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SHORT COMMUNICATION

PLAQUE FORMATION BY THREE VARIANTS OF THE MIYAMA STRAIN OF HERPES SIMPLEX VIRUS[1]

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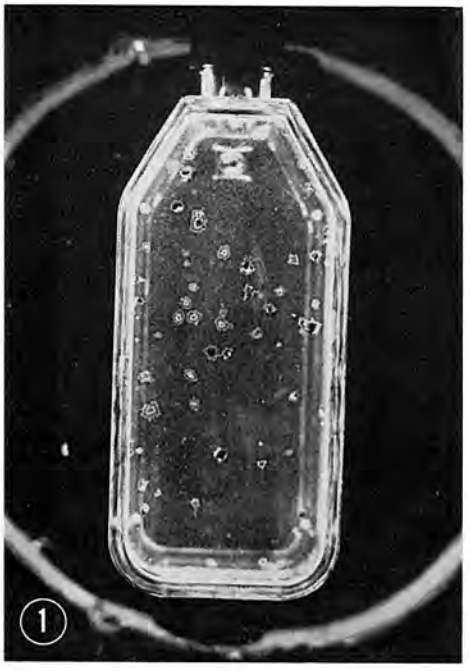
(Received September 16, 1969)

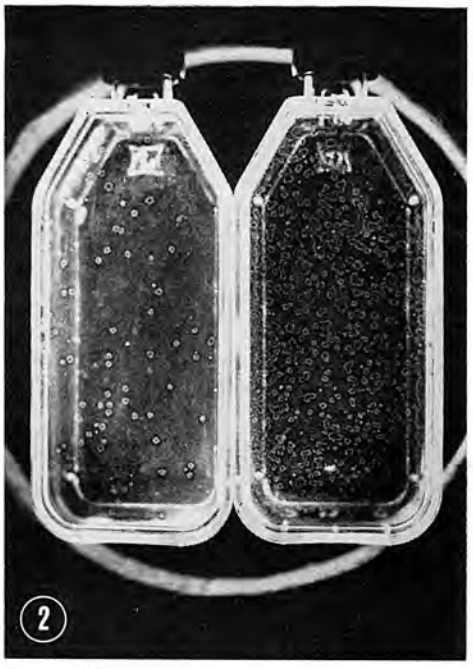
Differences in the cytopathic changes in tissue culture cells infected with different strains of herpes simplex virus have been reported by many investigators (Gray et al., 1958; Hoggan and Roizman, 1959; Nii and Kamahora, 1961a and 1961b; Kohlhage and Siegert, 1962; Munk and Donner, 1963 and Wheeler, 1964). The first report of Gray et al. (1958) was concerned with three types of cellular response to infection with this virus while all other reports, except one of Kamahora and myself, were on two virus strains, namely one forming syncytial giant cells and the other inducing cell rounding of single cells. Reports of Kamahora and myself described the isolation of a further strain causing agglutination and fusiform development of cells (Nii and Kamahora, 1961b) and and details of the cytopathic changes induced by the three variants of the Miyama strain (Nii and Kamahora, 1961c). The genetical interrelations among those variants were also discussed (Nii and Kamahora, 1962). The present report describes plaque formation by these variants correlated with the behaviour of the infected cells.

Viral samples of the the three variants of the Miyama strain, designated as +GC, $-GC_r$ and $-GC_f$, were obtained from the supernatants of FL cell cultures infected with one of these variants. The samples were serially diluted ten fold and a 0.2 ml aliquot of each was inoculated onto two to three BSC_1 monolayer sheets prepared in 30 ml Falcon plastic bottles. The BSC_1 cell line was used because herpes simplex virus forms larger plaques in this cell line than in several cell lines of FL, L, VERO and BHK_{21}. In these plaque experiments a liquid overlay method was employed using approximately 0.25% human γ globulin (NBC) in 199 medium supplemented with 5% calf serum. After one hour's adsorption period medium containing the antibody was added to the monolayer sheets.

