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Observations on *Trichomonas vaginalis* by Electron Microscopy*

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SUMMARY

Ultra-thin sections of *Trichomonas vaginalis* from V-bouillon (Inoki and Hamada, 1953) were examined by electron microscope, and the following results were obtained. The nucleus is surrounded by a nuclear membrane formed of two distinct layers, and its discontinuous profile suggests the presence of pores in the membrane. The periphery of the cytoplasm forms a distinct cell wall (periplast). Within the cytoplasm, several kinds of structures are very clear; Round granules ranging from 5 to 12 mμ in diameter are usually observed, some dense, and some less dense. The Golgi apparatus near the nucleus, and vacuoles of different size are also found. Collagen fibrils are clearly seen, which seem to connect the base of the flagellum with the surface of the body. No mitochondria are seen, and this is compatible with biochemical results. The parasite has an endoplasmic reticulum of the type described by Palade. A probably oblique section of the axostyle was made, in which both small granules and stripes were seen. The flagellum consists of an axoneme and covering sheath. The axoneme contains ten pairs of fibrils, one central and nine peripheral.

INTRODUCTION

In 1952, Inoki and Hamada first employed the electron microscope for comparing the *Trichomonas vaginalis* with its mouse-passed variant morphologically. In the following year, they also reported on the fine structure of *Trichomonas vaginalis* at the Annual Meeting of the American Society of Tropical Medicine and Hygiene held at Louisville, Kentucky, U. S. A. Their results were obtained by ordinary electron microscopy, and showed the superficial morphology of both flagellum and axostyle and their variations. Since then, however, no further reports have been published on this parasite. In this paper results are given of data from thin-section microscopy.

Material and Method

A stock culture of *T. vaginalis* (TV 12) was employed for this study. Cells were cultured in V-bouillon (Inoki and Hamada, 1953) for 48 hours, and

* An outline of this study was reported at the 27th Annual Meeting of Society of Japanese Parasitologists held in Gifu City, in May, 1958.

harvested by centrifugation at 1,500 r.p.m. for 5 minutes. They were then transferred 1% osmic acid buffer (pH 7.4 or 5.6), and kept in an ice box for 30 minutes for fixation. The cells were then dehydrated in alcohol without washing and rountinely embedded into methacrylate resin (containing 80% *n*-butylmethacrylate and 20% methylmethacrylate), using 3% *o*-chlorobenzoyl peroxide as a catalyst. Sections were prepared with a glass knife in a Porter-Blum Serial 401 microtome, and studied under an HU 6 type (under 50 KV) or HU 10 type (under 100 KV) electron microscope.

RESULTS

1. Nucleus

As shown in Fig. 1 (B), the nucleus has denser and finer granules than the cytoplasm. The nuclear membrane is formed of two distinct layers (See Fig. 1 (D)), and its discountinuous profile suggests the presence of pores (See Fig. 1 (arrows)) between the nucleus and the cytoplasm, (See Fig. 1 (D)). This structure is quite similar to that observed in mammalian cells (Watson, 1954). Inside the nucleus, some structures are differenciated. However, they are too indistinct to make it possible to determine their nature.

