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FINE STRUCTURE OF THE CONOID OF *TOXOPLASMA GONDII*

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SUMMARY

Using the technique of double fixation and thin sectioning and the technique of negative staining it has been observed that the conoid of *Toxoplasma gondii* has 3 ring-like structures. From the lowest of the rings (the polar ring) 22 microtubules run to the posterior region of the parasite where they end freely. Others run to the distal pole of the conoid in two different directions crossing each other. In the interior of the conoid the end portions of the rhoptries are observed. It is suggested that the microtubules in the wall of the conoid when contracting make the conoid act as sphincter that compresses the end portion of the rhoptries (toxonemes) liberating the proteolytic enzymes supposedly present in this organelles.

INTRODUCTION

The ultra-structure of *Toxoplasma gondii* has been studied in parasites deriving from infected animals as well as from tissue cultures [6, 7, 9, 15, 18, 24, 28]. The results obtained show that the parasite has all the structures which are essential to other protozoan or to metazoan cells. Besides these structures it has the conoid and the rhoptries or toxonemes [11] which are typical for *Toxoplasma gondii* and the whole group of related parasites. In spite of possessing all the essential structures, *Toxoplasma gondii* is an obligate intracellular parasite. In tissue cultures, when found in the intercellular spaces, just after having left a ruptured cell, it can be seen that the parasites move around with undulating, apparently contracting movements and enter the tissue cells by their own activity [17]. It is supposed that in order to enter a new cell the parasite uses certain proteolytic enzymes which would exist in the rhoptries and which would act on the membrane and on the cytoplasm of the future host cell [6, 24, 28].

Under the light microscope *Toxoplasma gondii* shows no structures, such as cilliae or flagellae, which could be made responsible for the movements of the parasites. Ultrastructural studies using double fixation with glutaraldehyde and osmium tetroxide and thin sectioning revealed the existence of tubular structures, especially in the anterior region of the parasites [6, 24]. With the technique of negative staining it was shown that *Toxoplasma gondii* has a system of 22 microtubules [3]. They originate in the anterior region of the parasite, running from there to the posterior region where they end freely. They are of different length (4-8 μm), show a width of 180-230 Å and a cross banding with a periodicity of about 78 Å. Their role has been discussed and it was suggested that they are contractile structures which could be responsible for the motility of the parasite. However, the possibility that they also play a role in sustaining the form of the parasite could not be excluded. Similar results have been obtained recently by VIVIER & PETITPREZ [28] with the same technique.

In the present work these structures were studied in more detail in order to find out about the origin of the tubules in the anterior part of the parasite.

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MATERIAL AND METHODS

The parasites were obtained from mice infected with *Toxoplasma gondii*. Small pieces of infected liver or spleen tissue from the infected mice were put in contact with pieces of normal embryonic chicken heart and cultured in a plasma clot of chick plasma and chick embryo extract with the hanging drop method which is used for the culture of protozoa in tissue cultures in this Laboratory [13]. When the cultures showed a rich infection under the light microscope, they were fixed in toto with glutaraldehyde 2.5% for 30 minutes and postfixed with 1% osmium tetroxide for one hour in the refrigerator. For dilutions of both fixatives the same Tyrode solution was used which had been employed for the culturing of the tissue cells. Both fixatives were buffered to a pH of about 6.8-7.2. Dehydration was done in acetone. After one night in pure acetone the interesting regions of the culture were cut out with a cataract knife, and dehydration of the small pieces was continued. They were stained with Uranyl acetate (0.3% in pure acetone) for one night. After repeated washings in pure acetone they were embedded in Epon resin shell. Sectioning was done with a Porter-Blum MT2-B ultramicrotome. The sections were stained with lead citrate [19] and the observations were done with an electron microscope AEI, Em6-B.

The technique of negative staining was the same used in a previous work [3].

RESULTS

A) Technique of double fixation and thin sectioning — The anterior region (apical complex) of *Toxoplasma gondii* is covered by the external membrane only. The inner membrane is not continuous, but ends just below the conoid in a thickened structure which forms the polar ring. The conoid has a cylindrical form in which 3 ring-like structures can be distinguished (Figs. 7, 9). Microtubules are seen originating apparently in the lowest of the rings. Sometimes in favorable sections it can be seen that they are arranged in a manner to form a lattice-like structure. Some microtubules (1 to 3) run straight over the whole structure, surpassing even the polar end of the conoid (Fig. 7). In cross sections the conoid appears as a circular structure with a diameter of about 3,000 Å in the center of which the small endings of the rhoptries can be seen, also in cross section (Fig. 8).

