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### The Difference in the Cytopathic Changes in FL Cells Infected with Different Strains of Herpes Simplex Virus

It has been shown that the nature of the cytopathic changes induced by herpes simplex virus depends on the strain of virus. Gray *et al.*<sup>1)</sup> differentiated three types of cellular response to infection with this virus and more recently Scott *et al.*<sup>2)</sup> reported on the biological characters of the GC(giant cell) strain and P (proliferative) strain. Similar results were presented by Hoggan *et al.* as well as by us.<sup>3)4)</sup> In the previous report we described two substrains of the Miyama strain of herpes simplex virus, which were distinguishable by their effects on FL cells.

This report describes another variant of the strain. This new substrain was obtained by the limiting dilution method, using the viral sample which since isolation had been passaged serially twice on FL cells, 5 times on HeLa cells, 36 times on FL cells and 21 times on L cells. The cytopathic changes produced on FL monolayers by this new variant are agglutination and fusiform development of infected cells.

At a high viral dose of  $3 \times 10^7$  TCID<sub>50</sub> per 2 ml on a monolayer having  $4.9 \times 10^6$  cells, the first change of the infected cells appeared to be cohesion and flattening. One or two days after infection, many cells looked fusiform, although round refractile cells were also observed among them. As infection proceeded, the fusiform cells became more elongate and did not form syncytia. Figs. 1 and 2 show the cytopathic effects on FL monolayers 3 days after infection. In Figs. 3 and 4 other cultures are shown, which were inoculated with about the same amount of strain -GC virus and incubated for the same period as the cultures exposed to the new substrain. In the latter cultures almost all cells look rounded and this type of cytopathic change has been recognized as a characteristic induced by the -GC strain, as described in the earlier report. With the new substrain, many infected cells became fusiform although some round cells were always seen among them. This conspicuous pattern of cellular morphology in the monolayer could be seen at any time after the cytopathic change had appeared. The number of round cells did not decrease when purer viral material, purified by the limiting dilution method, was used.

The morphology of the microscopic plaques was also found to be as reported by Farnham.<sup>5)</sup> At high dilutions of the virus, cytopathic effect became visible by the second day and a feature of the foci was clusters of agglutinated cells.

It was sometimes difficult to differentiate plaques induced by this substrain from those formed by the +GC virus, although syncytia were never formed in the former plaques.

FL cells were infected with high multiplicity inputs of the -GC and the new

substrain of virus and the number of cell nuclei of replicate cultures were counted at various times after infection. Neither strain induced proliferation of infected cells and this result is different from the data reported by Gray *et al.*

Now we have obtained three substrains of the Miyama strain of herpes simplex virus, which are distinguishable from each other morphologically on FL monolayers; one produces giant cells, another causes rounding of infected cells and the third causes to become fusiform. These strains are designated as strain +GCl, -GCr and -GCf.

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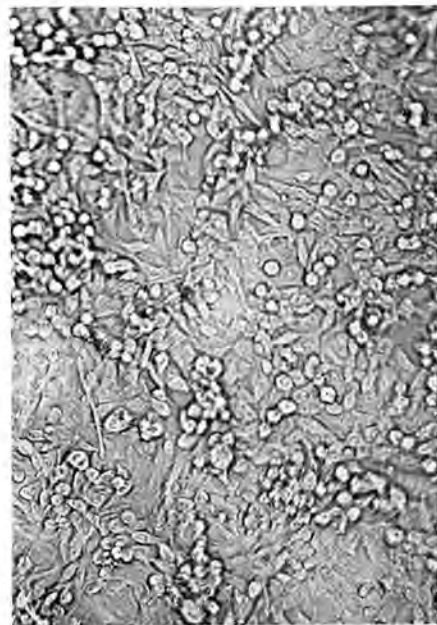
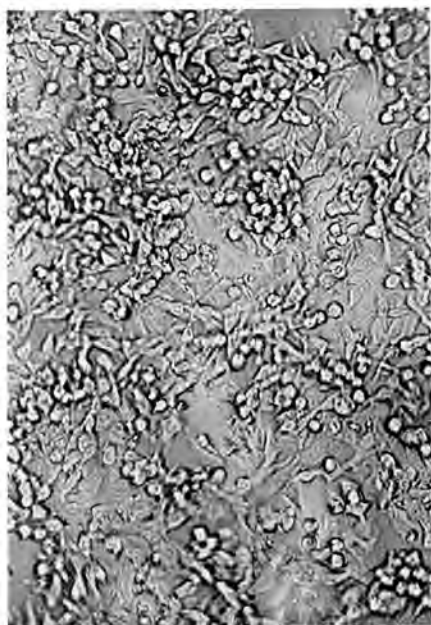


Fig. 1, Fig. 2. FL cells infected with the —GCf Miyama strain of herpes simplex virus ( $\times 120$ )

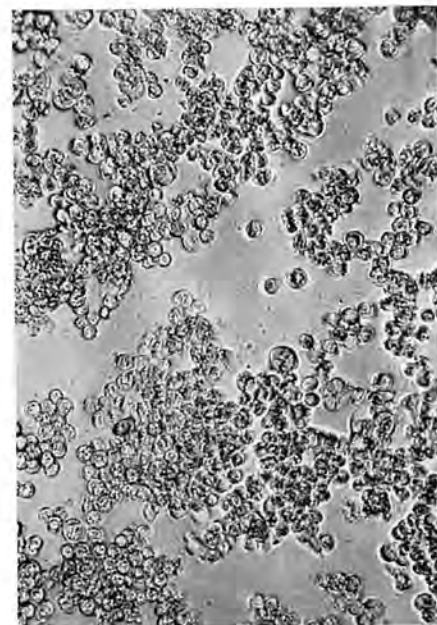
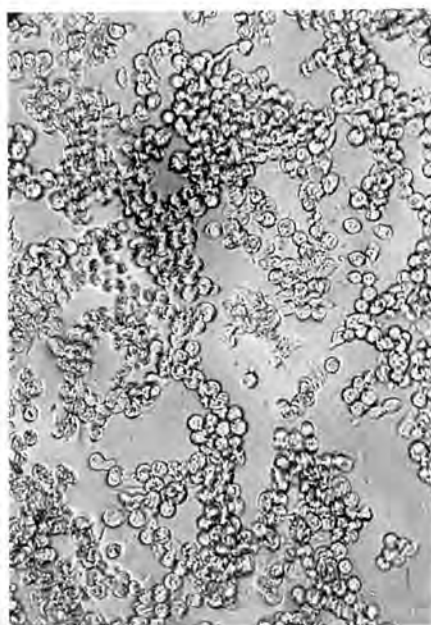


Fig. 3, Fig. 4. FL cells infected with the —GCr Miyama strain of herpes simplex virus ( $\times 120$ )