Ultrastructural characteristics of *Trichomonas vaginalis*

An electron microscopical study

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Infection with *Trichomonas vaginalis* is common among men and women in all parts of the world. Yet the significance of this organism in human pathology remains unclear. Most venereologists feel that it is responsible for certain inflammatory urogenital lesions and classify trichomoniasis as a sexually-transmitted disease (Teocharov, 1961). Some consider trichomonads to be innocuous commensals dwelling in pathologically altered tissues, and others regard them as facultative pathogens (Gnesdilov, 1959).

The organism is now attracting much attention, and advances in electron microscopy have led to the appearance of several reports on the ultrastructure of trichomonads (Anderson and Beams, 1959; Inoki, Nakanishi, and Nakabayashi, 1959, 1960; Hashimoto, 1964; Perju and Petrea, 1963; Ludvik, Stoklosová, and Weglarska, 1961; Panaitescu, Voiculescu, and Ionescu, 1971; Panaitescu, Voiculescu, Ionescu, and Petrovici, 1970; Filadoro, 1969; Ovcinnikov and Delektorskij, 1969).

Further study is needed, however, because the published data are incomplete and some of the observations have been interpreted incorrectly. Descriptions based on optical microscopy do not satisfy modern requirements and are often erroneous.

We have undertaken an electron microscopical study of ultrathin sections of trichomonads from a culture in Johnson-Trussell medium (Johnson, Trussell, and Jahn, 1945) and from secretions of patients with trichomoniasis.

Methods

A 3- to 4-day culture was used. 2 ml. 0.1 M cacodylate buffer were added to a centrifuge tube with 0.5 ml. medium taken from the area of greatest growth. The material was spun at 3,000 r.p.m. for 30 min. 2 ml. of a 4 per cent. glutaraldehyde solution in 0.1 M cacodylate buffer cooled to +4°C. were carefully layered onto the sediment. Pre-fixation lasted 2 hrs in the cold (+4°C.), and the material was then washed with 0.1 M cacodylate buffer plus 0.2 M sucrose and fixed with osmium tetroxide (Fernando and Movat, 1964).

When material of human origin was studied, a drop of urethral or vaginal secretion was placed on a slide. After the specimen had been examined under a phase-contrast microscope, the coverslip was removed and transferred into a buffered 4 per cent. glutaraldehyde solution. The opalescent film was detached from the coverslip. Fixation was done by the same procedure as that used for the culture material. The preparations were dehydrated in a battery of alcohols and embedded in an Epon-araldite mixture (Mollenhauer, 1964).

Polymerization proceeded during 48 hrs at 49°C. The sections were cut with an LKB-8801A ultramicrotome and stained with an aqueous uranyl acetate solution and lead hydroxide (Millonig, 1961).

We used Czechoslovak electron microscopes, models Tesla 513BS and 613BS, resolution 4.5 Å.

Results

When examined under the electron microscope by the negative contrast method, the trichomonads usually have an oval or bulbous shape, though some are amoeboid. The anterior (upper) end carries a bundle of flagella and an undulating membrane, which descends approximately halfway along the body of the organism (Fig. 1).

Ultrathin sections show a finely granular cytoplasm with occasional large osmiophilic particles (Figs 2 and 2a); aggregates of osmiophilic granules are located near the nucleus (Figs 2 and 3). The body is covered with an outer membrane presenting as an osmiophilic membrane (Fig. 3a), but some parts of it are indistinct.

The oval nucleus is situated in the anterior third of the body (Figs 2 and 3); its karyoplasm consists of finely granular chromatin, which is condensed peripherally (Fig. 19). In young specimens an electron dense nucleolus can be discerned. The nuclear membrane contains numerous small electron dense granules; here and there pores are clearly visible. The perinuclear zone is rich in polyribosomes (Fig. 3).

The flagellar apparatus in trichomonads includes four free flagella (Fig. 1) about 2,000 Å in diameter (Figs 3a, 6, 11, 20). The protozoan owes its motility

to the active movements of the flagella. One of them descends along the body of the organism and, together with the outer membrane, forms the undulating membrane (Figs 3, 3a, 5).