Microtubules are found easily in the anterior region of the parasite where sometimes they are cut transversally, in which case they show that they are hollow structures with a diameter of about 260 Å (Fig. 6). They are also found at the nuclear level immediatelly below the inner membrane.

B) Technique of negative staining — The results obtained with this technique show the presence of 22 microtubules which run from the apical complex to the posterior part of the parasite, ending before they reach the posterior pole (Fig. 1).

According to the position in which the parasite is observed, the aspect of the anterior region is different. When it is seen in front view from above, a position which is comparable to a transversal section through this region, it is seen that the 22 microtubules in the polar ring are regulary spaced, with a distance of about 700 Å between them. When this region is observed laterally (Figs. 3, 4 and 5), it is seen that in the conoid exists a complex arrangement of microtubules. From the analysis of the micrographs obtained it is seen that beginning at the polar ring the microtubules run to the distal end of the conoid in parallel array but bend right and left and cross each other to form a lattice-like structure. They meet a second ring and beyond this a third one (Fig. 5). The wall of this latter seems to be composed by microtubules in parallel array and in intimate contact, as if compressed (Figs. 4, 5).

The microtubules in the wall of the conoid have a width of 280 Å. The periodicity here does not show up very well. Also with this technique it can be seen that in many preparations 1-3 microtubules run straight over the conoid surpassing even the distal pole (Figs. 3, 4). Here they show the characteristic cross banding especially well (Fig. 3). These microtubules are continous with those of the posterior body part of the parasite (Fig. 4).

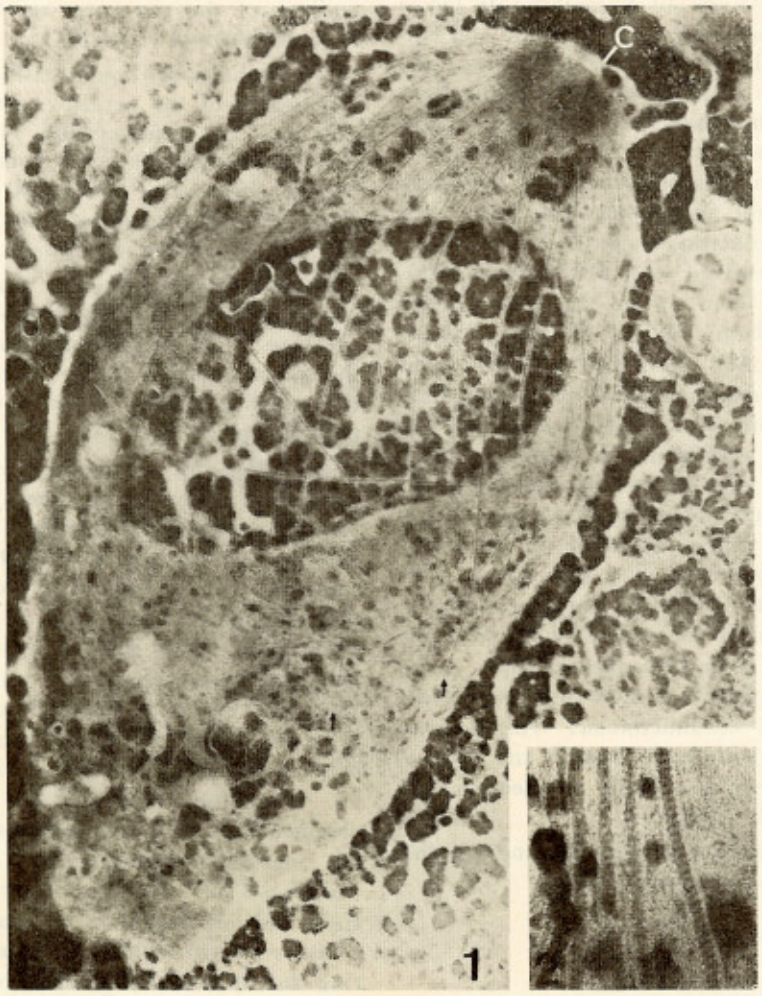

Fig. 1 — Free extracellular parasite showing microtubules running from the conoid (C) to the posterior half of the body ending before they reach the posterior pole (→). 30,000 ×. Negative staining.

