BIKEN'S JOURNAL
Letter to the Editors

Mode of Appearance of Reactivated Virus in Cells Superinfected with Active Ectromelia and Heat Inactivated Vaccinia Virus

It has been reported that heat inactivated vaccinia virus can be reactivated only after superinfection with other active pox viruses[1,2,3], and that in the process of reactivation of vaccinia virus some mechanism may be involved which differs from the cross-reactivation of bacteriophages[4]. This letter describes the characteristic features of the appearance of reactivated vaccinia virus in L cells superinfected with heat killed vaccinia and active ectromelia virus.

The viruses used, the cultivation of L cells and the method for heat inactivation were described in an earlier report[2]. The virus titer was estimated by plaque counting. Reactivated vaccinia virus could easily be assayed independently of ectromelia virus, since the former rapidly produces larger plaques on L cells than the latter.

About 2000 PFU* of active ectromelia was infected into monolayer cultures of L cells (5×10^6 cells/bottle) in three ways, simultaneously, before or after inoculation with 4×10^6 CRU** of heat inactivated vaccinia. Any infection, whether active or inactive, was followed by an adsorption period of two hours. After various periods the superinfected cells were disrupted by sonic oscillation and the intracellular, reactivated vaccinia virus was assayed. When active ectromelia and heated vaccinia were simultaneously inoculated, the mode of development of reactivated vaccinia virus differed both from that of active vaccinia and from that of ectromelia virus in the following respects. (1) The reactivated virus appeared 12 hours after inoculation, whereas growth curves for both vaccinia and ectromelia virus ($0.4\sim1\times10^3$ PFU) showed that these viruses multiplied logarithmically after a latent period of $4\sim6$ hours, as reported by Furness and Youngner[5]. (2) The reactivated virus multiplied rapidly after 12 hours as if all the heated virus had been reactivated symultaneously, and after remaining steady for about 10 hours the curve rose again, indicating another increase in intracellular virus. Measured at the time of the steady plateau, the number of reactivated vaccinia obtained from superinfected cells by sonic oscillation coincided with the number of infective centers of vaccinia obtained by plating the superinfected cells on L cell monolayers. Therefore one superinfected cell may produce one reactivated virus.

Pre-infection of ectromelia 4 hours prior to inoculation of heated vaccinia gave the same unique characteristic mentioned above of the synchronous appearance of the reactivated virus 12 hours after inoculation with inactivated virus. On the other hand, when the active virus was superinfected 4 hours or later after inoculation of the inactivated virus, reactivated vaccinia began to increase 8 hours after infection of the active virus, irrespective of when the inactivated virus was added. This depended only on the time of infection by the active virus. Moreover,

* PFU : Plaque forming unit
** CRU : Capacity to be reactivated[4])

in this case growth of the reactivated virus was logarithmic and asynchronous.

As a result of these experiments, the phenomena of reactivation may be analysed into two parts: one is the development of conditions in the inactivated vaccinia in which reactivation can occur, and the other is the maturation of active ectromelia virus. This explains why there is a certain period before the heat killed virus can be reactivated. It is likely that the appearance of reactivated vaccinia coincides with the maturation of ectromelia virus even when the change of the inactivated virus into a form which can be reactivated has been accomplished. More precise analyses of the growth characteristics of reactivated virus will be described in a succeeding paper.

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(Received August 24, 1960)