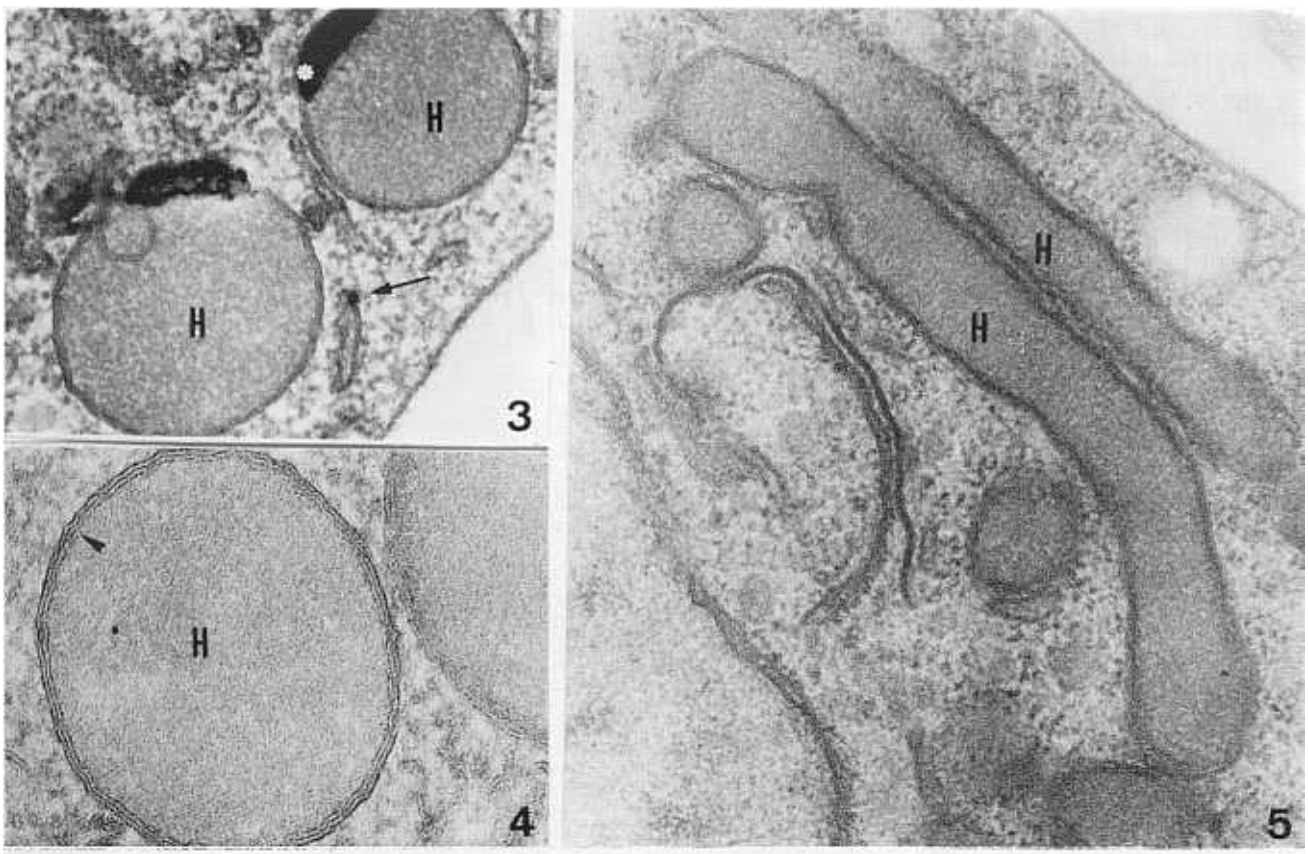
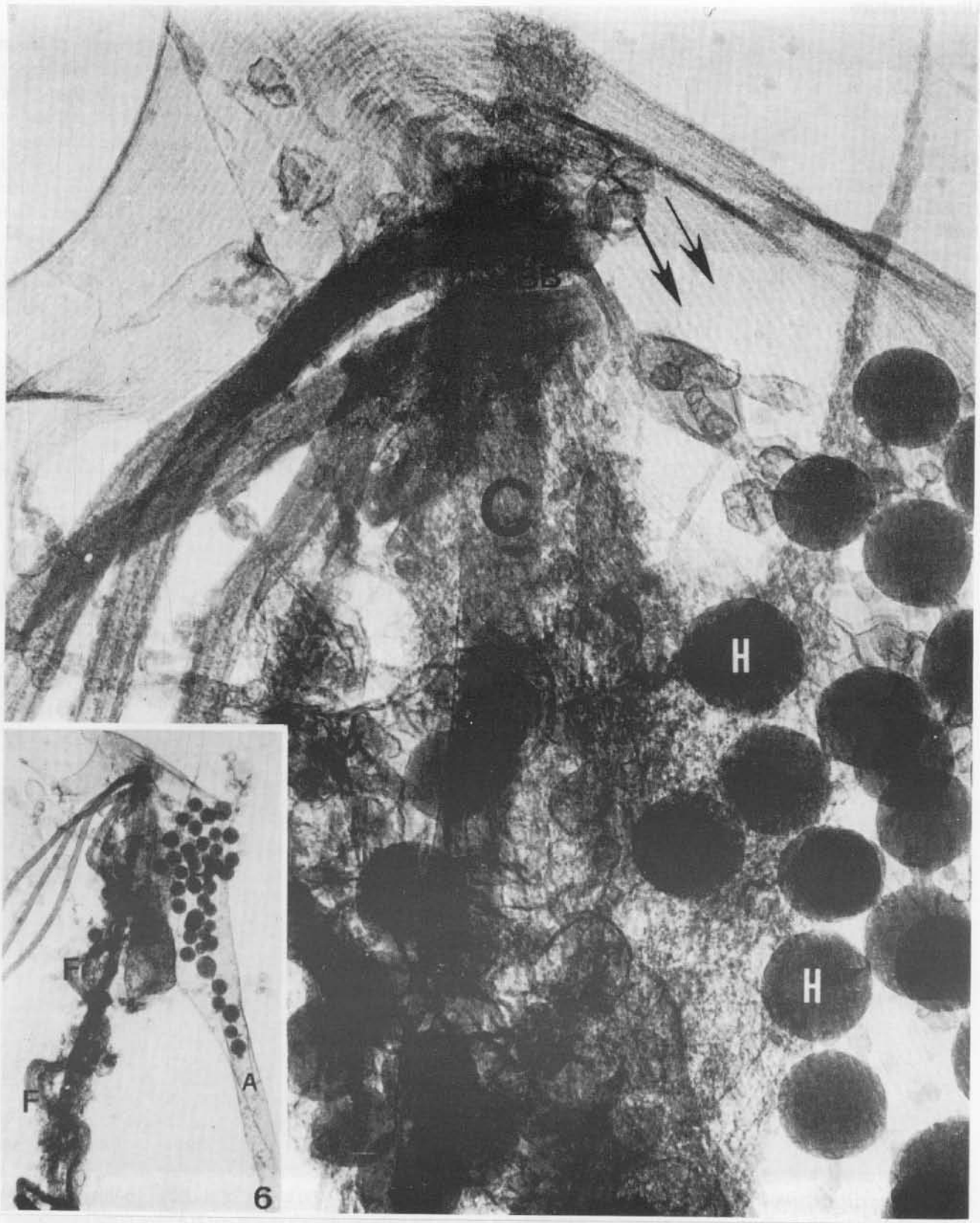


Fig. 3: *Hydrogenosomes of T. foetus fixed according to the glutaraldehyde osmium tetroxide - potassium ferrocyanide procedure with 5 mM $CaCl_2$ added to all solutions. Electron-dense calcium reaction product is seen in the hydrogenosome's vesicle and in profiles of the endoplasmic reticulum (arrow). X 30,000 (from Benchimol* et al., *1996 a).*
Fig. 4: *T. foetus hydrogenosome surrounded by two closely apposed unit membranes (arrowhead). Notice that there is no space in between the two membranes. X 50,000.*
Fig. 5: *Thin section of elongated hydrogenosomes (H) from Monocercomonas sp. X 72,000.*



Figs. 6-7: *General images of two Triton X-100 extracted Tritrichomonas foetus as seen with the high voltage electron microscope. The association of 1) the costa (C) with the basal bodies (BB), 2) the costa with the recurrent flagellum (F), 3) hydrogenosomes (H) with the costa and the microtubules which form the axostyle (arrows) are clearly seen in these micrographs. Fig. 6: X 7,500; Fig. 7: X 56,000 (from Benchimol and De Souza, 1987).*

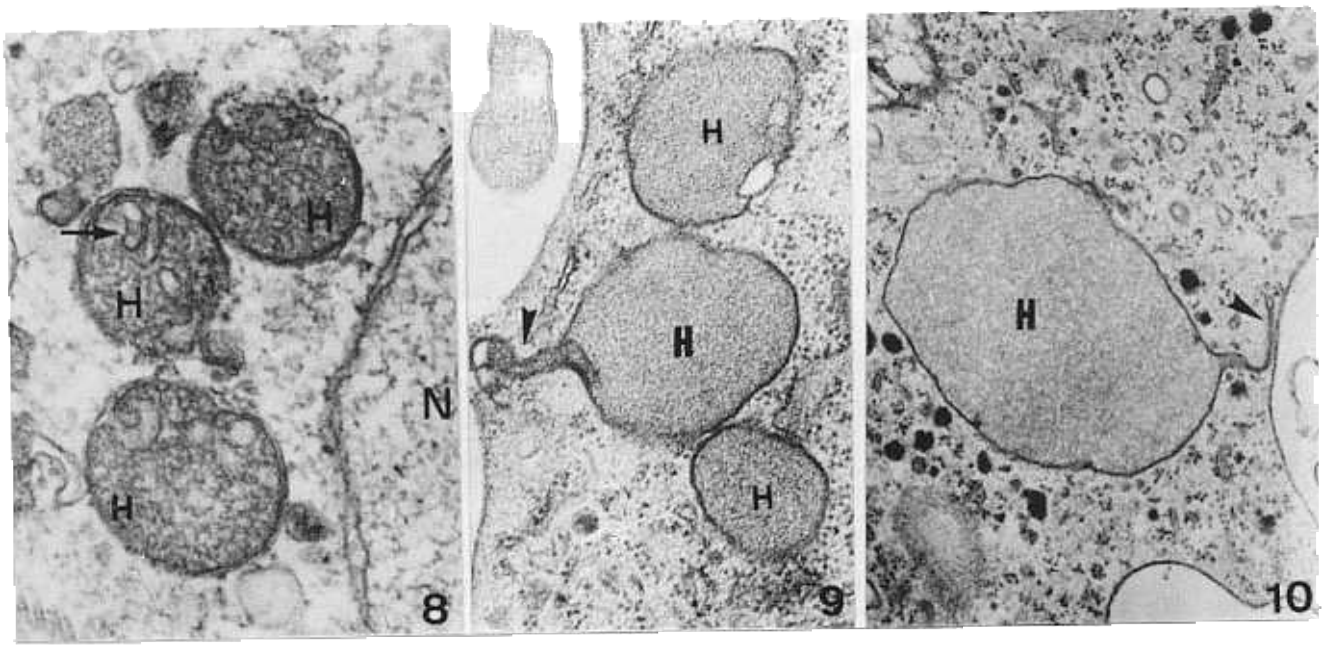


Fig. 8: *Invaginations (arrow) of inner hydrogenosome (H) membrane of Tritrichomonas foetus is shown in this figure. N, nucleus. X 35,000 (from Benchimol* et al., *1996 a).*
Figs. 9-10: *These figures show continuity of the hydrogenosome (H) envelope with a tubular extension (arrowheads) in T. foetus. Fig. 9 X 35,000; Fig. 10 X 45, 000 ((from Benchimol* et al., *1996 b).*

hydrogenosome (Figs. 3-5, 15). In most of the cases no space is observed in between the two membranes (Figs.4, 15). Each membrane had a thickness of 6 nm and presented a certain undulation (Benchimol *et al.*, 1996a) (Fig. 4, 15). The presence of $CaCl_2$ in the fixative solution, as well as the use of reduced osmium, is important to achieve good visualization of the two membranes. (3-5, 15, 22). When thicker sections are obtained the two membranes are hardly visualized (Fig. 47). Because this, other authors proposed that the hydrogenosome is enveloped by only one unit membrane (Honigberg *et al.*, 1971). Two closely apposed membranes, even in the anaerobic fungus *Neocalimastix* (Benchimol *et al.*, 1997) always bound the hydrogenosome (Fig. 31). Invaginations of the hydrogenosome membrane are occasionally observed, delimiting inner compartments. Some of the compartments had the same morphology and electron density of the hydrogenosomal matrix whereas others, however, had a lower density and presented tubular structures (Figs. 8-11) (Benchimol *et al.*, 1996a). Occasionally continuity of the hydrogenosome envelope with tubular extensions are found in *T. foetus* (Figs 9-10) as well surface protusion associated with a hydrogenosomal vesicle and containing concentric membranous structures (Fig. 12).