Fig. 2 — High magnification of microtubules showing periodic cross banding. 200,000 ×. Negative staining.

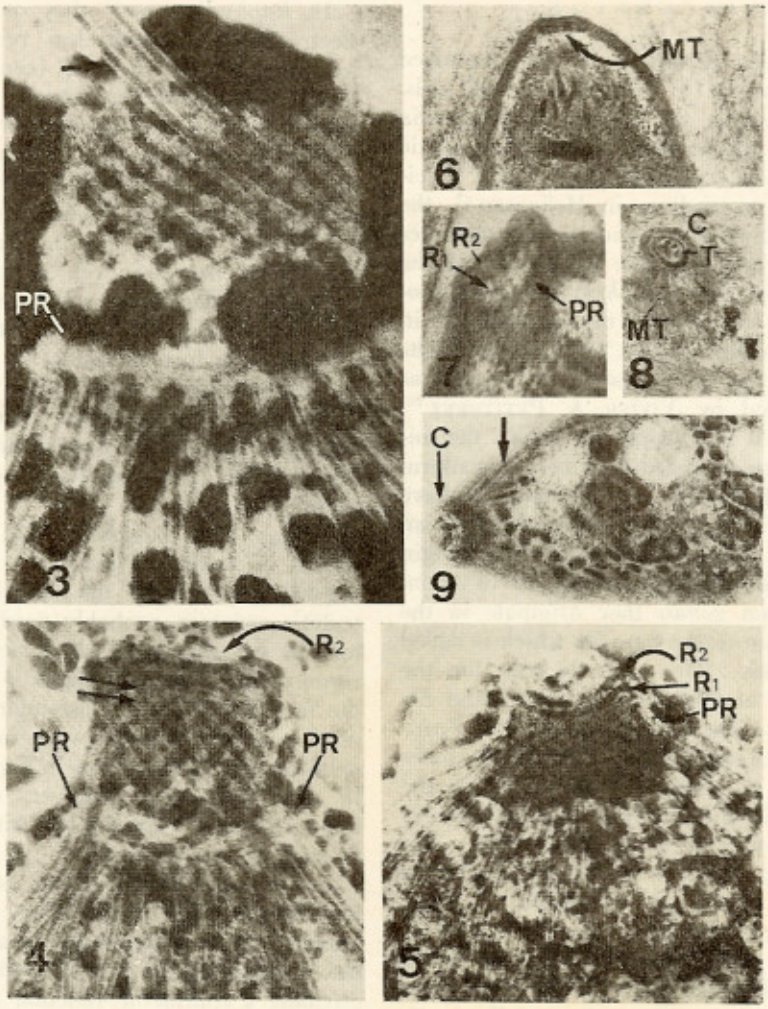

Fig. 3 — Apical complex of parasite showing lattice-like structure in conoid. Microtubules originating in polar ring (PR) run to posterior as well as to anterior pole of parasite. 2 microtubules (→) running over conoid ending beyond it, showing very clear cross striations. 140,000 ×. Fig. 4 — Apical complex of parasite showing lattice-like structure in the conoid. Polar ring (PR) is seen giving origin to microtubules. Another ring formation is seen at the end pole (R2) where microtubules seem to be compressed in intimate contact with each other. 80,000 ×. Fig. 5 — Apical complex of parasite showing lattice-like structure in the conoid. Three ring formations are observed: PR (polar ring), R1 and R2. In the last ring (R2) microtubules seem to be compressed in intimate contact with each other. 60,000 ×. Figs. 6-9 — Are from thin sections of infected tissue cultures. Fig. 6 — Extracellular parasite showing microtubules (MT) in cross section in the apical complex. 40,000 ×. Fig. 7 — Oblique section of conoid showing lattice-like structure in its wall. Three rings (PR, R1 and R2) are also observed. 60,000 ×. Fig. 8 — Extracellular parasite showing conoid (C) in cross section in the interior of which rhoptries (T) are seen. Microtubules (MT) apparently beginning in the polar ring. 25,000 ×. Fig. 9 — Extracellular parasite showing three ring-like structures in its conoid (C). Microtubules running from polar ring to posterior part of body (→). 30,000 ×. Figs. 3-5 — Negative staining.

