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ULTRASTRUCTURE OF TAENIA SAGINATA ONCOSPHERES CULTIVATED IN ARTIFICIAL MEDIA

J. SCHRAMLOVÁ, K. BLAŽEK, Z. MARHOUL and A. SINGHVI

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague

Abstract. Oncospheres of *Taenia saginata* cultivated in Leibovitz's medium on a cell monolayer from calf kidney developed up to 10th day of in vitro cultivation. Fine and often branched microvilli were observed on their surface. On day 5 of cultivation, the muscle system of hooks turned to a granular matter and on day 7 neither hooks nor their muscle system were found. The tegumental cytoplasm was not formed in the period of 7—60 days of cultivation. On days 7 and 10 of cultivation, a contact of oncospheres with the cells of monolayer was visible. On day 10 of cultivation, an extrusion of a portion of cytoplasm of surface cells filled with minute vesicles occurred. The bonds among cells, particularly in the centre of the oncosphere, were loosened in a 30-day-old culture and the cells were interconnected by long cytoplasmic bridges. Degenerative changes occurred on about day 40 of cultivation. The oncospheres cultivated in pure Leibovitz's medium lost the microvilli on their surface and the original tegumental cytoplasm turned to a fibrous layer already on day 5 of cultivation. Dystrophic changes in cells occurred from day 7 of cultivation and gradually increased.

The first experiments with in vitro cultivation of *Taenia saginata* oncospheres in artificial medium were performed by Heath and Elsdon-Dew (1972), but the development of the oncospheres was not documented and the authors just mentioned that a cavity bordered with cells was formed on day 10 of cultivation. The same topic was further studied by Wikerhauser et al. (1971, 1978), Rickard and Adolph (1976), Rickard et al. (1977), Rickard and Brumley (1981) and Rickard et al. (1981). The authors used *T. saginata* oncospheres maintained in artificial media or antigens released into the medium for cattle immunization. A description of the changes in the oncosphere structure during the early postoncospheral development is lacking in all of the above papers.

In our opinion, the in vitro cultivation of *T. saginata* oncospheres not only enables to obtain antigens for a possible cattle immunization, but also provides an information on the early phases of development of *T. saginata* larvae which are very difficult to study in the intermediate host.

MATERIAL AND METHODS

The eggs were treated with 0.5 % peracetic acid for 4 min and then washed in physiological saline. No culture contamination occurred in the eggs treated in this way.

The oncospheres were released from the embryophore and activated after Silverman (1954). Their activity was assessed by staining with neutral red (0.1 %) or trypan blue (0.1 %) after Heath and Smyth (1970). Active oncospheres were cultivated in artifical Leibovitz's medium (L-15; Gibco) containing 10 % of bovine serum and in the same medium containing 2 % of bovine serum in the presence of cell monolayer. The cell monolayer consisted of calf kidney cells. The cultures of oncospheres were maintained at 37 °C. The medium was changed every three days and the monolayer every six days. The cultures were observed with an IDM 30 inverse microscope. The samples were fixed in 3 % glutaraldehyde in 0.1 M cacodylate buffer, postfixed in 1 % OsO_4 in 0.1 M cacodylate buffer, dehydrated with ethanol and embedded in EPON 812 and Vestopal resin. Ultrathin sections were stained with uranyl acetate and Reynold's solution and then examined with the JOEL 100B microscope.

RESULTS

The oncospheres cultivated in pure medium containing 10 % of bovine serum were compared with those cultivated in the same medium containing 2 % of bovine serum in the presence of cell monolayer consisting of calf kidney cells. The development of the oncospheres was different under the different conditions. The number of oncospheres gradually decreased and this phenomenon was more pronounced in the cultures in pure medium than in those grown above cell monolayer. The oncospheres exhibiting most marked changes in a given time period are described below.

Day 1 of cultivation: The oncospheres in both media preserved their hexacanth shape with conspicuous hooks. A refractile "halo" was visible around the oncospheres. As it was observed with the electron microscope, the halo was formed by a zone of microvilli extending from the surface of the oncospheres.

Day 3 of cultivation: The oncospheres cultivated in the pure medium remained unchanged.

The oncospheres cultivated above the cell monolayer became spherical. The study of their ultrastructure revealed that their surface was covered with a thin layer of tegumental cytoplasm containing small electron-dense granules and projecting in numerous microvilli. Muscles of still distinctly visible hooks and somatic cells of the oncosphere among them were observed under the tegumental cytoplasm. The somatic cells possessed a spherical nucleus with chromatin clods near nuclear membrane and without nucleolus, surrounded by a thin band of cytoplasm with electron-dense granules (Plate I, Fig. 1). At that time, the dominating structure in the oncospheres was the secretion of penetration gland cells.

