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THE ULTRASTRUCTURE OF THE VILLI-LIKE STRUCTURE IN *TRYPANOSOMA CRUZI*¹

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SUMMARY Electron micrographs of *Trypanosoma cruzi* which have been incubated for 7 days with HeLa cells, show intracellular trypanosomes, with the *crithidia* form, which come out from the host cell. On some of these trypanosomes an unknown structure is seen covering the surface membrane. This structure covers the entire body and flagellum, and consists of a thick compact layer of long, slender, hollow tubules. They are arranged serially but are irregular in size, and some appear like the fuzzy layer described by Sanabria (1968, 1971). Each tubule has a central core. This appearance is very different from that of the outer thick layer described by Kubo (1968), the "surface coat" reported by Vickerman (1969) or the "pellicula" observed by Fuge (1968), so the whole structure is here provisionally termed a "villi-like structure".

INTRODUCTION

Pathogenic trypanosome, have an additional layer outside the periplast or cell membrane. This layer is approximately uniform in thickness and appearance and covers the entire body and flagellum. Kubo (1968) described this layer on *Trypanosoma lewisi* and *T. gambiense* as the outer thick layer. Sanabria (1968 and 1969) named it a "fuzzy layer" in *T. cruzi* found in mouse brain and liver. Fuge (1968) called it a "pellicula" in *T. brucei* and *T. gambiense*. It has also been called a "surface coat" by Vickerman (1969) who studied its

structure in many species of bloodstream trypanosomes, including *T. brucei*, *T. rhodesiense*, *T. congolense*, *T. vivax*, *T. equinum*, *T. equiperdum* and *T. evansi*. Thus the thick layer outside the periplast in different species has been variously named but always has a rather similar appearance, being rather thin and uniform in thickness with either a compact or loose structure. Inoki et al. (1971) observed a different type from that described before and called it a "villi-like structure" around the agglomerated *crithidia* form of *T. cruzi* in tissue culture.

Inoki et al. (1971) also reported that this structure appeared when *T. cruzi* was inoculated into a culture of HeLa cells and seemed to disappear just before the parasite penetrated

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into the host cell. Sooksri and Inoki (1972) also observed the villi-like structure in a culture of *T. cruzi* which had been incubated for 7 days with HeLa cells. This paper describes the ultrastructure of the villi-like structure in more detail.

MATERIALS AND METHODS

The materials and methods used were described previously (Sooksri and Inoki, 1972).

RESULTS

The villi-like structure appeared on some *T. cruzi* which had come out from HeLa cells 7 days after inoculation. This structure covers the entire body and flagellum of the *crithidia* form (Fig. 1, 3, and 4) or probably the *leishmania* form (Fig. 2) but it has not been found on the *trypanosoma* form. Apparently, different structures combine to form the villi-like structure. The most easily seen component of the structure is the group of elongated tubules, with an average diameter of 716Å (525Å–870Å), which are arranged in parallel in some areas (arrow A in Figs. 1, 2, 3, and 4) and irregular in other areas (arrow B in Fig. 2). Some of the tubules have free ends while others have blind ends. Each tubule is seen as two dark lines with a less electron dense area

in between and an elongated line as the central core. Hollow, round or oval granules of 525–1050Å diameter, which appear as a dark circle surrounding a less electron dense area are seen with the tubules. These two structures mix together forming what Sanabria (1968, 1971) called the “fuzzy layer”. This fuzzy mass in Fig. 2 and 4 seems to be regular in thickness and is rather loose in Fig. 2 and compact in Fig. 4. Trypanosomes with the villi-like structure show no evidence of abnormal organelles but are apparently agglutinated together. Some of them even show the beginning of cell division (Fig. 1).

