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TOXOPLASMA GONDII IN TISSUE CULTURES
A MICROCINEMATOGRAPHIC STUDY IN PHASE CONTRAST

(RESEARCH NOTE)

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SUMMARY

Life cycle of *Toxoplasma gondii* maintained in tissue cultures has been recorded in a motion picture taken in phase contrast. The film shows all the development steps of the parasite in the cell, from active penetration in fibroblasts after rupture of the final phase of rosettes, division in two, four, eight and so on until new rupture and liberation of parasites, with intermediate images of mitotic divisions of parasitized cells. Macrophages filled with a great number of parasites retracted but did not rupture, possibly giving the impression of pseudo-cysts described by the pathologists in fixed and stained *Toxoplasma* material.

INTRODUCTION

The life cycle of *Toxoplasma gondii* maintained in tissue cultures has been recorded in a motion picture taken in phase contrast. The parasite was derived from mice infected with *Toxoplasma*. Small pieces of the infected tissues, liver and spleen, were cultured in contact with normal cells of the heart muscle of the chick embryo (3), (1), and also with blood macrophages of the chick. The cells were grown in a clot consisting of chick plasma and chick embryo extract. In order to obtain optimal photographic conditions, the plasma was diluted with Tyrode solution (1:2) and the clot spread out to a thin layer. The recording was done with a microcinematographic installation "Zeiss", using a 100x objective. The speed was 30-15 pictures per minute which gave a final acceleration of about 30-45 times. The photographic film used was Agfa Isopan ISS reversible (16 mm).

In the beginning the film shows a cell, filled with parasites, which soon ruptures. The liberated parasites are seen to move with contractile, undulating movements towards surrounding fibroblasts which they enter by their own activity.

The next sequence of the film shows a cell with one recently entered parasite surrounded by a vacuole (Fig. 1). Such vacuoles can be seen a few minutes after the entrance of a parasite into a cell. The same parasite is seen to divide into two daughter parasites (Fig. 1a). Before the parasites divide they become very large. Only binary division has been observed. The process of endodyogeny did not show up "*in vivo*" with the magnification we used. Two lighter areas, considered by us to be the nuclei, were always seen in the middle of the body of large parasites before they divided, always into two new, smaller forms. It was often observ-

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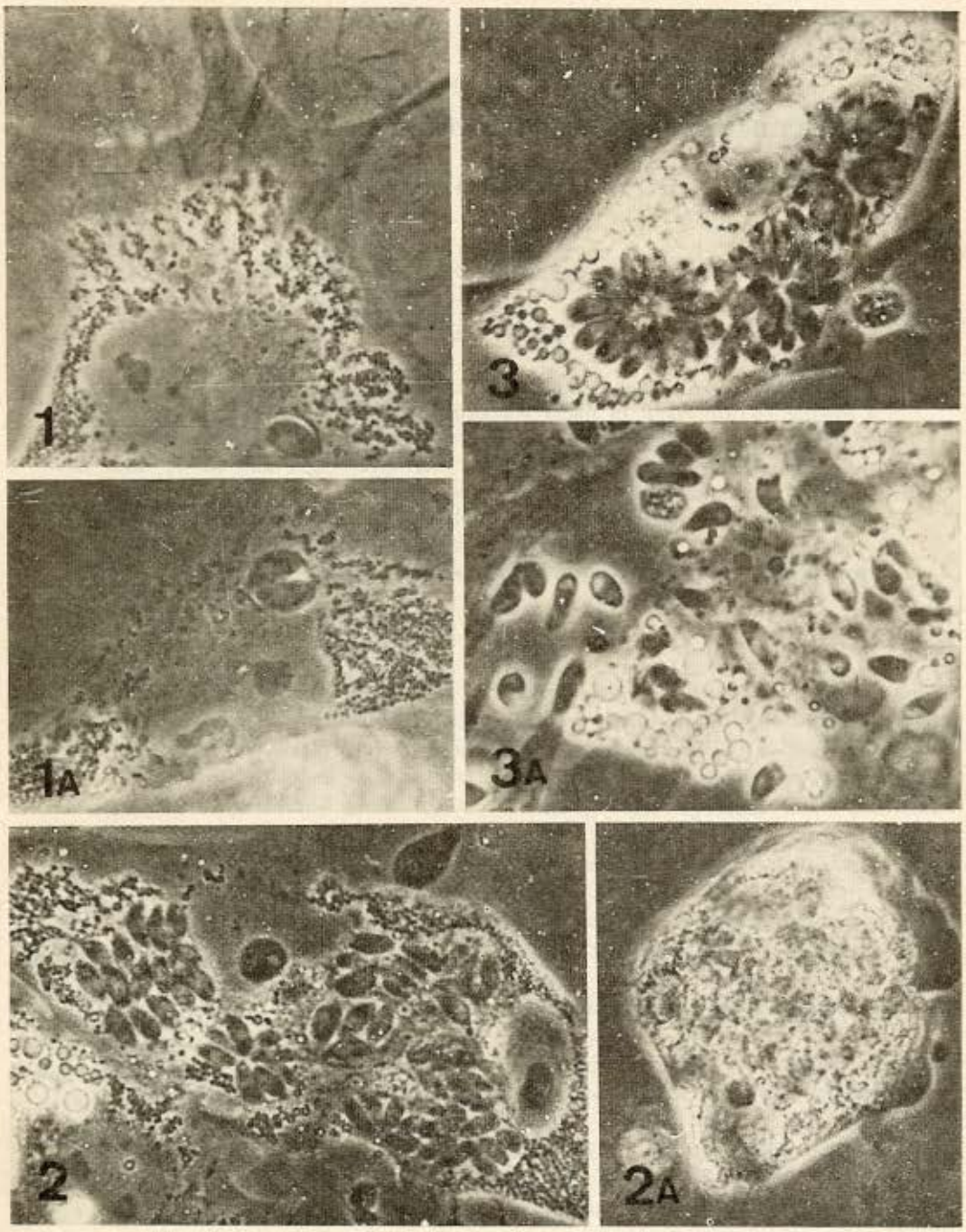

