Dual Infection of Single Cells with Ectromelia and Herpes Simplex Virus

Many instances of dual infections of host cells have been reported.[1,2,3] Double infections of single cells in tissue culture preparations have also been studied[4]

This report is on the dual infection of FL cells with ectromelia virus and herpes simplex virus.

The Hampstead strain of e. v. (ectromelia virus) was used throughout the present work. The whole liver of an infected mouse was ground in a glass mortar with 3-4 ml of Hanks' BSS and centrifuged for 10 minutes at 2,500 rpm. The supernatant was used as the source of virus and contained about 10^8 $TCID_{50}$.

A strain of h. s. v. (herpes simplex virus) was isolated on FL monolayers in this laboratory on the 2nd of Aug. 1958 from the vesicle fluid of a patient with herpes labialis. A neutralization test was used for identification with rabbit antiserum obtained from Dr. Yoshino. This strain was designated as the Miyama strain. To the present, more than 40 serial passages have been made on FL monolayers in 200 ml prescription bottles containing about 5×10^6 cells. This strain produced small pocks on the chorioallantoic membrane and 10 passages have been made on this membrane. Intracerebral inoculation into adult mice caused convulsions and death. Seven passages have been made intracranially. The cytopathogenic effects of this strain on FL monolayers are severe. When the destructive effect of the monolayers became severe, the culture fluids were collected and centrifuged at 2,500 rpm for 10 minutes. The supernatant contained about $10^8 - 10^7$ $TCID_{50}$ and was used as the viral sample.

FL monolayers for morphological observation of infected cells, were prepared on 10×50mm coverslips in tubes or in 50ml square bottles. The nutrient medium was a mixture of 0.5 per cent lactalbumin hydrolyzate in Earle's solution (L-E), and 15 per cent bovine serum.

Viral samples of e. v. and h. s. v. were mixed in a ratio of 1 : 1 and used for experiments on dual infection. The unmixed samples of e. v. and h. s. v. were diluted in a ratio of 1 : 1 with L-E medium so as to have the same $TCID_{50}$s as the mixtures.

For plating of virus, the nutrient medium was removed from the tube. Two ml of viral sample were inoculated into the tubes and incubated at 37°C. Two hours later the inoculating fluid was removed, and the infected cells were washed twice with L - E medium, and then mixed with the nutrient medium. 17 hours after viral inoculation, the coverslips were washed twice with PBS, fixed with Bouin's fluid and stained with Harris' hematoxylin and eosin stain.

Coverslips infected only with e. v. are shown in Figs. 1 and 2. All the cells contained inclusion bodies. Formation of two kinds of inclusion bodies in cells infected with e. v. has been extensively studied in this laboratory.[5,6,7] Figs. 1 and 2 show many round "A" type bodies (the so called Marchal bodies) which stained densely with eosin, and also "B" type inclusion bodies which are diffusely

located in the cytoplasm and stained slightly with hematoxylin.

The development of intranuclear inclusion bodies in FL cells infected with the Miyama strain of h. s. v. was found to be as described by Scott *et al.*[8] Infected cells, 17 hours after inoculation of h. s. v., are shown in Figs. 3 and 4. All cells contained intranuclear inclusion bodies. Some nuclei show shrunken type "A" inclusions surrounded by an unstained "halo" and some fully formed complete inclusions with marginal chromatin.

The following observations were obtained on cells infected with mixed viral samples. Some cells contained only the intranuclear inclusion bodies of h. s. v. or only the intracytoplasmic inclusion bodies of e. v. A very few cells contained the inclusion bodies of both viruses in a single cell (Fig. 5). In still other cells it was difficult to distinguish what type of inclusions, if any, there were. Polynucleated giant cells often had nuclei containing the Cowdry "A" type bodies of h. s. v. and some apparently intact nuclei, while Marchal bodies (designated as "A" bodies by Kato *et al.*) and "B" bodies of e. v. were observed in the cytoplasm. (Fig. 6)

To see if one virus interferes with the other in the dual infection, the following experiments were performed. H. s. v. were inoculated 3 hours before e. v. infection. 12 hours after e. v. inoculation, the coverslips were fixed with meth anol, stained with Giemsa solution and the "B" type inclusion bodies counted. 97.9 per cent of the cells infected only with e. v. contained inclusion bodies. However only 8.0 per cent of the cells which were infected with h. s. v. before e. v. inoculation had inclusion bodies in them, and many of the inclusions were rather compact in form. The above data shows that the immunologically unrelated viruses, e. v. and h. s. v. are able to initiate infection in a single cell but complete propagation and development of inclusion bodies of both viruses in a single cell seems to depend upon delicate timing and dosage of the two agents.