Carbohydrates could be cytochemically detected in the membrane of the hydrogenosome (Benchimol *et al.*, 1996a) (Fig. 17) using the periodic acid - thiosemicarbazide - silver proteinate technique (Thiéry, 1967). The membrane of the hydrogenosomal vesicle (see description below) is also labeled. Gold-labeled WGA binds to the flat vesicle membrane, indicating the presence of N-Acetyl-glucosamine (Fig. 19).

Conventional freeze-fracture replicas also revealed the presence of two membranes enveloping the hydrogenosome (Figs. 27-32). Each membrane face presented a different density and distribution of intramembranous particles. Four fracture faces could be identified: two concave faces representing the P faces of the outer and the inner membranes, and two convex faces representing the E faces of the two membranes. It is very common to find the P (Fig. 27, 29-30) and E (Figs. 28-32) faces of the outer membrane. Although no quantitative analyses was carried out the P face seemed to have a higher density of particles than the E face. The E face of the inner membrane is observed in a few cases (Figs. 28). However, the P face was not visualized in any of the replicas examined.

Johnson *et al.*, (1993) have provided evidence that hydrogenosomal proteins in *T. vaginalis* are synthesized in free ribosomes and post-translationally inserted into the organelle. These proteins present leader sequences, absent in the mature protein, and which are very similar to mitochondrial presequences. Our present cytochemical observations show that carbohydrates are found in the membrane of the hydrogenosome (Fig. 17). It is possible that they are associated to membrane proteins as glycoproteins. At present we do not know how the glycoconjugates reach the hydrogenosome. One possibility is the fusion of ER-derived vesicles with pre-existing hydrogenosomes during the growth phase of this organelle before division (Benchimol *et al.*, 1996b). Further studies in this area are necessary, especially to explain the specific presence of N-Acetyl-glucosamine residues, detected in cryosections using gold-la-

beled WGA (Fig. 19), in the membrane of the hydrogenosomal vesicle (Benchimol *et al.*, 1996a).

The Peripheral Vesicle:

A special compartment is found at the periphery of almost all hydrogenosomes (Figs. 3, 11-15). In some hydrogenosomes two and even three peripheral vesicles are seen (Figs. 22, 39). Most of the times they are flat but spherical and even unshaped ones can be seen (Figs. 3,13-14, 48-50). They vary in size and electrondensity. In *T. foetus* this vacuole has been described as a flat vesicle. Other observations, specially using 3D reconstruction, show that this vesicle varies significantly in shape and size (Benchimol *et al.*, 1996a). When fixed in a glutaraldehyde solution containing Ca^{++} and post-fixed in a osmium solution containing potassium ferricyanide and Ca^{++}, an electron dense product appeared within this vesicle of the hydrogenosome of *T. foetus* (Figs..3, 15). Treatment of ultrathin sections with EGTA removed this electron-dense reaction product (Fig. 14), suggesting that it could represent sites of Ca^{++} accumulation (Benchimol and De Souza, 1983). Chapman *et al.*, were able to locate Ca^{++} in isolated hydrogenosomes of *Trichomonas vaginalis* using energy-dispersive X-ray microanalysis (Chapman *et al.*, 1985). Electron spectroscopic imaging (ESI) is a highly sensitive method for localization

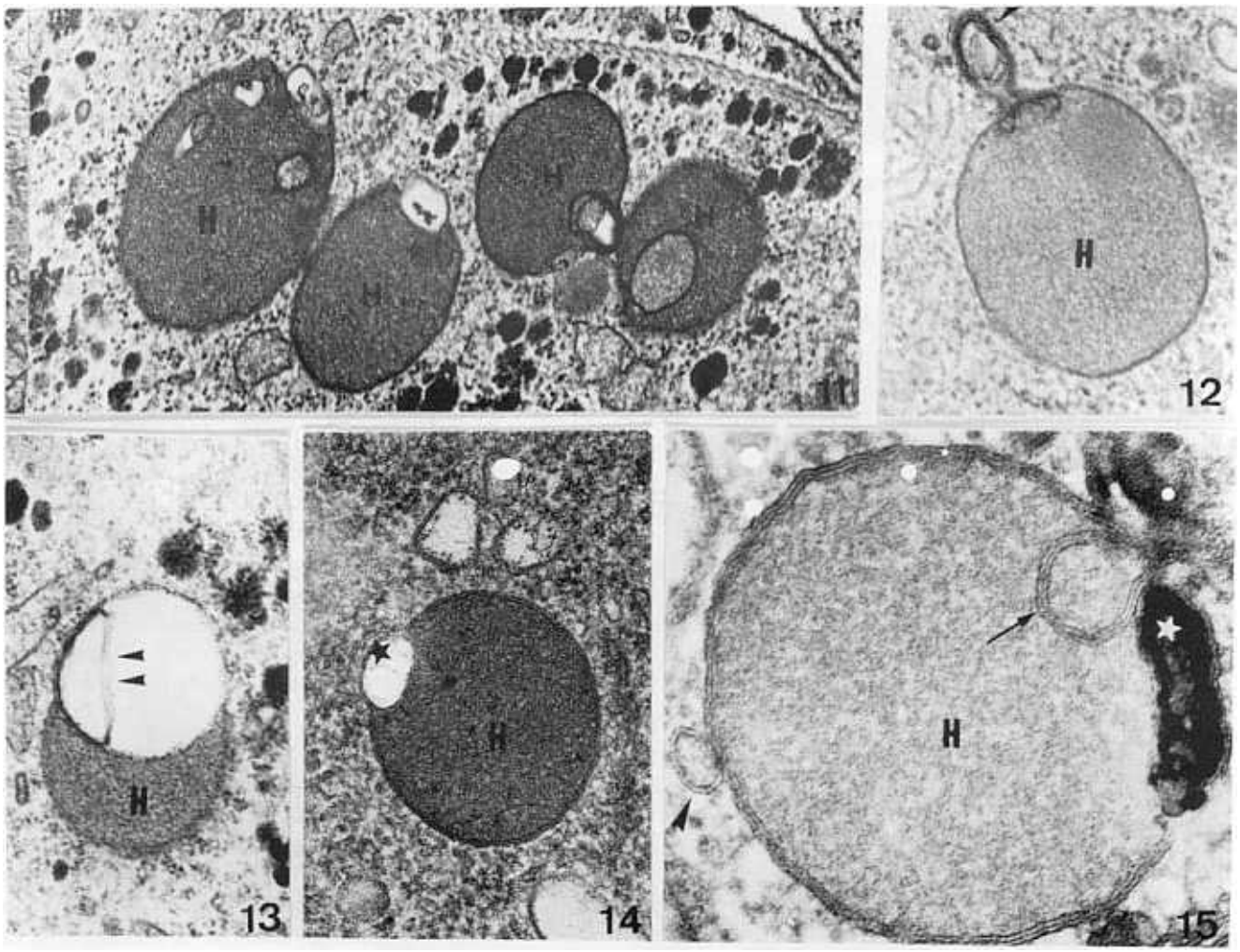


Fig. 11: *Different views of hydrogenosomes (H) of T. foetus presenting internal membranous structures and vesicles. X 28,000 (from Benchimol et al., 1996 a).*
Fig. 12: *View of hydrogenosome (H) of T. foetus presenting a surface protrusion associated with a hydrogenosomal vesicle and containing concentric membranous structures (arrowhead). X 30,000.*
Fig. 13: *View of the internal compartment of hydrogenosome (H). A double membrane is seen lining the vesicle (arrowheads). X 25,000 (from Benchimol et al., 1996 a).*
Fig. 14: *Thin section of a T. foetus fixed according to the glutaraldehyde osmium tetroxide - potassium ferrocyanide procedure with 5 mM $CaCl_2$ added to all solutions and treated with EGTA. The electron-dense reaction product associated with the vesicle-like structure of the membrane of the hydrogenosome (H) was dissolved by the treatment, leaving an electron-transparent area (star). X 60,000.*
Fig. 15: *T. foetus fixed according to the glutaraldehyde osmium tetroxide - potassium ferrocyanide procedure with 5 mM $CaCl_2$ added to all solutions. The two closely apposed outer and inner unit membranes of the hydrogenosome (H) are clearly seen. The vesicle-like structure is seen presenting a electron-dense deposit (white star). Membrane invaginations and vesicles fusing with hydrogenosome membranes are depicted (arrow and arrowhead). X 80,000 (from Benchimol et al., 1996 a).*