DISCUSSION

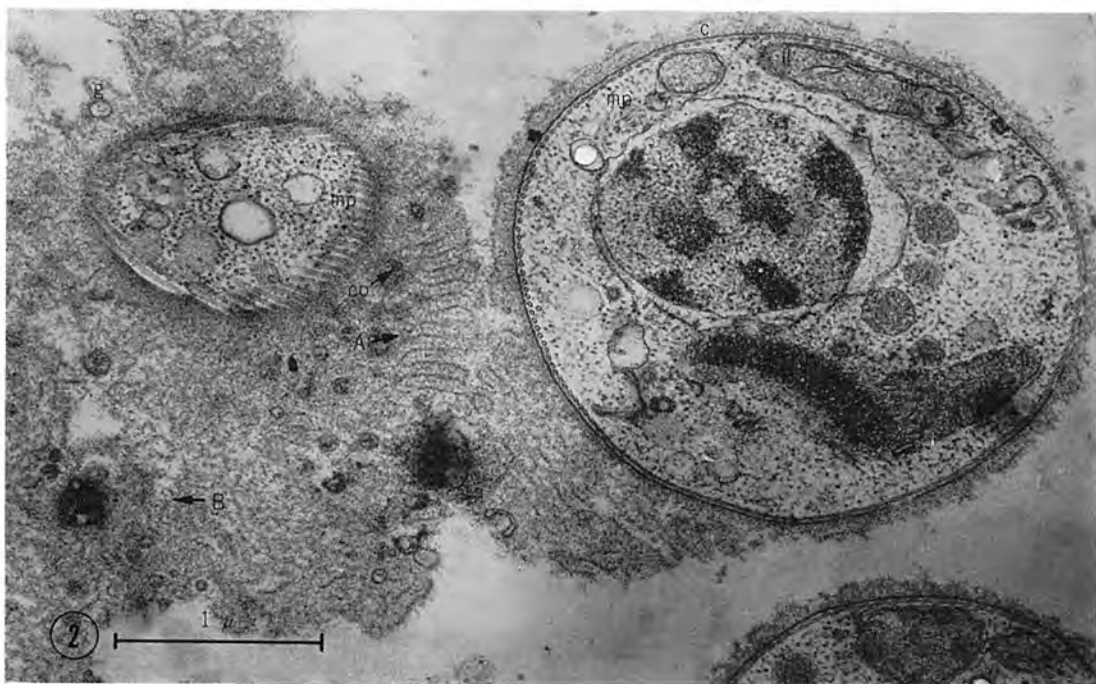
Normally, the cell membrane of *T. cruzi* in the culture medium (*crithidia* form) is smooth and as thick as that of the *trypanosoma* form in the blood. A fuzzy mass covering the cell membrane has been seen in micrographs of the bloodstream form of *T. cruzi* (Inoki et al., 1971) and other bloodstream trypanosomes (Vickerman, 1969). Seven days after inoculation of the parasite into a HeLa cell culture, a villi-like structure appeared on some parasites. These parasites appeared to be the *crithidia* form, which seems to come out from the host cell (Sooksri and Inoki, 1972). In Fig. 3,



FIGURE 1. The *crithidia* form of *Trypanosoma cruzi* surrounded by a villi-like structure. Groups of tubules each with a central core are arranged serially (arrows A), with hollow granules in a fuzzy mass. Two basal bodies are seen.



FIGURE 2. Trypanosomes surrounded by a villi-like structure. One is covered mainly with a loose, fuzzy mass while another is mainly surrounded by tubules arranged disorderly. Arrow A indicates an area of well-arranged tubules while arrow B indicates an area of irregular arrangement.



the mitochondria of a degenerated HeLa cell can still be seen near a *crithidia* form of parasite with the villi-like structure. Other micrographs showed the villi-like structure around intracellular parasites trapped in a degenerated HeLa cell, the membrane of which could still be seen. The villi-like structure has not yet been found on intracellular *T. cruzi* in active HeLa cells (Sooksri and Inoki, 1972). This suggests that the villi-like structure is produced by the trypanosome itself to protect it from autolytic enzymes liberated from dead HeLa cells. Vickerman (1969) also thought that the surface coat is a cellular secretion from the parasite.

The authors (Inoki et al., 1971) reported that the villi-like structure appeared when the culture form of *T. cruzi* was inoculated into HeLa cells. This is supported by the finding of Sooksri and Inoki (1972) that there is no villi-like structure around *T. cruzi* (*trypanosoma* form) which are penetrating the cell but it is uncertain whether this structure is formed on trypanosomes before penetration. Sooksri and Inoki (1972) observed an unknown structure a *trypanosoma* form of *T. cruzi* at about the time of its penetration into a HeLa cell membrane. This unknown structure

did not resemble the villi-like structure, fuzzy layer (Sanabria 1968, 1971), or the surface coat (Vickerman, 1969) but was quite similar to the finger-like structure (microvilli) of a HeLa cell. The longitudinal tubular arrangement of the villi-like structure seems very like to the so-called myelin structure of the nerve cell, but the spacings of myelin membranes is much closer (~ 120 Å) than that of this structure (525–870 Å). Moreover many round, oval, hollow granules, which were probably cross sections of tubular structures, were seen in the villi-like structure but these have not been found in the myelin structure.

From this study, the villi-like structure seems to appear on intracellular *T. cruzi* which are either trapped in degenerating HeLa cells or have already come out from degenerated cells. The structure is probably formed by the trypanosome cell itself.