2. Cytoplasm

The periphery of the parasite appears to form a thin layer or membrane (See Fig. 1 (E)), which has no such striations as are found in *Leishmania donovani* (Nakanishi, 1956; Pyne, 1958). Of particular interest is the fact that several round granules ranging from 5 to 12 mμ in diameter are found in the cytoplasm, some of which are dense (See Fig. 1 (C), Fig. 2 (A), and Fig. 5 (C)), while others are less dense (See Fig. 2 (B)). At present, it is unknown whether these two kinds of granules are similar, and what they are. As judged by their shape however, they are not mitochondria. Vacuoles of different sizes are also seen in the cytoplasm (See Fig. 1 (F)). Fig. 1 (A) shows that the Golgi apparatus is also present close to the nucleus. It consists of lamellar components of about 20 mμ width. Furthermore, endoplasmic reticulums are demonstrated in the cytoplasm, of 40 to 270 mμ diameter. As previously described, they consist of a relatively light center surrounded by a dense, thin membrane with fine granules on the outside (See Fig. 1 (G)). Ribbon-like structures stretching through the cytoplasm with a number of dense transverse stripes at regular interval of about 30 mμ have been found (See Fig. 5 (B), Fig. 6 (B), and Fig. 7 (A) (B)). These structures seem to arise from the base of the flagellum (See Fig. 6 (B)) and extend to the peripheral layer of the body (See Fig. 7 (B)). From their fine structure, these organella can be assumed to be the collagen fibrils which probably play an important role in the movement of this parasite. However, further studies are needed for elucidation of this point. In this study, mitochondria-like bodies have not been found, although many sections were examined. This is contrary to observations on *L. donovani* (Inoki *et al.*, 1957)

3. Axostyle

For some reason, it is difficult to find the axostyle in the section. Fortunately we succeed in demonstrating a probable axostyle surrounded by a clear limiting

membrane and containing fine, dense granules and stripes (See Fig. 2 (C)).

4. Flagellum

The flagellum consists of a central axoneme with a sheath covering it. In the former, 20 fibrils arranged in 10 pairs run along the logitudinal axis; one pair is central, while the other nine surround it (Inoki *et al.*, 1958). The connection between the flagellum and the body also appears to be like that in *L. donovani.* (Inoki *et al.*, 1957). (See Figs. 3 (A), 4 (A), 5 (A), and 6 (A)).

DISCUSSION

In this study, major attention was directed to elucidating the inner structure of this parasite from ultra-thin sections.

From these studies the demonstration of mitochondria, such as has been described in metazoan cells, was unsuccessful, although a number of sections were carefully examined. This observation is in contrast to Anderson's report (1955) in which he describes the so-called mitochondria of *Trichomanas muris.* The mitochondria-like bodies found by Anderson in *T. muris* are unlike typical mitochondria demonstrated in *Paramecium caudatum* (Powers *et al.*, 1955) and *L. donovani* (Inoki *et al.*, 1957). This is supported by biochemical studies on carbohydrate metabolism in trichomonads. Ninomiya and Suzuoki (1952) reported that their strain of *T. vaginalis* is incapable of utilizing succinate, citrate, and glutamate. Wirtschafter *et al.* (1956) state that krebs cycle activity was not found in Strain 5 A of *T. vaginalis.* In *Trichomonas foetus*, Ryley (1955) stated, no evidence for the presence of cytochromes could be obtained by spectroscopic examination, by the use of respiratory inihbitors, or by the use of cell homogenates with *p*-phenylenediamine. Accordingly, it is reasonable to consider that the trichomonas have no mitochondria of similar structure to those in metazoa.

It was very interesting to find a ribbon-like structure with many transverse stripes, which apparently arises at the base of the flagellum, runs through the cytoplasm, and probably terminates at the surface of the parasite. From its fine structure, this organella must correspond to the collagen fibrils in metazoa (Gross, 1956) and play some role in movement.

A group of lamellae was observed in close contact with the nucleus. These bore stricking similarities to the Golgi apparatus already reported in metazoan cells (Dalton and Felix, 1956). However, further studies are needed to see if the organella observed are a true Golgi apparatus. In this study, an endoplasmic reticulum was found in the cytoplasm, showing the features that Palade and Porter (1954) described for identification of an endoplasmic reticulum.

There is no fundamental difference between the fine structure of the flagellum of *T. vaginalis* and *L. donovani.* However, the flagellum of *T. vaginalis* is formed from ten pairs of fibrils, - one central and nine peripheral. The connection between the flagellum and the body is similar to that in *Leishmania donovani* (Inoki *et al.*, 1957.) Two types of large granules were always found in the cytoplasm. However no studies on their nature have been made.

This type of study will be necessary to relate the physiology and morphology of the protozoa.