Two days later the plaques of 2 to 3 mm diameter produced by the +GC variant were clearly observed, some appearing as two irregular concentric circles. Plaques produced by the $-GC_f$ and $-GC_r$ variants were still pinpoint size although the ones formed by the $-GC_f$ variant were slightly the larger. In the next couple of days the plaques formed by the +GC variant increased in size and severely affected areas of infected cells became detached from the bottle wall. Five days later most of the plaques of the $-GC_f$ variant were about 1 mm in diameter (Fig. 2) while those

1 This work was presented at the Annual Meeting of the Society of Japanese Virologists, in Fukuoka, on October 11, 1968.





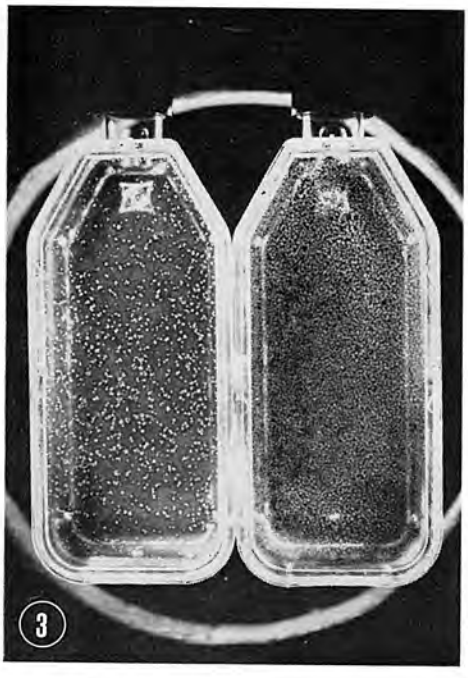


FIGURE 1. *Plaques produced by the +GC variant of the Miyama strain on a BSC_1 monolayer in a 30 ml Falcon plastic bottle. Two days after virus inoculation.*

FIGURE 2. *Plaques produced by the $-GC_f$ variant of the Miyama strain on BSC_1 monolayers prepared in 30 ml Falcon plastic bottles. Five days after virus inoculation.*

FIGURE 3. *Plaques produced by the $-GC_r$ variant of the Miyama strain on BSC_1 monolayers prepared in 30 ml Falcon plastic bottles. Five days after virus inoculation.*

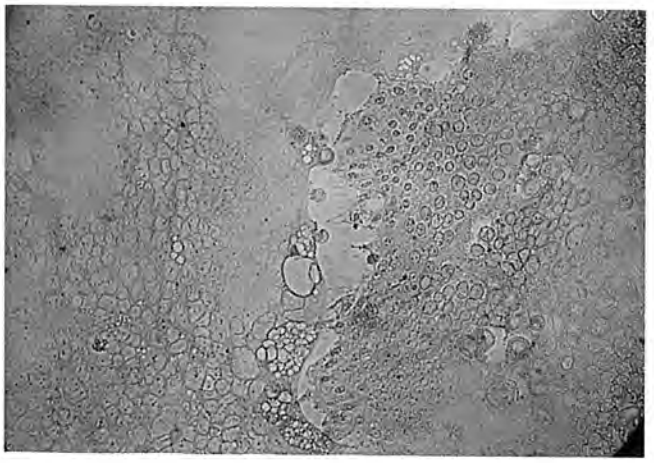


FIGURE 4. *Part of a plaque formed by the +GC variant of the Miyama strain.* ×120

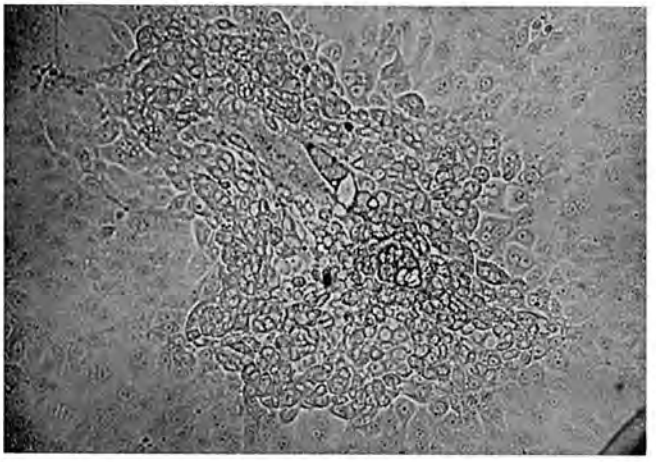


FIGURE 5. *Plaque produced by the $-GC_t$ variant of the Miyama strain. Non-proliferative type of CPE is shown.* ×120

