DNA SYNTHESIS AND MITOTIC ACTIVITY OF A CULTURE OF RES CELLS (CLONE 1) INFECTED WITH VACCINIA VIRUS

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Conflicting results have been obtained from the study of DNA synthesis in cells infected with vaccinia virus: in some experiments an increase of DNA synthesis has been observed, but in others—a decrease [3, 6]. The differences may probably be explained by differences in the experimental conditions. The authors' observations made on HeLa cells infected with vaccinia virus have shown that 1 h after infection the incorporation of thymidine-H^3 into the nuclear part of the cells is increased and remains at this level for 2 h. The intensity of incorporation of the isotope then falls to reach the control values. Cells with label in the cytoplasm begin to appear 2 h after infection, and their percentage increases as the period of infection grows longer (3-5 h).

The object of the present investigation was to study the relationships between changes in DNA synthesis and changes in the mitotic activity in RES cells (clone 1) infected with vaccinia virus.

EXPERIMENTAL METHOD

Experiments were carried out on 3-day cultures of RES cells (clone 1) grown in tubes with cover slips in medium No. 199 with 5% bovine serum. The cells were infected with vaccinia virus (dermovaccine strain, series 113, dose 12 ID_{50} per cells).

DNA synthesis in the infected cells was studied by autoradiography. The tritium-labeled DNA precursor, thymidine, was used for this purpose in a dose of 0.5 μCi/ml medium. The isotope was added to the cells at various times after infection (15 and 30 min, 1, 2, 3, 4, 5, 6, 7, and 8 h). The number of labeled cells in 100 cells was counted. In addition, the mean number of granules per cell, i. e., the intensity of incorporation of isotope into its nucleus and cytoplasm, was determined.

Changes in the mitotic activity under the influence of vaccinia virus were investigated in RES cells (clone 1) infected in test tubes with cover slips. At various times after infection (1, 2, 4, 6, 8, 10, 12, 18, 24, and 48 h) the cells were fixed in Shabadash's neutral mixture and stained with Carazzi's hematoxylin or with iron hematoxylin–eosin. The mitotic activity was expressed as the number of dividing cells per thousand cells counted. Pathological forms of mitosis were recorded by the system suggested by I. A. Alov [1].

EXPERIMENTAL RESULTS

The study of the action of vaccinia virus on DNA synthesis in RES cells (clone 1) showed that, as also in HeLa cells, stimulation of synthesis of nuclear DNA took place during the first hours after infection. An increase in the incorporation of isotope, shown both by the number of labeled cells and by the intensity of incorporation in each cell, was observed 2 h after addition of the virus, and it continued above the control level until 6-7 h after infection. By 9 h the intensity of incorporation had fallen to the control values. However, incorporation of isotope into the cytoplasm was not observed sooner than 2-3 h after infection.

To study the relationship between the changes in mitotic activity and the dynamics of DNA synthesis in infected cultures, a clonal line of transplantable RES cells (clone 1), more balanced in the karyologic respect than HeLa, SOTs, HEp-2, and other transplantable lines used in virology, were used. This line was obtained by cloning, using the method of V. I. Gavrilov and R. G. Zmieva [2], from a "parent" population of

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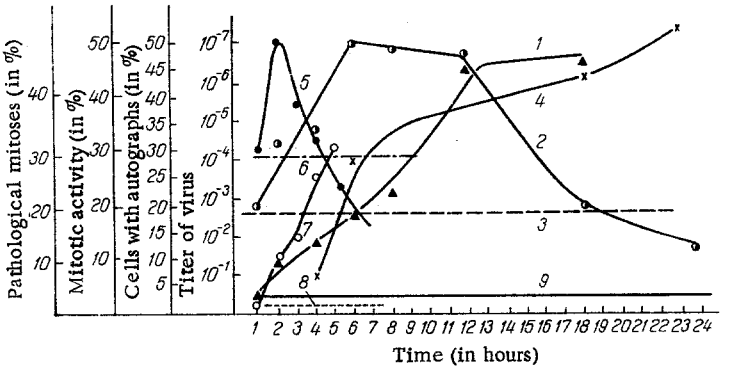

Fig. 1. Relationship between DNA synthesis, reproduction of virus, and mitotic activity. 1) Pathological mitoses; 2) mitotic activity of infected cultures; 3) mitotic activity of uninfected cultures; 4) titer of virus; 5) incorporation of thymidine H^3 into nucleus of infected cells; 6) incorporation of thymidine-H^3 into nucleus of uninfected cells; 7) incorporation of thymidine-H^3 into cytoplasm of infected cells; 8) incorporation of thymidine-H^3 into cytoplasm of uninfected cells; 9) uninfected cells (control).

line RES. RES (clone 1) cultures have a purely epithelioid, monomorphic character. The cell boundaries are sharply defined; the number of binuclear cells is very small (8-12%), and even fewer cells have a larger number of nuclei.