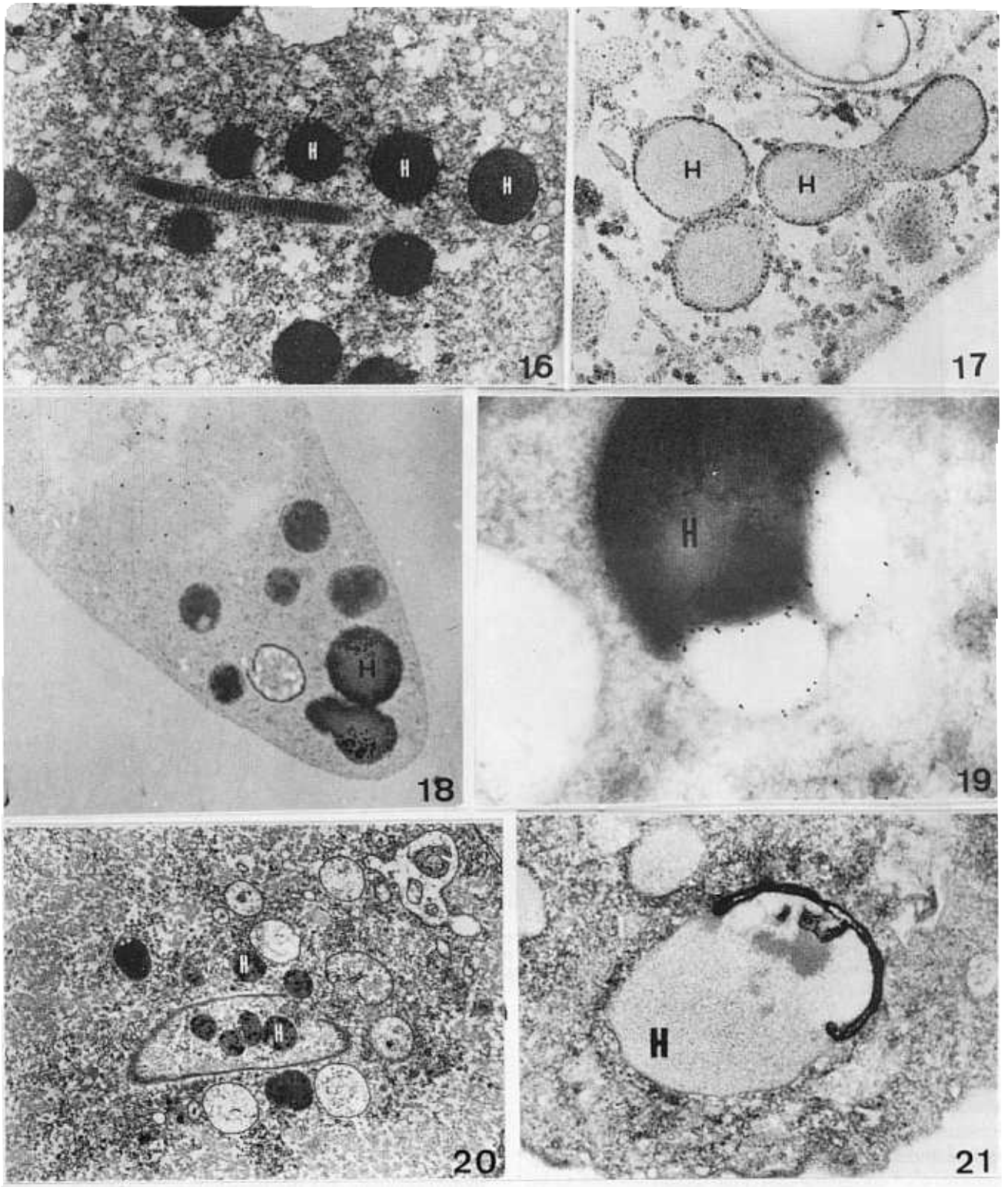
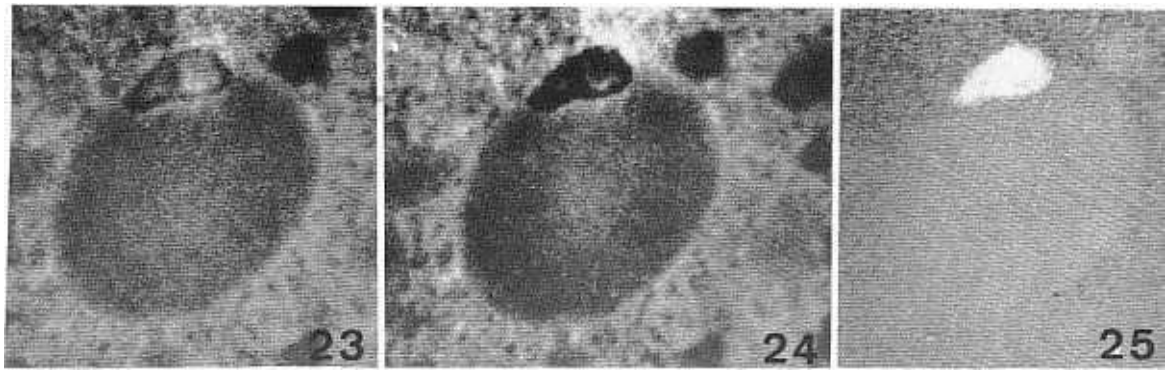


Fig. 16: *Electron microscopy of T. vaginalis treated with BCECF, DAB and UV-iluminated, show electron-dense deposits in the hydrogenosome matrix (H). Glycogen was metabolized during the course of the treatment and are not seen. C, costa. X 35, 000.(from Scott et al., 1998).*
Fig. 17: *T. foetus after Thiéry s technique used for demonstration of carbohydrates shows strong positive reaction in the hydrogenosomes (H) membranes. X 35,000.*
Fig. 18: *T. foetus submitted to the ethanolic phosphotungstic acid (E-PTA) technique shows strong positive reaction for basic proteins in the hydrogenosomes (H). X 25,000 ((from Benchimol et al., 1982 b).*
Fig. 19: *Hydrogenosome cryosection (H) was incubated with the lectin WGA. The membrane lining the peripheral vesicle but not other portions of hydrogenosome is labeled. X 100,000 (from Benchimol et al., 1996 a).*
Fig. 20: *T. foetus after pyroantimonate treatment. Hydrogenosomes (H) are seen heavily labeled. X 10,000.*
Fig. 21: *Glucose-6-phosphatase cytochemistry. Electron dense reaction product is present in a membranous profile partially surrounding the hydrogenosome from normal cell cultures. X 80 000 (from Benchimol 1999).*



Figs. 23-25: *ESI (electron spectroscopic imaging) of calcium in the hydrogenosomes of* Tritrichomonas foetus, *processed as described in Fig. 22. The net distribution of Ca^{++} (figure 25) was obtained by computer-assisted imaging processing of the difference between an ESI taken above the Ca^{++} edge (Fig. 23) at DE=360 eV and below the edge (Fig. 24) at DE=320 eV. X 50,000. (from De Souza and Benchimol, 1988).*

product appears within the vesicle when cells are fixed in conditions to reveal Ca^{++}, indicating that the vesicle compartment is involved in the uptake of Ca^{++}. Morphometric analysis showed that it represented a mean volume of 8.6% of the whole organelle.

In freeze-fracture and deep-etched cells few particles are seen in the membranes of the vesicle. Recent observations also show that Zn^{++} is accumulated within the vesicle when cells are incubated in the presence of $ZnCl_2$ (Benchimol *et al.*, 1993).

The Matrix of the Hydrogenosome:

The matrix of the hydrogenosome is described in previous studies as homogeneously granular, occasionally presenting a dense amorphous or crystalline core (Honigberg and Brugerolle, 1990). Our observations indicate that the core is not a usual structure, appearing however either when the protozoa are incubated in the presence of drugs or when good fixation is not achieved. The electrondense core is frequently seen in those not well preserved cells, a situation in which the hydrogenosome proteins could coagulate and precipitate, leading to the formation of the core. The density of the matrix may vary between hydrogenosomes of the same cell.

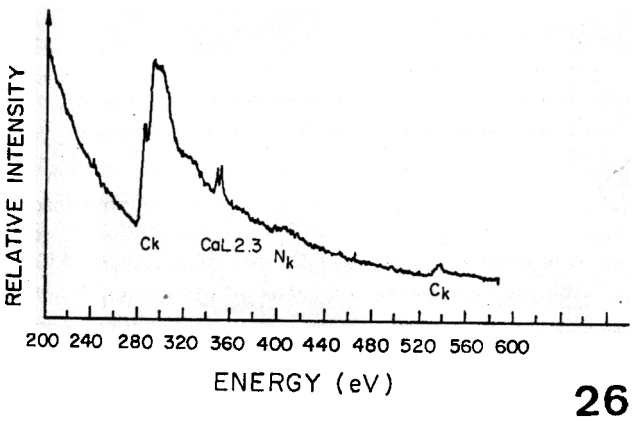


Fig. 26: *Electron energy loss spectrum of the calcium-containing region show in previous figures, with E varying from 200 to 600 eV. Peaks for C, Ca, N and O were detected (from De Souza and Benchimol, 1988).*

It is very unusual to find an electrodenser amorphous core or a paracrystalline one in trichomonads hydrogenosome (Benchimol *et al.*, 1996a).

Electron dense spots are observed in the matrix of some hydrogenosomes when cells are processed for the localization of Ca^{++} (Figs. 20, 36). The granular structure of the matrix of the hydrogenosome is clearly visualized in replicas of quick-frozen, freeze-fractured, deep-etched and rotary replicated cells (Fig. 33). When the fracture plane exposed the internal portion of the hydrogenosome a large number of particles is seen. Most of them had a diameter of 6 nm. Some, however, are larger, with a diameter of 20 nm. These particles are not randomly distributed. A certain orientation in their array is noted (Fig. 33).

Hydrogenosomes and the Cytoskeleton:

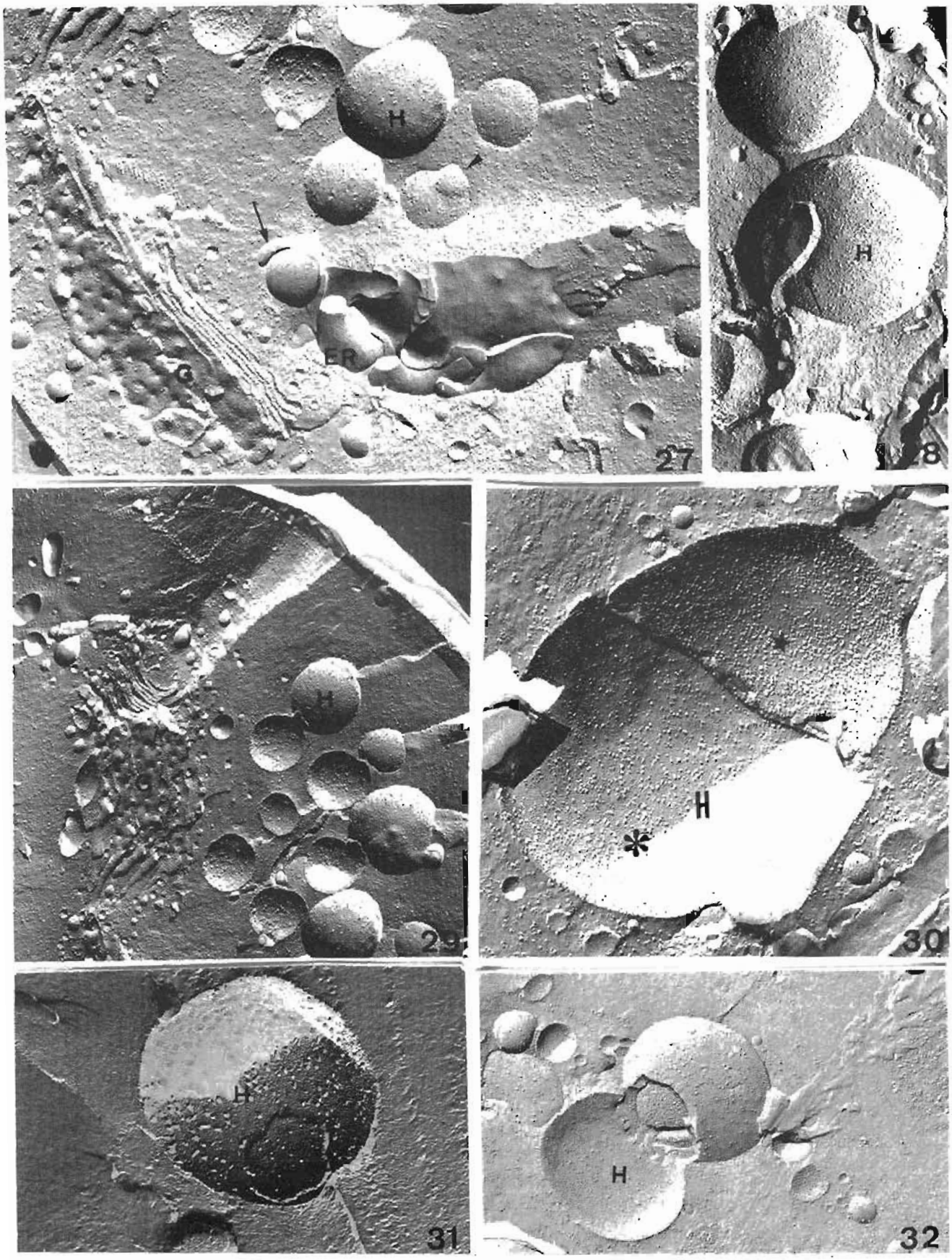
Studies performed by Benchimol and De Souza (1987) using the high voltage electron microscope showed clearly the association of hydrogenosomes and the microtubules of the peltar- axostylar system (Figs. 6-7). It was also demonstrated the presence of hydrogenosomes at the posterior tip of the protist *Tritrichomonas foetus* close to microtubules.

