ULTRA STRUCTURE OF *BALANTIDIUM*

V. ZAMAN

Department of Parasitology, Faculty of Medicine, University of Singapore.

Parasitic ciliate *Balantidium* has a very wide distribution in nature and has been reported from 50 or more species of animals (Moulton, *et al*, 1961). The parasite is also of medical importance as it is the only ciliate which infects man. This is the first publication on the ultra structure of the parasite.

In this study, a species of *Balantidium*, isolated from turtle, has been used as this species grows luxuriantly in vitro. The exact taxonomic position of the species is, however, uncertain. *Balantidium* has been described previously in tortoise by Chagas (1911) and Wenyon (1926).

MATERIAL AND METHODS

The parasite was first isolated in hsm + S (Jones, 1946) and maintained in this medium. This is a simple monophasic medium containing horse serum and yeast autolysate. The parasite was kept at room temperature (25-30° C) and subcultured at intervals of 7 days. The cultures contained a mixed bacterial flora and also *Entamoeba terrapinae*. The *Entamoeba* grew luxuriantly along with the *Balantidium* and neither parasite adversely affected each other's growth. For electron microscopy, sediment from 3—4 day-old cultures was pooled from a number of tubes and washed by centrifugation in phosphate buffered saline (pH 7.2). The washed parasites were fixed in buffered glutaraldehyde (pH 7.2) at 5° C for 12 hours and subsequently post-fixed in buffered osmium tetroxide, dehydrated in graded alcohols and embedded in Araldite. Sections were cut on a Porter-Blum microtome with glass knives and mounted on formvar coated grids. The examination was made in a Hitachi HS-8 microscope. For light microscopy, the ciliates were transferred to starch-free medium for 7-8 days and then examined under phase-contrast. Starch containing parasites were quite unsuitable for this purpose as starch grains obscured the internal structure of the parasite. The parasites were photographed using a Leitz "Orthomat" microscope, with an electronic flash.

RESULTS

The surface of the parasite is covered with thousands of cilia arranged in linear manner and the parasite has a striped appearance under phase-contrast. (Fig. 3). The lines of cilia start from the oral cavity and extend backwards over the body of the parasite to the posterior end.

In the electron microscope the surface of the parasite shows alternating ridges and furrows both in the longitudinal and cross sections (Fig. 1, 2). The ridges are an extension of the ectoplasm of the parasite and the cilia arise from the furrows (Fig. 2-4). The cell membrane or the pellicle is double-layered and covers the ridges and the furrows. The outer layer of the cell membrane is reflected to the protruding cilium at each ciliary exit (Fig. 5). The cilia on cross-section display the universal 9+2 pattern of tubular fibrils (Fig. 4). The basal region of the cilium or kinetosome is tubular and opens proximally (Fig. 5) and maintains an almost constant diameter throughout its length. In the centre of the distal part of the kinetosome is an electron dense oval body or the central corpuscle. From this central corpuscle 2 central fibrils of the cilium extend up to the base of the kinetosome. In some sections,