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inclusion body. *J. Immunol.* **71**, 385–396.

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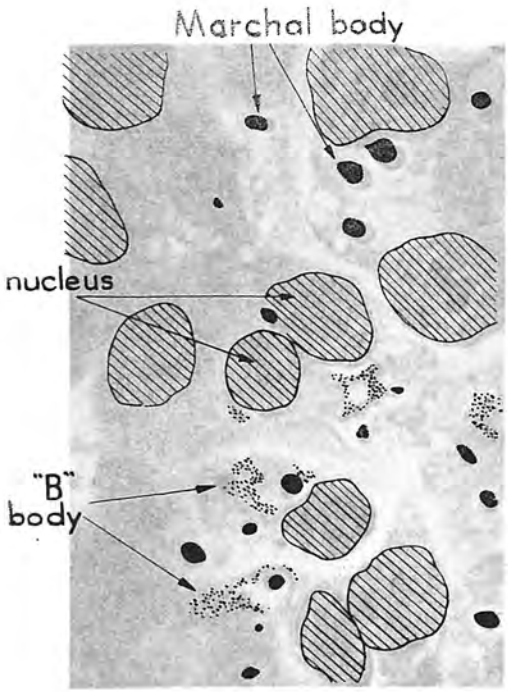


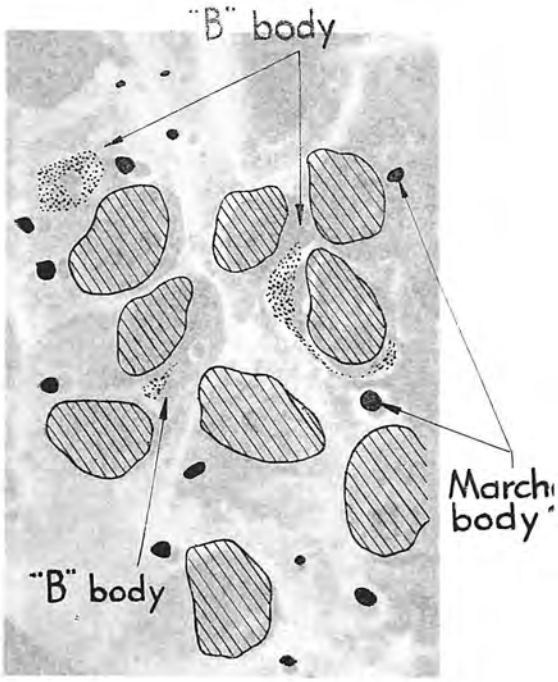
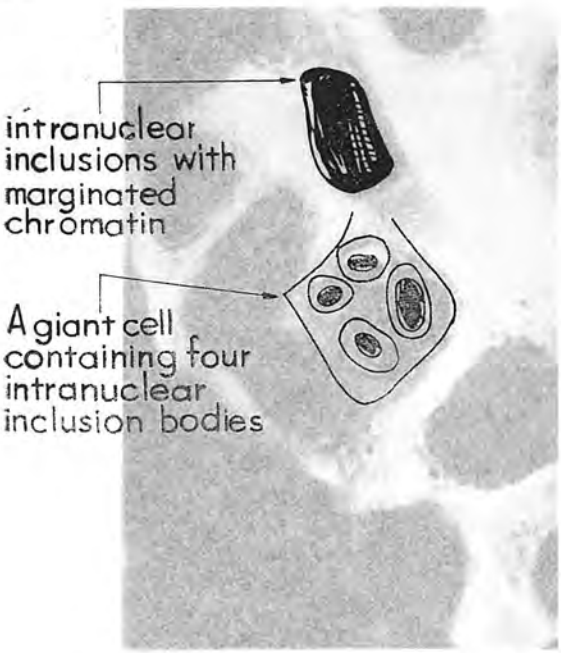


Fig. 1, Fig. 2. FL cells infected with actinomella virus.
Marchal bodies (like "B" bodies) (cf. Kato et al.)



