BIKEN JOURNAL
Preliminary Report

Localization of RNA and DNA Synthesis in Measles Virus-Infected Cells

The significance of cytoplasmic inclusions in cells infected with measles virus has not yet fully clarified with regard to virus multiplication. Toyoshima *et al.* (1960) reported that perinuclear cytoplasmic inclusions appearing in the early stage of infection were rich in RNA and considered them to be the site of virus multiplication. Conversely cytoplasmic inclusions in the late stage of infection, were found to be poor in RNA and viral antigen. Kallman *et al.* (1959) and Tawara *et al.* (1961) reported that cytoplasmic bodies have a particulate appearance and have definite arrangement or crystalline pattern. However, they were uncertain whether these particles represented the infective agent of measles.

The present autoradiographic study was on the relationship of cytoplasmic inclusions of measles virus to the localization of RNA synthesis and DNA synthesis.

FL cells grown on coverslips in Leighton tubes (2×10^5 cells) were infected with the Toyoshima strain of measles virus (10^3 $TCID_{50}$). Three days after infection, when cytopathic changes were readily detectable, infected cells were exposed to a 30 min-pulse of ^{3}H-uridine (2 μC/ml) in the presence of a 1000 fold excess of unlabelled thymidine. The latter prevented the uptake of label into DNA. In another experiment, infected cells were exposed to ^{3}H-thymidine (2 μC/ml) for 30 min. Then the cells were immediately washed with cold Hanks' solution and fixed with Bouin's fluid. They were extracted with 2 per cent $HClO_3$ for 40 min at 4°C and thoroughly washed with water. Dipping autoradiography using the Eastman Kodak NTB2 emulsion was carried out according to the techniques described previously (Kato *et al.*, 1963a). As a drying agent, silica gel was used during the exposure period (3 days). Cells were stained with hematoxylin-eosin (H-E) stain after development of the autoradiograms. Control uninfected cells were subjected to the same procedures. With ^{3}H-uridine, the specificity of the silver grains for RNA was confirmed by disappearance of the grains by the pretreatment with RNase.

With H-E staining, most cells showed characteristic changes due to measles virus infection, *i. e.* giant cell formation, spindle cell formation, enlargement of the nucleolus and change in the nucleus. Cytoplasmic inclusions of various sizes and shapes and surrounded by halos were detectable in most giant cells and single cells.

The uptake of ^{3}H-uridine was generally less in inclusion-bearing cells than in noninclusion-bearing cells (Table 1). Most of the silver grains were found in the nuclei. No labelling was observed in cytoplasmic inclusions of any size or shape (Figs. 2, 3). This suggests that cytoplasmic inclusions of measles virus are not sites

Table 1. Uptake of ^{3}H-Uridine into Normal and Measles Virus-infected FL Cells

	Mean Number of Grains per Cell		
	Nucleolus	Nucleus	Cytoplasm
Control Cells* (Noninfected)	15.1	43.8	9.7
Giant Cells** (Infected)	6.7	28.4	4.4
Inclusion-bearing single Cells* (Infected)	12.6	28.0	4.8

* Counts were made in 100 cells.
** Counts were made in 100 nuclei.

of active RNA synthesis unlike the inclusions of meningopneumonitis virus (Kato *et al.*, 1963b).

With ^{3}H-thymidine also there was no labelling of cytoplasmic inclusions unlike the "B" type inclusions of pox virus. The silver grains were found exclusively in the nuclei. The extent of incorporation of ^{3}H-thymidine into the nuclei of inclusion-bearing cells was less than that in noninclusion-bearing cells (Table 2). The incorporation of ^{3}H-thymidine into the nuclei in giant cells was suppressed.

Our experiments suggest that cytoplasmic inclusions of measles virus are not sites of active RNA and DNA synthesis. It would be one possibility that the incorporation of tritiated precursors into cytoplasmic inclusions was not seen because of this slow rate or small amount of synthesis of nucleic acids of measles virus. However, there is also the fair possibility that the cytoplasmic inclusions of measles virus may belong to the "secondary virus inclusions" (Kato, 1963) which do not play any role in virus multiplication, like the "A" type inclusions of poxvirus. However, it is still not clear whether the nuclei which show only active nucleic acid synthesis in inclusion-bearing cells, are the sites of synthesis of viral nucleic

Table 2. Uptake of ^{3}H-Thymidine into Nuclei of Normal and Measles Virus-infected FL Cells

	Mean Number of Grains per Nucleus			
	0~9	10~29	30~49	>50
Control Cells* (Noninfected)	50 %	9.5%	8.5%	32 %
Giant Cells* (Infected)	70 %	22 %	8 %	0 %
Inclusion bearing single Cells* (Infected)	53 %	19.5%	18 %	9.5%

* Counts were made in 200 nuclei.

acid or not.

REFERENCES

Kallamn, F., Adams, J. M., Williams, R. C. and Imagawa, D. T. (1959). Fine structure of cellular inclusions in measles virus infections. *J. Biophys. Biochem. Cytol.* **6,** 379-382.

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

Kato, S., Ogawa, M., Miyamoto, H. and Kamahora, J. (1963b). Autoradiographic study on the dual infection of single cells with psittacosis-meningopneumonitis virus and poxivirus. *Biken J.* **6,** 165-169.

Kato, S. (1963). Host-virus interaction in poxvirus infection. *Acta Pathol. Japonica.* in press.

Tawara, J. T., Goodman, J. R., Imagawa, D. T. and Adams, J. M. (1961). Fine structure of cellular inclusions in experimental measles. *Virology* **14,** 410-416.