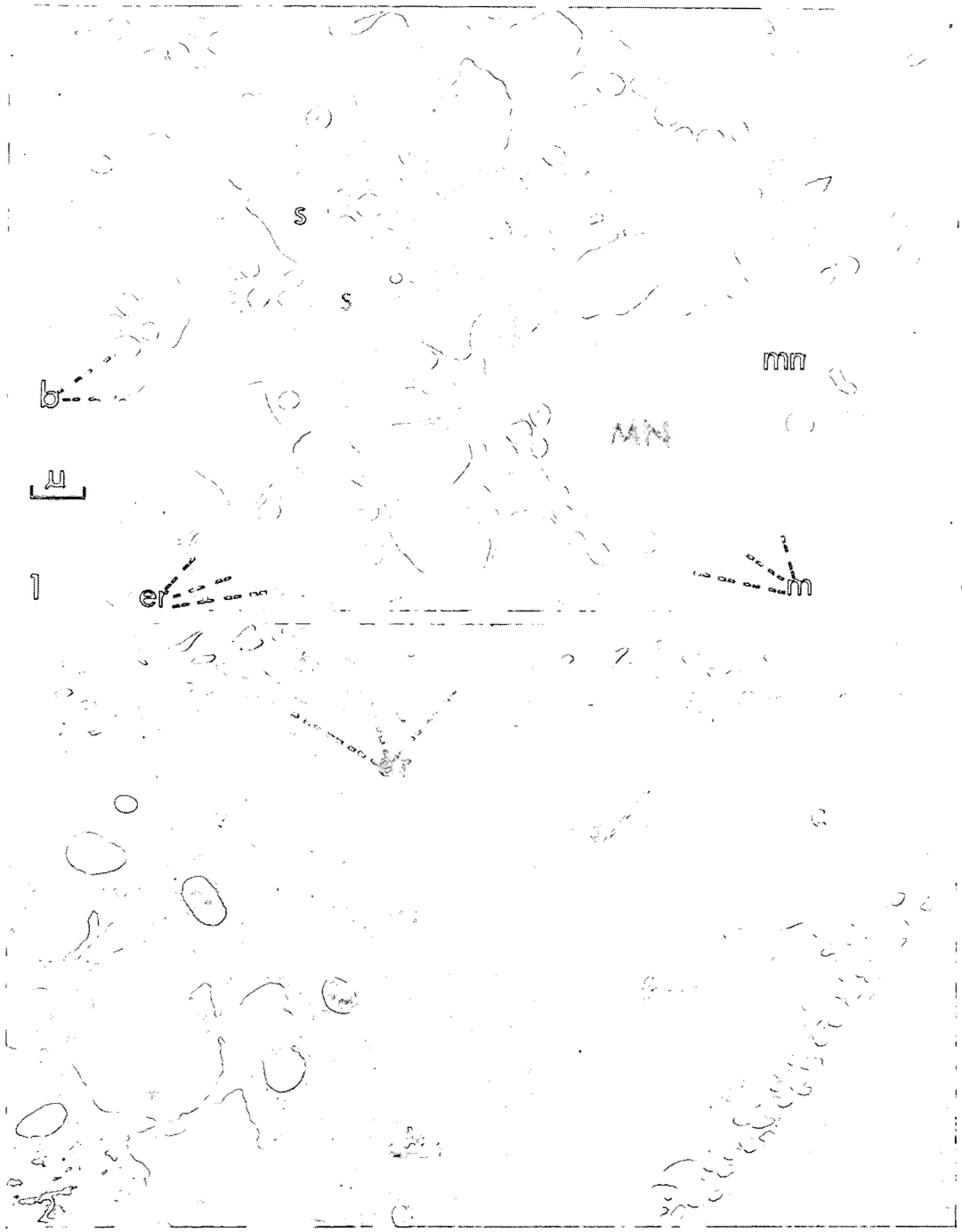
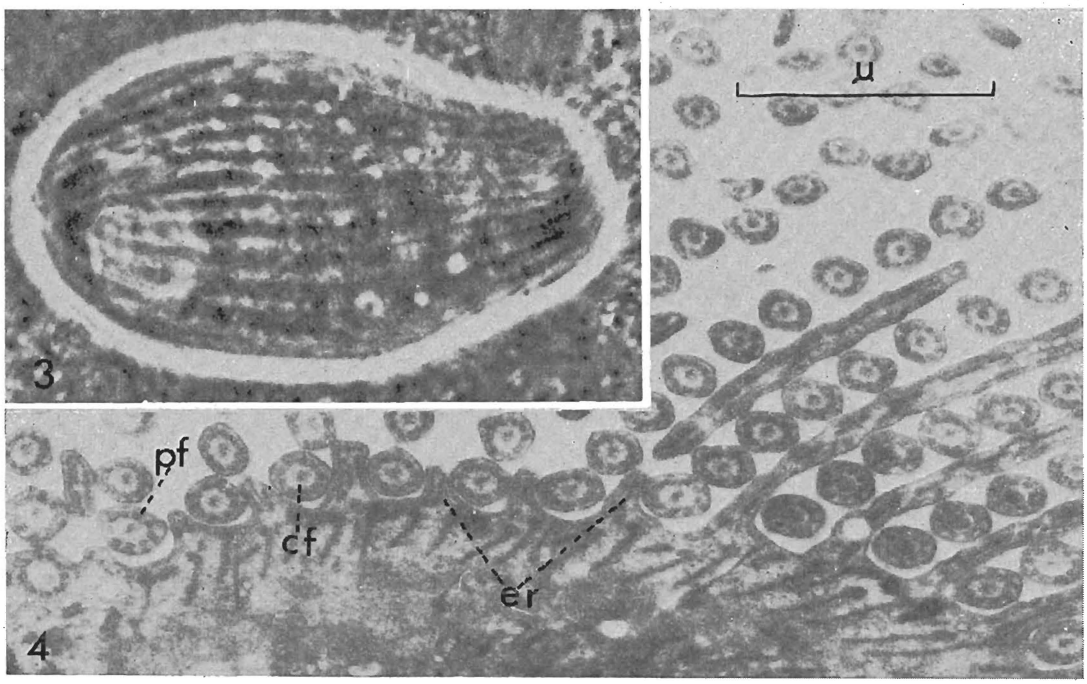


