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Studies on the Carrier Culture of Rabbit Fibrama and Myxoma Virus

Recently virologists have had great interest in the study of the persistent infection or carrier culture of viruses. We found rabbit myxoma virus (MV) and rabbit fibroma virus (FV) multiply in cultured human amnion cells forming viral incusion bodies (Kato et al., 1959a, Takahashi et al., 1958, Kato et al., 1959b). This letter describes the establishment of the carrier culture of the fibroma and myxoma virus in the cultured human amnion cell (FL strain) and possible analysis of the states using the FV and FL systems.

While studying the growth of MV and FV in FL cells in test tubes, it was noticed that unlike ectromelia virus or vaccinia virus, these viruses never caused total cell degeneration or total cell desquamation and many cells remained which were apparently normal even 10 days or more after virus inoculation. To study the growth capacity of these cells, they were transferred to a prescription bottle. The cells continued to grow and within about 10 days covered almost all the surface of the bottom of the bottle. These cultures have been passed serially for many cell generations in both the FV-FL system and MV-FL system these 3 months. The FV-FLsystem was used for analysis of this state.

The following possibilities may be considered to explain the experimental findings. 1) Virus multiplication gradually decreases or ceases. 2) Cells resistant to the virus appear. 3) A lysogenic state exists in this system. 4) Simultaneous

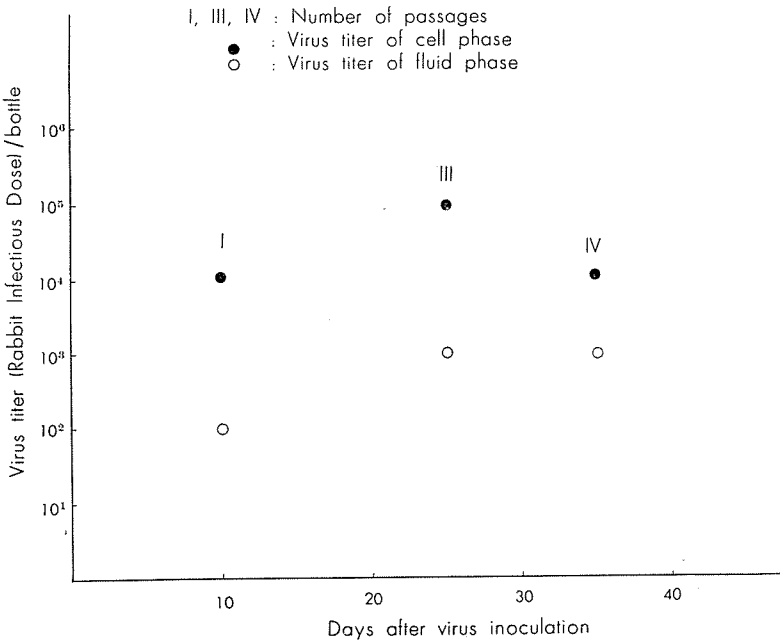

Fig. 1 Virus titer of fibroma virus-FL cell system in successive culture.

