THE USE OF DIFFERENT TYPES OF CELLS FOR CULTURING TOXOPLASMA GONDII

G. T. Akinshina

From the Laboratory of Toxoplasmosis, Division of Endemic Diseases, of the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences, USSR, Moscow
(Presented by Active Member of the AMN SSSR N. N. Zhukov-Verezhnikov)
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The method of tissue culture, widely used in virological investigations, has recently been applied just as successfully in the study of certain pathogenic protozoa, in particular, toxoplasma. Up until now, it has been impossible to culture toxoplasma in a medium free of cells. However, it has been shown that they multiply in different types of tissue cultures.

We used trypsinized tissues from chick embryos, and certain transplantable lines of the cells SOTs, HEp-1, HEp-2, HeLa, human amnion cells, Chang cells, monkey kidney cells, and mouse embryo cells.

EXPERIMENTAL METHOD

The tissue was cultivated according to the widely accepted methods, on strips of glass in test tubes or penicillin flasks. The cell cultures of varying age were inoculated with toxoplasma (Toxoplasma gondii, strain RH) from the peritoneal exudate of white mice [1,2]. Different dilutions of the exudate were prepared in culture fluid, and 0.1 ml of the inoculate was introduced into the prepared test tubes containing the cells. After a 30-40 min contact with the toxoplasma, the cells were covered with fresh nutrient medium, consisting of medium 199 supplemented with 2% bovine serum. Cultivation was carried out at 37°, or for special purposes—at 4-5°.

The control consisted of non-infected cell cultures of the corresponding lines and ages. At the same time, biological tests were set up on mice, with the culture fluid of the control and infected cultures.

The preparations were fixed in a mixture of Nikiforov's and Carnoy's solution, at varying intervals—from 1 to 12 h and from 1 to 20 days after the inoculation. The preparations were stained according to Romanowsky-Gimsa, with Heidenhain's iron hematoxylin, and with hematoxylin-eosin. For demonstration of DNA in the toxoplasma and the cell hosts, in the course of infection, we used the Feulgen reaction.

EXPERIMENTAL RESULTS

We traced, dynamically, the characteristic cytopathogenic action of toxoplasma on types of cells of varying origin. We did not observe any radical differences in the capacity of the toxoplasma to infect the various types of cells. However, toxoplasma infection of the transplantable cells, SOTs, Hep-1 and HEp-2 was focal in character. The infection in these numerous foci extended from a single center in such a manner that, in the center of the focus, we encountered cells almost or completely destroyed by the parasites, while closer to the periphery the cells were less damaged. The cultures of chick fibroblasts were infected more uniformly throughout the entire monolayer, and there were no such clearly manifested foci.

The cytological changes described below are common to all the types of cells which we used.

Only one h after the inoculation, we observed individual parasites in the cells. The toxoplasmas lay freely in the cytoplasm or in clearly delineated vacuoles. In the majority of cases, the vacuoles contained a thin network of fibers. The intracellular parasites were larger than the extracellular ones, and both their ends were rounded. In interphase, the nucleus of the toxoplasma cell looked like a nuclear vacuole, with peripheral granules of DNA (according to Feulgen's reaction). During division of the parasite, the DNA in the nucleus collected in the center in a compact

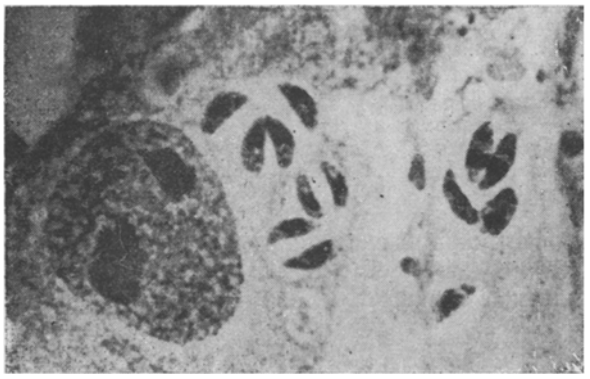

Fig. 1. Fibroblasts from a mouse embryo, 3 h after the inoculation: paired and single toxoplasmas in clearly delineated vacuoles. Stained according to Romanowsky-Gimsa. Obj. 100 ×, ocul. 8 ×.

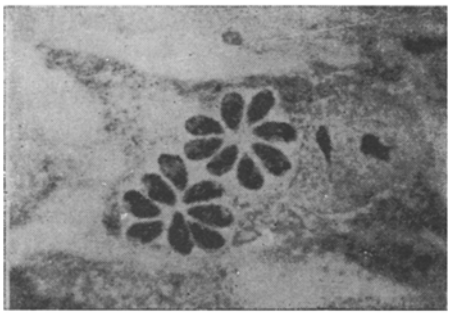

Fig. 2. Typical accumulations of the toxoplasmas in the form of rosettes, within a chick embryo fibroblast 24 h after the inoculation. Stained according to Romanowsky-Gimsa. Obj. 100 ×, ocul. 8 ×.

mass, and after division, it was distributed evenly in the two daughter nuclei. In the culture, at early intervals after the inoculation, we often encountered cells which divided mitotically, despite the presence of parasites in their cytoplasm.