The undulating membrane is not derived exclusively from the outer membrane. Figs 6, 9, 11, 14, and 15 show that both the outer membrane and the cytoplasm participate in the formation of its basal part and that it contains vesicles (Figs 9 and 11) and sometimes lysosomes. This indicates that the undulating membrane cooperates with the flagella in entrapping food particles. Its basal part is lined with a flagellum joined by a costa (Fig. 12). Sometimes the impression is given that there are two (Figs 9 and 11) or even three (Fig. 12) undulating membranes. The end of the undulating membrane is marked with dark transverse bands characteristic for spirally symmetrical structures, which suggest that the membrane has independent contractile activity (Fig. 7). The relation of the undulating membrane to the flagellum is well illustrated in Figs 10 and 13. In a few sections the undulating membrane seems to lie in a cavity within the organism (Fig. 8). The undulating membrane does not serve only for locomotion; its movements draw in food particles which are filtered through a kind of 'sieve' at the base of the membrane (Fig. 4).

Like Hawes (1947), we have observed in a liquid medium by the phase-contrast technique how a vaginal trichomonad, continually distending and twisting its body, attracts a food particle by the ceaseless action of the flagella and the undulating membrane. Sometimes the latter are actively beating in one direction while the organism remains stationary.

One mode of nutrition is represented by the movements of the flagella drawing food particles into the body of the parasite. On the other hand, the trichomonads are undoubtedly capable of absorbing food particles (Figs 8, 20, 23), bacteria (Figs 9, 25, 34), etc., without the assistance of the flagella. Phagocytosis and intracellular digestion are confirmed by the presence of numerous lysosomes (Figs 12, 14, 19), lipid granules, vesicles, and digestive vacuoles (Figs 20 and 23). We do not agree with Jirovec (1960), who claimed that the occurrence of digestive vacuoles in the cytoplasm was exceptional. Digestive vacuoles are present in considerable numbers both in culture specimens and in trichomonads isolated directly from patients (Figs 8, 17, 20, 23, 34, 35). Fig. 36 clearly shows how food particles are entrapped.

All the flagella have a similar structure. In cross-section, they consist of nine pairs of ringlets arranged circumferentially and one central pair. The ringlets are surrounded by a common membrane (Figs 3a

and 6). The flagella end in the blepharoplast complex consisting of annular formations (Figs 15, 17, 28, 29). In contrast to the flagella, the blepharoplasts are devoid of the central pair of ringlets. The blepharoplast complex is assumed to function as a regulator of motility. Besides the presence of annular structures, the blepharoplast zone is distinguished from the rest of the cytoplasm by greater electron density. Sometimes it contains osmiophilic inclusions (Fig 27).

The axostyle arises from the blepharoplast zone (Figs 20, 22, 24, 25). We disagree with those authors who suggest that the structure of the axostyle is that of another flagellum, which runs inside the body of the parasite. According to our observations, the axostyle is hollow and has a fundamentally different structure (Figs 22 and 25). Arising at the blepharoplast zone, it traverses the entire body and sometimes protrudes in the form of a spicule at the opposite end of the organism (Figs 22, 23). Its width varies; it is wider at the middle part and tapers towards each end. Its walls are formed by thin longitudinal filaments (Figs 22, 24). The internal cavity is filled with a finely granular mass throughout the length of the axostyle (Fig. 25). The exact significance of this structure is unclear. Many investigators believe that it has a supporting function.

The costae, formations with a specific structure, extend from each of the blepharoplasts to the lower third of the body (Figs 26, 27, 28). As in the axostyle, their upper and lower ends are thinner than the central portion. Because of longitudinal and cross striations, they somewhat resemble collagen fibres. Perju, Petrea, and Toader (1963) defined the costa as a collagen fibril. Its function is also debatable.