Toyoshima, K., Hata, S., Takahashi, M., Miki, T. and Okuno, Y. (1960). Virological studies on measles virus. III. Morphological changes and virus growth in FL cells. *Biken's J.* **3,** 241-248.

Michiaki Takahashi*
Hiroyuki Miyamoto**
Shiro Kato**

* *Department of Virology*
** *Department of Pathology,*
The Research Institute for Microbial Diseases,
Osaka University, Osaka
Received on October 3, 1963

EXPLANATION OF FIGURES

Autoradiogram with ^{3}H-uridine

Fig. 1. Noninfected control FL cells.
Incorporation mostly into the nuclei.

Figs. 2, 3. Measles virus-infected FL cells.
Incorporation into cells was reduced. No grains were observed in cytoplasmic inclusions at any developmental stage.

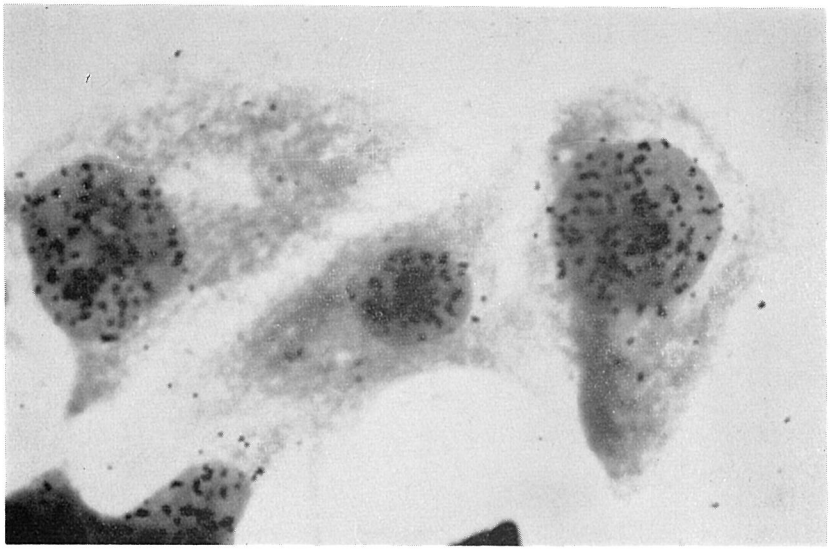

Fig. 1

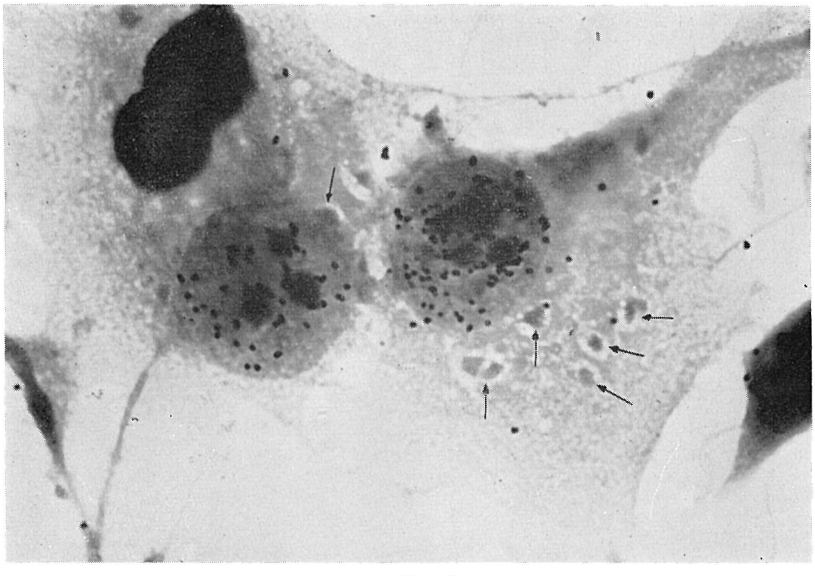

Fig. 2

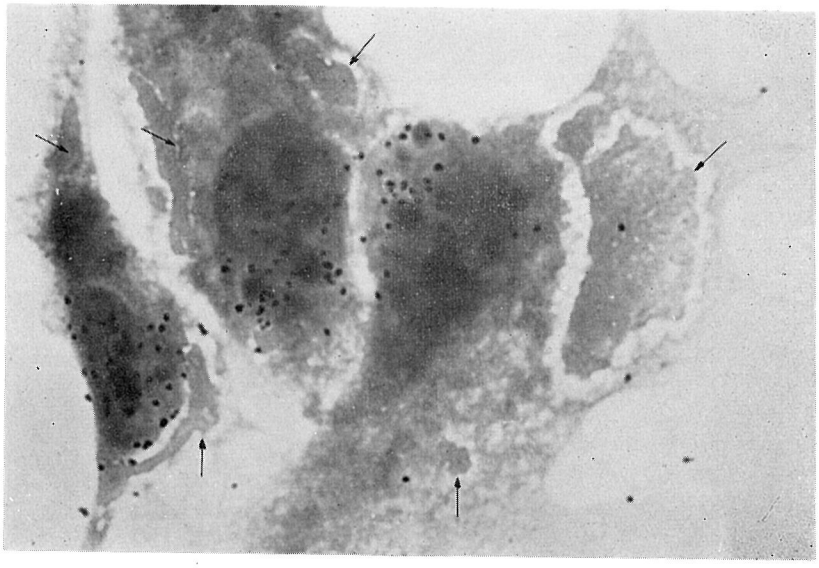

Fig. 3