Day 5 of cultivation: The oncospheres cultivated in the pure medium became spherical. Electron microscopical observations showed that the microvilli gradually disappeared from their surface and were completely lacking in many of them. The tegumental cytoplasm appeared as a layer consisting of fine fibrils only. Various vesicles, vacuoles and isles of an electron-dense substance arose in the cells inside the oncospheres.

The oncospheres cultivated in the presence of cell monolayer possessed tegumental cytoplasm with long, often branched microvilli. The hook muscles of the oncosphere gradually disintegrated: their structure became thin and muscle fibres gradually turned to a finely granular matter. The oncospheres consisted of a compact mass of cells. The cells possessed a large nucleus with nucleolus and chromatin clusters near nuclear membrane. Their cytoplasm contained dispersed ribosomes, endoplasmic reticulum and ribbon-shaped mitochondria with a dark matrix.

Day 7 of cultivation: The oncospheres cultivated in the pure medium were spherical, but no cells could be discerned inside them. There were only remnants of cell structures, various vacuoles and vesicles and an electron-dense matter the amount of which still increased.

The oncospheres cultivated above cell monolayer were already without hooks and their muscle system. No tegumental cytoplasm was formed and no somatic cells were found. The oncospheres consisted of cells with projecting microvilli shorter than those projecting earlier from the tegumental cytoplasm. Vacuoles originating from the cells of monolayer were found near the microvilli or in a close contact with them (Plate I, Figs. 2, 3). The cytoplasm of these cells contained dispersed ribosomes, ribbon-shaped mitochondria and endoplasmic reticulum forming narrow cisterns.

Day 10 of cultivation: The oncospheres cultivated in the pure medium underwent further degenerative changes and the electron-dense matter continued to accumulate in them.

In the oncospheres cultivated in the presence of cell monolayer the tegumental cytoplasm was not yet formed. The cells contained, in addition to the above-mentioned structure, spherical, dark vacuoles, probably lipid droplets, near the nuclei. Cells of the monolayer with adhering thin microvilli were often found in the vicinity of oncospheres (Plate II, Fig. 2). Processes of cytoplasm of superficial cells filled with small vesicles were observed at some sites on the surface of the oncospheres (Plate II, Fig. 1).

Day 30 of cultivation: The oncospheres cultivated in pure medium were completely filled with the electron-dense matter in which spherical structures of medium electron density started to appear. They resembled the lipid droplets formed by non-saturated fatty acids.

The oncospheres cultivated above cell monolayer possessed shorter microvilli. The cells inside the oncospheres were loosened, but remained interconnected by cytoplasmic bridges (Plate II, Fig. 3).

Days 40—60 of cultivation: If still some oncospheres remained in the cultures in the pure medium, then they were spherical and filled with an electron-dense matter with dispersed lipid droplets.

The structure of the oncospheres cultivated in the presence of cell monolayer did not undergo any substantial changes. Not even after 60 days of in vitro cultivation the tegumental cytoplasm was formed. The electron-dense matter with lipid droplets, observed in the cultures in pure medium already on day 5 of cultivation, started to appear. The cells became still more dispersed in the direction towards the centre of the oncosphere and their cytoplasmic bridges filled the intercellular spaces.

DISCUSSION

A comparison of the development of oncospheres cultivated in the pure medium with those cultivated in the medium in the presence of calf kidney cells revealed that the cell monolayer supported the development at least at the beginning. This seems to be due to the changes in the structure of the oncospheres, which were observed during the in vitro cultivation and compared with the changes occurring in vivo in early phases of postoncospheral development of *T. taeniaeformis* as described by Engelkirk and Williams (1982). These authors found that during three days after infection the oncospheral muscles and tegumental cytoplasm disappeared and the oncospheres consisted of a compact mass of cells with fine microvillar projections on their surface. During the further development the cells loosened and remained connected by cytoplasmic bridges. This phenomenon was observed also in our experiments in the oncospheres of *T. saginata* cultivated in the medium above cell monolayer, but 2—3 days later. Lawrence et al. (1980) compared the development of *T. ovis* oncospheres cultivated in the presence of cell monolayer with the development of oncospheres in the intermediate host. They found that the oncospheres in artificial medium developed more slowly. The stage to which the larva of *T. ovis* develops in vivo within 14 days was reached only on day 23 of cultivation.