The formation variously called 'parabasal fibril', 'parabasal body', or 'parabasal apparatus' is situated opposite the costae (Figs 2, 5, 15–18, 20, 21, 24, 26, 28–33). In our opinion, this is not a fibril, but a system of tubules, which arise in a semicircle at the blepharoplast zone (Figs 17, 18, 20, 21, 24) and go down alongside the nucleus, and for a certain distance beyond it. 'Parabasal apparatus' is therefore a more appropriate term. Some tubules of the parabasal apparatus may take an oblique direction (Figs 32 and 33), extending in a single or double row (Fig. 16). The zone delimited by the tubules contains polyribosomes (Fig. 33). The parabasal apparatus resembles in structure, and possibly in function, the subpellicular microtubules in *Leishmania* which seem to serve as a supporting frame. Some authors (Perju and others, 1963) consider that the parabasal apparatus plays a role in protein metabolism, since its granules consist of nucleic acids and are extremely sensitive to chemical toxins. The parabasal apparatus does not extend to the end of the body. Honigberg

Key to the Figures

a	— axostyle
b	— blepharoplasts
ch	— nuclear chromatin
d	— engulfed diplococcus
er	— endoplasmic reticulum
f	— flagella
f'	— filaments between body and undulating membrane
f''	— recurrent flagella
fo	— vacuoles with food residue
fu,sp	— dark transverse bands on undulating membrane
fv	— digestive vacuoles
g	— granules
k	— costae
l	— lysosomes
m	— outer membrane
mc	— phagocytosed microbial cell
mN	— nuclear membrane
N	— nucleus
o	— osmiophilic inclusions
p	— pores in nuclear membrane
pa	— parabasal apparatus (parabasal body)
pb	— parabasal apparatus (parabasal body)
pe	— spicule
ph	— phagosomes
phS	— phagosome with engulfed rod-shaped bacterium
pr	— polyribosomes
r	— ribosomes
t	— body of trichomonad
um	— undulating membrane
v	— vesicles, vacuoles
zb	— blepharoplast zone
zG	— Golgi zone
zp	— perinuclear zone

NOTE

*Figs 1, 2, 4, 20, 21, and 23 show trichomonads obtained from cultures; the other figures depict organisms obtained from secretions of female patients

*FIG. 1 *T. vaginalis* under the electron microscope. Negative contrast method. $\times 7,500$

*FIG. 2 General structure of *T. vaginalis*. $\times 7,500$
FIG. 2a Detail. $\times 24,000$

FIG. 3 Undulating membrane with a recurrent flagellum. $\times 21,000$

FIG. 3a Detail. Nine peripheral pairs of fibrils and one central pair are well outlined. $\times 81,000$

*FIG. 4 Undulating membrane with a recurrent flagellum. Thin filaments are visible between the body and the undulating membrane. $\times 34,000$

FIG. 5 Undulating membrane with a recurrent flagellum. $\times 24,000$

FIG. 6 Undulating membrane. Dark short transverse bands can be easily seen under the outer membrane. Cross-section of a flagellum is clearly demonstrated. $\times 5,000$

FIG. 7 Undulating membrane. The end of the undulating membrane is marked in the midportion with dark transverse bands having a definite period. They do not extend to the outer membrane. $\times 58,000$

FIG. 8 Atypical location of undulating membrane. The section is taken in such a plane that the undulating membrane and the recurrent flagellum seem to be inside the organism. $\times 17,000$

FIG. 9 The undulating membrane has two ends. Large vesicles are situated at its basal part. Smaller vesicles can be found elsewhere in the cytoplasm. A phagocytosed diplococcus is visible. $\times 17,000$

FIG. 10 Isolated undulating membrane. In this plane of cutting the undulating membrane is not connected with the body. A costa passes between its two ends. Recurrent flagella are adjacent to the undulating membrane. $\times 37,500$

FIG. 11 Two undulating membranes with recurrent flagella. $\times 22,000$

FIG. 12 Three undulating membranes with recurrent flagella. $\times 13,000$

FIGS 13, 14, 15 Region of undulating membrane. Note that it is formed by the cytoplasm, as well as by the outer membrane. $\times 22,000$