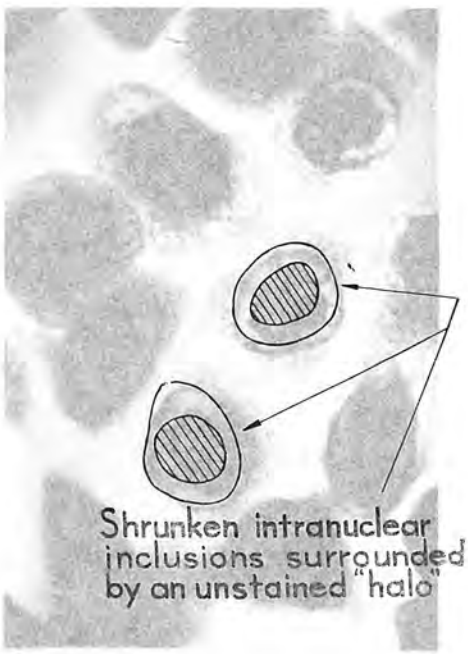
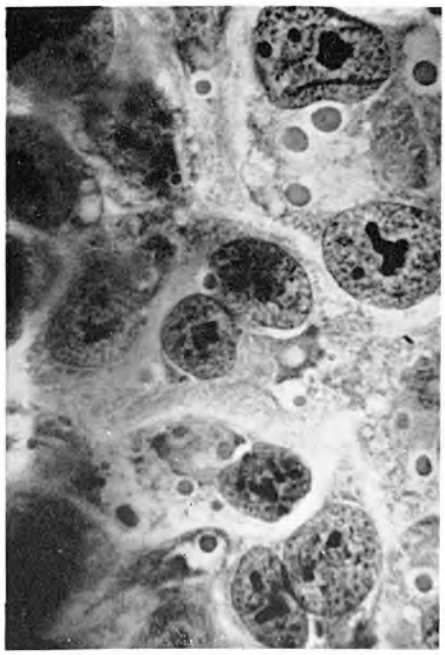


Fig. 3, Fig. 4. FL cells infected with herpes simplex virus.
all cells have intranuclear bodies



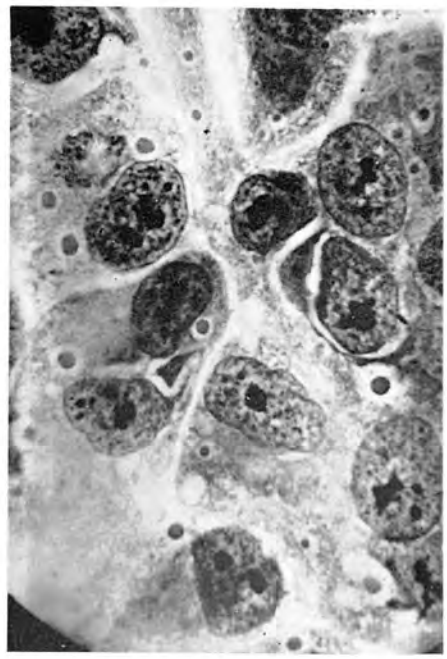
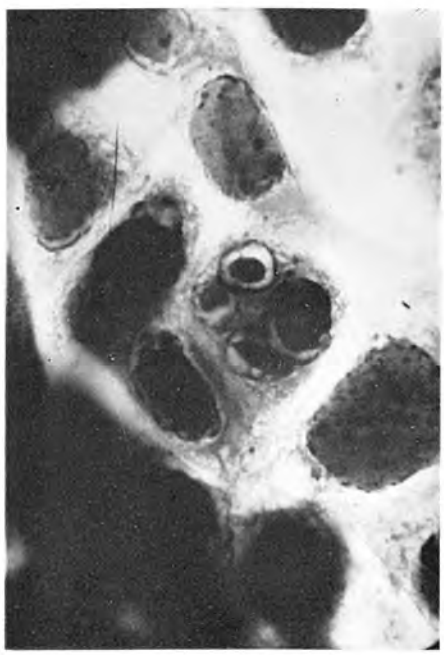
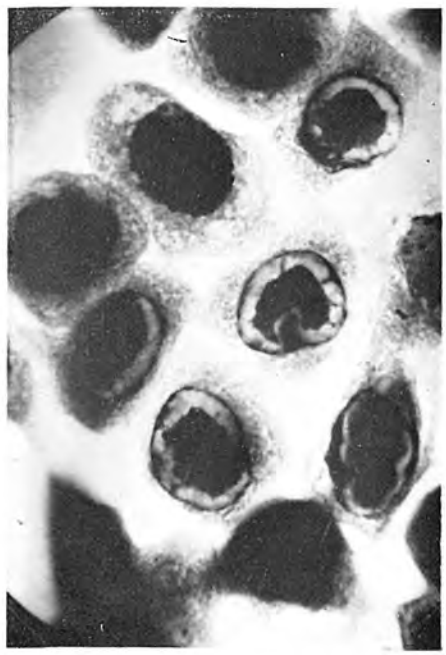