Hydrogenosome Cytochemistry:

T. foetus submitted to the ethanolic phosphotungstic acid (E-PTA) technique shows strong positive reaction in the hydrogenosomes (Fig. 18). Also when the cells are submitted to ammoniacal silver method, silver particles are seen only in the hydrogenosomes (Benchimol *et al.*, 1982 b). This organelle appears to be rich in basic proteins since it gives intense reaction product with either the E-PTA or the ammoniacal silver technique.

Proximity with other cellular structures:

Glycogen particles although distributed throughout the



Figs. 27-32: *Views of freeze-fracture images of hydrogenosomes (H) of T. foetus (Figs. 27-30), from the fungus Neocallimastix frontalis (Fig. 31) and from T. vaginalis (Fig. 32) showing different fracture planes. Figures 27 and 29 show profusion of the hydrogenosome (arrowheads). All figures show the different distribution of intramembranous particles on outer and inner membranes. Association with the endoplasmic reticulum (ER) is also observed (arrow in Fig. 27). Fig. 28 shows the E face of the inner membrane (arrow). Fig. 30 presents the E face of the outer membrane (star) and the P face of the inner membrane (astensk). Figure 31 shows an hydrogenosome double membrane from fungus and figure 32 an hydrogenosome in process of division. G, Golgi complex. Fig.27: X 15,000; Figs. 28 X 30,000; Fig. 29 X 15,000; Fig. 30 X 60,000; Fig. 31. X 35,000; Fig. 32 X 30,000.*

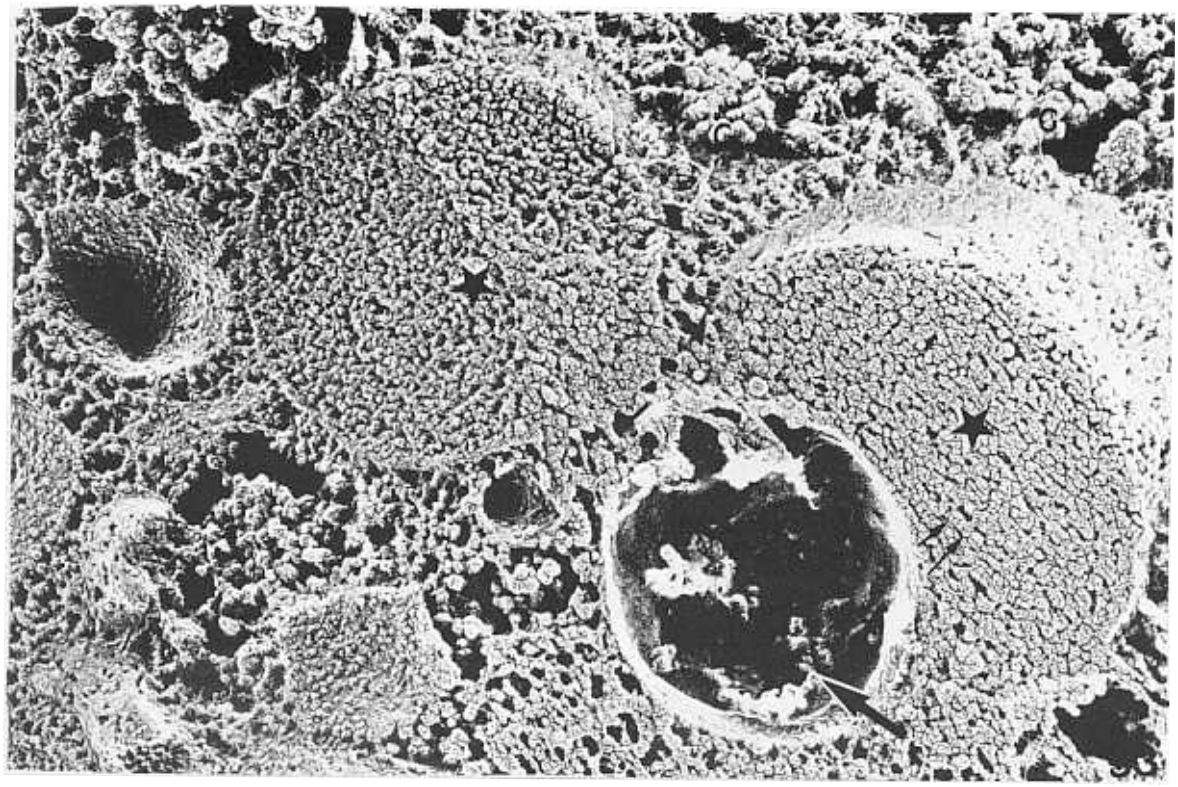


Fig. 33: *Freeze-etching view of the hydrogenosomes. It presents a granular matrix (star) and a peripheral compartment where deposits are seen (arrow). Filaments are seen projecting from the outer hydrogenosome membrane (arrowheads). Close proximity with glycogen granular (G) is observed. The internal portion of hydrogenosome shows particles of different sizes (thin arrow). A certain orientation in their array is observed. X 140,000 (from Benchimol et al., 1993).*

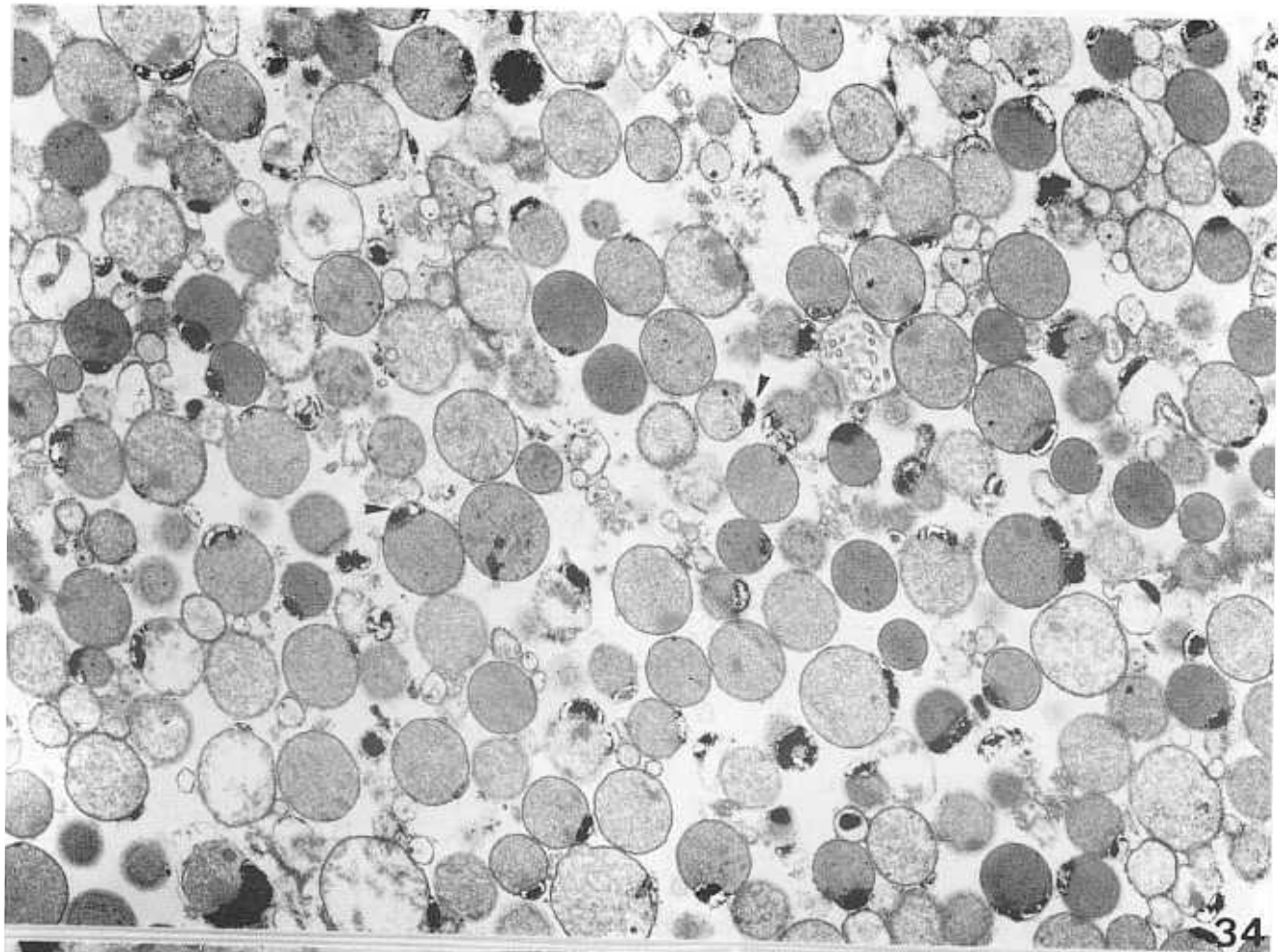
protist are concentrated in the region where hydrogenosomes are located (Figs. 1, 17, 33, 51). Microtubules are also observed in close association with hydrogenosomes.

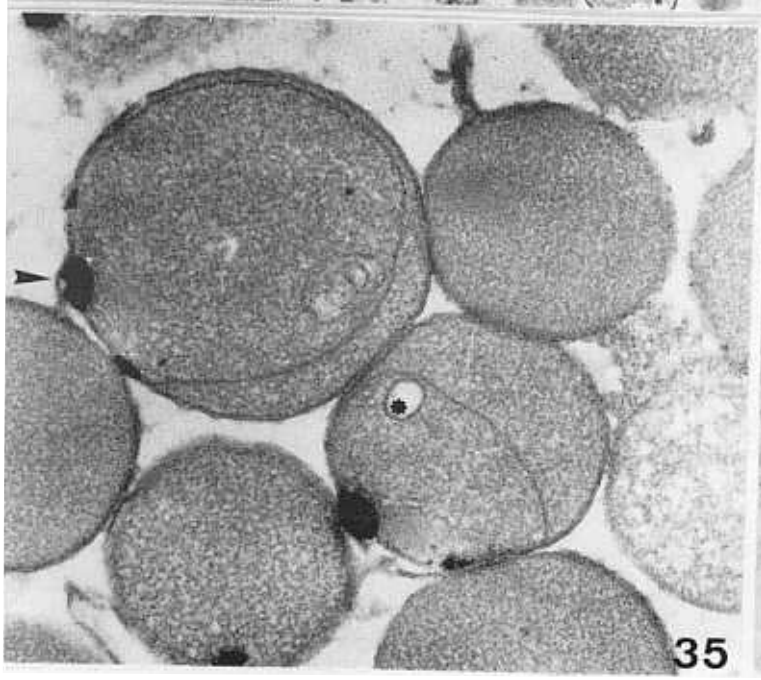
Hydrogenosomal Purification:

