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Preliminary Report

Characteristics of Growth of HVJ in PS Cells

Several workers (Bukrinskaya, 1958; Matsumoto and Maeno, 1959; Ishida and Homma, 1960; Traver *et al.*, 1960; Fukumi and Nishimura, 1961; Zhdanov *et al.*, 1961) have reported the multiplication of egg-adapted HVJ in tissue culture cell systems, but the production of HVJ with as high a ratio of (more than 6) EID_{50}/HA as is usually obtained in chorioallantoic cavity of chick embryos was never observed in their tissue culture systems, where this ratio was between 2 and 5. The author examined the multiplication of HVJ in a stable line of porcine kidney (PS) cells, the most convenient tissue culture systems for assay and propagation of Japanese B encephalitis virus (Inoue and Ogura, 1961), and found that infection of PS cells with HVJ produced a highly infectious virus for eggs ($EID_{50}/HA=6$), compared with that produced in other tissue culture systems. The successive passage of egg-adapted HVJ in PS cells was under investigation.

The HVJ used was the Z strain which had a history of more than 300 passages in the chorioallantoic cavity of developing eggs. PS cells was kidnly furnished by Dr. Inoue (Inst. for Virus Research, Kyoto Univ.), cultivated in YLH (0.1 % yeast extract, 0.5 % lactalbumin hydrolysate in Hanks solution) containing 5 % bovine serum and maintained in YLH containing 1% bovine serum.

Fig. 1 shows the growth of HVJ in PS monolayers of 10^6 cells/bottle at a multiplicity of 500 EID_{50}/cell. After adsorption of HVJ to the PS cells for 60 minutes, the cells were washed 4 times with PBS and incubated in the maintenance medium. At intervals during incubation, the fluids were removed and clarified and the cells were washed, disrupted by 4 cycles of freezing and thawing after additions of the original volume of YLH and clarified. Then the hemagglutinating titer and infectivity for eggs of both materials were determined.

The infective virus begins to grow within cells 5 hours after infection, increases approximately logarithmically and reaches a maximum after 11 hours. The infective virus begins to increase in fluids 6-7 hours after infection and increase logarithmically untill 12 hours after the begining of incubation. However, it is possible that if the residual virus titer is much lower, the first increase of HVJ in fluids is a little earlier, when at the similar time to the intracellular increase of HVJ, because the extra-and intracellular growth curves of infectivity almost coincide between 6 and 11 hours after the begining of incubation. The hemagglutinating activity is detectable within the cells 2 hours later than the production of infective virus, *i. e.*, 8 hours after infection, reaches a maximum 12 hours after infection, is liberated into the fluid after 10-11 hours and increases rapidly to 12 hours. The ratio of EID_{50}/HA of HVJ liberated in the fluid was between 6.1 and 6.4. Even at a highest multiplicity of 4,000 EID_{50}/cell, the virus produced in the fluid still had a ratio of 5.8. This mode of production of hemagglutinating and in-

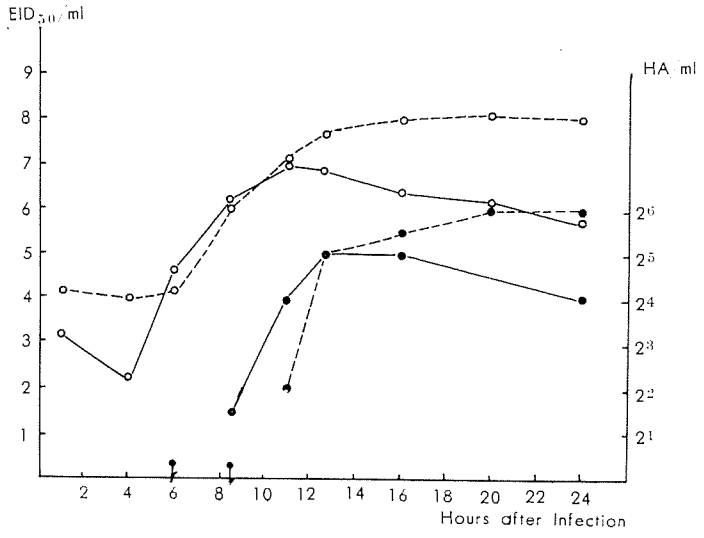

Fig. 1. Growth of HVJ in PS cells.

One ml of infected chorioallantoic fluid diluted with YLH ($10^{8.7}$ EID_{50}/ml) was added to PS monolayers (10^6 cells/bottle) and at intervals the titers of the fluid and cell associated virus were determined.

○——○ intracellular infectivity
○- - -○ extracellular infectivity
●——● intracellular hemagglutinating titer
●- - -● extracellular hemagglutinating titer

fective HVJ in PS cells is in contrast to those in other tissue culture cells (Ishida and Homma, 1960; Matsumoto and Maeno, 1959; Zhdanove *et al.*, 1961), in which the production of hemagglutinating particles was earlier than that of infective particles (in L or primary mouse lung cells) or at the same timc (in monkey kidney cells) and the HVJ produced had a lower ratio of EID_{50}/HA, that is, 5 at most. Hemoadsorption was detectable at the same time as the production of intracellular hemagglutinin, 8 hours after infection and became marked at the same time as the production of extracellular hemagglutinin, that is 10 hours after infection. After 24 hours the infected cells appeared rounded and contracted and after 48 hours cytopathic effects were marked and the destroyed cells became detached from the glass.

