STUDY OF THE RNA SYNTHESIS IN CELLS INFECTED WITH THE VIRUS OF FOWL PLAGUE VERA

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In studies devoted to the multiplication of myxoviruses in the cell, it has been shown that the virus subunits are synthesized before the appearance of infectious virus [2-4].

The virus of fowl plague vera (FPV), belonging to the group of myxoviruses, is of a ribonucleic character. Experiments with fluorescing antibodies have shown that the S-antigen of the virus FPV is detected in the cell after three-hour infection, while the GA-antigen is detected only after four-hours of infection [5]. The maximum RNA synthesis of the virus in the cell falls at three hours of infection [3].

In this work, an attempt was made to study the localization of the RNA synthesis of the virus of fowl plague vera in the cell, using radioactive isotopes.

PROCEDURE

In the experiments we used a preliminary trypsinized, surviving culture of fibroblasts of 10-day chick embryos. The experimental series was infected with the virus of fowl plague vera (Baybridge strain) in a dose of 10 EID_{50} per cell and incubated at 37° in lactalbumin hydrolyzate. An uninfected tissue culture was used as the control. At the end of the incubation period with the virus, the cellular material was placed for 30 min in a medium with the addition of adenine-C^{14} in a dose of 7 μCi per ml of medium, then repeatedly washed with cold physiological solution, frozen, and pulverized in a slightly ground down Potter homogenizer (20-25 vertical tractions). The cellular material was separated into nuclei and cytoplasm in sucrose [1]. The nuclei were repeatedly homogenized in a mixture of tris $MgCl_2$ buffer, pH 7.2. The RNA was isolated from the nuclear and cytoplasmic fractions by the phenol method, followed by precipitation and reprecipitation with ethanol. The nucleic acid precipitates were dissolved for purification in a 0.14 M solution of NaCl and dialyzed against a 0.14 M solution of NaCl for 24 h at 4°. The radioactivity was counted in an end-window counter, the amount of RNA determined in the ultraviolet in the SF-4, and the results expressed in counts of C^{14}/min · μg of RNA (Fig. 1).

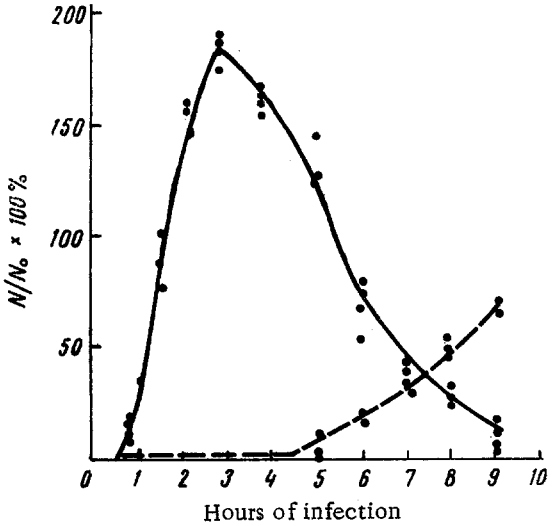

Fig. 1. Incorporation of adenine-C^{14} into RNA of cells infected with FPV virus; 1) nuclear RNA; 2) cytoplasmic RNA; N) specific activity in experimental; N_0) in control.

A method of autoradiography was used to study the localization of RNA synthesis. In the experiments we used a monolayer culture of cutaneomuscular cells of the human embryo. The cells were inoculated in amounts of $1 \cdot 10^6$/ml onto glasses and used on the fifth to sixth day of incubation at 37°. The experimental series was infected with the fowl plague virus (Baybridge strain) in a dose of 10 EID_{50} per cell.

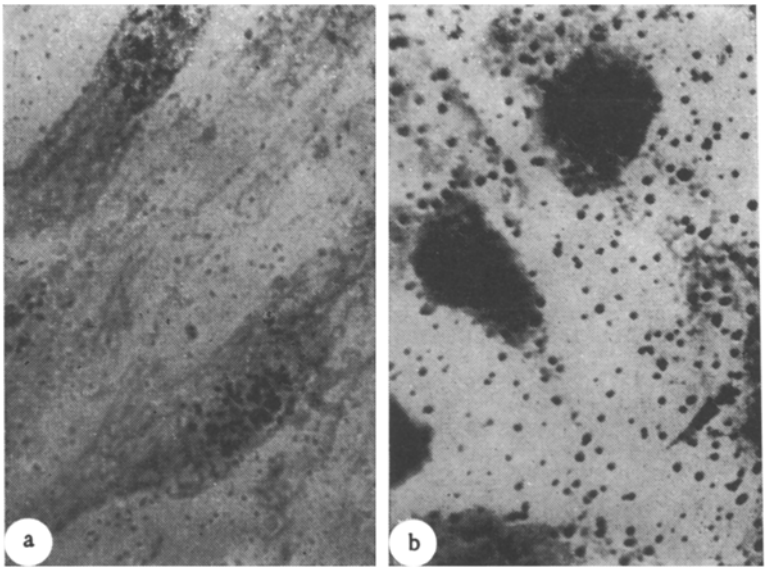

Fig. 2. Incorporation of uridine-H^3 into cells of thick fibroblast, infected with FPV virus: a) three hours after infection; b) after six hours.

After various periods of time, the cells of the tissue culture were placed for 30 min in nutrient medium with an addition of uridine-H^3 (1 μCi per ml of medium). Then the cells were thoroughly washed and fixed in Carnoy's mixture. The preparations were covered with nuclear emulsion of the M-type and developed in an amidol developer after 14-days exposure to darkness at 4° with silica gel, fixed with hyposulfite, stained, and examined microscopically.

RESULTS

The use of radioactive isotopes makes it possible to give a quantitative characteristic of the changes in the RNA synthesis of the cell during virus infection, as well as to observe this process graphically by the method of autoradiography. Since infectious RNA of myxoviruses has not been isolated (communications on this are contradictory), the study of the RNA synthesis of these viruses is accessible primarily to the isotopic method.

It has been shown [4] that the dynamics of the synthesis of virus RNA in thick fibroblasts infected with FPV virus is analogous to the incorporation of P^{32} into the RNA of infected cells in comparison with the uninfected cells. These results give a basis for considering it justified to judge the dynamics of the synthesis of virus RNA in cells according to the incorporation of radioactive metabolites into the RNA of the infected cells.

Figure 1 presents the results of an experiment on the incorporation of adenine-C^{14} into the RNA of infected cells within the limits of one cycle of multiplication of the virus. It may be seen that synthesis of the virus RNA begins in the cellular nucleus between the first and second hours after infection. The maximum virus RNA is synthesized after approximately three hours. These data agree with the results of experiments in which the production of S-antigen of the FPV virus was studied [2].

Evidently almost all the virus RNA is synthesized during the first three hours in the cellular nucleus.

From four hours after infection on, RNA synthesis is ascertained in the cytoplasm of the infected cells, increasing somewhat by six hours after infection. These data may probably be the result of the discharge of newly synthesized virus RNA into the cytoplasm, as well as an increase in the metabolism of the nucleic acids providing for the synthesis of the virus subunits.

Figure 2 shows the results of an autoradiographic investigation of the incorporation of uridine-H^3 into infected cells of a tissue culture. The autographs are visible in the form of black points. A graphic picture was obtained, showing that the radioactive precursor of nucleic acid is detected only in the cellular nucleus during the first three hours after infection, and in the cytoplasm during the subsequent hours.

The data that we obtained on the chemical fractionation and the results of autoradiographic investigations indicate that the RNA of the fowl plague virus is of nuclear origin.

LITERATURE CITED

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