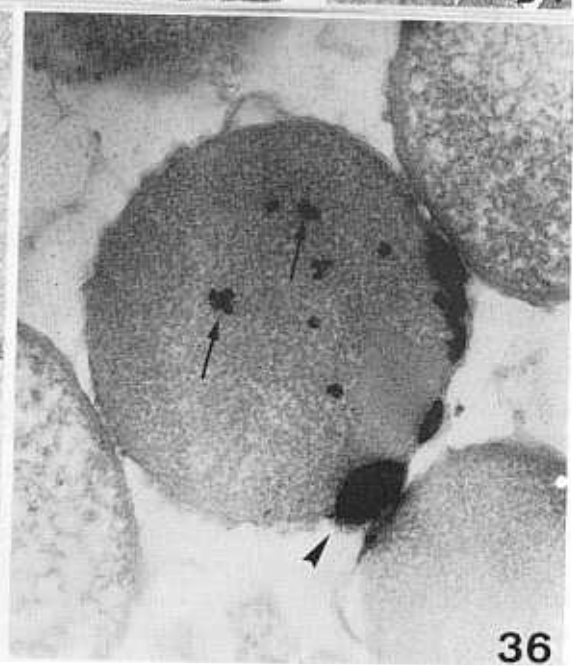
In the recent study of Morgado and De Souza (1997), a very pure hydrogenosomal fraction containing the peripheral vesicle was obtained (Fig. 34-36). The hydrogenosomes were isolated from *Tritrichomonas foetus*, showing the same morphology as observed in intact cells, including the presence of a peripheral vesicle with an electron-dense content. SDS-PAGE revealed the presence of several protein bands, with those of 120, 66, 60, 59, 48, 45 and 35 kDa as the major ones. The hydrogenosome membrane was solubilized with Triton X-100 leaving a fraction containing its matrix attached to the peripheral vesicle (Fig. 37). Further treatment with proteinase K solubilized the matrix components, leaving a pure peripheral vesicle fraction. Enzymatic assay during all procedures suggested that malate dehydrogenase was localized in the hydrogenosomal membrane. The isolated peripheral vesicles maintained their flattened morphology, suggesting that each individual vesicle have its own inherent structural framework. SDS-PAGE showed that proteins of 66, 45 and 32 kDa are localized in the peripheral vesicle. Western blot analysis revealed the presence of glycoproteins, with a major one of 45 kDa, in the peripheral vesicle of the hydrogenosome (Morgado and De Souza, 1997).

Hydrogenosome Autophagy:

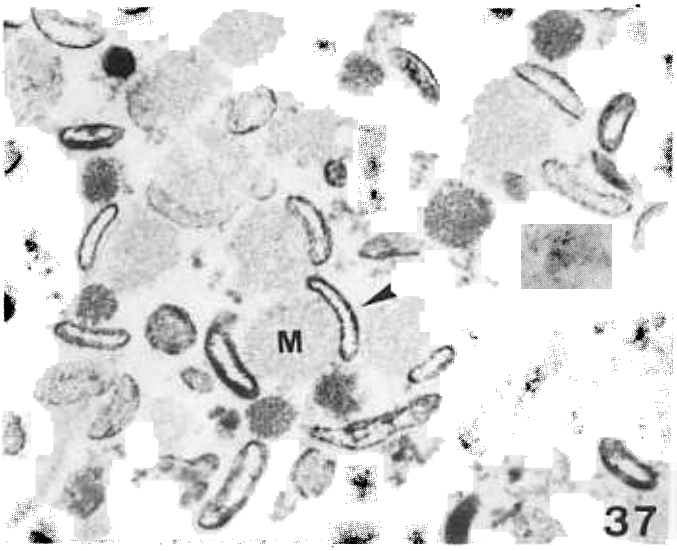
The process of autophagy was studied in *Tritrichomonas foetus* under nutrient deprivation, drug treatment (hydroxyurea, zinc sulfate), and also in normal conditions using routine electron microscopy, freeze-fracture, freeze-etching, freeze-substitution, and enzyme cytochemistry (Benchimol, 1999) (Figs. 39-46). It was also used latex beads coated with cationized ferritin to better characterize the participation of lysosomes on the process of hydrogenosome degradation. Apparently normal hydrogenosomes and also giant, abnormal and presenting internal cristae, are seen in the autophagic process (Figs.40-44). The first event observed is the rough endoplasmic reticulum surrounding (Fig. 40-41) and enclosing the hydrogenosome (Figs. 42-43), forming an isolation membrane. The hydrogenosomes sequestered from the remaining cytoplasm are then degraded within lysosomes (Fig. 44). Double or multiple concentric membranes limit the autophagic vacuoles and many contained recognizable hydrogenosomes (Figs.45). The hydrogenosome enclosed in the autophagic vacuole presented positive reaction for







Figs 34-38: *Hydrogenosomes obtained after cell frationation.*
Fig. 34: *Electron micrograph showing a general view of the purified hydrogenosomal fraction from Tritrichomonas foetus obtained using a Percoll gradient. The hydrogenosome matrix presents different electrondensities, probably as the result of fractionation treatment. The peripheral vesicle is seen in almost all hydrogenosomes (arrowheads). X 25,000.*
Figs 35-36: *Electron micrographs showing some of the purified hydrogenosomal fraction from Tritrichomonas foetus obtained using a Percoll gradient. The peripheral vesicles are seen presenting an electron-dense content (arrowheads). The hydrogenosomal matrix presents vesicles (asterisk) or dense spots (thin arrows) (Ca^{++} deposits).*



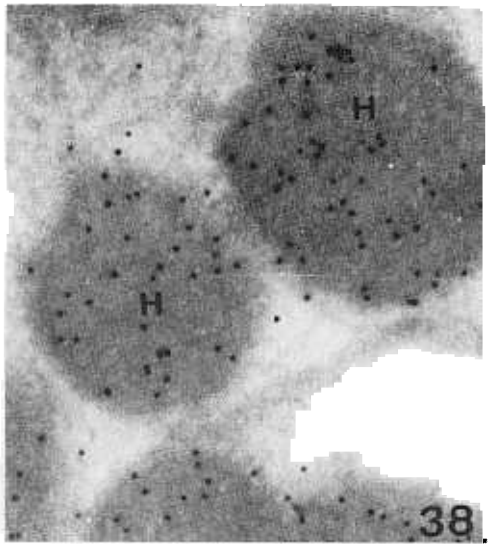


Fig. 37: *Electron microscopy of purified hydrogenosomes treated with Triton X-100. The components of the hydrogenosomal matrix remain attached to the peripheral vesicle (arrows). X 28,000 (from Morgado and De Souza, 1997, with permission).*
Fig. 38: *Immunocytochemical localization of bsuccinyl CoA synthetase in the T. foetus hydrogenosome. The gold particles are located in the hydrogenosomal matrix. X 50,000.*

malate dehydrogenase (decarboxylating) (Fig. 46), an enzyme previously detected in this organelle (Brugerolle and Méténier, 1973), suggesting that it was still a recognizable organelle. Hydrogenosomes are partially degraded, forming hydrogenosomal remnants (Benchimol, 1999).

Hydrogenosome Morphogenesis:

The morphogenesis of hydrogenosomes was analysed in several trichomonad species such as *Tritrichomonas foetus, Trichomonas vaginalis, Tritrichomonas suis, Trichomonas gallinae, Tritrichomonas augusta* and *Monocercomonas sp.* (Benchimol *et al.*, 1996b, Kulda *et al.*, 1987). The studies were carried out by transmission electron microscopy of thin sections and freeze-fracture replicas of whole cells or the isolated organelle (Figs. 32, 47-51). Close proximity, and even continuity, between endoplasmic reticulum and hydrogenosomes was observed (Figs. 3, 9-10, 27, 39-41). Morphological evidence was presented showing that in all the trichomonads studied, hydrogenosomes, like mitochondria, may divide by two distinct processes: segmentation and partition (Figs. 32, 47-51). In the segmentation process, the hydrogenosome grows, becoming enlongated with the appearance of a constriction in the central portion (Fig. 47, 49). Microfibrillar structures appear to help the furrowing process, ending with a total fission of the organelle (Fig. 47). In the partition process, the division begins by an invagination of the inner hydrogenosome membrane, forming a transversal septum, separating the organelle matrix into two compartments (Figs. 32, 48, 50-51). It was suggest that myelin-like structures seen either in close contact with or in the vicinity of the hydrogenosomes (Fig. 12) could be a source of membrane lipids for hydrogenosome growth.

Hydrogenosomal pH:

Hydrogenosomes share properties such as a transmembrane pH gradient, inside alkaline in relation to the cytosol, and a transmembrane electrochemical potential (Biagini *et al.*, 1997a, 1997b). These authors have suggested that the bright fluorescence of BCECF (2',7'-bis- (2-carboxyethyl)-5 (and -6)-carboxyflurescein, a fluorochrome widely used for the measurement of intracellular pH, labeled the hydrogenosomes of *Trichomonas vaginalis.* Biagini *et al.* (1997), claimed that this could be due to high pH in the organelle, since BCECF is more fluorescent at alkaline pH. However, this labeling might also be due to a high concentration of the dye. Scott *et al.* (1998) found that nigericin treatment did not affect the brightness of hydrogenosomes as observed by fluorescent microscopy. These authors showed that BCECF localizes to hydrogenosomes in intact *T. vaginalis* loaded with the AM ester (Fig.16), suggesting that the dye enters the organelle via an anion channel. Its hydrogenosomal location was confirmed by treatment of BCECF-loaded cells with diaminobenzidine and hydrogen peroxide together with UV ilumination. This produced an osmiophilic precipitate in the matrix of hydrogenosomes, observable by electron microscopy. Nigericin, being a proton ionophore, would collapse pH gradients across membranes and the pH inside organelles would become the same as the buffer pH, which in this case was pH 7.2.

Marvin-Sikkema *et al.* (1994) claimed that hydrogenosomes from *Neocallimastix* contained a novel

