Electron Microscopic Study of the Inclusions of Cowpox Virus*

Downie described large, round, eosinophilic inclusions in tissues infected with cowpox virus.[1] Two types of inclusions of cowpox virus were recently shown by Kato *et al.*[2] to be present in cytoplasm infected with cowpox virus. Their "A" type inclusions were shown to correspond to Downie type inclusions, and their "B" type inclusions were new discoveries. Their further investigations indicated that the "B" type inclusions were the site of DNA synthesis and a viral antigenic pool, while the "A" type inclusions had no relation to viral multiplication.[3, 4, 5]

This letter reports results of the first electron microscopic observations of "A" type inclusion (Downie type).

FL cells in tissue culture were employed as hosts, because they have been reported to be susceptible to cowpox virus.[2] FL cell monolayers (about 1 millions) which had been cultured on a plasma clot, were inoculated with cowpox virus, LB red strain**. Three days after virus inoculation, the cells which were still attached to the plasma clot were fixed with Osmium tetraoxide and were scraped off. These cells were prepared for electron microscopy by the standard methacrylate technique. Simultaneously a study was made of part of the samples by Giemsa staining with a light microscope.

There were sharply defined, round, homogeneous structures in the cytoplasm of most of the FL cells in tissue culture. The size of these structures varied. By comparison with the observations of Giemsa stained preparations, these structures were very similar to the "A" type inclusions which under the light microscope were round, sharply outlined bodies, taking on a pale blue tinge with Giemsa stain. Curiously enough none of these "A" type inclusions contained virus particles, while the virus particles were observed in the surrounding cytoplasmic area.

The results seem to indicate that the "A" type inclusions of cowpox virus are not virus colonies at any stage throughout their development and are presumably secondary products.

I am greatly indebted to Prof. Kamahora, Prof. Fukai and Asst. Prof. Kato for their helpful suggestions and to Mr. Tanaka for technical assistance.

REFERENCES

1) Downie, A. W. (1947). A study of the lesions produced experimentally by cowpox virus. *J. Path. Bact.* 48, 361-378.
2) Kato, S., Takahashi, M., Kameyama, S. and Kamahora, J. (1959). A study of new inclusion bodies of cowpox virus. *Biken's J.* 2, 93–96.
3) Kato, S., Takahashi, M., Kameyama, S. and Kamahora, J. (1959). A study on the morphological and cyto-immunological relationship between the inclusions of variola,

* The compendium of this letter was presented at the Annual Meeting of the Japanese Dermatological Association on April 1, 1960.
** This was received from Dr. Kato, the Research Institute for Microbial Diseases, Osaka University.

cowpox, rabbitpox, vaccinia (variola origin) and vaccinia IHD and a consideration of the term "Guarnieri body." *Biken's J.* **2,** 353–363.

4) Kato, S., Kameyama, S. and Kamahora, J. (1960). Autoradiography with tritium-labeled thymidine of pox virus and human amnion cell system in tissue culture. *Biken's J.* **3,** 135–138.

5) Kato, S., Kameyama, S. and Kamahora, J. (1960). Autoradiography of cells infected with variola and cowpox viruses with H^3–thymidine. *Biken's J.* **3,** 183～189.

KANEMITSU MORITA

Department of Dermatology
Osaka University Medical School
Osaka
(Received on June 27, 1960)

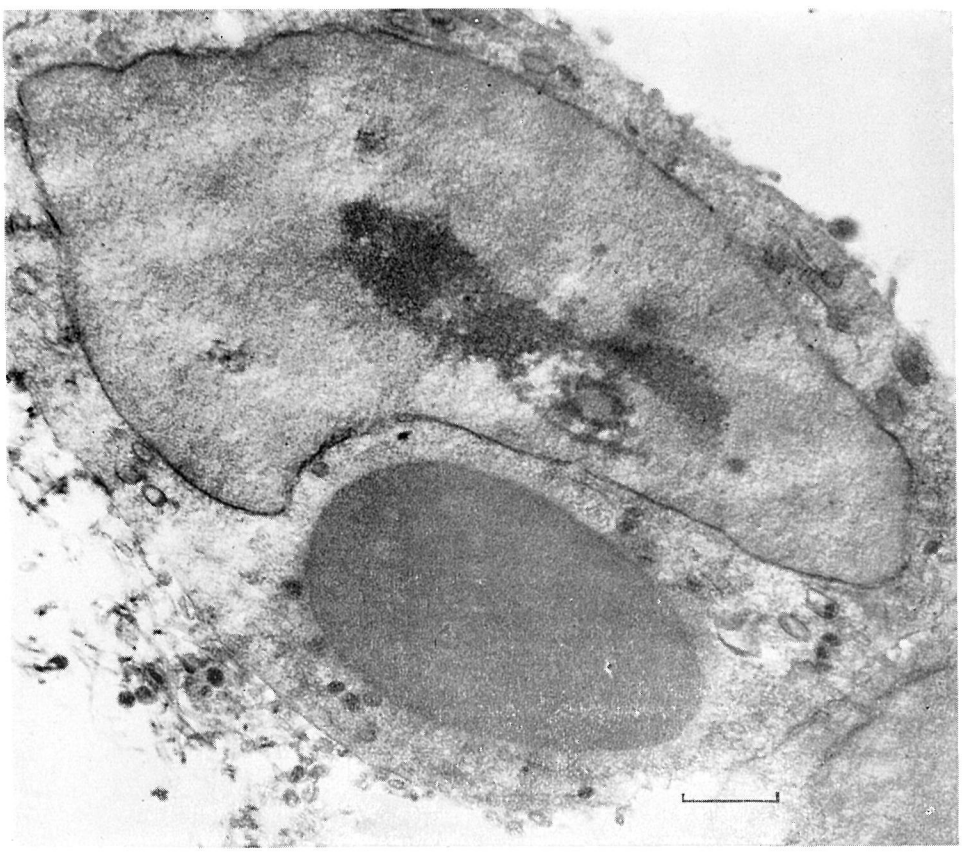

Fig. 1. Section of FL cells infected with cowpox virus. An "A" type inclusion is shown as a homogeneous and sharply outlined structure which does not contain virus particles. Virus particles are seen in the surrounding cytoplasm. Magnification ; ×12,500