FIG. 16 Parabasal apparatus. This Figure convincingly shows that the parabasal apparatus consists of tubules arranged in a single or double row. Their shape is annular, if cut crosswise, or tubular, if cut obliquely. The granular endoplasmic reticulum is clearly visible. $\times 34,000$

FIG. 17 Parabasal apparatus and Golgi zone. The parabasal apparatus encircles the blepharoplast zone and goes downwards. The Golgi zone is represented by tubules and small vesicles. A phagosome with food residues is displayed. $\times 15,000$

FIG. 18 Parabasal apparatus and Golgi zone. Undulating membrane with a vesicle in its basal part and a recurrent flagellum. $\times 25,000$

FIG. 19 Structure of nucleus. $\times 25,000$

*FIG. 20 Zone of flagella. The parabasal apparatus surrounds in a semicircle the blepharoplast zone. $\times 15,000$

*FIG. 21 Blepharoplast zone. $\times 15,000$

FIG. 22 Structure of axostyle. Almost the entire length of the axostyle is shown. Note its hollow shape. Above Longitudinal filaments forming its walls. Below spicule. $\times 9,500$

*FIG. 23 Section of spicule displaying its inner cavity. $\times 16,000$

FIG. 24 Axostyle. Filaments in its wall are distinctly visible. Origin of the costae arising from blepharoplasts is also demonstrated. $\times 16,000$

and King (1964) and Smith and Stewart (1966) claim that it is identical with the Golgi complex, but this opinion is incorrect. Figs 17 and 18 show that trichomonads have an independent and well-developed Golgi zone.

According to Dalton (cited by Policard and Bessis, 1968), the Golgi zone includes a dictyosome, represented by a stack of double membranes, a variable amount of small vesicles derived from the dictyosome, and some considerably larger vacuoles. The dictyosome is the central component of the Golgi complex. It is formed by a stack of flattened sacs arranged in parallel and having in section the typical structure of a double membrane. The exact function of the Golgi complex has not been elucidated, but it is supposedly concerned with the general metabolic activity of the cell.

The zone between the nucleus and the parabasal apparatus contains large electron dense granules against a background showing an extremely fine granular structure.

Opinions differ as to the mode of nutrition of *T. vaginalis*. Most investigators deny the existence of a cytostome and believe that nutrition proceeds endosmotically and through phagocytosis of particulate material (Ludvik and others, 1961). However, in the past, some authors (Schmit and Kamniker, 1926) have asserted that a cytostome existed. We are of the opinion that the parasite absorbs nutrients endosmotically and by entrapping food particles, and that possibly some region of its body also serves as a cytostome.

T. vaginalis has no mitochondria. As suggested by Inoki and Hamada (1953), their function is performed by the microgranules contained in the cytoplasm, the parabasal apparatus, etc. Various vesicles are present in the body. The endoplasmic reticulum is poorly developed, with small numbers of ribosomes attached to it (Figs 16 and 18).

Trichomonads isolated directly from patients are

more active; they contain many digestive vacuoles with entrapped particles, including microbes, and numerous vesicles of various sizes; their phagocytic activity is more marked; the organisms often assume an amoeboid shape with deep invaginations which also contain food particles.

The trichomonads reproduce by division. At first, all the elements are reduplicated and then the cell divides longitudinally into halves. Budding has also been noted.

When observing living trichomonads in vaginal secretions with a phase-contrast microscope, we could see multiple fission besides division into halves. Young organisms appeared as twisted strands radiating from a kind of granular mass located in the centre, and finally they separated completely. We may add that, during a prolonged period of observation, trichomonads were seen to assume an amoeboid shape and to put out pseudopodia.

The above findings show that the structure of *T. vaginalis* is much more complicated than was previously considered.

Summary and conclusions

Ultrathin sections of *Trichomonas vaginalis* were studied by electron microscopy. Both culture material and specimens obtained from urethral and vaginal secretions of patients were examined.

(1) The following structures were observed: outer membrane, flagella, blepharoplasts, undulating membrane, nucleus and nuclear membrane, parabasal apparatus, axostyle, costae, lysosomes, ribosomes, digestive vacuoles, and vesicles.