At an extremely high multiplicity (40,000 EID_{50}/cell), giant cell formation of PS cells occurred. Fig. 2 shows the process of giant cell formation of PS cells caused by HVJ. In the case of monolayers of PS cells, the process of the giant cell formation with HVJ was later than in the system of HVJ-free cells (Okada, 1958); the giant cell formation of PS cells with HVJ was detectable about 2 hours after infection and subsequently giant cells increased in number and size.

The characteristics of the HVJ (PS-HVJ) thus produced in PS cells were in-

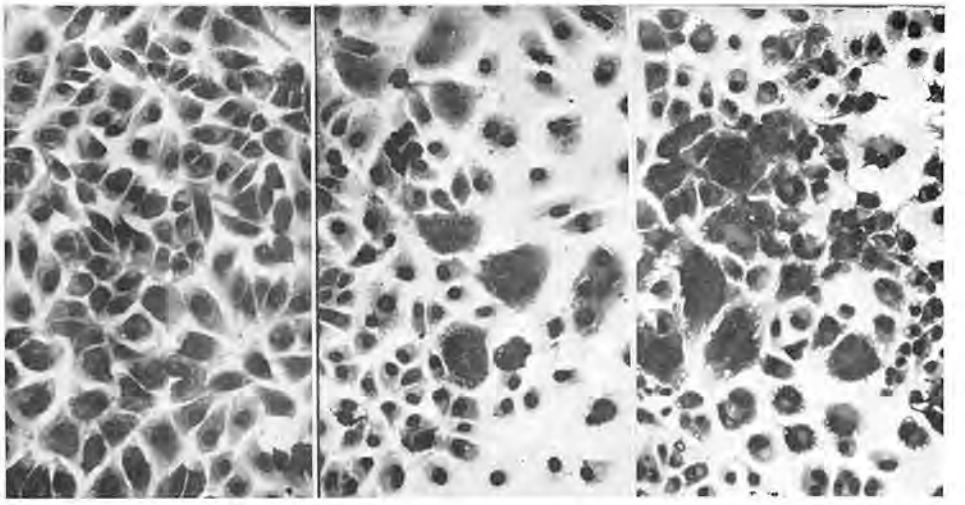

Fig. 2. Formation of Polynuclear Giant Cells by Fusion of PS Cells by HVJ Giemsa Staining × 100

a, normal cells

b and c. 5 and 10 hours after infection at a multiplicity of 40,000 EID_{50}/cells; cell sheets containing 10^5 cells infected with 1 ml of HVJ (4×10^9 EID_{50}/ml) in YLH containing 0.5% bovine serum.

vestigated, and so far results are as follows:

1) PS-HVJ retains complete infectivity for eggs, but shows little infectivity for PS and no infectivity for L cells, (Table 1) although unpurified PS-HVJ was inoculated.

Table 1. Secondry Passages of PS-HVJ in PS and L Cells

Inoculum (total)		Virus titer/ml in PS cells 24 hours		Virus titer/ml in L cells 24 hours	
EID_{50}	$10^{7.7}$	EID_{50}	$10^{4.7}$ $10^{4.4}$	$<10^{3.2}$	$<10^{2.7}$
HA	32	HA	<1 <1	<1	<1

0.5 ml of PS-HVJ was added to monolayers of PS cells (10^6 cells/bottle). After one hour adsorption, the cells were washed 2 times with PBS and then incubated in 5 ml of maintenance medium. 24 and 48 hours after infection, infectivity and hemagglutinating titer of the fluid virus were determined. In 3rd passages in PS cells, the infectivity of fluid virus was $<10^{3.2}$ EID_{50}/ml 48 hours after infection.

The slight infectivity in the secondary passage of PS-HVJ in PS cells seems to be due to the first multiplication in PS cells of a trace of egg-HVJ residual in the inoculum preparation of PS-HVJ. The employed PS-HVJ was the fluid virus, not purified, produced in PS cells.

2) There was no detectable hemolytic activity for fowl red cells or fusing activity for Ehrlich's tumor cells (Okada, 1958) with purified PS-HVJ at a

concentration of 1,000 HA/ml although the hemolytic activity of PS-HVJ became slightly evident on the treatment of freezing-thawing, but these were restored by an egg passage.

3) The size and shape of PS-HVJ and its subunits are in principle similar to those of egg-adapted HVJ (Horne and Waterson, 1960; Hosaka *et al.*, 1961); some pieces of the disrupted envelope with projections have a hemagglutinin like appearance, and the inner structure consists of helical structures of 170 - 180 Å width with a periodicity of 50 A. (Fig. 3) The presence of a great number of helical structures within the virus particle supports the idea that PS-HVJ is a highly infective virus. Consistent with the low susceptibility of PS cells to PS-HVJ, the production of HVJ in PS cells, regardless of the incubation period (48-72 hours), was only dependent upon the inoculum size. Therefore in PS cells, only one step growth seems to occur under the condition employed.