The results of karyologic investigation of cultures of line RES (clone 1) showed that about 60% of cells of this line contain a number of chromosomes corresponding to a diploid set of chromosomes in somatic cells of the domestic pig (2n = 38). In addition, RES (clone 1) cultures contain cells with a hypodiploid number of chromosomes. The number of tetraploid and hypotetraploid cells is only 2-5%.

A study of the mitotic activity showed that 1 h after infection no statistically significant differences were present between the number of mitoses in the infected and control cultures. Starting from 2 h, mitotic activity began to increase in the infected cultures. The difference between mitotic activity of the infected and control cultures reached a maximum 6 h after infection, when the mitotic activity in the infected cultures was 50.2 ± 1.2%, compared with 14.4 ± 0.7% in the controls ($P < 0.001$). Highly significant differences were also found 8-12 h after inoculation of virus. However, by 18 h the mitotic activity of the infected cultures had fallen sharply and the difference was no longer significant (18.4 ± 0.61% in the infected and 17.8 ± 0.92% in the control culture; $P > 0.1$). Finally, after 24 h a statistically significant decrease was observed in the mitotic activity of the infected cultures by comparison with the controls (13.9 ± 0.34 and 18.4 ± 0.88% respectively). Destruction of a considerable part of the cell layer was observed 48 h after infection, and comparative investigation of the mitotic activity was by that time practically impossible.

Determination of the percentage of pathological mitoses among the dividing cells of the infected and control cultures showed that the proportion of pathological mitoses in the infected cultures increased continuously starting from the second hour of the experiment, whereas the percentage of pathological mitoses in the uninfected cultures remained almost unchanged compared with the original low values.

Differential counting of the pathological mitoses revealed the following forms: pathological prophase (premature separation of chromatids), dispersal of chromosomes in the metaphase, colchicine-like metaphase (K-metaphase), deletion of single chromosomes in metaphase, 3-group metaphase, multipolar metaphase, deletion of chromosomes or their fragments in anaphase, chromosomal and chromatid bridges, irregular separation of chromosomes, multipolar anaphase. The commonest form of pathology of cell division in the cultures infected with vaccinia virus was deletion of 1, 2, or several chromosomes in metaphase. Second in order of frequency was 3-group metaphase, and third—deletion of chromosomes in anaphase. Starting with 4-6 h after infection, the first two pathological forms accounted for more than 90% of all anomalies of mitosis. It was also found that the number of cells with 2, 3, or more nuclei increased between the 4th and 24th hour of infection (up to 35% of binuclear and 10% of trinuclear cells). In addition, it was found that as the period of infection increased, the number of cells with micronuclei in the cultures also increased (up to 15%). Starting from 4-6 h after infection, the difference between the infected and control cultures in terms of these indices was highly significant ($P < 0.001$).

The following conclusions may be drawn from a comparison of the dynamics of DNA synthesis and the data for reproduction of vaccinia virus with changes in the mitotic activity (see Fig. 1).

The increase in mitotic activity of the infected cultures was the result of an initial increase in synthesis of cell (nuclear) DNA. However, this factor could hardly be directly responsible for the appearance of pathological forms of mitosis.

The appearance of pathological forms of mitosis and, in particular, of metaphases with deletion of single chromosomes, and also of 3-group metaphases was connected with commencing reproduction of the virus. As reproduction of the virus increased, the number of pathological mitoses increased still further. The character of the pathology of mitosis suggested that initially the achromatin spindle and, perhaps, the kinetochore were affected. It is evident that the micronuclei arising in RES (clone 1) cultures infected with vaccinia virus are organized from deleted chromosomes or from small groups of them.

The decrease in synthesis of cell DNA and subsequent disturbance of mitotic activity were probably connected with an increase in the synthesis of virus DNA, which induces specific repressors influencing these processes. This influence also extends to the nuclei formed from chromosomal groups passing through mitosis without loss, and to those which, having lost 1, 2, or more chromosomes, entered a new interphase.

The decrease in mitotic activity taking place immediately after its rise was the result of depression of synthesis of cell DNA by reproduction of vaccinia virus. At the same time, the increase in the percentage of pathological mitoses among cells still in process of division was due to the continued action of virus reproduction on the achromatin apparatus of these cells.

LITERATURE CITED

1. I. A. Alov, Vestn. Akad. Med. Nauk SSSR, No. 11, 58 (1965).
2. V. I. Gavrilov, Transplantable Cells in Virology [in Russian], Moscow (1964).
3. J. Backer and W. Joklik, Proc. Nat. Acad. Sci. Washington, 51, 577 (1964).
4. J. Cairns, Virology, 11, 603 (1960).
5. C. Cocito, Arch. Ges. Virusforsch., Bd 14, S. 201 (1964).
6. D. Dubbs and S. Kit, Virology, 22, 214 (1964).