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REFERENCES

Anderson, E. (1955) The electron microscopy of *Trichomonas vaginalis*. *J. Protozool.*, **2**, 114-124.

Dalton, A. J. and M. D. Felix (1956) A comparative study of the Golgi complex. *J. Biophys.* and *Biochem. Cytol.*, **2** (Suppl.), 79-84.

Gross, J. (1956) The behavior of collagen units as a model in morphogenesis. *J. Biophys.* and *Biochem. Cytol.*, **2** (Suppl.), 261-274.

Inoki, S. and Y. Hamada (1952) Inheritable variation appeared in the mouse-passed *Trichomonas vaginalis*. *Jap. Jour. Genet.*, **27**, 219-220, (in Japanese).

Inoki, S. and Y. Hamada (1953) Experimental transmission of *Trichomonas vaginalis* (pure culture) into mice. *J. Inf. Dis.*, **92**, 1-3.

Inoki, S., K. Nakanishi, T. Nakabayashi, and M. Ohno (1957) Fine structure of the flagellum of *Leishmania donovani* revealed by electron microscope. *Med. J. Osaka Univ.*, **7**, 719-729.

Inoki, S. (1957) Expèriences d'immunolgie, de biochemie, de génétique sur *Trichomonas vaginalis*. Les Infestation A Trichomonas (Premier Symposium Européen), 265-269.

Inoki, S., K. Nakanishi, and Y. Nishi (1958) Observation of *Trichomonas vaginalis* by electron microscope (2). Proceeding of the 27th Annual Meeding of Japanese Parasitologists held in Gifu City, in May 16-17. (in Japanese).

Nakanishi, K. (1959) On the fine structures of *Leishmania donovani* as shown by electron microscope. *Kiseichu-gakkai Zasshi*, **5**, 329-337 (in Japanese).

Ninomiya, H. and Z, Suzuoki (1952) The metabolism of *Trichomonas vaginalis* with comparative aspects of trichomonads. *J. Biochem.* (Japan), **39**, 321-331.

Palade, G. E. and K. R. Porter (1954) Studies on the endoplasmic reticulum. I. Its identification in cells in situ. *J. Exp. Med.*, **100**, 641-656.

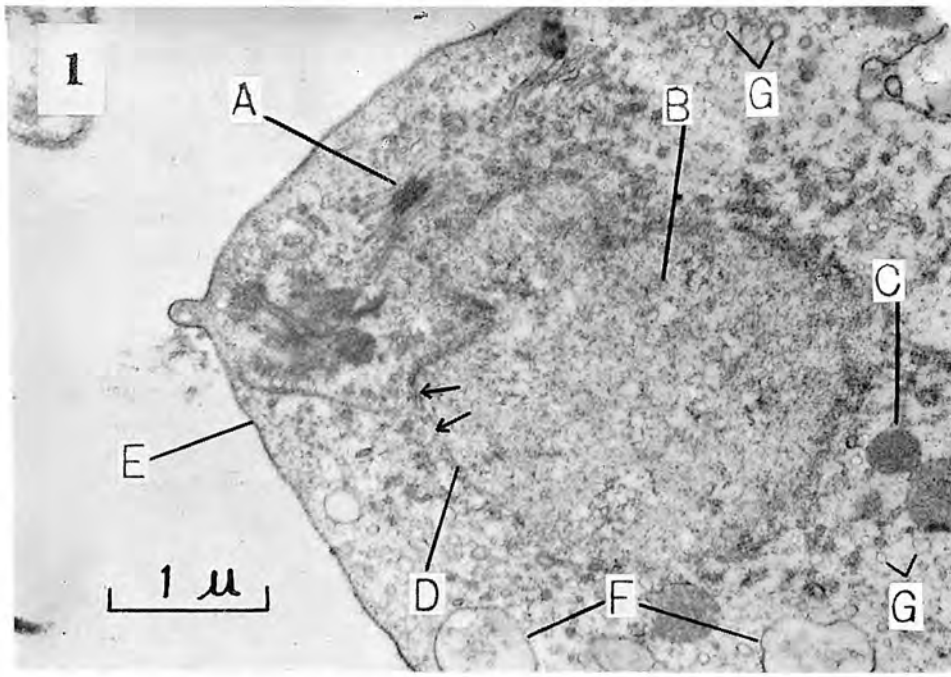
Powers, E. L., C. F. Ehret and L. E. Roth (1955) Mitochondrial structure in Paramecium as revealed by electron microscopy. *Biol. Bull.*, **108**, 182-195.