Fig. 1 — b = bacteria
er = ectoplasmic ridges
MN = macronucleus
mn = micronucleus
s = starch
m = mitochondria

Fig. 2 — er = ectoplasmic ridges
f = fibrils
c = cilia



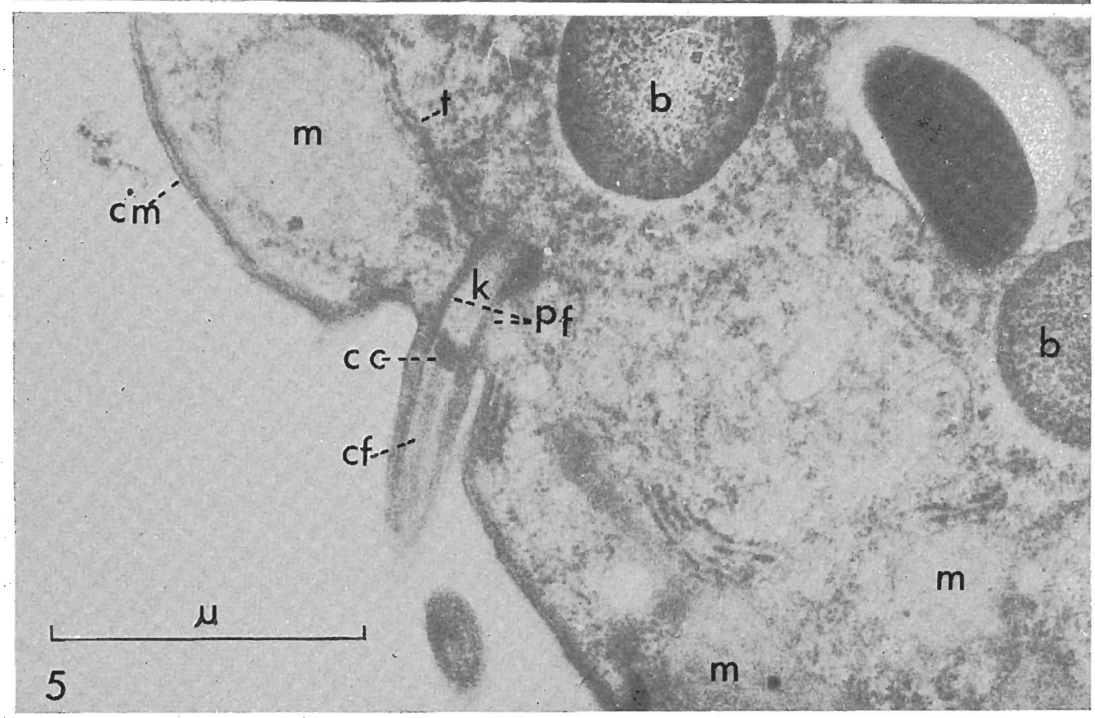
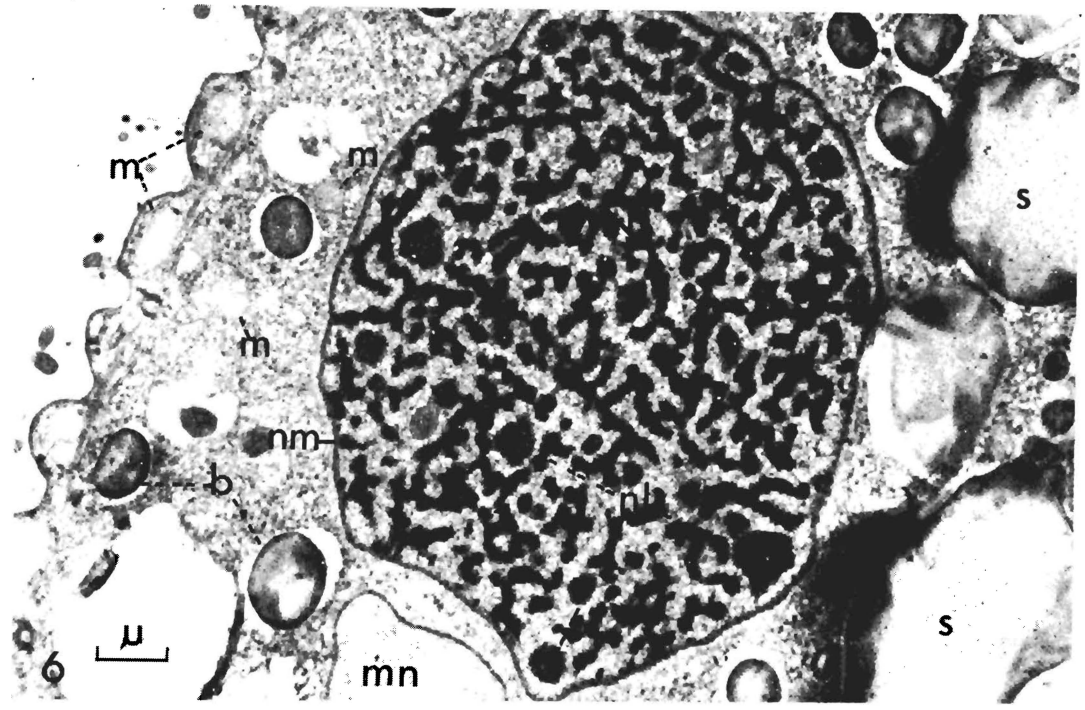
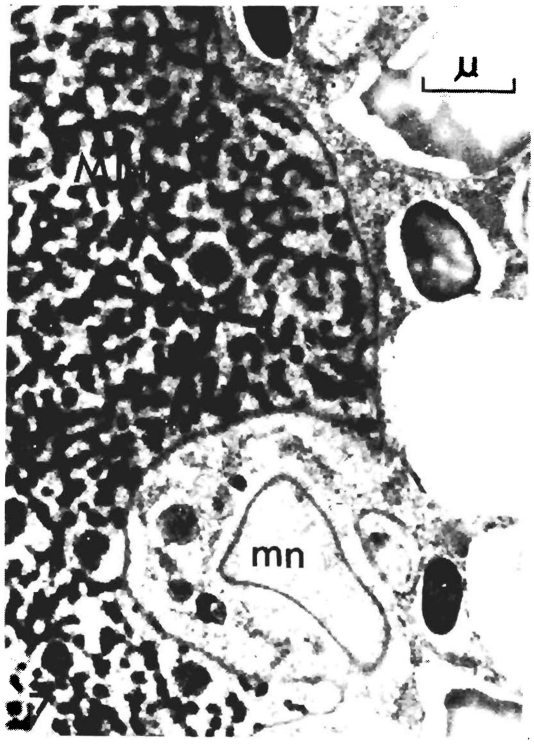