After 3 h, the number of parasites in the cells increased (Fig. 1), and they often lay in pairs. The protoplasm of the cell was slightly frothy, but the nucleus retained its normal structure. We noted substantial changes in the cells in the course of the infection, which appeared after 1-2 days. At this time, the main mass of cells was infected with the parasites, and the characteristic collections of toxoplasmas appeared in the cells, forming rosettes that consisted chiefly of an even number of toxoplasmas (Fig. 2). Characteristically, the toxoplasmas were very frequently arranged close to the nucleus, sometimes surrounding it on all sides. Some of the cells contained up to 20-25 "rosettes" of parasites (Fig. 3). The rossettes were located in vacuoles, and were bounded by membranes, whose presence was confirmed by electron microscope investigations [4]. In some of the preparations it was clearly apparent that the volume of the vacuole depended on the number of multiplying parasites.

At later periods after the inoculation, there were many cells in the culture that were literally "stuffed" with toxoplasmas, both singly and in pairs (Fig. 4). The cell-hosts also exhibited marked morphological changes: large vacuoles filled the entire cell, and the nucleus was intensely deformed, frequently fragmented and pushed toward the periphery. The contours of many of the cells became fringed and the cells gradually underwent lysis. However, despite complete cell lysis, the nuclei retained their membranes and could be detected, for a long time, among the toxoplasmas discharged from the cell. The moment of complete destruction of the cells coincided with mass death of the parasites.

It is interesting to note that, beginning with the 3rd-4th day after inoculation, numerous cells were observed in the preparations stained with iron hematoxylin that contained black inclusions of varying size and irregular form. It is still not clear whether they lay in vacuoles, or whether the clear aureole around them was a result of fixation.

Following inoculation, a certain portion of the cultures of chick fibroblasts was incubated at 5°. In this case, the toxoplasma was gradually freed into the nutrient medium and retained there over the course of a month.

In performing the biological tests with culture fluid from infected cultures, using mice, it was noted that the infectiousness of the culture fluid for mice was lower than the infectiousness of the peritoneal exudate, containing the same number of toxoplasmas. This is probably explained by a gradual loss of activity by the toxoplasmas in the culture fluid, a medium that is not favorable for their survival.

On the basis of the obtained results, and the data of foreign authors, it can be postulated that toxoplasma is rather indifferent in regard to infecting different types of cells. Certain data indicate that multiplication of the toxoplasmas depends on the metabolic level of the cell-host. A study of cells treated with colchicine [3] showed that this dependency was not related to the energy of mitotic division, which is disrupted by colchicine.

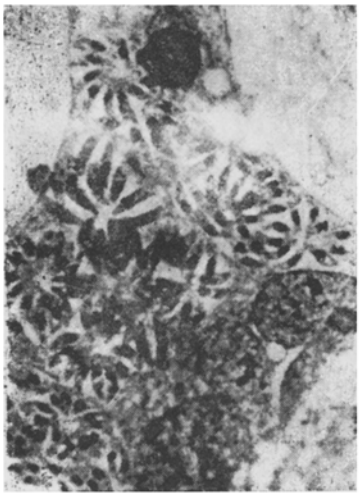

Fig. 3. Numerous parasites, arranged in the form of "rosettes", fill the entire cell (4th day after inoculation). Stained according to Romanowsky-Gimsa. Obj. 100 ×, ocul. 6 ×.

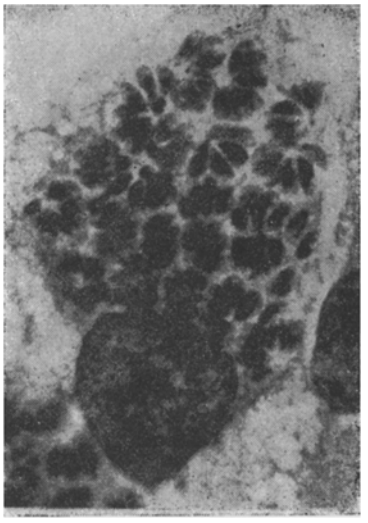

Fig. 4. Monkey kidney cell "stuffed" with parasites (8th day after inoculation). Stained according to Romanowsky-Gimsa. Obj. 100 ×, ocul. 8 ×.

Degeneration of the cells under the influence of the toxoplasma occurred, as we saw, by the same routes. However, we observed a certain delay in the degeneration of the transplantable cells of various lines—SOTs, HEp-1, HEp-2 and Chang cells, which was probably caused by peculiarities in their metabolism.

Cook and Jacobs [3] noted the same characteristic in relation to the degeneration of epithelial cells from human conjunctiva and intestine under the influence of toxoplasma. The authors observed that the slow rate of multiplication of the toxoplasmas was dependent upon reduction of the temperature. In our experiments with cultivation of the toxoplasma at 5°, their activity was somewhat lowered. The parasites were gradually freed into the medium and retained there over the course of a month. This technique could be convenient for preserving strains without uninterrupted serial passages in mice. For these purposes, one could use culture fluid from inoculated cultures, that were incubated at 37°, with constant replacement of the medium every 7-10 days [5].

SUMMARY

In culturing Toxoplasma gondii, it was demonstrated that they could invade different types of cells. Primary and transplantable cell cultures were used; they were obtained from man (from the liver, uterus, amnion, pharyngeal, epithelium), monkey (from the heart and kidney), chick embryo and mouse embryo.

Degeneration of the various types of cells under the influence of toxoplasma is much the same; however, some variations were noted in the rate of their degeneration.

By culturing toxoplasma in the tissue cultures at 5° C, it was possible to keep them viable without transplantation for a month. This may prove to be convenient for preserving the toxoplasma strains without serial passages in mice. For this purpose, it is possible to use the culture fluid of infected tissue cultures that have been incubated at 37°, with a constant replacement of the medium every 7-10 days.

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