EFFECT OF HERPES SIMPLEX VIRUS (STRAIN $L2_{K5}$) AND ITS MUTANTS ON MITOTIC ACTIVITY OF CELL CULTURES

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Herpes simplex virus (strain $L2_{K5}$) and its mutants mt_1 and fpp_{10} influence different indices of mitotic activity of PS cultures (a line of transplantable hog kidney cells). Strain $L2_{K5}$ and mutant mt_1 cause the appearance of colchicine-like metaphases and inhibit mitotic activity of the cell culture. Mutant fpp_{10} has a weak colchicine-like action and stimulated mitotic activity in the later periods of the experiment.

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The effect of herpes simplex virus on mitosis of infected cell cultures and on chromosome structure has been demonstrated by several investigators [7, 8, 11-13, 15-17]. In some investigations [2, 7, 10] the similarity between the action of this virus and that of colchicine was emphasized. Reports have also appeared of the colchicine-like action of other viruses included in the herpes group [9,14].

We studied the effect of herpes simplex virus strain $L2_{K5}$ (1st antigenic group) and mutants mt_1 and fpp_{10} obtained from it on the mitotic activity of cell cultures of line PS.

EXPERIMENTAL METHOD

The following strains of herpes simplex virus were used in the investigation: 1) $L2_{K5}$ after five passages through a culture of chick embryonic fibroblasts and cloned three times by the plaque method [3]; 2) mt_1, a mutant of strain $L2_{K5}$ forming "speckled" plaques on a culture of human embryonic fibroblasts, producing intensive syncytium formation in this culture and also in cultures of transplantable cells A_1 and PS [5, 6]: 3) fpp_{10}, a mutant of strain $L2_{K5}$ obtained with the aid of hydroxylamine, having lost its ability to form plaques on a culture of chick embryonic fibroblasts in the absence of DEAE-dextran, and also by the proliferative character of its cytopathic action on PS cells [4].

Two-day monolayer cultures of line PS grown on slides in penicillin flasks were infected. To each flask 0.2 ml of virus suspension containing 1 PFU/cell was added. The infected and control cultures were fixed with Dubosc-Brazil-Bouin mixture 4, 8, 12, 16, and 20 h after inoculation and stained by Unna's method.

Mitotic activity was expressed by the number of dividing cells per thousand cells counted. In each case from 5000 to 10,000 cells were counted. The percentage of pathological forms of mitosis among dividing cells was calculated. The classification of Alov [1] was used with slight additions, and the predominant forms of pathological mitoses were determined. Statistical analysis was carried out by the Fischer-Student method.

EXPERIMENTAL RESULTS

Control cultures of line PS consisted mainly of polygonal cells and were epithelioid in character. An absolute majority of cells in the layer were mononuclear. The number of binuclear cells was 10-15 $^0/_{00}$. Polynuclear cells were much more rarely seen. In PS cultures infected with strain $L2_{K5}$, cells with small intranuclear eosinophilic inclusions appeared by about 16 h. The number of these cells and the size of the inclusions increased appreciably until 20 h. Meanwhile margination of the chromatin took place. In some cells the inclusions occupied nearly the whole of the nucleus, and were separated from the nuclear membrane by a colorless zone. A few conglomerates of round, degenerating cells and a few syncytia also were observed. In cultures infected with mutant fpp_{10}, neither intranuclear inclusions nor syncytia were found.

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TABLE 1. Comparative Study of Mitotic Activity of PS Cultures Infected by Strains of Herpes Virus $L2_{K5}$ and its Mutants mt_1 and fpp_{10} and of Control Cultures (value of P given relative to control at the same period)

Material tested	Mitotic activity (in $^0/_{00}$) at various periods of experiment				
	4 h	8 h	12 h	16 h	20 h
Control (uninfected cultures)	33.6 ± 1.04	37.0 ± 0.79	39.0 ± 1.3	32.0 ± 1.03	41.1 ± 1.23
Cultures infected with strain $L2K_5$	33.3 ± 0.89	21.0 ± 0.62	16.2 ± 0.97	5.0 ± 0.4	9.2 ± 0.6
	P > 0.1	P < 0.001	P < 0.001	P < 0.001	P < 0.001
With mutant fpp_{10}	36.2 ± 1.2	23.8 ± 1.26	41.3 ± 1.29	32.2 ± 1.12	55.6 ± 3.5
	P > 0.1	P < 0.001	P > 0.1	P > 0.1	P < 0.01
With mutant mt_1	–	27.4 ± 1.1	14.6 ± 1.45	13.6 ± 1.25	12.7 ± 1.2
	–	P < 0.001	P < 0.001	P < 0.001	P < 0.01