Pyne, C, K. (1958) Electron microscopic investigations on the leptomonad form of *Leishmania donovani*. *Expl. Cell Res.* **14**, 388-397.

Ryley, J. F. (1955) Studies on the metabolism of the protozoa. 5. Metabolism of the parasitic flagellate *Trichomonas foetus*. *Biochem. J.*, **59**, 361-369.

Watson, M. L. (1954) Pores in the mammalian nuclear membrane. *Biochim.* et *Biophys. Acta*, **15**, 475-479.

Wirtshafter, S., P. Saltman and T. L. Jahn (1956) Metabolism of *Trichomonas vaginalis*: The oxdation pathway. *J. Protozool.*, **3**, 86-88.



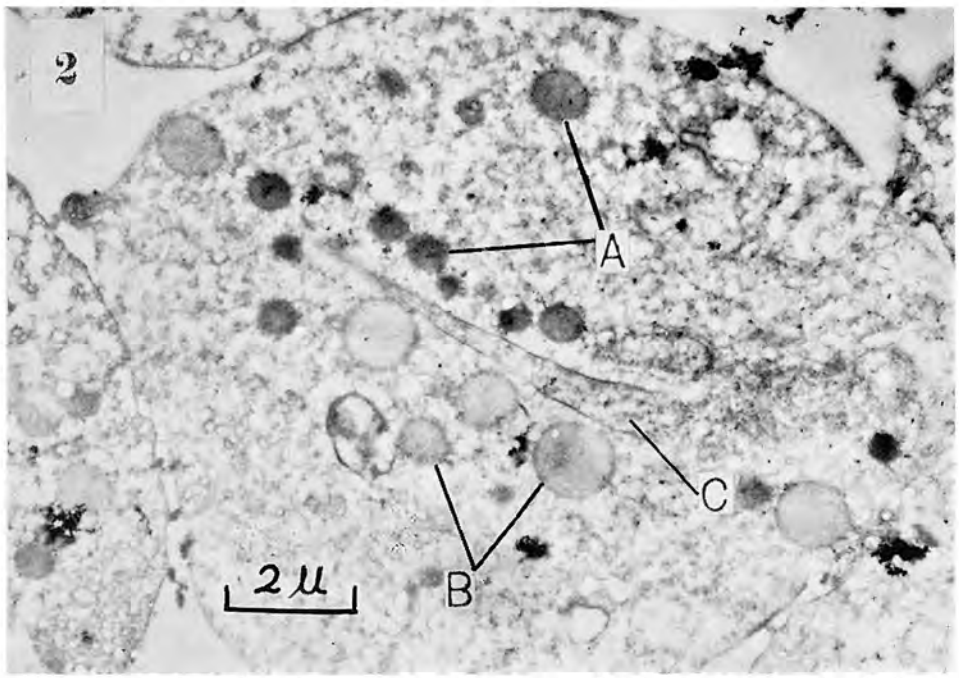
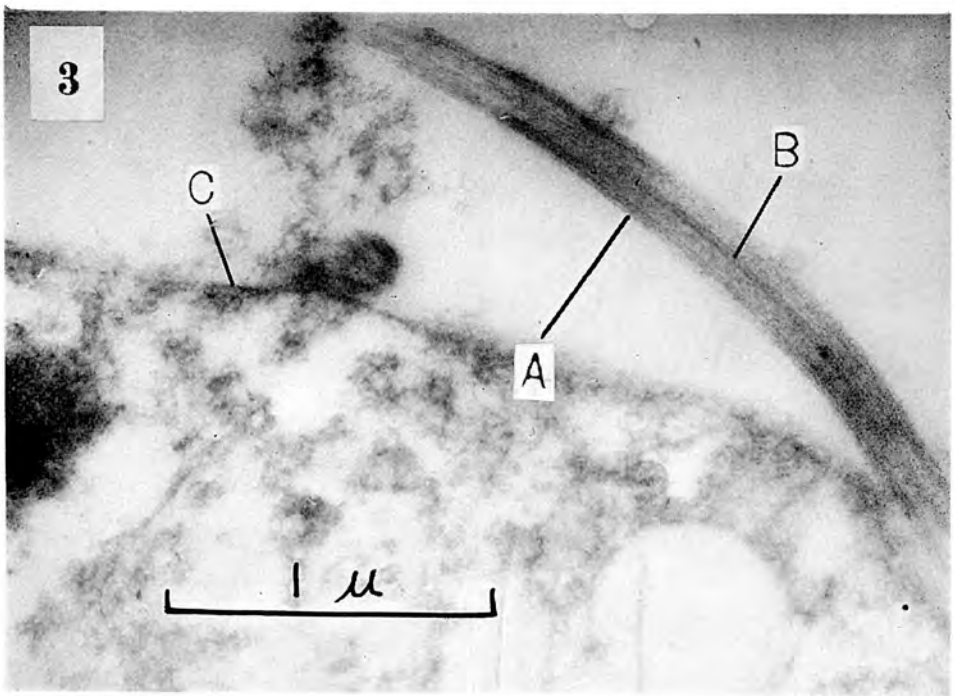


