# MITOTIC INDICES IN RES (CLONE 1) CULTURES INFECTED WITH ACUTE HUMAN ENCEPHALOMYELITIS VIRUS

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Human acute encephalomyelitis virus causes ill-defined changes in mitotic activity in RES cultures (clone 1). Mitotic activity in cultures infected with the "Reznik" strain persisted longer than in control cultures. No increase in the number of pathological mitoses was found in infected cultures.

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Changes in the mitotic indices are among the morphological manifestations of the action of viruses on cell cultures. Recently this has been proven for several viruses [3, 4, 9-11]. The extent to which qualitative and quantitative changes in mitoses depend on the process of virus reproduction in the chosen cell system and how much they depend on the cytopathogenic action of the virus on the cell culture is of considerable interest.

This communication is concerned with the study of some indices of mitosis in RES cultures of clone 1 (a line of transplantable hog embryonic kidney cells [2] characterized by the almost complete absence under normal conditions of pathological mitoses), inoculated with the virus of acute human encephalomyelitis (AHEM) [5-7].

## EXPERIMENTAL METHOD

AHEM virus of strain "Mam" (isolated from the blood of a patient with meningoencephalitis) strain "Feok" (obtained from the cerebrospinal fluid of a patient with disseminated sclerosis), and "Reznik" (isolated from the spinal cord of a woman dying from disseminated sclerosis) was used in the experiments. RES cell cultures (clone 1) were infected with a 10% virus-containing brain suspension of albino mice prepared in physiological saline. The multiplicity of infection was 0.24 $LD_{50}$/cell. The cells were grown in medium No. 199 with 10% normal bovine serum on cover slips placed in penicillin flasks. After contact for 1 h at 37° the virus was removed and the cell layer washed with Hank's solution after which maintenance medium No. 199 with 5% bovine serum was added to the flasks. The cover slips with infected and control cultures were fixed daily for 9 days in Shabadash's neutral mixture or in Carnoy's fluid. The preparations were stained with hematoxylin and eosin and by Carazzi's hematoxylin. The presence of virus in the culture fluid was determined by titration in albino mice weighing 6-7 g infected intracerebrally. Observations were made on the animals for 12-14 days. Infected and control cultures were studied cytologically. The mitotic activity of the cultures was expressed as the number dividing cells per thousand (in each case 5000-10,000 cells were counted). The percentage of pathological mitoses was determined among the dividing cells of the infected and control cultures (by Alov's method [1]). The results were analyzed statistically by the Fisher–Student method.

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TABLE 1. Mitotic Activity of RES Cultures (clone 1) Infected with AHEM Virus (in %) M ± t

| Strain | Time after inoculation with AHEM virus (in days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 change of medium | 5 | 6 | 7 | 8 | 9 |
| "Reznik" | 24,4±0,7 | 19,2±1,1 | 17,6±1,1 | 27,8±0,7 | 39,4±0,6 | 22,8±0,9 | 19,3±0,8 | 7,5±0,8 | 15,6±0,7 |
| "Feok" | 21,4±0,7 | 19,3±0,8 | 18,3±0,9 | 4,1±0,8 | 37,3±0,9 | 7,2±0,6 | 0 | 0 | 0 |
| "Mam" | 14,2±0,5 | 12,6±0,9 | 11,3±1,3 | 33,6±1,2 | 9,0±0,8 | 12,4±0,7 | 7,0±0,7 | 0 | 0 |
| Control | 19,0±0,7 | 23,2±0,9 | 30,4±1,2 | 8,5±1,7 | 16,4±0,6 | 5,3±1,3 | 2,3±1 2 | 0 | 0 |

## EXPERIMENTAL RESULTS

After infection of RES (clone 1) cultures with AHEM virus, strains "Mam" "Feok," and "Reznik," no signs of a cytopathogenic action were observed. Strains "Mam" and "Feok" did not multiply in the chosen cell system under the experimental conditions used. Strain "Reznik" was found in the culture fluid 24 h after infection in low titer (1.5 log $LD_{50}$). During the next 4 days, only traces of virus were found in the undiluted culture fluid. Starting from the 6th day, no virus could be found.

Data showing changes in mitotic activity of the control and infected cultures are given in Table 1. They can be divided into two groups: the first relating to 3 days after addition of virus, the second to the period after changing the medium. During the first 3 days the mitotic activity of the control cultures increased to 30.4 ± 1.2%, while that of the infected cultures gradually fell, the differences by the third day being highly significant ($P < 0.001$). During the 5th-8th days the mitotic activity of the control cultures gradually fell to zero. In cultures infected with strain "Feok," a temporary burst of mitotic activity was observed on the 5th day, followed by a sharp decline; starting from the 7th day no mitoses could be found. A similar pattern was observed in cultures infected with strain "Mam," but the burst of mitotic activity in this case was recorded on the 4th day and mitoses disappeared 8 days after addition of the virus (at the same time as in the control cultures). The dynamics of the changes in mitotic activity were different in cultures infected with strain "Reznik." Mitotic activity remained high (15.6 ± 0.7%) even on the 9th day after infection. The phenomenon of its preservation may be explained by the protective power of strain "Reznik" relative to RES cultures.

The number of pathological mitoses in the infected cultures was not greater than in the control. In both cases the number of abnormal mitoses varied from 0 to 9%. The differences were not statistically significant ($P > 0.1$). No particular type of pathological mitoses predominated.

From data in the literature and the writers' own observation [3, 4, 9-11] it is possible to consider the possible link between reproduction of virus in cell cultures, manifestations of the cytopathogenic action (in the wide meaning of this term), and changes in the quantitative and qualitative indices of mitosis. For instance, strains of herpes simplex virus, belonging to the first antigenic group [8] ("Tolstova," "Berezina," $L_2$) and multiplying in cultures of human diploid cells with the development of a cytopathogenic effect, not only modify the mitotic activity of these cultures but also lead to the appearance of numerous pathological mitoses (mostly colchine-like metaphases) [9]. Meanwhile, strain K of herpes simplex virus, belonging to the second antigenic group and multiplying in the same cell cultures without the development of a cytopathogenic action, causes changes in mitotic activity of these cultures without the appearance of pathological mitoses. Propagation of viruses of the smallpox group [3, 4] in a system of RES cells (clone 1) is accompanied by characteristic cytological changes—the formation of inclusions in the cytoplasm of the infected cells, changes in mitotic activity, and the appearance of pathological mitoses, principally metaphases with deletion of chromosomes and three-group metaphases.

The absence of increase in the number of a typical mitoses and the ill-defined changes in mitotic activity in RES cultures (clone 1) infected with AHEM may probably be associated with the insensitivity of this cell system to AHEM virus. The longer preservation of mitotic activity in cultures infected with the "Reznik" strain may perhaps be explained by the preservation of a small quantity of virus in RES (clone 1) cells.

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