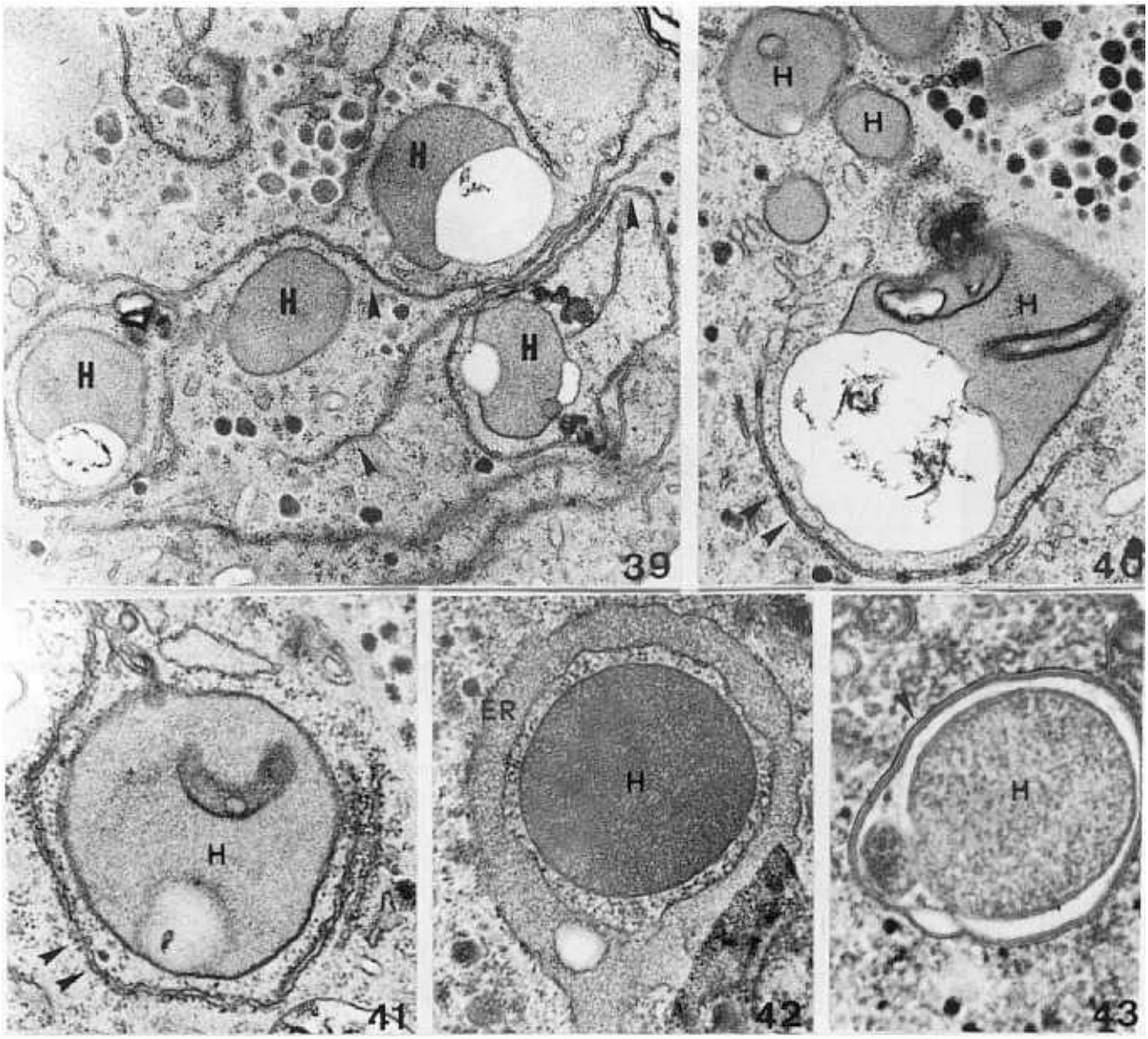


Fig. 39-42: *Images of association of hydrogenosomes with endoplasmic reticulum.* **Fig.39:** *shows a general distribution of hydrogenosomes (H) in a cell treated with hydroxyurea. In* **Fig. 40** *a giant hydrogenosome presenting an enlarged peripheral vesicle. In this micrograph cristae-like membranous structures are seen partially surrounded by cisternae of the rough endoplasmic reticulum (arrowheads).*
Fig. 41 *shows gradual enclosure of hydrogenosome by the endoplasmic reticulum, which is studded by ribosomes (arrowheads). Fig. 39-40, X 15,000; Fig. 41 X 40,000. (from Benchimol, 1999).*
Fig. 42: *In this figure the hydrogenosome (H) is seen trapped by the endoplasmic reticulum (ER). Some ribosome-free regions of the rough ER is seen. X 80, 000. (from Benchimol, 1999).*
Fig. 43: *Observation of a nascent double-layered autophagic vacuole (arrowhead), containing an intact hydrogenosome (H) after high-pressure freezing and freeze-substitution. The cell was grown under normal conditions. X 45, 000. (from Benchimol, 1999).*

ATPase, believed to act as a proton translocating ATPase, in contrast to trichomonads which do not appear to contain F_0 F_1 (Lloyd *et al.*, 1979), although other ATPases may be present (Biagini *et al.*, 1997a and 1997b).

The Hydrogenosomal Genome:

It has been postulated that the hydrogenosome evolved from mitochondria by the concomitant loss of their respiration and organellar genomes (Müller, 1993, Palmer, 1997). Until recently, it was believed that it does not present DNA, RNA or ribosomes. However, Akhmanova *et al.*, (1998) claimed that they provided evidence for the hydrogenosomal genome of mitochondrial descent in the anaerobic heterotrichous ciliate *Nyctotherus ovalis*, found in the hindgut of the cockroaches *Periplaneta americana* and *Blaberus spp.* The numerous hydrogenosomes are intimately associated with endosymbiotic methane-producing Archea, which

use hydrogen produced by hydrogenosomes. These authors (Akhmanova *et al.*, 1998) claimed that in the above cited microorganisms the hydrogenosomal matrix presents ribosome-like particles of the same size as the numerous 70S ribosomes of the endosymbiotic methanogenic Archea. However, there is no agreement with these findings. *Trichomonad* hydrogenosome does not appear to contain DNA, apparently due to a complete transfer of their genes into the nucleus of an ancestral host cell, so that the genes for all hydrogenosomal proteins are encoded in the nucleus (Müller, 1993; Palmer, 1997). These proteins have been shown to be synthesized in the cytosol on free ribosomes which implies a post-translacional targeting route to their final hydrogenosomal destination (Lahti and Johnson, 1991). Experiments carried out using DAPI and other DNA markers did not present reaction in the hydrogenosome, although strong reaction was seen in the nucleus (Turner and Müller,1983; van der Giensen *et al.*, 1997b).

Hydrogenosomal Protein Import:

Import assays carried-out with a protein lacking the 8 amino acid N-terminal extension of ferredoxin clearly show that this protein neither binds to the surface of hydrogenosome nor is imported and cleaved (Bradley *et al.*, 1997). In this way, these results confirm the hypothesis that the N-terminal extension serves as a presequence which is absolutely necessary for protein targeting to hydrogenosome. Hydrogenosomal presequences present several common features: a Leu follows the start Met in all but two of the 15 hydrogenosomal presequences indicating a potential targeting requirement for Leu at +2 position.

The translocation of a precursor protein from one side of a membrane to the other requires energy in the forms of temperature, ATP, and where it exists, a transmembrane electrochemical potential (Schatz and Dobberstein, 1996; Plümper *et al.*, 1998). Hydrogenosome neither possess membranous electron transporters nor an F_0F_1-ATPase activity (Müller, 1993). Nevertheless, a weak electrochemical membrane potential across the hydrogenosomal membranes seems to be maintained (Yarlett *et al.*, 1987). There are requirements of factors other than ATP in hydrogenosomal protein import (Plümper *et al.*, 1998). With these characteristics hydrogenosomal protein import most closely resembles protein import into chloroplasts and mitochondria, as opposed to protein translocation across other cellular membranes (Schatz and Dobberstein, 1996).

The Hydrogenosome and Evolution:

Recent studies (Horner *et al.*, 1996) have searched for molecular clues on the modern genome of *Trichomonas* for evidence of a past association with the mitochondrion endosymbiont. The target gene was the highly conserved cpn-60 gene product (chaperonin) which plays a key role in mitochondrial function, through the maintenance of correct protein conformation (Hartl *et al.*, 1994). Cpn-60 is located on the inner mitochondrial membrane, but among eukaryotes is typically encoded by the host nuclear genome, presumably as the result of an ancient gene transfer from symbiont-organelle to host. Chaperonin sequences are particularly useful because there is an extensive and relevant database of bacterial sequences for phylogenetic inference (Horner *et al.*, 1996). These authors showed that the protist

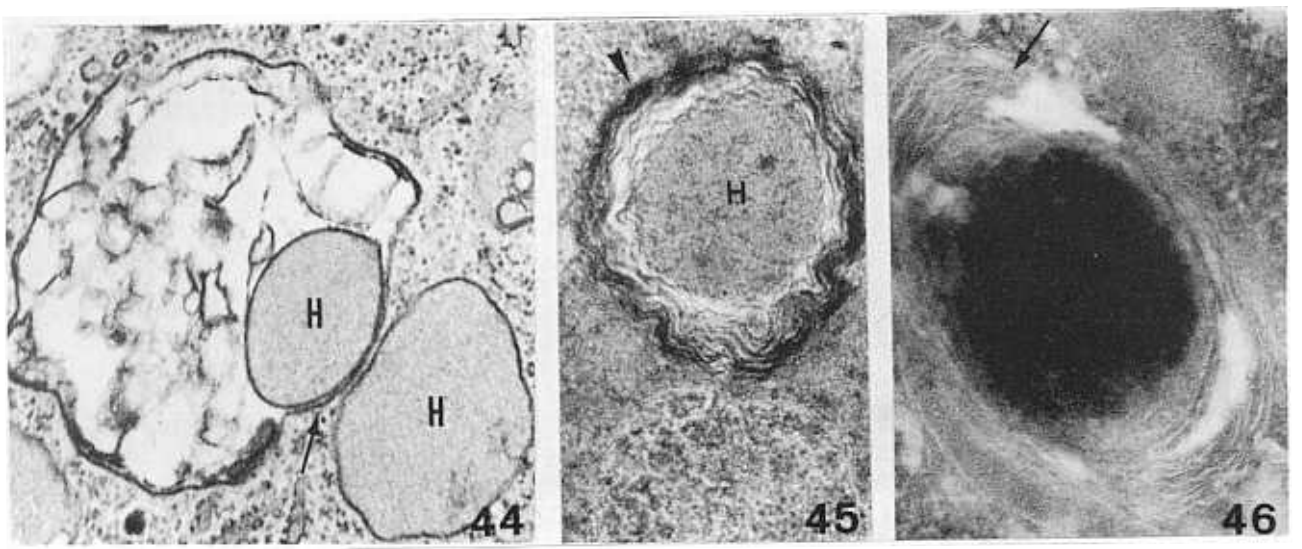


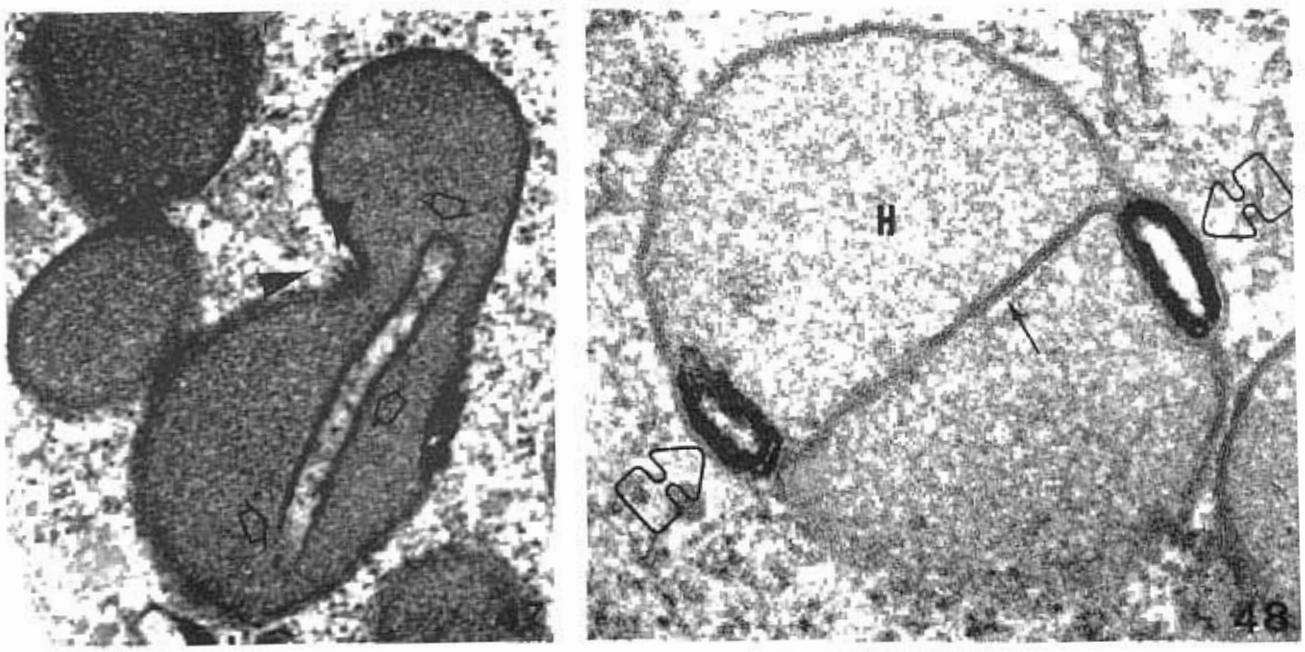
Fig. 44: *Hydrogenosome (H) inside digestive vacuole (arrow). Routine preparation of T.foetus, without any treatment. Other hydrogenosome is seen vacuole outside, while one (arrow) is seen inside of a lysosome-like structure. X 33 000. (from Benchimol, 1999).*
Figs. 45-46: *Observation of multiple concentric membranes limiting the autophagic vacuole (arrows). Figure 46 contains an intact hydrogenosome (H), still positive for malate-dehydrogenase cytochemistry. Fig. 45 X 50 000; Fig. 46 X 70, 000. (from Benchimol, 1999).*

Trichomonas vaginalis contains a cpn-60 gene sequence from the same endosymbiont that subsequently gave rise to mitochondria, potentially placing this endosymbiosis much earlier in eukaryote evolution than was previously thought.