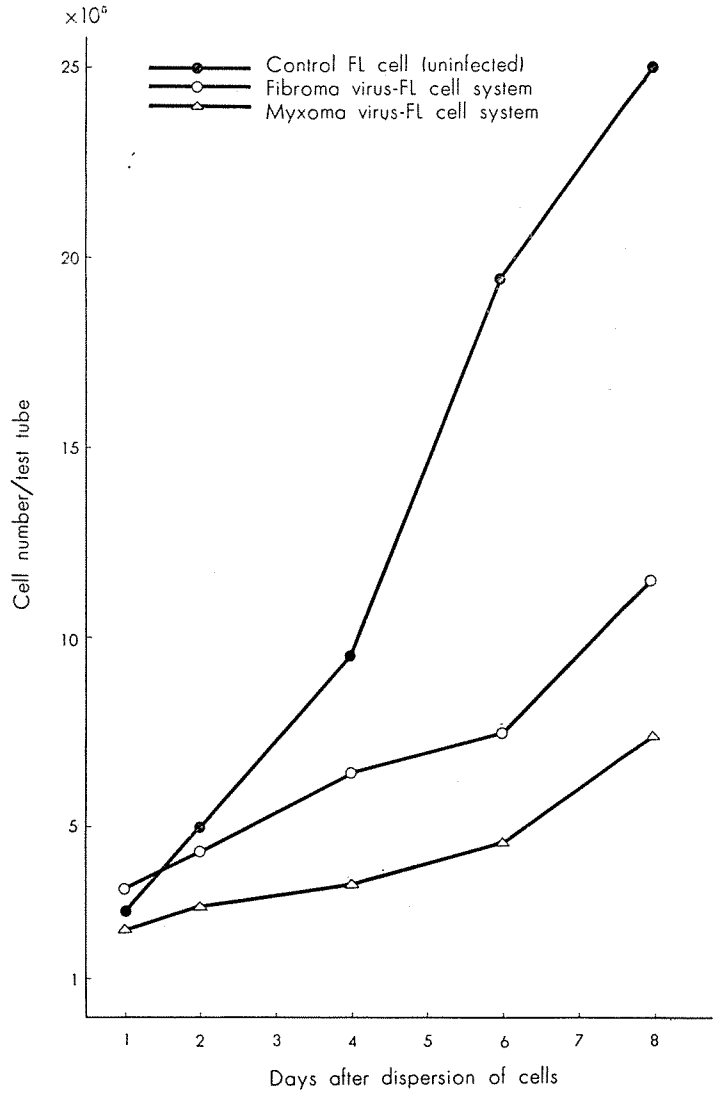

Fig. 2 Comparison of cell growth curves.

multiplication of both cells and virus occurs. The fourth possility should be further analysed as follows ; a) The cells multiply with virus multiplication in them but without virus release from the cells. b) The cells multiply with virus multiplication and with virus release from the cells. c) Only uninfected cells multiply and once a cell is infected it goes through the normal cycle of virus multiplication and cell death, but all the cells are not infected simultaneously due to small virus yield or slow virus growth or rapid attenuation of the virus infectivity in the fluid.

The most unique characteristic of this system is the " B " type inclusion body

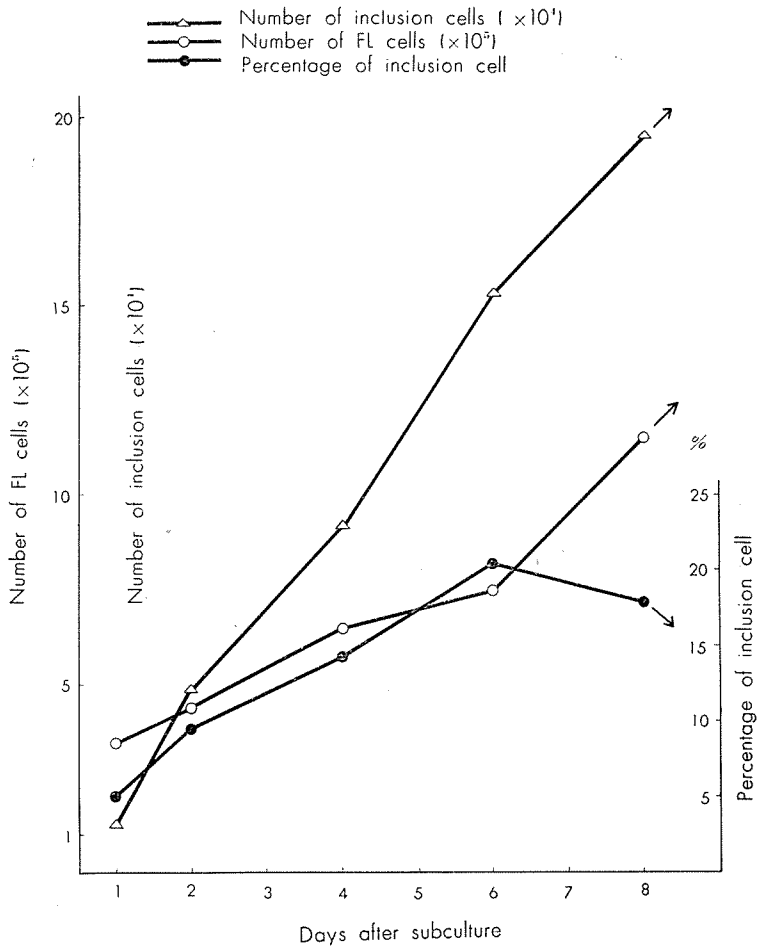

Fig. 3 Inclusion cell curve of carrier subculture. Cells in the third passage of carrier culture were dispersed in a series of test tubes. After various times intervals, the number of the cells and the percentage of the inclusion cells were calculated.

