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Preliminary report

Autoradiographic Studies on Rabbit Corneal Cells Infected with Herpes Simplex Virus

The first autoradiographic studies on herpes infected cells were reported by Nii *et al.* (1960, 1961), using a cultured cell line (FL). A characteristic pattern of incorporation of ^{3}H-thymidine into the nuclei was recognized in the earlier stage of infection, indicating that the DNA synthesis of herpes virus begins in the nucleus. Recently, a similar report has appeared by Munk *et al.* (1964). Hanna and Wilkinson (1964) described the uptake of ^{3}H-thymidine in the rabbit corneal epithelium infected with herpes virus. These reports are especially interesting, if we compare them with the data obtained with poxvirus which contains DNA and multiplies in the cytoplasm. Extensive studies on the autoradiography of cells infected with poxvirus have been carried out using cultured cells (Kato *et al.* 1960), mouse tissue (Kato *et al.* 1963) as well as rabbit cornea (Hara *et al.* 1964). One of the points they emphasized is the dissociation between host nuclear DNA synthesis and viral cytoplasmic DNA synthesis, that is, viral cytoplasmic DNA synthesis can be initiated regardless of host nuclear DNA synthesis.

This work was to show the occurrence of intranuclear viral DNA synthesis in rabbit corneal epithelial cells and endothelial cells which show little or no DNA synthesis under physiological conditions.

A strain of herpes simplex virus (-GCr Miyama) was used for these experiments (Nii and Kamahora, 1961). The supernatant fluid of infected FL cell cultures used for inoculation and it contained about 10^{8} $TCID_{50}$ /ml.

Virus was administered in two ways. The corneas of adult albino rabbits were scratched with the tip of a needle. A drop of the virus suspension was then put on the corneal surface, and the eyelids were closed and rubbed for a while. The other way was as follows. Two tenth ml of chamber fluid was withdrawn from the limbus with a needle. Then the same quantity of virus suspension was inoculated intracamerally. Control rabbits were treated in the same way, except that normal saline was used in place of the virus sample. The former route was used to study infected epithelium and the latter to study infected endothelium.

One or three days after virus inoculation, 10μC per 0.1 ml of ^{3}H-thymidine was injected intracamerally. Two hours later the eye balls were enucleated. Smear preparations of the corneal epithelium and stretch preparations of the corneal endothelium were fixed with methanol. Dipping autoradiography with liquid emulsion NR-M1 (Sakura) was carried out according to the technique described previously (Kato *et al.* 1963). After dipping, preparations were kept in light-free boxes at 4°C for 4 weeks. After development and fixation, the preparations were stained with Giemsa solution.

Macroscopically the infected cornea of the rabbit became opaque and showed dendritic ulcer within 2 day after virus inoculation. Two weeks later only haziness of the cornea was observed.

Autoradiographically, the incorporation of ^{3}H-thymidine into the infected epithelial cells of the cornea were found exclusively in the nucleus and not in the cytoplasm. Grains were found in localized areas of the nucleus (Figs. 1 and 2). Marginated chromatin was generally free from silver grains. This peculiar pattern of distribution of silver grains is identical with that found in FL cells (Nii *et al.* 1961) but differs from the rather diffuse distribution of silver grains in the normal nucleus, though labeled nuclei were rarely encountered in the epithelium under physiological conditions.

Silver grains appeared in the nuclei of many infected endothelial cells of the cornea. Their distribution was similar to that in the infected epithelial cells (Figs. 3, 4 and 5). In control autoradiography experiments on noninfected cornea with ^{3}H-thymidine, no labeled nuclei were found in the endothelial cells.

These findings show that DNA synthesis of herpes virus is initiated in localized areas of the nuclei of epithelial and endothelial cells of rabbit cornea. Herpes simplex virus can initiate DNA synthesis in the nucleus of cells which show little or no DNA synthesis under physiological conditions, just as poxvirus initiates its synthesis in the cytoplasm.

REFERENCES

Hanna, C. and Wilkinson, K. P. (1964). Uptake of tritium-labeled thymidine in the rabbit cornea infected with herpes simplex. *Exp. Eye Res.* **3,** 36-41.

Hara, J., Mizukawa, T. and Kato, S. (1964). Nucleocytoplasmic interaction in poxvirus-infected cells. II. Relationship between nuclear DNA and inclusion in rabbit cornea. *Biken J.* **7,** 57-63.

Kato, S., Kameyama, S. and Kamahora, J. (1960). Autoradiography of cells infected with variola and cowpox viruses with ^{3}H-thymidine. *Biken J.* **3,** 183-189.

Kato, S., Aoyama, Y. and Kamahora, J. (1963). Autoradiography of the tissues of mice infected with ectromelia virus using ^{3}H-thymidine. *Biken J.* **6,** 9-16.

Munk, K. and Sauer G. (1964). Relationship between cell DNA metabolism and nucleocytoplasmic alterations in herpes virus-infected cells. *Virology* **22,** 153-154.

Nii, S., Kato, S., Kameyama, S. and Kamahora, J. (1960). A study of herpes simplex virus infection of FL cells using autoradiography. *Biken's J.* **3,** 355-356.

Nii, S., Kato, S., Kameyama, S. and Kamahora, J. (1961). Further studies on herpes simplex virus infection of FL cells using autoradiography. *Biken's J.* **4,** 51-58.

Nii, S. and Kamahora, J. (1961). Cytopathic changes induced by herpes simplex virus. *Biken's J.* **4,** 255-270.