Fig. 1. An ultra-thin section of *Trichomonas vaginalis*, showing Golgi apparatus (A), nucleus (B), large granules (C), nuclear membrane (D), periplast or cell wall (E), vacuoles (F), endoplasmic reticulums (G) and pores through the nuclear membrane (arrows).

Fig. 2. An ultra-thin section of *Trichomonas vaginalis*, showing dense granules (A), less dense granules (B) and axostyle (C).



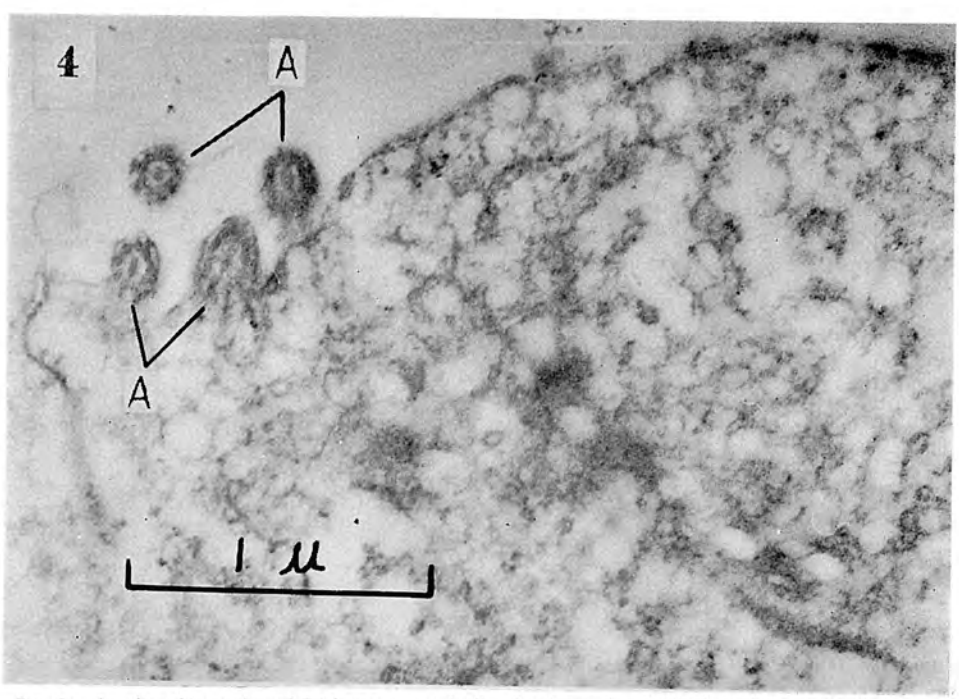
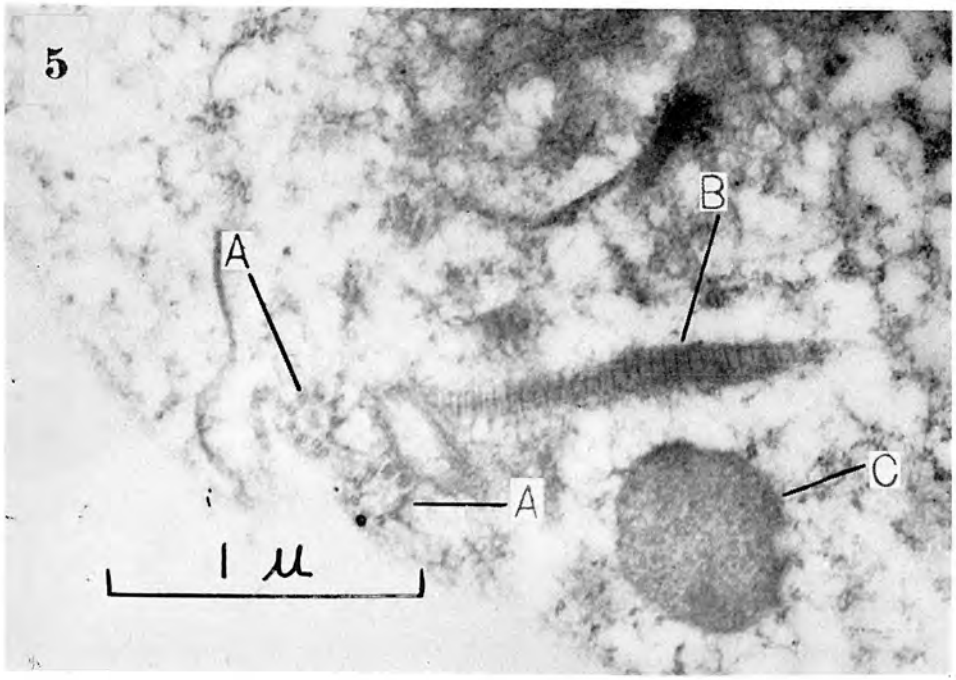


Fig. 3. An ultra-thin section of *Trichomonas vaginalis*, showing a longitudinal cut face of the flagellum. A. : flagellum, B. : fibril, C. : periplast.

Fig. 4. An ultra-thin section of *Trichomonas vaginalis*, showing the transverse cut faces of the flagella (A).