Two main hypotheses have been proposed for the origin of the trichomonad hydrogenosome: it is either the product of an independent endosymbiosis of an anaerobic *Clostridium*-like Gram positive eubacterium (Müller, 1993) or a highly modified mitochondrion adapted to an anaerobic lifestyle (Cavalier-Smith, 1987). The latter hypothesis is strongly supported by the demonstration that *Trichomonas* nucleus carries genes for one or all three of the mitochondrial heat-shock proteins Hsp 10, Hsp60, and Hsp70 (Horner *et al.*, 1996, Germot *et al.*, 1996). These Hsps are among the most reliable tracers of the eubacterial ancestry of both the mitochondrion and choroplast, and all three *Trichomonads* Hsps ally firmly with mitochondrial Hsps in phylogenetic analyses (Horner *et al.*, 1996; Germot *et al.*, 1996). By immunocytochemistry, Bozner verify that Hsp60 and Hsp 70 proteins reside in the *Trichomonas* hydrogenosome. The Hsps are evolutionarily conserved molecules with a high degree of interspecies homology. The 70-kDa heat shock proteins (Hsp70s) are among the most highly conserved proteins present in all organisms, Archaebacteria, Eubacteria, and Eukaryota. They are involved in the correct folding of mature proteins and in the translocation of newly synthesized polypeptides across membranes. In eukaryotes, they constitute a multigenic family, the members of which are located in cytosol, endoplasmic reticulum, mitochondria, and chloroplasts. Germot at al. (1996) found in *Trichomonas vaginalis* two genes of eubacterial affinity. A detailed phylogenetic analysis demonstrated that these genes consistently emerged with the mitochondrial group. These authors claimed that these data could indicate that the hydrogenosome could be a highly derived mitochondrion.

T. vaginalis genes encoding for hydrogenosomal Hsp70, Hsp60, and chaperonin-60 cofactor Hsp10 have been identified, cloned, and sequenced by (Bui *et al.* 1996; Bozner, 1997). In addition, Hsp60 has been purified from the hydrogenosomal fraction and its amino-terminal end sequenced. The results of these molecular studies have also provided evidence for the common evolutionary origin of mitochondria and hydrogenosome. A recent study demonstrated that *Trichomonas mobilensis* and *T. augusta* posses a cpn-60 related protein which apparently colocalizes with hydrogenosomes (Bozner, 1996).

Biologic affinities and the evolutionary origin of hydrogenosome are interesting but unresolved problems. The ability of eukaryotic cells to produce hydrogen might be linked in a yet unknown way to the existence of a membrane-limited organelle. The functional and biochemical similarities of hydrogenosome to certain groups of anaerobic bacteria suggest their origin from such bacteria for some authors (Müller, 1980). The origins of the key enzymes which are responsible for hydrogen production are currently



Figs. 47-51: *Views of the division process of hydrogenosomes.*
Fig. 47: *View of the segmentation process of dividing hydrogenosomes. The organelle is elongated showing a constriction in the central region. Microfibrillar (arrowheads) and tubular (open arrow) structures are associated with the division process. X 60,000. (from Benchimol* et al.,*1996 b)*
Fig. 48: *Hydrogenosome dividing via a partition process.* Tritrichomonas suis *presenting an hydrogenosome (H) with a transversal "septum", which is formed by an invagination of the inner hydrogenosomal membrane (arrow). Two opposite peripherical calcium positive compartments are indicated by arrowheads. X 135,000. (from Benchimol* et al.,*1996 b)*

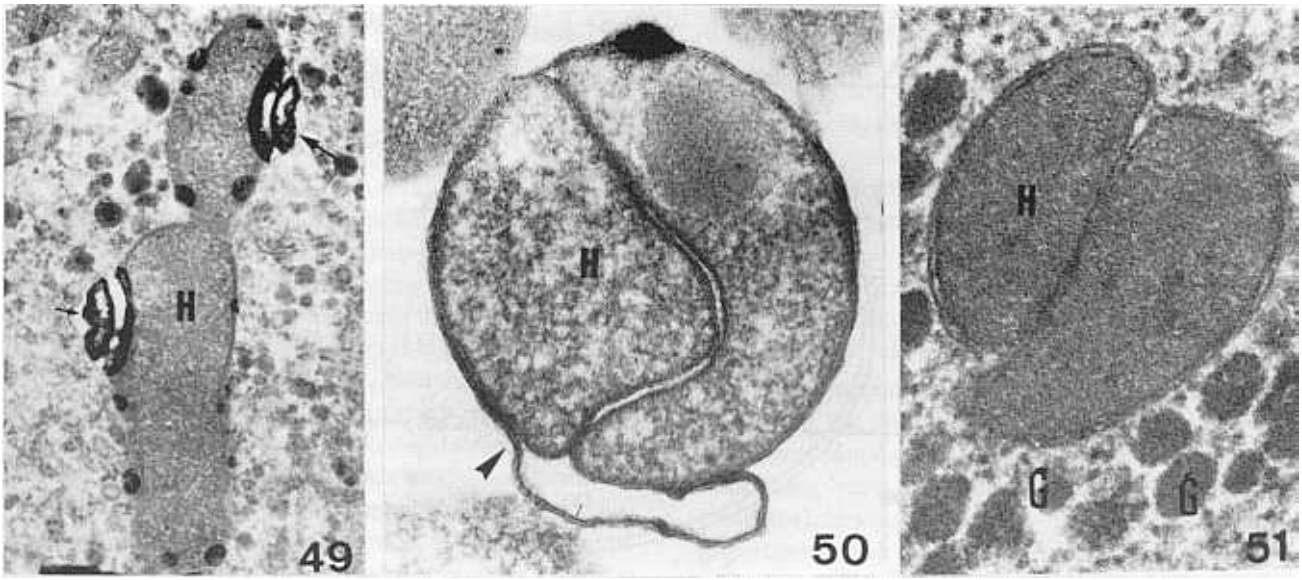


Fig. 49: *T.vaginalis showing an hydrogenosome at the end of segmentation process. The thin arrows point to calcium deposits. X 40,000. (from Benchimol et al.,1996 b)*
Figs. 50-51: *Progresive separation of the hydrogenosome into two compartments. Figure 50 shows that initially the inner membrane separates the hydrogenosome (H) in two compartments, but they are still joined by the outer hydrogenosomal membrane (arrowhead). This figure shows a purified hydrogenosome isolated by Percoll-sucrose density centrifugation. Figure 51 shows glycogen rosettes (G) surrounding the hydrogenosome. Fig.50 T.vaginalis, X 105,000. Fig.51, Trichomonas gallinae, X 100,000.*

obscure but they are not considered to be typical of most eukaryotes (Embley *et al.*, 1997).

A new hypothesis for the origin of eukaryotic cells was proposed, based on the comparative biochemistry of energy metabolism. Eukaryotes are suggested to have arisen through symbiotic association of an anaerobic, strictly hydrogen-dependent, strictly autotrophic archaebacterium (the host) with eubacterium (the symbiont) that is able to respire, but generate molecular hydrogen as a site product of anaerobic heterotrophic metabolism. The host's dependence upon molecular hydrogen produced by the symbiont is put forward as the selective principle that forged the common ancestor of eukaryotic cells (Martin and Müller, 1998).

What Martin and Müller propose is:

1. There is indeed an ancient symbiosis involving an α-proteobacterium as one partner and a relative or member of Archae as the other. The relevant activity of the α-proteobacterium is not, however, respiration, as in Margulis's hypothesis, but the excretion of H_2 and CO_2, which are the site products of anaerobic fermentation of externally available, reduced organic compounds in some contemporary proteobacterium. The Archean partner, like modern methane-producing archeans, used H_2 and CO_2 as its sole sources of energy and carbon. Both metabolisms could go on simultaneously and independently in anaerobic environments like many known today, and there are many examples of mutual relationships based on their coupling.
2. In the absence of an outside source of hydrogen, the archean (nominally the host) became dependent on the proteobacterium (the symbiont). Selection on host genes produced tighter physical association and greater surface contact between the two.
3. Gene transfer from symbiont to host provided the latter with membrane proteins for the import of the substrates and enzymes for glycolysis, the process by which ATP is generated anaerobically. So the host could begin to feed its symbionts, and thus surround them completely.
4. The effect of this is conversion of the host from autotrophy (using H_2 and CO_2 as substrates) to heterotrophy (able to use complex organic molecules). The symbiont is then lost, converted to a hydrogenosome, or became a mitochondrion (in the evolutionary line leading to all complex cells).

The strongest support for this remains the presence of α-proteobacterium genes in eukaryotic genomes.

The hydrogen hypothesis accounts for this α-proteobacterial presence, and for the kinds of genes concerned, more naturally than does the endosymbiont theory. It more readily explains the energy metabolism of eukaryotes that lack mitochondria (which, however, were little known when Margulis formulated her hypothesis).