(2) In cross-section the flagella consist of nine peripheral pairs of ringlets and one central pair. The ringlets are enveloped in a common membrane.

The cytoplasm participates in the formation of the undulating membrane. Judging from its structure,

FIG. 25 Axostyle. Its granular contents can be seen. A phagosome with an engulfed rod-shaped bacterium is shown. $\times 18,000$

FIG. 26 Costa. Osmiophilic inclusions are present in the centre of the parabasal apparatus. $\times 18,000$

FIG. 27 Costa arising from a blepharoplast. The blepharoplast complex is clearly displayed. $\times 16,500$

FIG. 28 Blepharoplast zone. The blepharoplasts are sectioned in various planes. The costa arising from a blepharoplast has a characteristic structure somewhat reminiscent of a collagen fibre. $\times 28,000$

FIG. 29 Blepharoplast zone. $\times 37,500$

FIG. 30 Blepharoplast zone. $\times 24,000$

FIG. 31 Blepharoplast zone. Osmiophilic inclusions are evident. $\times 25,000$

FIG. 32 Parabasal apparatus. $\times 31,000$

FIG. 33 Parabasal apparatus. Its tubules course in different directions. $\times 21,000$

FIG. 34 Digestive vacuoles with distinct walls. They contain food residues and an entrapped bacterial cell. $\times 15,000$

FIG. 35 Large digestive vacuole with food residues. $\times 15,000$

FIG. 36 Engulfment of food. An invagination formed in the body wall (arrow) contains amorphous material. Its edges are drawing together. $\times 9,000$

the undulating membrane may have an independent contractile function.

(3) The nucleus contains chromatin and has a finely granular structure.

(4) The parabasal apparatus is represented by a system of tubules which form a semicircle round the blepharoplast zone and go down alongside the nucleus. This region contains a considerable amount of polyribosomes.

(5) The hollow axostyle traverses the entire body of the parasite, protruding in the form of a spicule at its lower end. The walls of the axostyle consist of longitudinal filaments and its central cavity is filled with a granular mass.

(6) The costae extend from the blepharoplast zone. They have a specific structure resembling that of collagen fibres.

(7) Trichomonads absorb nutrients endosmotically and by phagocytosis, and are capable of engulfing bacteria. However, the existence of a cytostome is not excluded.

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Caractéristiques ultra-structurales du *Trichomonas vaginalis*. Étude au microscope électronique

SOMMAIRE

Des coupes ultra-fines de *Trichomonas vaginalis* furent étudiées au microscope électronique. On examina à la fois du matériel de culture et des spécimens provenant de sécrétions urétrales ou vaginales de malades.

(1) On observe les structures suivantes: membrane externe, flagelles, blépharoplastes, membrane ondulante, noyau et membrane nucléaire, appareil parabasal, axostyle, côtes, lysosomes, ribozomes, vacuoles digestives et vésicules.

(2) En coupe transversale, les flagelles sont représentées par neuf paires de boucles périphériques et une paire centrale. Les boucles sont enveloppées dans une membrane commune.

Le cytoplasme participe à la formation de la membrane ondulante. À en juger par sa structure, la membrane ondulante peut avoir une fonction contractile indépendante.

(3) Le noyau contient de la chromatine et a une structure finement granulaire.

(4) L'appareil parabasal est représenté par un système de tubules qui font un demi cercle autour de la zone blépharoplastique et descendent le long du noyau. Cette région contient une quantité considérable de polyribosomes.

(5) L'axostyle creux traverse tout le corps du parasite, se terminant en forme de spicule à son extrémité inférieure. Les parois de l'axostyle consistent en filaments longitudinaux et sa cavité centrale est remplie par une masse granulaire.

(6) Les côtes s'étendent à partir de la zone blépharoplastique. Elles ont une structure spécifique ressemblant à celle des fibres de collagène.

(7) Les *Trichomonas* absorbent les éléments nutritifs par endosmose et phagocytose; ils sont capables de capter les bactéries. Cependant, l'existence d'un cytostome n'est pas exclue.