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Letters to the Editors

A Study of Myxoma Virus Inclusions by Fluorescein-labeled Antibody

Several authors (Splendore, 1909; Rivers, 1930; Lewis and Gardner, 1932) who studied morphologically the infected rabbit tissue using Giemsa staining or eosin-methylene blue staining, called the inclusion bodies of rabbit myxoma virus acidophilic or eosinophilic. Chaproniere (1956) described the formation of eosinophilic inclusions in cultured cells of rabbit tissue and at the same time depicted growth curves of the virus. Chaproniere and Andrewes (1957) reported the cultivation of the virus in tissues of nonsusceptible hosts. The susceptibility of the human amnion cells (A 203) (Strain established by Zitcer and Dunnebacke) to the myxoma virus was found by Kato and Cutting (1958, 1959). They found inclusion bodies in these human amnion cells as well as in the cells from the abdomen and from tumor tissues in the rabbit. Histochemically the inclusions were all Feulgen positive. Neither polysaccharide, nor fatty substance could be demonstrated in them. The inclusions took up hematoxylin and became reddish purple with Giemsa stain. The above mentioned appearance and the histochemical characteristics were the same as those oi the inclusion of rabbit fibroma virus, the so-called Guarnieri body of vaccinia virus, the "B" type inclusion of ectromelia virus (Kato, 1955; Kato et al, 1955) and fowlpox virus (Kamahora et al., 1955).

This letter describes the relationship between the localization of the myxoma virus antigen and its inclusion in tissue culture cells: a relationship which has up to the present been quite obscure. Human amnion cells (FL strain) were used throughout the experiments. About 3×10^5 cells were cultured in a series of test tubes. The culture medium cosisted of 10% ox serum and 90% YLH (Hanks' balanced salt solution containing 0.1% yeast extract and 0.5% lactalbumin hydrolysate).

The virus material was added after 3 days culture. The growth curve of the virus until seventh day is shown in Fig. 1.

To stain with fluorescein-labeled antibody, cover slip culture was also carried out simultaneously. Myxoma antiserum was prepared by repeated inoculation of ultraviolet ray irradiated viruses into the rabbits. Complement fixation titres up to 640 X were obtained. Fluorescein-isothiocyanate (kindly given by Dr. Seiwald of San Francisco University) was used for coupling. At various time of growth, the cover slips were taken from the test tubes and treated as described by Coons and Kaplan (1950). The immunological specificity of this staining was established by (a) lack of staining in uninfected cultured cells and (b) inhibition of staining by prior exposure of infected cells to unconjugated homologous antiserum, whereas no inhibition occurred by prior exposure to normal rabbit serum. As shown in Fig. 2-5, almost all the inclusion bodies ranging from initial small round bodies to large network bodies correspond to the fluorescent area. So far nowhere else in the cell, the nucleus or the cytoplasm other than the inclusions

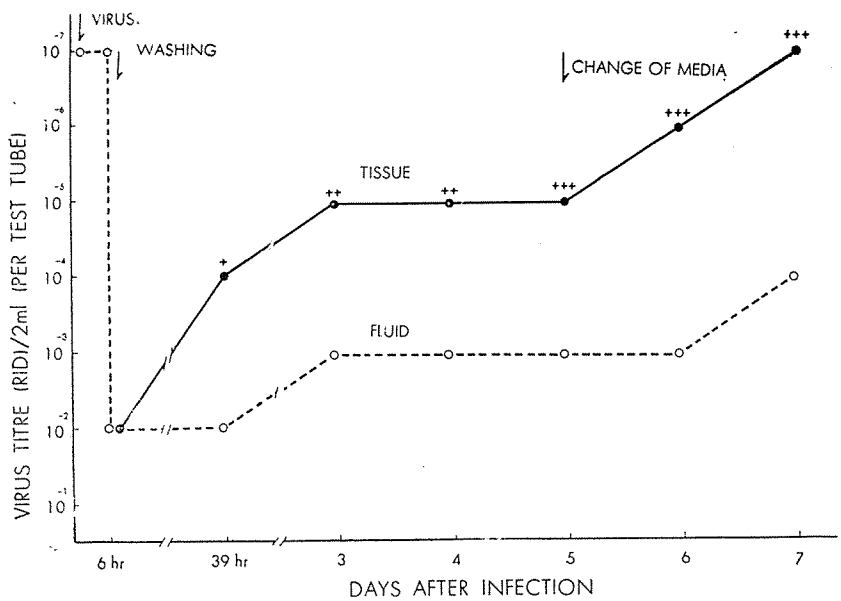

Fig. 1. The growth curve of rabbit myxoma virus in cultures of human amnion cells (FL strain). Broken line: virus in fluid phase; unbroken line: virus in freeze-thawed tissue. Plus signs (+, ++, +++) indicate the approximate amount of inclusions. RID is Rabbit Infectious Dosis determined by the tumor formation on the rabbit skin.

has revealed a distinct fluorescence.