It remains uncleared what is the principle of the effect of cell monolayer on the growth of the oncospheres or on the inhibition of their degeneration. The cell monolayer may either supply essential nutrients directly to the medium or be in a contact with oncospheral microvilli, as it was observed in our experiments. Lawrence et al. (1980) assume that the cells of cell monolayer may influence favourably the physical environment of the culture or act as an enzymatic substrate preventing the secretion of the parasite from actively destroying its own tegument. This effect of the parasite's secretion was not observed in the oncospheres cultivated in the pure medium, though their penetration gland cells were active in the first days of in vitro cultivation. The

tegumental cytoplasm remained undivided even at the time when a degenerative process already took place in the oncospheres. On the other hand, at the same period (day 7 of cultivation) the tegumental cytoplasm of the oncospheres cultivated in the presence of cell monolayer was no more preserved and the oncospheres consisted of only a compact mass of cells with microvilli. We assume therefore that the cell monolayer cannot be regarded as a substrate neutralizing the secretion of the parasite and that the change in the superficial layer of the oncosphere (i.e., loss of the original tegumental cytoplasm) is a regular process of development. This hypothesis is supported also by the paper by Engelkirk and Williams (1982), who observed a temporary disappearance of the tegumental cytoplasm. Similarly also other authors (Heath and Smyth 1970, Heath 1973, Heath and Lawrence 1976), who cultivated the oncospheres of other *Taenia* species in pure media, did not observe any lytic effect of the parasite's secretion on its own tegument. In our opinion, the presence of the cell monolayer enables the cultivation of those oncospheres, the postoncospheral development of which occurs inside the organs, at the site of their definitive localization in the intermediate host. The larvae, which remain inside the organs for a short time and the definitive localization of which is the cavity system of the intermediate host, probably do not require the cell monolayer for their development in vitro.

The fact that even after 60 days of cultivation in the medium above cell monolayer the cysticerci were not developed may be caused by several reasons. The development of the oncospheres may be affected, e.g., by the composition of the bovine serum, if it does not contain a sufficient amount of stimulating substances. Heath and Elsdon-Dew (1972), who studied the role of serum in the cultures of *T. saginata* and *T. taeniaeformis* oncospheres, showed that not only the host specification should be considered while chosing the serum, but also the bovine sera markedly differed in their ability to support the development of *T. saginata* oncospheres. An analysis of the bovine fetal sera revealed that in addition to substances stimulating the development of the oncospheres also inhibitory factors occurred in the sera. The ratio of these substances is not standard. We assume that some substances necessary for a good development of the oncospheres were lacking in the used culture medium, but they have not yet been specified. Our own observations of the development of oncospheres and early stages of cysticerci in vivo indicate that a rapid development of the larva is stimulated by a high metabolic activity of cells of the surrounding tissue, particularly macrophages and fibroblasts. It will be therefore necessary to imitate the host tissue in the in vitro medium. The above results suggest that even cells of the organism of the specific intermediate host, *T. saginata*, might be used for this purpose.

УЛЬТРАСТРУКТУРА ОНКОСФЕР ЦЕСТОДЫ *TAENIA SAGINATA*, КУЛЬТИВИРУЕМЫХ В ИСКУССТВЕННЫХ СРЕДАХ

Я. Шрамлова, К. Блажек, З. Маргоул и А. Сингви

Резюме. Онкосферы цестоды *Taenia saginata*, культивируемые в среде Лейбовица на клеточном монослое из почек теленка, развивались до 10-го дня культивирования in vitro. На их поверхности образовались тонкие и часто разветвленные микровилы. На 5-й день культивирования мускулярная система крючков переменялась в зернистое вещество и на 7-й день культивирования крючки и их мускуляная система исчезли. Тегумент цитоплазмы не образовался через 7—60 дней культивирования. На 7-й и 10-й дни культивирования наблюдался контакт онкосфер с клетками монослоя. На 10-й день культивирования встречалась экструзия части цитоплазмы на поверхности клеток, выполненной мелкими везикулами. Связи между клетками, особенно в центре онкосферы, расслаблялись через 30 дней культивирования и клетки были соединены друг с другом долгими цитоплазматическими мостиками. На 40-й день культивирования встречались дегенеративные изменения. Онкосферы, культивируемые в чистой среде Лейбовица, потеряли микроворсинки на поверхности и их тегументальная цитоплазма превращалась в волокнистый слой уже на 5-й день культивирования. Дистрофные изменения наблюдались на 7-й день культивирования и постепенно увеличались.