formaton which is considered as a site of virus multiplication, because it is always Feulgen positive (Kato et al., 1959a) and has been shown by means of fluorescein-labelled antibody technique to be full of specific antigen (Takahashi et al., 1958, Kato et al., 1959b, Takahashi et al., 1959). Such an inclusion body can be an indicator of the infection of single cell, unless the cell is infected with a virus particle which has entered the dark period or eclipse and does not show any inclusion yet.

The analysis was made quantitatively by inclusion cell count and by observing the mode of distribution of the inclusion cells in a monolayer in the test tubes using both Giemsa staining and the fluorescein-labelled antibody technique. Samples taken from the various stages of serial passage always showed a virus titre in both fluid and cell phases, as shown in Fig. 1. The growth rate

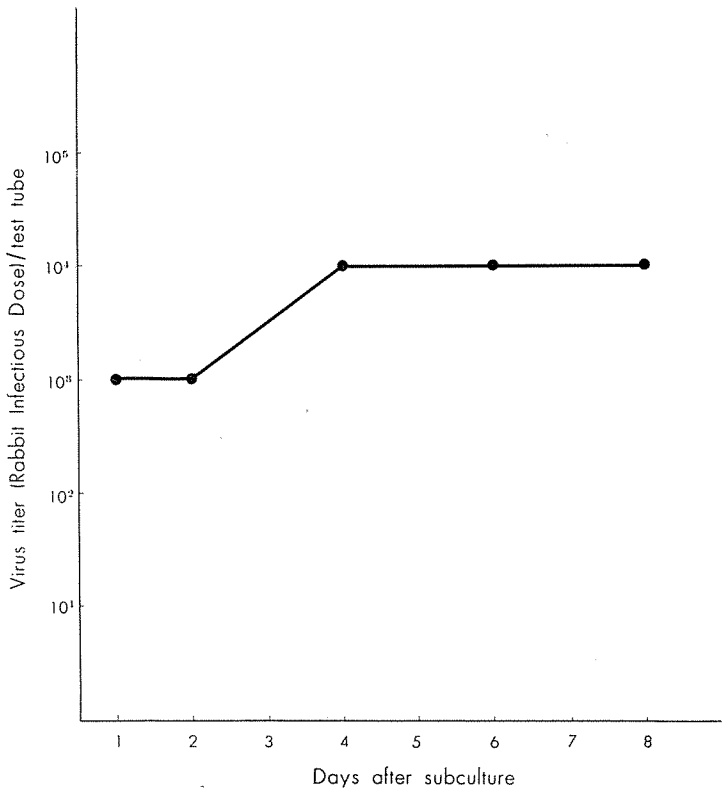

Fig. 4 Virus growth curve of cell phase in the same carrier subculture as Fig. 3

of the cells was compared with that of control uninfected FL cells and the MV-FL system. The result is shown in Fig. 2. For more detailed analysis infectivity titrations and inclusion counts were made at each stage. As shown in Fig. 3 and Fig. 4, the inclusion cell number and infectivity increase very steadily and not abruptly. However once the total cell number is increasing logarithmically the inclusion percentage decreases again to 2% or less. These facts were confirmed by the repeated experiments. The disribution of the inclusion cells resembles a colony, with a rather even distribution as easily demonstrated by the fluorescein antibody technique. Mitosis in the inclusion cells can hardly be seen. There are always some degenerating cells which have fully developed inclusions. The facts mentioned above may suggest that possibility no. 4 c) is most likely to explain the carrier culture of this system. Details of the results and discussion will be published in the Biken's Journal soon.

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