Thus the HVJ-PS cell system provides a convenient one for studies on the infectious growth of HVJ in culture cells, which have been disturbed by defective production of HVJ in other tissue culture cell systems.

Furthermore, it was found that the infection of PS cells with influenza virus (Al type, Omachi strain and B type, Lee strain) produced non-infectious hemagglutinins and the infection with NDV (Osaka strain) resulted in infective particles.

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EXPLANATION OF FIGURES

Fig. 3. Structure of PS-HVJ shown by the negative staining technique.

A. Intact particles. Particles of various shapes and sizes of between 1500 and 5000 A of particles were observed. Projections of 100-130 A long and 10-20 A wide are arranged on the surface of the particles

B. Partially disrupted particles. Inner helical structures of 170-190 A with a periodicity of approximately 50 A can be seen. The appearance of the structures suggests a somewhat regular arrangement of the helical structures in the intact particles. The disrupted envelopes appear as hemagglutinin-like structures. (Hosaka *et al.*, 1961)

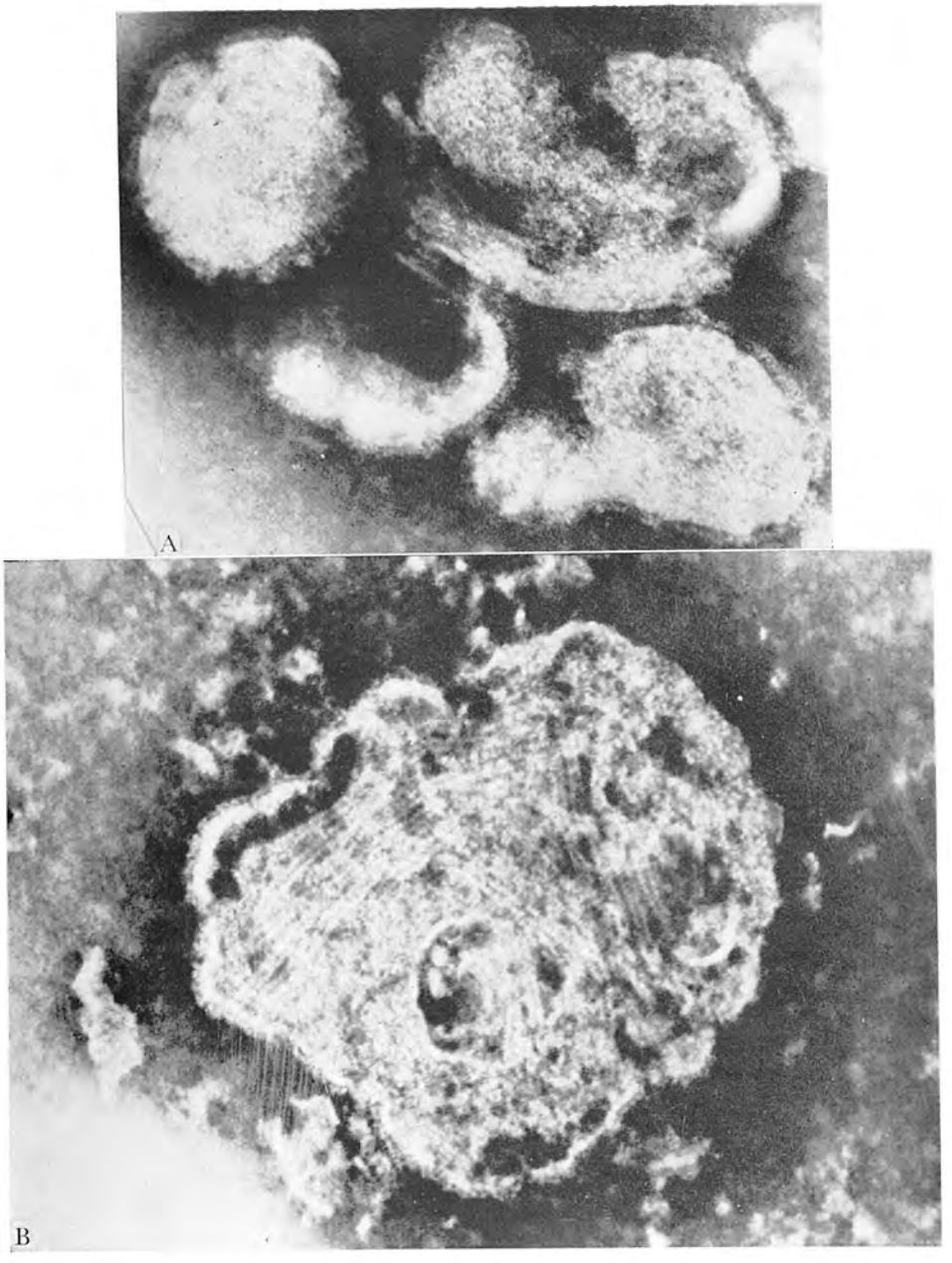

Fig. 3.