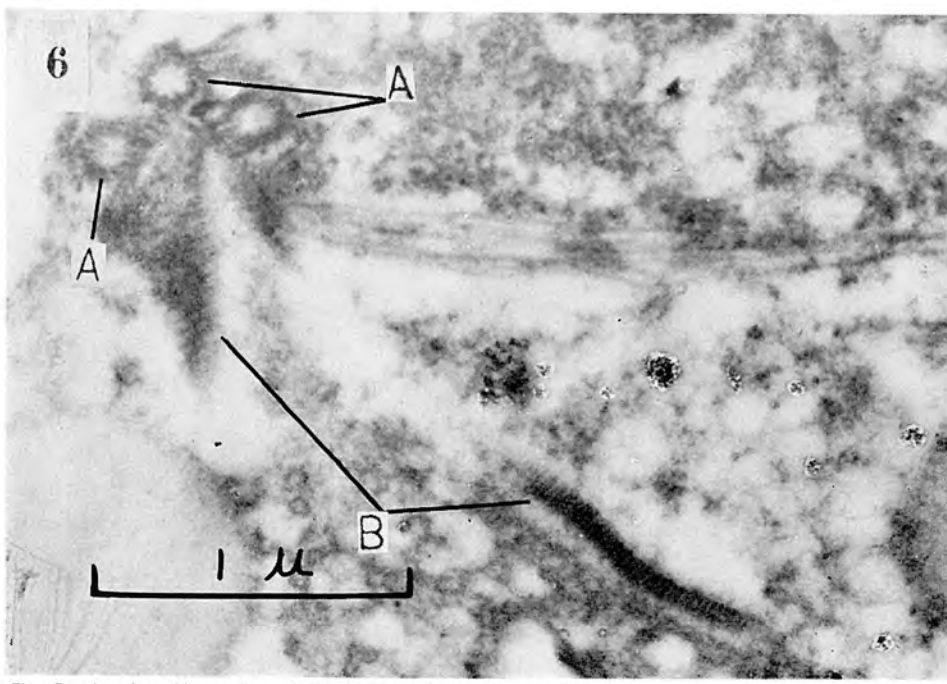


Fig. 5. An ultra-thin section of *Trichomonas vaginalis*, showing flagella (A), collagen fibril (B) and enlarged dense granule (C).

Fig. 6. An ultra-thin section of *Trichomonas vaginalis*, showing flagella (A) and collagen fibrils (B).

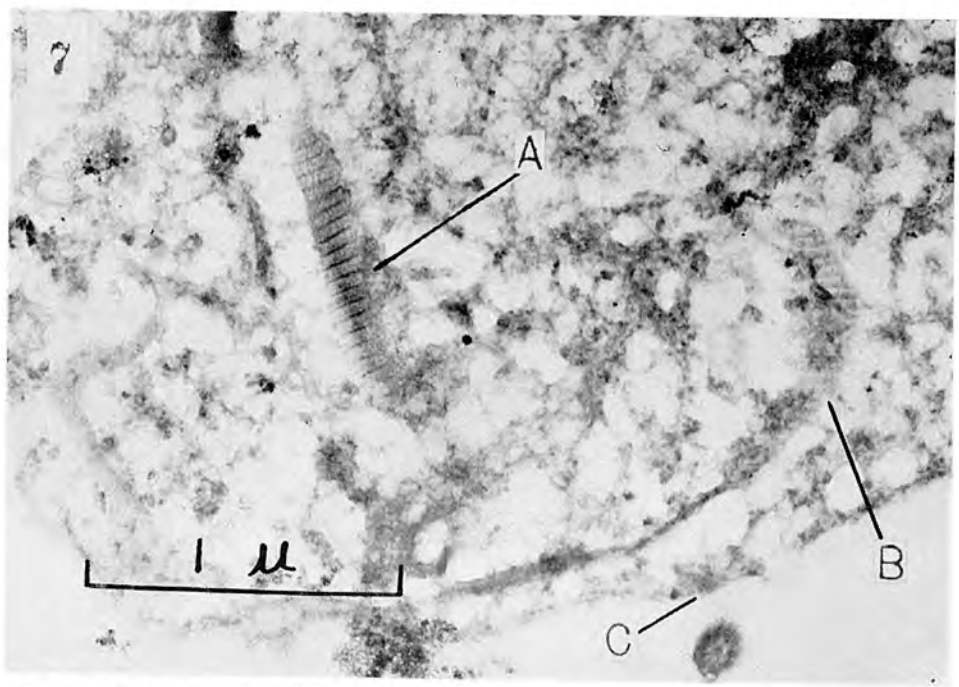


Fig. 7. An ultra-thin section of *Trichomonas vaginalis*, showing collagen fibrils.
A. and B. : collagen fibril, C. : periplast or cell wall.