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J. Sch., Parasitologický ústav ČSAV, Flemingovo n. 2, 166 32 Praha 6, ČSSR

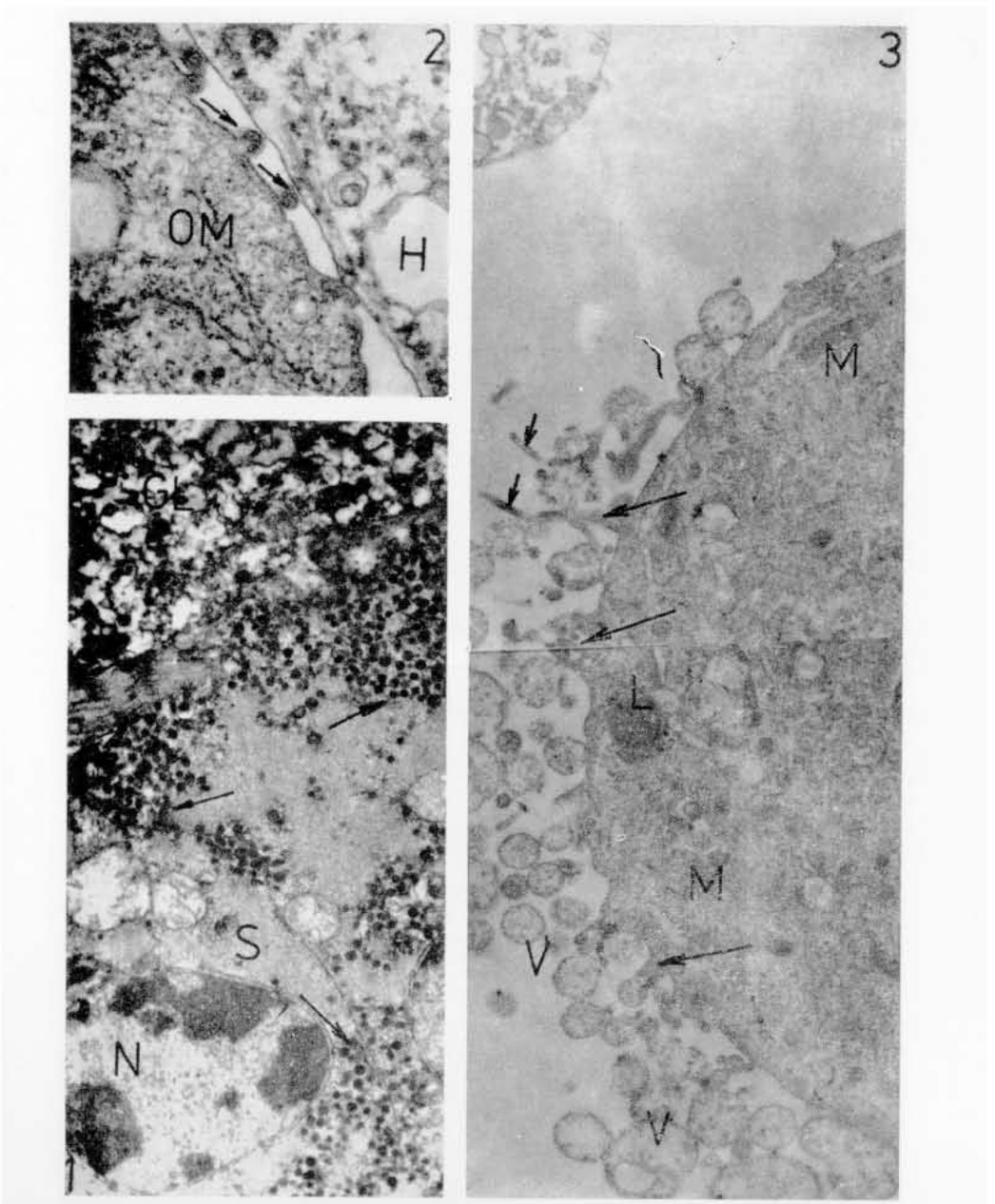

Fig. 1. Oncosphere of *Taenia saginata* on day 3 of cultivation. Note somatic cells (S) with spherical nucleus (N) surrounded by cytoplasm filled with electron-dense granules (arrows) in the vicinity of secretion of penetration gland cell (GL) (×6000). **Fig. 2.** Oncosphere of *T. saginata* on day 7 of cultivation (detail). The oncosphere (OM) is situated in the vicinity of monolayer cells (H): microvilli (arrows) (×10 200). **Fig. 3.** Oncosphere of *T. saginata* on day 7 of cultivation. Microvilli (arrows) project from the cell surface, vacuoles of monolayer cells (V) are situated near the microvilli. Cells of the oncosphere contain mitochondria (M) and lipid droplets (L) (×10 200).