As the hydrogenosome occur in a number of phylogenetically distinct groups, as *Trichomonads*, ciliates and chytrid fungi, it appears that they have evolved repeatedly and independently. Most theories of hydrogenosome origin have assumed that they are of endosymbiotic origin. Bio-

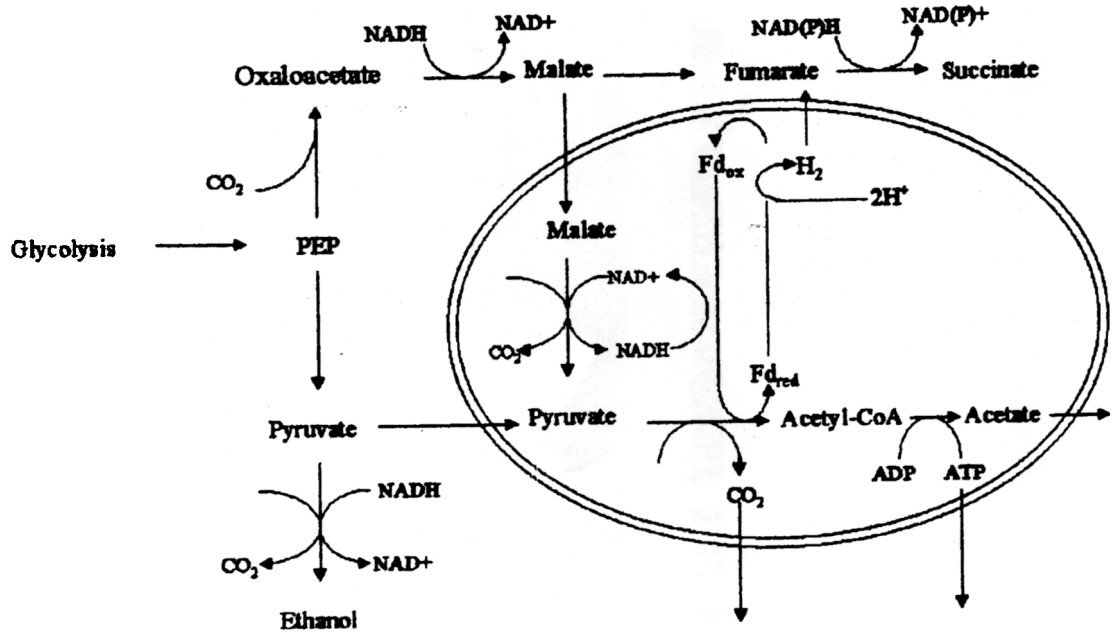


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Fig. 52: *Schematic metabolic map of T. vaginalis hydrogenosome. The double line denotes the double-membrane surrounding the hydrogenosome. After glycolysis, pyruvate is formed, enters the hydrogenosome where it is oxidatively decarboxylated thus forming acetyl-CoA from which acetate is liberated. The CoA is transferred to succinate forming succinyl-CoA. This serves as substrate for a substrate-level phosphorylation reaction leading to ATP production. Reducing equivalents derived from the oxidation of pyruvate are transferred to protons with the formation of H_2 under anaerobic conditions. The enzyme key of the organelle, responsible for pyruvate oxidation is pyruvate:ferredoxinn oxidoreductase (Fd). In T. foetus, malate might be the substrate for hydrogenosomes. (According to Müller, 1980).*

chemical similarities in their pathways of hydrogen evolution first led Müller (1993) to suggest that *Trichomonad* hydrogenosome might originate from endosymbiotic Gram-positive bacteria of the genus *Clostridium*. On the other hand, Cavalier-Smith suggested that *trichomonad* hydrogenosome is biochemically modified mitochondria because they possess a double membrane (Cavalier-Smith, 1987).

The most direct route to investigate organelle origins is to sequence genes, which are encoded by the organelle genome. Understanding how hydrogenosome has repeatedly evolved is a major challenge for biologists interested in eukaryote evolution and metabolic diversity.

The amitochondrial flagellates are considered the most ancient protists and the early descendents of the first eukaryotic cell or organisms. They comprise Archamoeba, the metamonads and the parabasalids. The parabasalids comprise a group of microorganisms that present hydrogenosome. The problem of knowing whether these organisms have never had mitochondria or instead have secondarily lost them remains, and molecular data are lacking for the Archamoeba as well as for the retortamonads and oxymonads. In ciliates, for example, most of species have mitochondria, except those living in anaerobic conditions, which have hydrogenosomes (Fenchel and Finlay, 1991).

The hydrogenosome also presents some characteristics common to peroxisomes, as the general form and electron density, especially when seen at low magnification. These organelles are thought to acquire their protein by a similar process of selective import from the cytosol (Fahimi *et al.*, 1993, Johnson *et al.*, 1993). Peroxisome and hydrogenosome thus resemble the ER in being self-replicating membrane-bounded organelles that exist without genomes of their own.

The available data for hydrogenosomes in trichomonads, ciliates and chytrid are consistent with them being biochemically modified mitochondria. The origin of hydrogenosomal enzymes such as hydrogenase and PFOR (ferredoxin-oxidoreductase), not found in mitochondria, could shed some light in the evolution puzzle.

Similarities between hydrogenosomes and mitochondria, such as the division process (Benchimol *et al.*, 1996b; Larsen, 1970), calcium incorporation, internal membranes and the presence of mitochondrial/hydrogenosomal type aminoterminal extensions in two sequenced proteins were demonstrated (Plümper *et al.*, 1998; Gasser *et al.*, 1982). The hydrogenosome presents some characteristics common to mitochondria: (a) it is surrounded by two unit membranes, (b) presents internal membrane specialization, (c) seems to be involved with the regulation of Ca^{++}, and (d) is involved in ATP production. Similarities between hydrogenosomes and peroxisomes also exist, such as (a) the general shape and electron density of the matrix, and (b) the association with the endoplasmic reticulum. The presence of enzymes not found in mitochondria such as pyruvate: ferredoxin oxi-

doreductase and hydrogenase, the absence of DNA and oxidative phosphorilation underscored the hydrogenosome as a special organelle that deserves further studies.

Fungal Hydrogenosome:

In the fungus *Neocallimastix sp. L2,* Marvin-Sikkema *et al.* (1992) found a single membrane lining the hydrogenosome. They also found a relationship between hydrogenosome and peroxisome concerning the presence of the characteristic peroxisomes targeting SKL signal in some hydrogenosomal proteins (Marvin-Sikkema *et al.*, 1993). After these findings some authors claimed that the hydrogenosome presented different morphological characteristics which could reflect fundamental differences in the biological nature of hydrogenosomes of various organisms. A polyphyletic origin for this organelle was proposed (Müller,1993; Cavalier-Smith, 1987). However, recent molecular studies demonstrated that primary sequences of the hydrogenosomal protein β-succinyl CoA synthetase (Brondijk *et al.*, 1996) and malic enzyme (van der Giensen *et al.*, 1997a) have targeting sequences that present some common features with mitochondrial targeting signals. Also, Benchimol *et al.*(1997) demonstrated that fungal hydrogenosomes are enveloped by two closely apposed membranes, in disagreement with Marvin-Sikkema findings (1993).

The rumen anaerobic fungus *Neocallimastix frontalis* (Figs. 2, 31) presents a two-stage life cycle, the motile zoospore, and the zoosporangium fixed to the cell wall plant particles by filamentous rhizoid. Previous studies carried out in *Neocallimastix patriciarum* demonstrated the presence of hydrogenosome-like organelles in all stages of the life cycle: in zoospores, in the vegetative form and also in the sporangia (Yarlett *et al.*, 1986).

Recent data now suggest that a mitochondrial origin is also plausible for fungal hydrogenosomes. It is known that different organelles require different targeting signals for the correct translocation of nuclear-genome encoded proteins and analysis of these signals is a potentially promising approach for inferring organelle origins. Both enzymes possess a 27- amino-acid leader-sequence, which is enriched in alanine, arginine, leucine and serine, and has an arginine at position-2. relative to aminoacid 1 of the mature protein. All of these features are typical for mitochondrial-targeting signals including those from *Saccharomyces* (von Heijne *et al.*, 1989). Detailed examination of both gene sequences failed to reveal any of the targeting signals which are characteristic of microbodies, providing no support for a peroxisomal origin for fungal hydrogenosomes (Embley *et al.*, 1997). Re-examination of the ultrastructure of *Neocallimastix frontalis* hydrogenosomes revealed a double membrane (Benchimol *et al.*, 1997).

Biochemical Characterization and Function:

The hydrogenosome participates in the ATP production. It compartmentalizes the fermentative metabolism of pyruvate, leading to the production of molecular hydrogen (Steinbüchel and Müller, 1986) (Fig. 52). Pyruvate generated by glycolysis or by conversion from malate is decarboxylated by pyruvate: ferredoxin oxidoreductase to the iron-sulfur protein ferredoxin and subsequently to protons forming molecular hydrogen, a process catalyzed by hydrogenase. Acetyl-CoA is converted to acetate with the concomitant conversion of succinate to succinyl-CoA synthetase. The generation of succinyl-CoA is coupled to ATP production via substrate-level phosphorilation (Fig. 52).

The hydrogenosome is also the site of activation of metronidazole, the drug used for treatment of trichomoniasis. Hydrogenosomal proteins convert metronidazol in a cytotoxic form, via a direct interaction with hydrogenosomal proteins (Johnson, 1993). Thus, in addition to its metabolic importance, the hydrogenosome plays an essential role in the susceptibility of trichomonads to therapy.

Hydrogenosomes have no tricarboxylic acid cycle enzymes and cytochromes and do not carry out oxidative phosphorylation. Similarly to mitochondria, however, these organelles use pyruvate as a major substrate and oxidize it to acetyl-CoA. Ferredoxin and succinate thiokinase are similar to mitochondrial ones. But pyruvate:ferredoxin oxidoreductase and hydrogenase are specific to the hydrogenosomes. The electrons produced in this process reduce protons and are eliminated as molecular hydrogen. Some hydrogenosomal enzymes are similar to mitochondrial ones, but some others are specific.

For biochemical characterization a highly purified hydrogenosomal fraction has to be obtained. Müller (1993) and Morgado and De Souza (1997) have obtained purified hydrogenosomes by different methodology (Fig. 34). This last group used differential and Percoll gradient centrifugation.

Isolated intact hydrogenosomes of *Tritrichomonas foetus* (Figs. 34-36) and *Trichomonas vaginalis* produce under anaerobic conditions approximately equimolar amounts of CO_2, H_2 and acetate from pyruvate (Müller, 1993). The two unit membranes surrounding the hydrogenosome represent a permeability barrier, as indicated by the structure-bound latency of several enzymes (Lindmark and Müller, 1973; Steibüchel and Müller, 1986). It is likely that a number of transport systems are involved in the metabolism of the organelle *in situ*. The presence of an adenine nucleotide transporter, exchanging ADP for ATP was shown (Steinbüchel and Müller, 1986), in isolated hydrogenosomes. This transporter is moderately susceptible to inhibition by atractyloside (Ckerkasov et al., 1978). Other transporters have not been studied to date (Müller, 1990).

Hydrogenosomes function in intact *T. vaginalis* under both anaerobic and aerobic conditions, as reflected by the production of acetate and CO_2. In fact, the absence or presence of O_2 does not significantly affect the carbon flow through the organelle, even though the fate of electrons lib-

erated through pyruvate oxidation is quite different. In the first case protons and in the second, O_2 serve as the terminal acceptor (Müller, 1990). So, under aerobic conditions, hydrogenosomes can use molecular oxygen as a terminal electron acceptor, thus acting as respiratory organelles. Respiration of these organelles is supported by only a few substrates: NADH, sn-glycerol 3-phosphate, pyruvate, and malate plus NAD (Müller, 1990).

Interconversion of malate and pyruvate is catalysed in hydrogenosomes by a malate dehydrogenase (decarboxylating) which in *T. foetus* can utilize either NAD^+ or $NADP^+$ (Hrdý and Mertens, 1993). Malate dehydrogenase has also been detected in *T. foetus* hydrogenosomes (Fig. 46) (Hrdý and Mertens, 1993; Benchimol *et al.*, 1996b). Intermediates of the tricarboxylic acid cycle and other organic acids are without effect (Ckerkasov *et al.*, 1978). Cyanide or rotenone, standard inhibitors of mitochondrial respiration (Ckerkasov *et al.*, 1980), do not inhibit respiration by *T. foetus* hydrogenosomes.

Hydrogenases of *Trichomonads* are oxigen-sensitive enzymes which utilizes reduced ferredoxin as an electron donor (Lindmark and Müller, 1973). The nature of the oxidase involved in hydrogenosomal respiration remains unknown. It is not susceptible to inhibitors of mitochondrial respiration (Ckerkasov et al., 1978) and has a high affinity for oxygen (Yarllett et al., 1986).

Acknowledgments

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