PROLONGED PRESERVATION OF TOXOPLASMA IN TISSUE CULTURES

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Recently tissue cultures have been used successfully for the study of many problems related to protozoa which cause leishmaniasis, Chagas' disease, trichomoniasis, toxoplasmosis and other diseases. A review of this subject, relating to toxoplasmosis, has been published by us earlier [1].

In laboratory investigations, different strains of Toxoplasma have to be maintained in experimental animals, which is expensive and unwieldy.

Some investigators have proposed that Toxoplasma strains could be maintained in tissue cultures [2, 4, 5]. This has been successfully done by Shukhova et al. [7], who for almost 4 years have been maintaining the "CB" strain of Toxoplasma gondii in HeLa cell cultures. However, this method could not be widely used in practice because it is necessary to transfer the organisms very frequently from infected cell cultures to fresh cells.

Cook and Jacobs [5] maintained Toxoplasma-infected tissue cultures not at 37°C as is customary, but at room temperature. This decreased the rate of growth of the parasites, and transfers had to be made less frequently.

Balducci and Tyrrell [3] have called this phenomenon "artificial hibernation in tissue cultures" considering observations of Rodhain [6] who found that Toxoplasma infections in hibernating marmots were more prolonged than those in nonhibernating animals. By maintaining Toxoplasma-infected tissue cultures at a low temperature, these authors were able to demonstrate parasites in cultures after as long as 7 weeks post infection.

The present investigation had as its aim the elaboration of a method for a prolonged preservation of Toxoplasma in tissue cultures, suitable for wide use in different laboratories.

METHODS AND RESULTS

Forty-eight-hour-old cultures of chick embryo fibroblasts, grown by the usual method in test tubes of flasks, were infected with Toxoplasma gondii, RH strain. The original material was derived from the peritoneal exudate of white mice infected with Toxoplasma and killed 3-4 days after infection. The old culture medium was pipetted off from the selected tubes containing tissue cultures. Each tube received 0.1 ml of the exudate, which was diluted 8-10 times. After the parasites had been in contact with the cells for 30-60 min, the cultures were overlaid with fresh medium and incubated at 37°c for 24-48 h.

Under these conditions the parasites divided actively and infected more and more cells. Following incubation, the cultures were placed in a refrigerator at 4°C, where they were kept for 3-8 weeks. The refrigerated cultures were transferred after 3, 4, 5, 6, 7, and 8 weeks to fresh 48-hour-old chick embryo fibroblast cultures. Before this was done the tubes were vigorously shaken until the cells became completely detached from the walls, and 0.1 ml of the suspension thus obtained was introduced into each of the new fibroblast cultures. The cultures were again incubated for 24-48 h at 37°C, refrigerated at 4°C and the whole procedure repeated again. The Toxoplasma strain was maintained

during 5 months through 5 and 7 serial passages. It was found that the best period for transferring from the refrigerated cultures was 3-4 weeks after the beginning of refrigeration. The parasites remained viable only in the presence of the host cells. In control cultures which contained only the culture medium with parasites none remained alive after as little as 5-8 days of refrigeration. Mice inoculated with material from these cultures did not become infected. Apparently Toxoplasma is able to remain alive under conditions which lower its rate of metabolism only within host cells.

Thus, the decreased rate of growth of Toxoplasma in tissue cultures at a low temperature allowed the lengthening of transfer periods to 3-4 weeks. This method may be found suitable for maintaining strains of Toxoplasma in many laboratories.

SUMMARY

Cultures of chick toxoplasma-infected fibroblast were stored at +4°C. Toxoplasma were reinoculated into non-infected cell cultures 3-4 weeks following the incubation of infected cultures at +4°C. Toxoplasma strain was maintained for 5 months, 5-7 serial passages having been required. This method allows prolonging the time limits for toxoplasma reinoculation into tissue cultures up to 3-4 weeks, and may, consequently, prove convenient for storing the toxoplasma strains in laboratories.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
