A Study of Herpes Simplex Virus Infection of FL Cells Using Autoradiography *, **

Since Lipschütz found intranuclear inclusion bodies in cells infected with herpes simplex virus, many cytochemical studies have been made on them.[1,2,3] Lebrun reported the appearance of herpes virus antigen in infected cells using fluorescent antibody staining, and considered the findings corroborated cytochemical studies.[4]

Studies on other DNA type viruses have been made using autoradiography. In this laboratory the incorporation of H^3-thymidine into the "B" type inclusions of pox viruses has been reported by Kato *et al.*[5,6]

This paper is a preliminary report of cytological changes of FL cells after herpes simplex virus infection, studied by autoradiography.

A strain (+GC Miyama) of herpes simplex virus isolated from a patient on a FL monolayer was used for these experiments.[7,8] It had been serially passaged and when the cytopathic effect on FL cells was advanced, the supernatant fluid of the culture contained about 10^8 $TCID_{50}$/ml.

FL monolayers were prepared on 10×50 mm coverslips in square tubes. The growth medium was composed of 90 parts of Earle's saline containing 0.5 per cent lactalbumin hydrolyzate and 10 parts of bovine serum.

Tritiated thymidine (4.4 C/mM) was used at a level of 1 μC/ml. The infected cells on coverslips were washed with saline, fixed with methanol and dried. Autoradiographic -stripping plate AR 10 (Kodak, England) was applied and the dry coverslips were stored in sealed boxes in the dark for one week. Then the autoradiographs were developed.

For plating the virus, the growth medium from the tube with a monolayer on a coverslip, was replaced by 2 ml of virus sample and the tube incubated at 37°C for 1 hour. H^3-thymidine in the medium was introduced for a duration of 2 or 3 hours at various times after infection. At the end of the exposure period the medium was removed and the coverslips were washed with saline and fixed.

As shown in Figs. 1 and 2 the distribution of silver grains was characteristic in the infected cells even in earlier stages of infection. The cells shown in these figures were exposed to the medium containing H^3-thymidine for a duration of 3 hours in the early stage after infection.

Autoradiographs of control cells had diffusely distributed silver grains in correlation with the chromation network in the nuclei, while infected cells showed concentrated incorporation of H^3-thymidine which appeared like a ring or an island of silver grains in the nuclei in autoradiographs.

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The infected cells which were exposed to H^3-thymidine for 2 hours four hours after infection, showed 95 per cent incorporation of thymidine, while in control cells 40 per cent was incorporated.

The site of the silver grains was not associated with the nucleolus. It is of interest to note that we could see definite spots of silver grains in the nucleus before any remarkable morphological changes such as homogeneous body or Cowdry A type inclusion formation were observed by usual staining method.

Further details of this work will be published later in the Biken's Journal.

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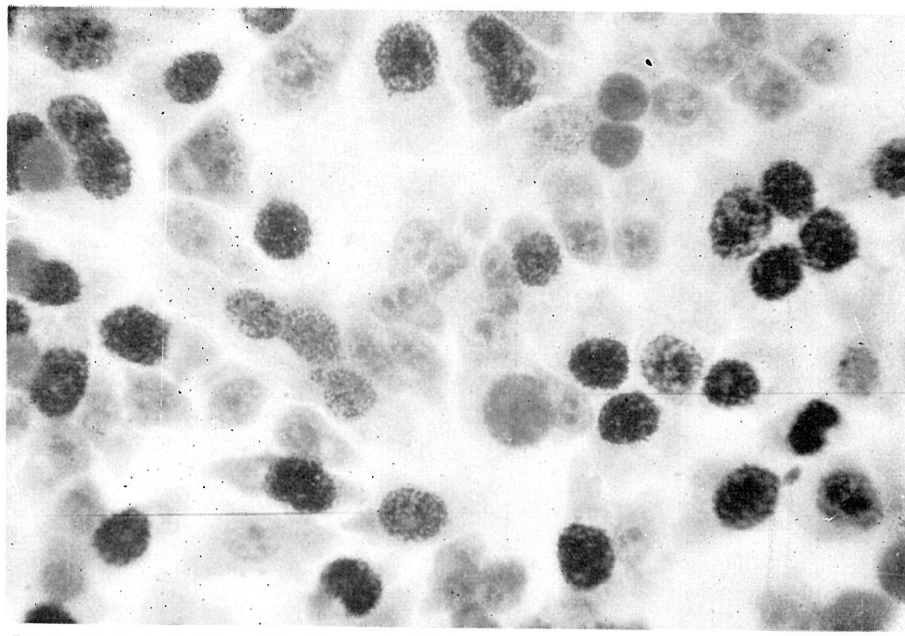

Fig. 1. Control cells labeled for 3 hours with H^3-thymidine. Some cells are labeled while others are not. A diffuse distribution of silver grains is shown in the nuclei. ×1000

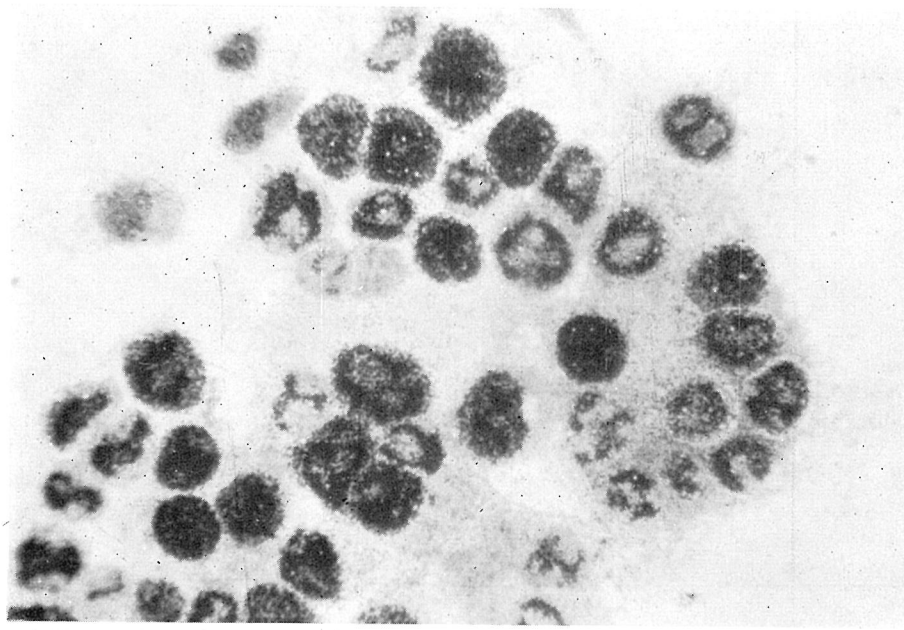

Fig. 2. Infected cells labeled for 3 hours, in the early stage after infection. Almost all cells are labeled. Silver grains in the nuclei show a peculiar distribution and have a ring or "S" shaped form.
At this stage of infection, breakdown of the cytoplasmic membrane is shown. This will finally lead to syncytium formation. ×1000