Thus rabbit myxoma inclusion bodies have been proved to contain a great deal of virus antigen as well as DNA material. It seems likely that myxoma inclusion bodies are an important site for virus multiplication.

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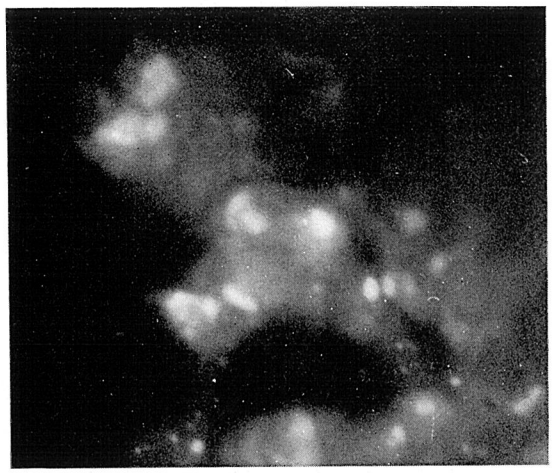

Fig. 2. FL strain cells, 4 days after infection. The bright yellow green fluorescent areas in various forms are exclusively in cytoplasm. Magnification : X 400.

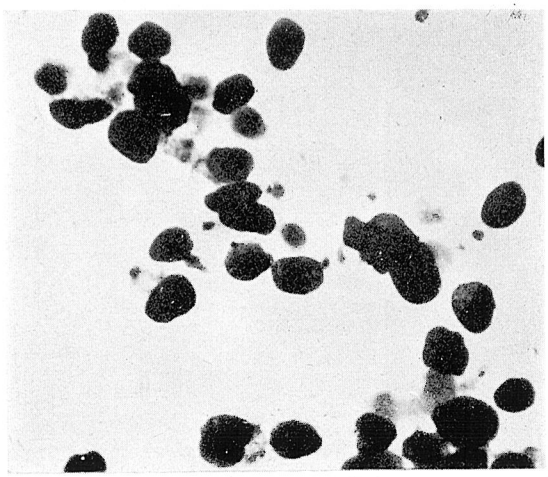

Fig. 3. The same area as Fig. 1 after staining with Giemsa solution. Cytoplasmic RNA almost entirely disappeared probably due to long exposure to ultraviolet rays. Distinct fluorescent areas correspond to inclusion bodies. Degenerated nuclei can be easily distinguished under the microscope. Magnification: X 400.

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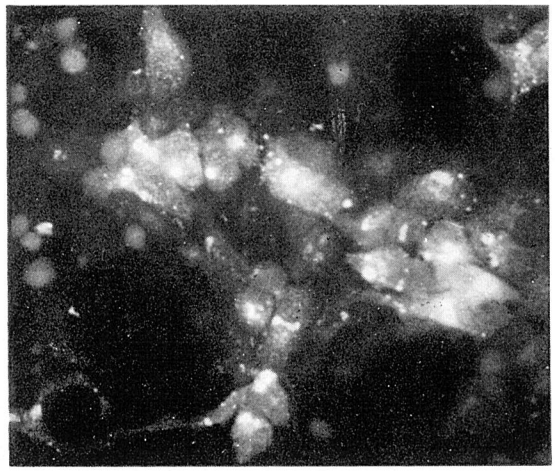

Fig. 4. Two days after infection. Some cells have numerous small fluorescent areas, suggesting that multiple infection had occurred. Magnification : X 200.

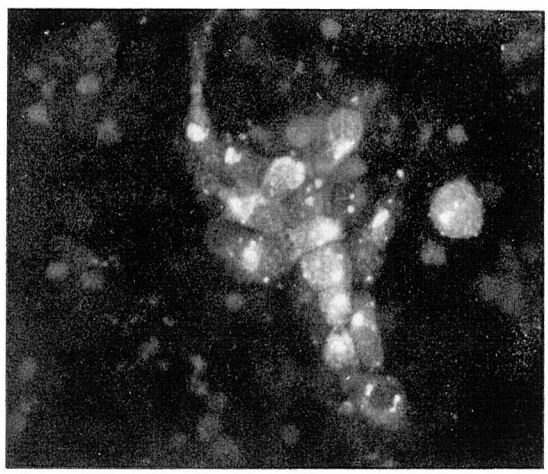

Fig. 5. Two days after infection. Upper cell clearly shows that virus antigens exist around the nucleus. Magnification : X 200.

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