Fig. 1 — Cell from chick embryo heart muscle with one recently entered parasite. Beginning of vacuole formation.
Fig. 1a — Same cell after division of parasite.
Fig. 2 — Bi-nucleate blood macrophage filled with parasites.
Fig. 2a — Same cell retracted.
Fig. 3 — Cell from chick embryo heart muscle with parasites in rosette formation.
Fig. 3a — Same cell ruptured, liberating parasites.

Fig. 1-3a — Final magnification. 1,000 × (objective 100 ×) Zoom-1.

ed that the two daughter parasites had not divided completely, but were bound together at their posterior pole, where a residual piece of cytoplasm united them.

The following sequences show repeated divisions of two parasites into four, four into eight, eight into sixteen, which lead to the formation of the well known rosettes.

In the beginning the cells seem not to suffer from the parasites. They migrate and divide mitotically, as described earlier [2]. The mitotic process, filmed of several cells, appears normal. The parasites in the resulting daughter cells are distributed at random. In one cell, however, which contained a great number of parasites, the mitotic process was delayed very much and resulted in a bi-nucleate cell.

In another sequence a bi-nucleate macrophage of the blood is shown, filled with a great number of parasites (Fig. 2). This cell, in spite of the heavy infection, moved around normally until eventually it retracted. It was then observed for many hours, but did not rupture (Fig. 2a). It seems possible to us that such cells, when found in fixed and stained histological preparations, may give the impression of "cysts" or "pseudocysts", always described by the pathologist in *Toxoplasma* material.

The rupture of some other cells is then recorded, followed by the exit of the liberated parasites (Figs. 3 and 3a) and their entrance into a new host. It was seen that several parasites may enter the same cell, and that there are always various parasites which soon stop moving and degenerate rather rapidly.

RESUMO

Toxoplasma gondii *em culturas de tecido. Estudo microcinematográfico em contraste de fase*

O desenvolvimento intracelular do *Toxoplasma gondii* mantido em culturas de tecido foi observado e filmado em contraste de fase. Todas as fases do ciclo são apresentadas desde a penetração ativa do parasito em fibroblastos após ruptura de rosáceas como a divisão binária em dois, quatro, oito etc. até formação de nova rosácea que se rompe e libera novamente parasitos que irão penetrar em outras células completando o ciclo.

Imagens intermediárias mostram divisões mitóticas de células parasitadas e macrófagos de sangue intensamente parasitados que se retraem mas não rompem durante a observação, sugerindo os pseudocistos descritos pelos patologistas em tecidos fixados e corados.

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