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EXPLANATION OF FIGURES

Figs. 1 and 2 Smear preparation of epithelial cells (3 days after herpes simplex virus inoculation). Grains are located in the nuclei but not on the marginated chromatin.

Figs. 3, 4 and 5 Flat preparation of endothelial cells (24 hours after herpes simplex virus inoculation). Note grains accumulated in localized foci of the nuclei.

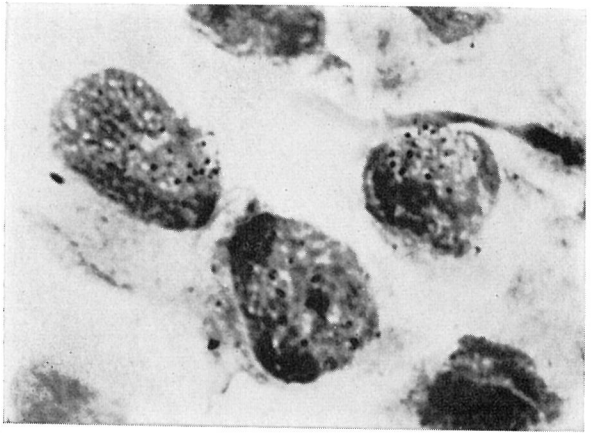

Fig. 1

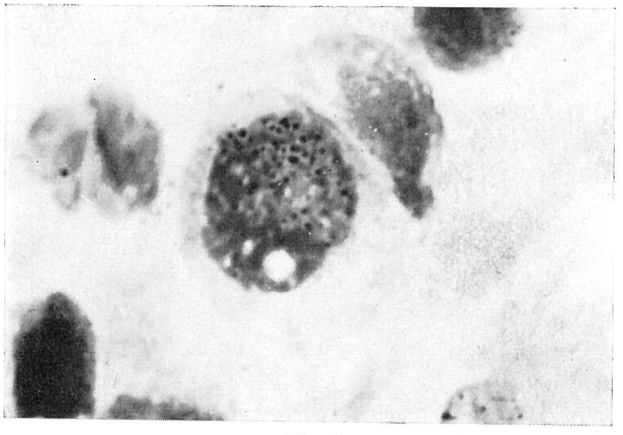

Fig. 2

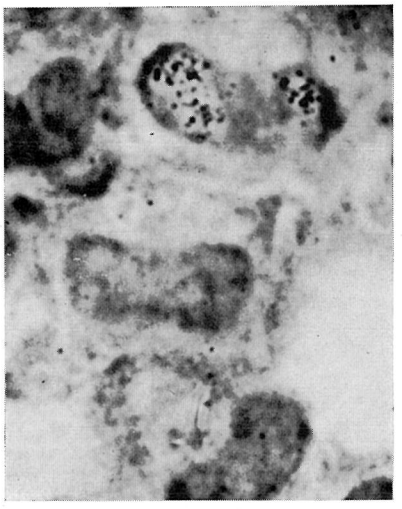

Fig. 3

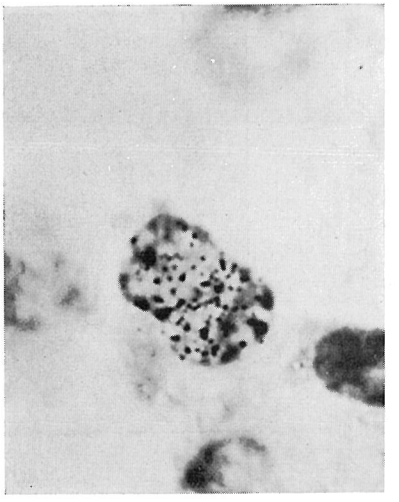

Fig. 4

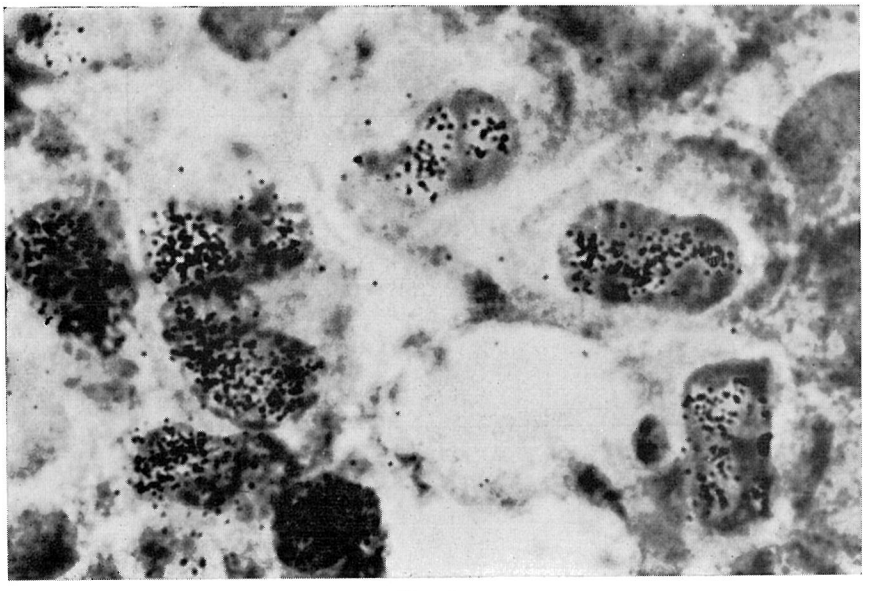

Fig. 5