Fig. 3 — Phase-contrast. Showing the striped appearance of *Balantidium*.

Fig. 4 — er = ectoplasmic ridges
cf = central fibril
pf = peripheral fibril

Fig. 5 — cm = cell membrane
cc = central corpuscle
cf = central fibril
pf = peripheral fibril
k = kinetosome
m = mitochondria
t = tubules
b = bacteria





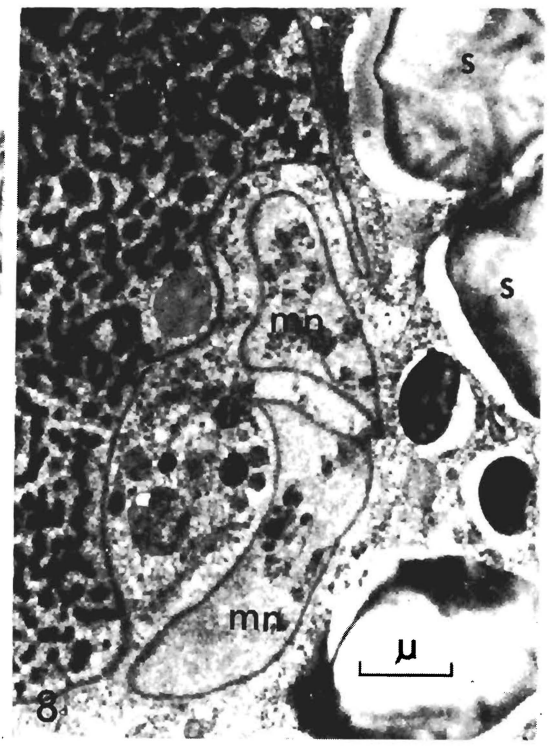


Fig. 6 — m = mitochondria
b = bacteria
mn = nuclear membrane
MN = macronucleue
nl = nucleoli
mn = micronucleus
s = starch

Fig. 7 — MN = macronucleus
mn = micronucleus

Fig. 8 — MN = macronucleus
mn = micronucleus
s = starch

fibrils are also seen arising from the base of the kinetosome and running posteriorly (Fig. 2). In the cross-section tubules are seen in the peripheral region of the parasite (Fig. 5). In the cytoplasm the starch grains are clearly visible as large crystalline bodies. The bacteria are seen as smaller dense electron bodies having a smooth outline (Fig. 1, 6). The mitochondria appear as structures with very low electron density situated mainly in the ectoplasm or the periphery of the parasite (Fig. 1, 6, 5). The mitochondrial contents are homogenous and do not display any cristae or microvilli. The most prominent structure in the cytoplasm of the parasite is the macronucleus (Fig. 1, 6). This is surrounded by a nuclear membrane which does not have pores. The nucleoplasm contains a very distinct network of electron dense chromatin. In between this network are a number of electron dense oval or slightly irregular bodies which are identified as nucleoli. The micronucleus is found close to the macronucleus in a depression on its surface (Fig. 1,6,7,8). The internal structure of the micronucleus is of low electron density and it lacks the chromatin network seen in the macronucleus. The micronucleus tends to appear triangular in cross sections (Fig. 1,7,8). In a few sections 2 micronuclei are seen lying close to each other (Fig. 8).