DISCUSSION

Microtubules arranged in an organized pattern occur in a wide variety of cells. Examples include the mitotic spindle, cilia and flagella, the axostyle of certain parasitic flagellates [8], the cytopharingeal basket of ciliates [26], the axopodium in Heliozoan [21], the tentacles of Suctoria [22] and the microtubules which have origin in the centroplast of the centrohelidian, *Raphidiophrys* [25]. Many protozoan have also a row of microtubules in the subpellicular region [5, 14]. The results obtained during this investigation, as well as those obtained by VIVIER & PETITPREZ [28], show that also in *Toxoplasma gondii* the microtubules are arranged in a certain pattern.

In spite of the fact that microtubules have been found in a great number of protozoan or metazoan cells, the point of their origin in the cell is not the same in all of them. In *Toxoplasma gondii* they originate in the anterior region of the parasite which is called apical complex. The same is true for the microtubules of *Plasmodium fallax* [1] and *Eimeria* [4, 20, 23]. The results obtained in *Toxoplasma gondii* suggest that the microtubules originate in the first of the 3 rings, in the polar ring. In *Eimeria* they also have origin in an electron-dense ring-like structure. The results in *Plasmodium fallax* are similar to those found in *Toxoplasma gondii*.

The lattice-structure seen in the conoid of *Toxoplasma gondii* is similar to that found in the conoid of *Eimeria* [4, 20]. Such structure was not found in *Plasmodium fallax* [1]. It might be due to collapsing of the specimen during its preparation, as has been suggested by Roberts and Hammond for *Eimeria* [20], or to a real crossing of the tubules in the wall of the conoid. This last hypothesis is supported by the fact that the lattice structure has been observed also in some of the thin sections of the same material [20, 24, 28].

The significance of the few (1 to 3) microtubules which run over the whole conoid, ultrapassing the end pole of this structure, is not clear. They have also been found in *Eimeria* [4, 20].

Biosynthesis of microtubules is a central problem in cell biology today [2, 10, 27]. In cilliae and flagellae they are formed by the basal body. The centriole forms the microtubules of the mitotic spindle.

In *Raphydiophrys* [25] they originate in an electron-dense, structureless region, situated in the center of the cell (centroplast). In *Toxoplasma gondii* the anterior region of the parasite shows an electron dense ring-like structure, the polar ring, which seems to be the point of their origin. Also centriole-like structures have been found in the region of the nucleus, connected with microtubules. It is however not clear yet, whether these represent spindle fibers or the new microtubules which will compose part of the new parasite which is formed inside the mother form by endodiogeny (unpublished data). Centrioles have recently been demonstrated in *Toxoplasma gondii* [28] without however, showing spindle microtubules associated with them.

It has been suggested that *Toxoplasma gondii*, in order to penetrate into a tissue cell liberates through the conoid certain proteolytic enzymes, probably produced or existent in the rhoptries, which act on the cell membrane [6]. This hypothesis is sustained by the fact that hyaluronidase added to the suspension of the parasites when infecting tissue culture cells gives a much higher infection in the cultures [12]. However, very little is known about the mechanism with which these enzymes are liberated.

As we saw in thin sections, as well as with negative staining of the whole parasites, the terminal parts of the rhoptries are present in the interior of the conoid cylinder. If we attribute a contractile function to the microtubules the images obtained, especially those obtained with the technique of negative staining, suggest that the conoid acts like a sphincter. Its diameter would be controlled by the contraction of the microtubules which form its wall. When in the state of contraction it is possible that they compress the final portions of the rhoptries allowing in this way the liberation of the proteolytic enzymes supposedly existing in these organelles.

RESUMO

Ultra-estrutura do conóide do Toxoplasma gondii

Utilizando-se a técnica de dupla fixação e cortes ultrafinos e a técnica de coloração negativa verificou-se que o conóide do *Toxo-*

plasma gondii apresenta 3 anéis. O mais inferior corresponde ao anel polar. Vinte e dois microtúbulos partem do anel polar para a região posterior do parasito onde terminam livremente. Outros partem para a área mais distal do conóide, em duas direções de modo que a parede do conóide apresenta aspecto quadriculado. No interior do conóide encontram-se as porções terminais das roptrias.

Sugere-se que os microtúbulos na parede do conóide se contraem e comprimem as porções finais das roptrias permitindo a liberação das enzimas proteolíticas supostamente existentes nessas organelas.

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