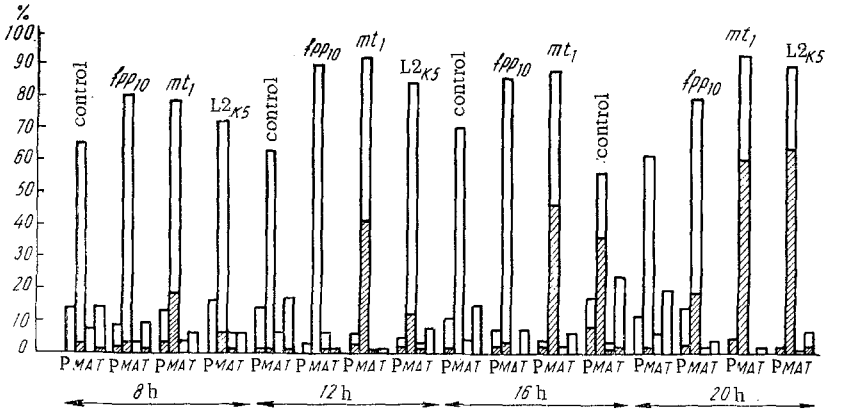

Fig. 1. Relative percentages of phases of mitosis in PS cultures infected with herpes virus (strain $L2_{K5}$ and mutants fpp_{10} and mt_1). P) Prophase; M) metaphase; T) telophase. Shaded part of columns represents percentage of pathological mitoses.

Cytologically speaking, the infected cultures were very similar to the controls. It was only toward 20 h that focal collections of proliferating cells began to appear. In PS cultures infected with mutant mt_1, syncytia began to appear after 12 h, and their number and size continued to increase after 16 and 20 h. The nuclei of cells participating in syncytium formation sometimes contained eosinophilic inclusions; their chromatin structure was coarsened and their nuclei became vesicular in appearance.

As the results given in Table 1 show, strain $L2_{K5}$ of herpes virus and mutants mt_1 and fpp_{10} isolated from it significantly affect the mitotic activity of PS cultures. Strain $L2_{K5}$ and mutant mt_1 inhibited their mitotic activity starting from 8 h after infection. Mutant fpp_{10} in the early periods had no significant effect on this index, but by 20 h after infection marked stimulation of mitotic activity of the infected cultures was observed.

The ratio between the phases of mitosis in infected and control PS cultures is illustrated in Fig. 1. In cultures infected by strain $L2_{K5}$ and mutant mt_1 the proportion of pathological mitoses gradually began to increase after 8 h. The great majority of them were metaphases similar to those produced by colchicine, but in a large proportion of the cells the metaphase chromosomes showed swelling, agglutination, and pulverisation. In PS cultures infected with mutant fpp_{10}, the number of pathological mitoses was much lower. The proportion of abnormal metaphases reached 19% only by 20 h.

The results thus confirm the view expressed in the literature concerning the effect of herpes simplex virus on mitotic activity of infected cultures and also on the colchicine-like action of this virus [2, 7, 9, 10, 14]. It must be emphasized, however, that the number of pathological metaphases (mainly similar to those produced by colchicine) was very high only in the case when mutant mt_1 and the initial strain $L2_{K5}$

were used, producing an early cytopathic effect. It may be postulated that injuries to the achromatin apparatus and chromosomes caused by these two variants of the virus are lethal in a high proportion of cases (correlating with the decrease in mitotic activity and increase in cytopathic action). The fpp_{10} mutant gives a more marked colchicine-like effect. The accumulation of dividing cells in metaphase is not accompanied by growth structural changes in the division spindle and chromosomes incompatible with survival of the cells and their progress into a new interkinesis.

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