Fig. 1, Fig. 2. FL cells infected with ectromelia virus.
Marchal bodies (the "A" bodies of Kato *et al.*)





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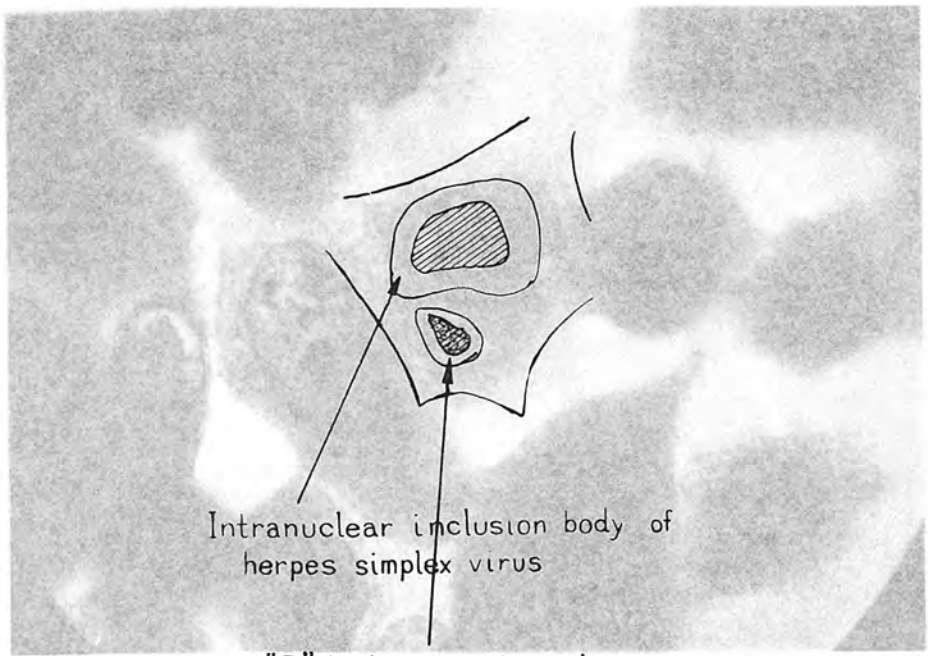


Fig. 5. FL cells infected with adenovirus and herpes simplex viruses. Intranuclear inclusions of herpes simplex virus and intracytoplasmic inclusions of ectromelia virus are shown.

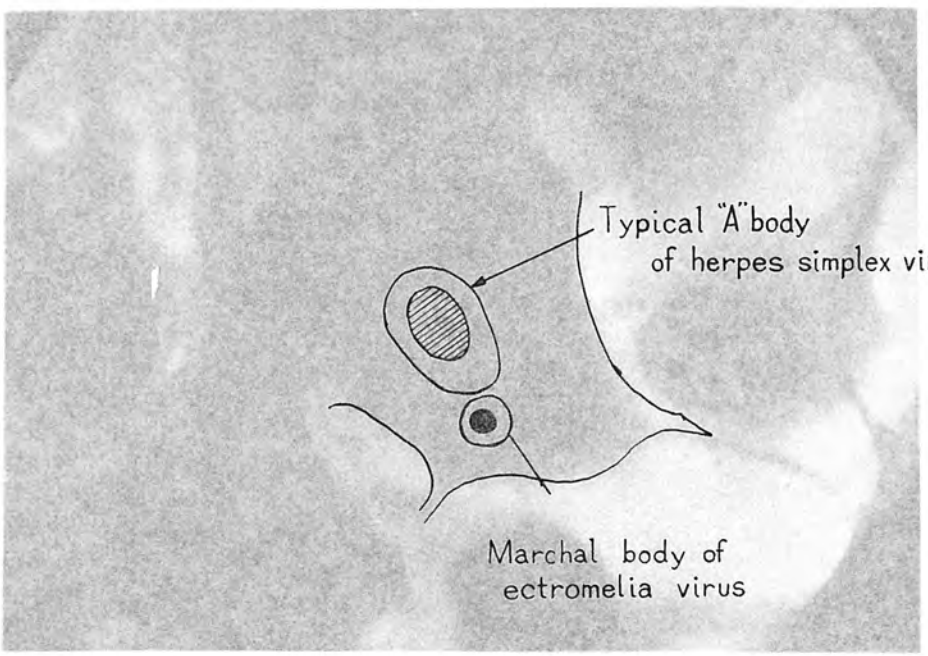


Fig. 6. Polynucleated giant cells containing intranuclear inclusion body of herpes simplex virus and Marchal body of ectromelia virus.