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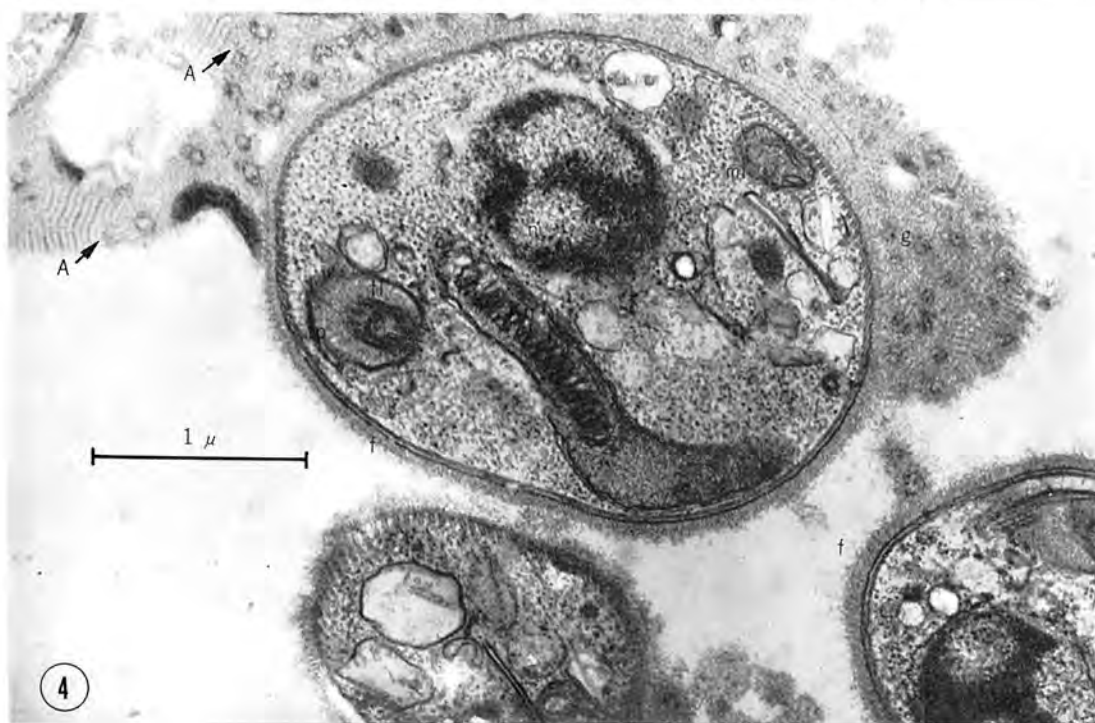
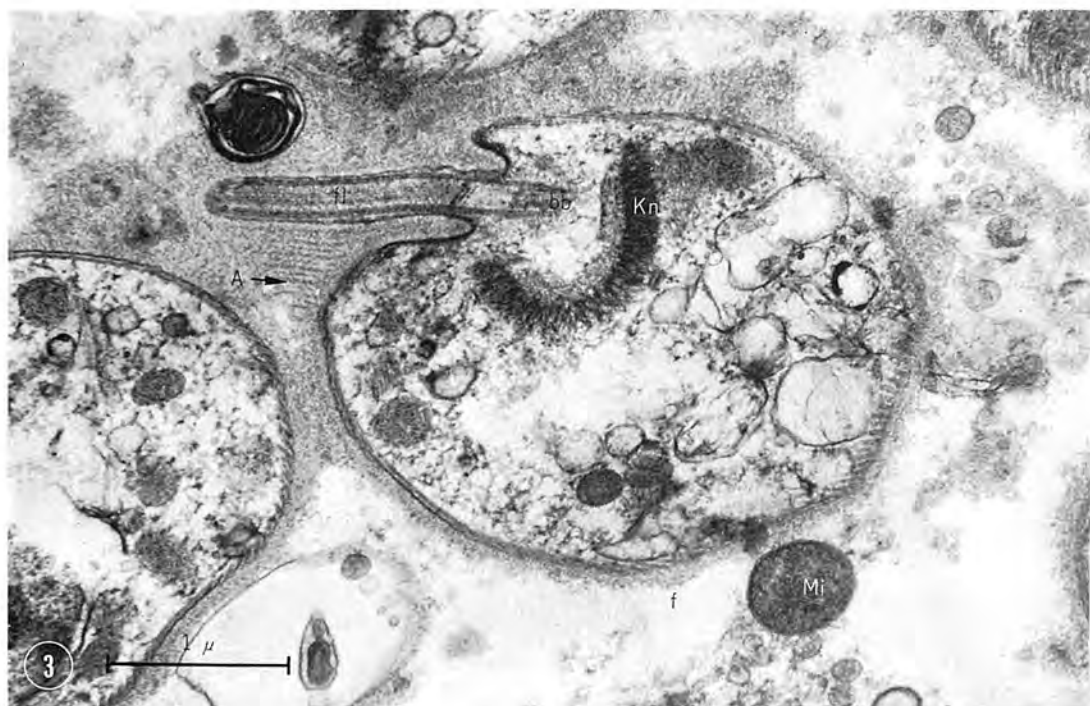
FIGURE 3. A *crithidia* form of *T. cruzi* surrounded by a villi-like structure. Well-arranged tubules (arrow A) run paralleled with the flagellum. The mitochondria of a degenerating HeLa cell is seen near the trypanosoma cell.



FIGURE 4. A trypanosome with coiled kinetocuclear fibers surrounded by a dense, compact fuzzy mass of the villi-like structure. The cross section of the flagellum in the flagellar pocket is seen. Arrow A shows an area of well-arranged tubules.

Abbreviations in figure

bb, basal body (blepharoplast); c, cell membrane; co, central core; em, envelope membrane; f, fuzzy mass; fl, flagellum; fp, flagellar pocket; g, granule; kn, kinetoculus; Mi, mitochondria of HeLa cell; mi, mitochondria of *T. cruzi*; mp, periplastic microtubules; n, nucleus; v, villi-like structure.



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