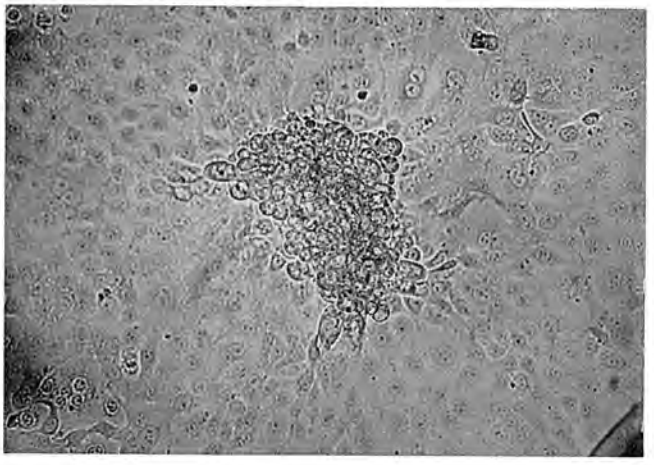


FIGURE 6. *Plaque produced by the $-GC_r$ variant of the Miyama strain. Proliferative type of CPE is shown.* ×120

of $-GC_r$ had enlarged slightly but were still pinpoint size (Fig. 3). Thus, the +GC, $-GC_f$ and $-GC_r$ variants produced plaques of large, medium and small size, respectively.

These plaques on BSC_1 monolayers were examined under a light microscope. Fig. 4 shows part of a plaque produced by the +GC variant with marked fusion of infected cells. The gap seen dividing the photograph into two parts is presumably formed by contraction of syncytial cell masses. The left part corresponds to a marginal portion of the plaque and contains cells in a rather early stage of infection while the right part is an area near or at the center of the plaque and the fused cells in this area are in the most advanced stage of infection, many nuclei being pyknotic. These outer and inner parts of the plaques with a gap between explains the macroscopic appearance of two concentric circles.

A plaque formed by the $-GC_f$ variant is shown in Fig. 5 with non-proliferative type cytopathic changes while Fig. 6 shows a proliferative type of lesion caused by the $-GC_r$ variant (Gray et al. 1958).

The appearances of plaques produced by the three variants in FL and VERO cell lines were also examined. The plaques of these variants on monolayers of these cells were in all cases slightly smaller but were of the same relative order of size: $+GC > -GC_f > -GC_r$.

These data indicate that viral spread of the three variants on monolayer cells is much influenced by the interactions between infected and non-infected cells. The +GC variant actually causes fusion between infected and non-infected cells and capsid particles with a core containing infectious DNA or macromolecules with viral infectivity can stream through the fused cytoplasms. The possibility of this rapid spreading of virus infection through the cytoplasms of fused cells explains why the largest plaques are formed by the + GC variant. The $-GC_f$ variant endows infected cells with an adhesive character. Although formation of giant cells was not induced by the virus, it is conceivable that interaction of surface membranes of infected and non-infected cells is comparatively frequent with fusion of microvilli of infected and non-infected cells enabling capsid particles to stream into the latter. The $-GC_r$ variant induced the least interaction between infected and non-infected cells and between neighbouring infected cells and infection proceeded very slowly in monolayers inoculated with a low virus input.

These three kinds of reaction of infected cells with the three variants of the Miyama strain are probably caused by different modifications of the cellular membranes on infection with these variants. Recently, Ejercito et al. (1968) classified established strains as well as freshly isolated strains of herpes simplex virus into four groups depending on their effects on infected Hep-2 cells. However, there have been no reports on the isolation of three variants of a single original strain of herpes simplex virus except those of Kamahora and myself on the Miyama strain. The existence of these three kinds of variant suggests some genetical interrelations between the strains and at least one direction of variation from $-GCr$ to +GC seems possible (Nii and Kamahora, 1962; Nii, unpublished data).

Besides the variants of the Miyama strain, three variants of the freshly isolated Iwa strain causing different sized plaques and different cytopathic effects were obtained. The correlation between the plaque sizes and cytopathic effects of the variants of the latter strain were essentially similar.

Conclusion: Comparative studies were made on the plaques formed by three variants of the Miyama strain of herpes simplex virus. The +GC varaint caused syncytial formation and produced the largest plaques, the $-GC_f$ variant caused fusiform development and adhesion of infected cells and induced plaques of medium size while the $-GC_r$ variant caused rounding of infected cells and small plaques of pinpoint size.

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