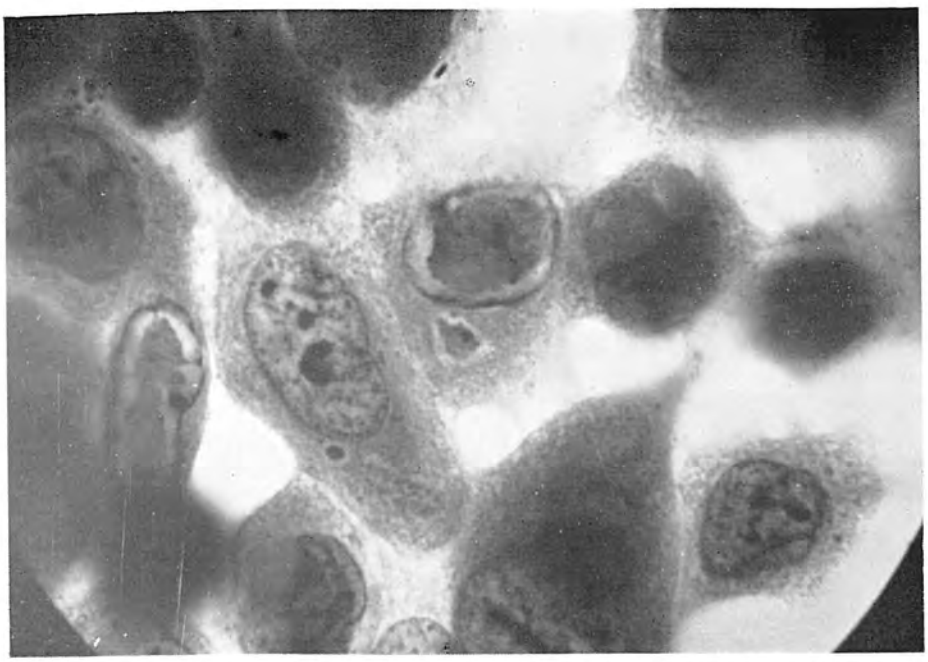
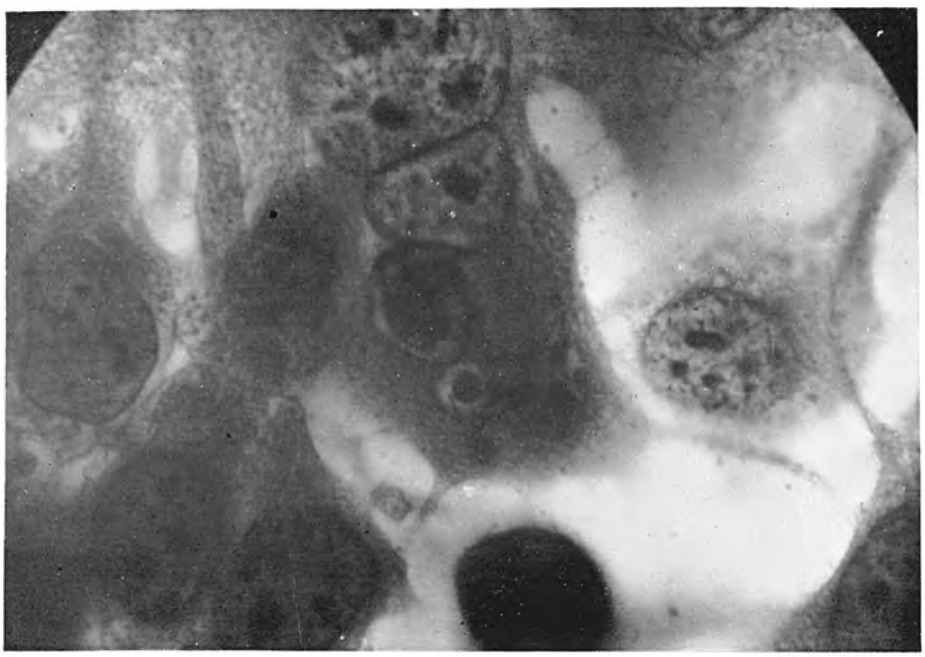


Fig. 5. FL cells infected with a mixed sample of ectromelia and herpes simplex viruses. Intranuclear inclusions of herpes simplex virus and intracytoplasmic inclusions of ectromelia virus are shown.



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