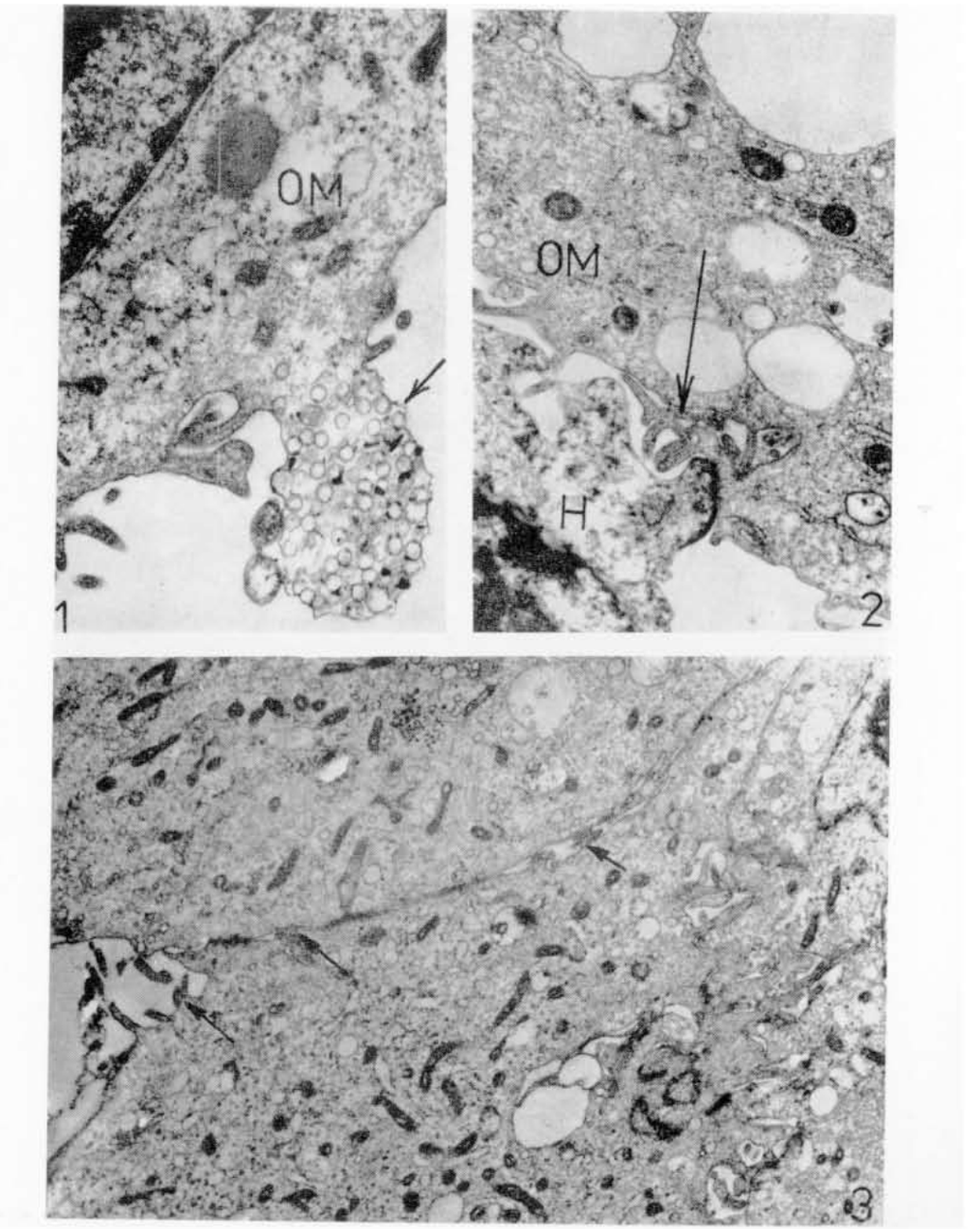

Fig. 1. Oncosphere of *T. saginata* on day 10 of cultivation. Processes of the cytoplasm are filled with small vesicles (arrows) (×10 200). **Fig. 2.** Oncosphere of *T. saginata* on day 10 of cultivation. The oncosphere (OM) is situated in the vicinity of monolayer cell (H) to which microvilli (arrows) adhere (×10 200). **Fig. 3.** Oncosphere of *T. saginata* on day 30 of cultivation. The cells inside it are loosened and remain interconnected by cytoplasmic bridges (arrows) (×6000).