DISCUSSION

The study shows a number of features which are common to other free-living and parasitic ciliates. These include the ridges and furrows on the surface, the structure of the cilium consisting of 9+2 pattern of the peripheral and central fibrils, the double-layered cell membrane and the tubular structure of the kinetosome (Randall, 1956; Grimstone, 1961; Pitelka, 1963).

There are, however, some unusual features not previously recognized. The macronucleus is large and displays a very dense rope-like network of chromatin, unlike many other ciliates where the chromatin material is arranged as oval or round bodies. A number of nucleoli are seen in between this network of chromatin, a feature which had not been recognized in previous light microscopic studies. The structure of the micronucleus is also unusual as it does not contain a great deal of electron dense material usually seen in other ciliates (Roth, 1957; Seshachar, 1964; Tsujita, *et al*, 1957). In some sections 2 micronucleoli have been observed, a finding which has not been recorded in previous light microscopic studies. The structure of the mitochondria in *Balantidium* is also of particular interest as it shows no internal structures such as microvilli or cristae. As is well-known mitochondria are of great importance in the oxidative phosphorylation in aerobic cells. *Balantidium* is essentially an anaerobic cell living in the caecum and the large intestine. Even in cultures, it grows at the bottom of the tubes in relatively anaerobic conditions. All the free-living protozoa have very distinct mitochondria but in *Balantidium* the structure has probably lost its significance during the course of evolution from the free-living to parasitic life. Mitochondria with poorly developed internal structure have also been described previously in other parasitic ciliates (Rudzinska, *et al*, 1966). *Entamoeba* which has similar cultural requirements and lives in the same habitat as *Balantidium*, mitochondria are completely absent (Deutsch and Zaman, 1959; Miller, *et al*, 1961). In this respect *Balantidium* seems to occupy an intermediate position between the free-living protozoa and *Entamoeba*.

SUMMARY

The ultrastructure of *Balantidium* has been studied. It was found that the surface consists of alternating ridges and furrows. The cilia arise from the furrows and display the univer-

sal 9 + 2 pattern of internal fibrils on cross section. The cytoplasm consists of food vacuoles containing starch grains and bacteria. The mitochondria which are located mostly at the periphery are indistinct and do not have any organized internal structures. The macronucleus shows electron dense rope-like network of chromatin with interspaced nucleoli. The micronucleus is situated in a depression on the macronucleus. Occasionally two micronuclei were observed.

ACKNOWLEDGEMENTS

During the study, technical assistance was provided by Mr. Loh Ah Keong. The ultramicrotome was obtained through the China Medical Board of New York.

REFERENCES

CHAGAS, C., (1911). *Mem. Inst. O. Cruz.*, 3 : 136.

DEUTSCH, K. and ZAMAN, V., (1959). *Exptl. Cell Res.*, 17:310.

GRIMSTONE, A.V., (1961). *Biol. Rev.*, 36:97.

JONES, W.R., (1946). *Ann. Trop. Med.Parasit.*, 40:130.

MILLER, J.H., SWARTZWELDER, J.C. and DEAS, J.E., (1961). *J. Parasit.*, 47:577.

MOULTON, J.E., HEUSCHELE, W.P. and SHERIDAN, B.W., (1961). *Cornell Vet.*, 51:350.

PITELKA, D.R., (1963). Electron-microscopic structure of Protozoa. Pergamon Press. London.

RANDALL, J.T., (1956). *Nature*, 178:9.

ROTH, L.E., (1957). *Biophys. Biochem. Cytol.*, 3:985.

RUDZINSKA, M.A., JACKSON, J.G. and TUFFRAU, M., (1966). *J. Protozool.*, 13:440.

SESHACHAR, B.R., (1964). *J. Protozool.*, 11:402.

TSUJITA, M., WATABE, K. and TSUDA, S., (1957). *Cytologia.*, 22:322.

WENYON, C.M., (1926). *Protozoology*. Bailliere, Tindall. and Cox. London.