SOME ASPECTS OF TOXOPLASMA CULTIVATION IN CULTURES OF TRANSPLANTABLE HOG EMBRYONIC KIDNEY CELLS (RES- and RES-La)

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UDC 576.893.192.1.093.35

Toxoplasmas have been successfully cultivated in many primarily trypsinized cell cultures and in heteroploid cultures of transplantable cells obtained either from normal tissues or from malignant tumors [1, 2].

Because of the karyologic stability of transplantable hog embryonic kidney cells of the line RES and its variant RES-La [4], it was decided to study whether any change takes place in the set of chromosomes of these cells when toxoplasmas are cultivated in them, and also to examine some aspects of the cytopathic action of toxoplasmas on RES cell cultures.

EXPERIMENTAL METHOD

The object for infection with toxoplasmas (Toxoplasma gondii, strain rh) consisted of 5- or 6-day-old RES cultures grown on cover slips in penicillin flasks. The RES cells were cultivated in medium No. 199 with 10% normal bovine serum, and the RES-La cells in medium with 0.5% lactalbumin hydrolysate and 10% normal bovine serum. A peritoneal exudate of albino mice infected with toxoplasmas and laparotomized on the 3rd-4th day after infection was used for inoculation. The toxoplasmas, in a dose of $6 \cdot 10^4$ cells, were introduced into flasks containing the cultures after preliminary aspiration of the nutrient medium. Contact between the parasites and cells took place for 1.5 h at 37°. The cell layer was then covered with fresh nutrient medium. The material was incubated at 37°. Every 24 h for 10-12 days some of the material was fixed in Nikiforov's mixture and stained by the Romanovsky–Giemsa method. Another portion of the material was treated with colchicine. The final concentration of colchicine was 0.5 μg/ml; contact with the cultures lasted 2-4 h. Subsequent treatment (hypotonic shock, fixation, and drying in air) was carried out by a slight modification of the method of Rothfels and Siminovitch [7]. Preparations fixed by Shabadash's method were stained by Feulgen's method. Chromosomes were counted in the cells in metaphase, and the results were compared with the control. Karyograms and histograms were plotted by the usual methods.

EXPERIMENTAL RESULTS

Intracellular parasites could be seen 3 h after inoculation. The rhythm of propagation of the toxoplasmas for 3-4 days was identical with that observed in other (heteroploid) cell cultures [1, 2, 3]. The parasites lay in pairs in the cells (Fig. 1), at random, or in characteristic groups forming "rosettes." Often the toxoplasmas lay next to the nucleus, which sometimes appeared deformed. Ultimately the nuclei of the infected cells underwent pycnosis. The picture of karyorrhexis also was observed. After multiplying the toxoplasmas almost filled the cell. The process of degeneration in the later period after inoculation (6th day and later) was much slower. Numerous groups of parasites showed changes – the toxoplasmas were apparently stuck together, becoming more tightly packed and losing the clarity of their outlines (Fig. 2). These clusters of parasites were described as cyst-like, for there was no sign of the clearly distinguishable membrane present in toxoplasma cysts or of certain other details typically found in them.

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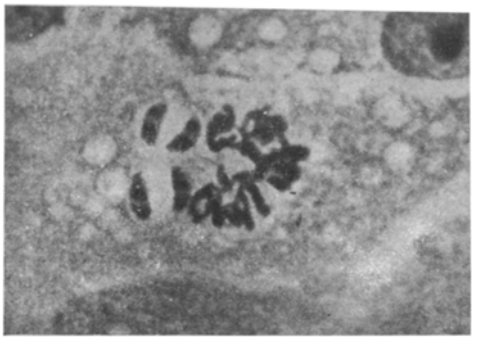

Fig. 1. A dividing cell of an RES culture with toxoplasmas in the cytoplasm. Fixation in Nikiforov's mixture, stained by the Romanovsky–Giemsa method. Objective 100, ocular 10.

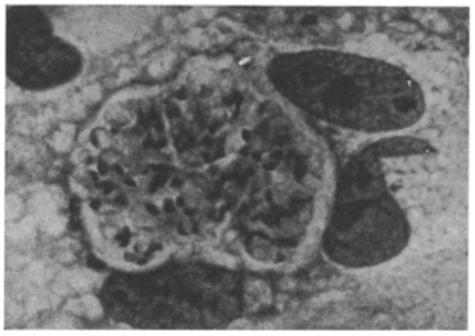

Fig. 2. Cyst-like cluster of toxoplasmas (10th day after inoculation). Fixation in Nikiforov's mixture, staining by the Romanovsky–Giemsa method. Objective 100, ocular 10.

While the cyst-like clusters of toxoplasmas were being formed, the RES cell cultures were becoming "spontaneously disinfected." The number of proliferative forms of the clusters (pairs, "rosettes") became much smaller: they had practically disappeared by the 9th-10th day after inoculation.

The changes in the RES-La cultures under the influence of infection with toxoplasmas was indistinguishable from the changes in the RES cells.

The results of the karyologic study of preparations of RES and RES-La cells infected with toxoplasmas, after treatment with colchicine and staining by Feulgen's method, showed that from 68 to 85% of the metaphase cells contained 38 chromosomes. The number of tetraploid and hypotetraploid forms did not exceed their number in the control cultures (2-4%). The remaining cells contained a hypodiploid number of chromosomes – from 34 to 37.

The frequency of appearance of abnormal forms of chromosomes (dicentrics, annular forms, etc.) did not exceed the number of chromosomal anomalies in the control cultures.

Certain comparisons may justifiably be made with the results obtained in other experiments on this stable cell system. First, during adaptation of the RES line to a medium with 0.5% lactalbumin hydrolysate (the trophovariant RES-La) no morphological changes in the karyotype were found from that in the control RES line. The RES-La line was characterized by the same modal number of chromosomes as the RES cells, 2n = 38 [5]. Second, RES cultures chronically infected with monkey adenovirus of types M2 and M4 retained their karyotype substantially unchanged despite the long persistence of the virus in them [6]. Third, the authors attempted to induce a disturbance of the karyotype of the RES line by means of colchicine, a polyploidizing factor, which was added to medium No. 199 in the course of 20 passages (for a period of 2.5 months). In this case also the attempt to induce distrubances of the karyotype structure was unsuccessful.

Hence, in widely different conditions (adaptation to a new medium, chronic adenovirus infection, prolonged treatment with colchicine, acute infection with toxoplasmas) the karyotype of the RES cells underwent no visible changes, demonstrating its great stability. In the light of these results, it is premature to reject the occurrence of structural modifications to the chromosomes of cells infected with toxoplasmas, more especially because the periods of observation were limited to the conditions of an acute infection.

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