NATURE OF THE INTRACELLULAR INCLUSIONS OF PAROTITIS VIRUS

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After infection of tissue cultures with parotitis virus, intracellular inclusions are formed in them. The greater the infecting dose of virus and the higher the sensitivity of the culture, the more numerous these inclusions. They do not contain specific antigen and are a structure of cellular origin.

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Although the agent of epidemic parotitis has been extensively studied by immunofluoroscence and cytological methods, its reproduction in tissue culture and the nature of the inclusions thus formed have not yet been adequately explained [2-5, 7].

The object of the present investigation was to examine the correlation between reproduction of the virus and formation of the inclusions.

EXPERIMENTAL METHOD

A strain of parotitis virus isolated from a sick child, in monkey kidney tissue culture and then subcultured a further ten times in the same tissue was used. Transplantable lines of cells from human kidneys and monkeys' kidneys (LLC-MK_2), primary tissue cultures from guinea pig and dog kidneys, fibroblasts from chick and duck embryos, grown in test tubes and on slides by introducing cells to the number of 100,000-150,000 into 1.5-2 ml of medium No. 199 with 5-10% calf serum, were infected with parotitis virus in a dose of 10-100,000 TCD_{50}/ml, and reproduction of the virus was subsequently estimated at various times from 6 to 144 h thereafter from the result of the hemadsorption reaction, the development of cytopathogenic changes, and by separate or combined use of a histochemical method and the fluorescent antibody technique.

The quantity of virus in the infected tissue cultures was determined by titration in chick fibroblasts, on the basis of determination of the results of the hemadsorption reaction with a 1% suspension of chicken erythrocytes.

The number of inclusions and luminescent cells was counted and expressed as a percentage of the total number of cells in the culture.

Isolation, purification, and conjugation of the globulins of the guinea pig parotitis antiserum were carried out as described previously [1, 6].

For the cytological examination, cells fixed in Carnoy's mixture were stained with methyl green-pyronine, and for combined staining they were fixed in acetone in the cold and then stained with methyl green-pyronine and the specific conjugate.

The controls were: a) uninfected culture of cells treated with specific conjugate; b) tissue culture infected with parotitis virus and treated with heterologous conjugate (against type 2 parainfluenza virus); c) uninfected culture of cells stained with methyl green-pyronine and treated with ribonuclease for 1 h at 37°; d) tissue culture infected with virus, stained with methyl green-pyronine, and treated with ribonuclease for 1 h at 37°. The stained and treated preparations were examined in the ordinary optical and the ML-2 luminescence microscopes.

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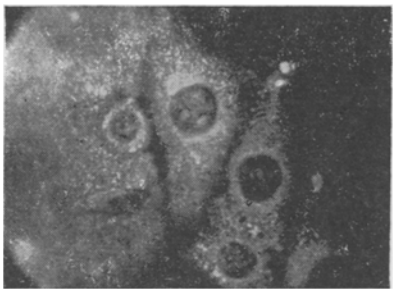

Fig. 1. Culture of LLC-MK_2 cells 48 h after infection with parotitis virus in a dose of 1000 TCD_{50}/ml. Uniform distribution of antigen throughout cytoplasm. Pyroninophilic nonspecific fluorescent inclusions can be seen. Stained with methyl green-pyronine and specific conjugate simultaneously. 630×.

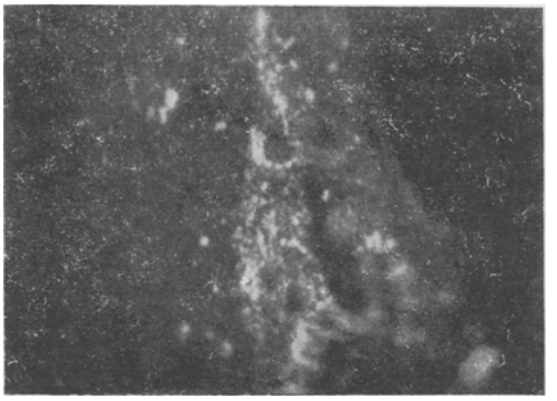

Fig. 2. Culture of chick embryonic fibroblasts 72 h after infection with parotitis virus in a dose of 1000 TCD_{50}/ml. Granular accumulations of specific antigen can be seen, localized mainly in lines in the cytoplasm, and also nonspecific fluorescent pyroninophilic inclusions. Staining and magnification as in Fig. 1.

EXPERIMENTAL RESULTS

The study of the relationship between formation of inclusions in LLC-MK_2 cells and the dose of virus introduced and the sensitivity of the tissue cultures to it showed that inclusion formation is directly proportional to the size of the infecting dose and inversely proportional to the sensitivity of the tissue cultures to reproduction of parotitis virus. Introduction of parotitis virus into a culture of LLC-MK_2 cells in a dose of 1,000-10,000 TDC_{50}/ml led to the regular formation of intracellular inclusions on the first day of incubation. Their number reached a maximum after 2-3 days and then diminished because of destruction of the infected cells.

With a decrease in the infecting dose of virus to 10-100 TCD_{50}/ml, no inclusions could be found even on the 9th day of incubation, despite an adequate concentration of virus (1.9-3.9 TCD_{50}/ml).

The same pattern was observed in a series of tissue cultures differing in their degree of sensitivity to reproduction of the virus, although in less sensitive tissues (duck embryonic fibroblasts and dog kidney cells) the number of inclusions was greater than in highly sensitive cultures (chick embryonic fibroblasts and guinea pig kidney cells), giving maximal accumulation of virus (6.9-7.3 TCD_{50}/ml) in a minimal period of incubation.

Because of the luminescent property of methyl green-pyronine, the histochemical method could be combined with the fluorescent antibody technique.

Optimal quantitative proportions of specific conjugate and methyl green-pyronine for simultaneous staining were 6 : 1. Staining continued for 30 min. For successive staining with methyl green-pyronine and conjugate or conjugate and methyl green-pyronine, the ratio 1 : 1 was essential. The optimal staining time for preparations with methyl green-pyronine was 5-30 min, and with conjugate 30 min.

In the blue rays of the luminescent microscope methyl green-pyronine when used by the combined staining method fluoresces from yellow-orange to red and picks out the cell structure distinctly. Specific fluorescence of an emerald green color is prolonged by the combined staining method until 10 min, enabling the localization and dynamics of morphological changes in the cytoplasm and nuclei of the cells to be assessed simultaneously (Figs. 1 and 2).

With a standard infecting dose (1000 TCD_{50}/ml) immunofluorescent activity of the virus appeared after 14-16 h, before hemadsorption properties and cytopathic changes were present, and when the content of virus in the cell fraction did not exceed 1.3 log TCD_{50}/ml.

The intensity of specific antigen reached a maximum after 2-4 days, when nearly 100% of the cells gave specific fluorescence. On these same days the concentration of virus in the cell fraction also reached its maximum.

Hemadsorption activity of the virus as a rule was detected later than the formation of inclusions, and much earlier than its cytopathogenic action.

Introduction of massive infecting doses (100,000 TCD_{50}/ml) of virus resulted in its more rapid reproduction in the tissue cultures cells and in earlier syncytium formation, but the syncytia discovered after 14-16 h did not contain specific antigen.

Reproduction of parotitis virus in tissue culture, as the results of cytological and immunofluorescence investigations showed, is thus accompanied by the formation of inclusions and by accumulation of antigen not only in the cytoplasm but also in the cell nuclei. Intracellular inclusions discovered in this way do not contain specific antigen and are structures of cellular origin.

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