BIKEN JOURNAL
Preliminary Report

High Frequency Appearance of Amitotic Nuclear Divisions in PS Cells Induced by Herpes Simplex Virus

It has been reported that phenomenon of amitotic nuclear division sometimes occurs in cells infected with herpes virus.[1,2,3] However, only Reissig *et al.*[4] have studied this subject experimentally. These authors considered that amitotic nuclear division of pseudorabies infected cells might be the result of an increased synthesis of DNA coupled with an inhibition of mitosis.

This is a preliminary report on consistent induction in high frequency of amitotic nuclear division in porcine kidney cells.

Two kinds of porcine kidney cells (PS cells) maintained in this Institute were used for our experiments. These two cell lines were originally derived from the same parent PS cells maintained by Dr. Inoue in the Institute for Virus Research in Kyoto University.[5] The line which was given to us first was cultured on YLH solution with added bovine serum (PS_I) and the line which we received recently was cultured on LE solution containing serum (PS_{II}).

The -GCr Miyama strain of herpes simplex virus was used. This virus causes rounding of cells after infection and does not cause formation of syncytia.[6,7] Monolayers of both lines of PS cells were prepared on 10×40 mm coverslips in Leiton tubes. Two or three days after plating, monolayers, each containing approximately 2×10^5 cells, were obtained on the coverslips. One ml of virus containing approximately 10^8 $TCID_{50}$ was inoculated into each tube. After a one hour adsorption period, the inoculation fluid was replaced by maintenance medium. At appropriate intervals after infection cells were fixed with Bouin's fixative and stained with hematoxylin and eosin.

The results obtained using the PS_I cells are as follows: Five hours after infection cells began to contract and their nuclei which before infection were normally in the center of the cytoplasm, seemed to shift to the cellular surface and most of the nuclei began to elongate around a central cytoplasmic mass, which could be differentiated from the rest of the cytoplasm by its denser staining with eosin. Subsequently, the nucleus became more elongated, often with constrictions which indented the nuclei or almost divided them into smaller lobulated nuclei. Sometimes the constrictions appeared very fine and a slender strand connected neighbouring nuclei together. In this case the nuclei looked like a horseshoe (Figs. 1, 2) and some of the cells with this type of nucleus closely resembled polymorphonuclear leucocytes (Figs. 3, 4). Occasionally, ringshaped or doughnut shaped nuclei (Fig. 5) and cauliflower shaped nuclei (Figs. 6, 7) were found. At about eight hours after infection the nuclei of almost all cells showed some of these

morphological changes. Each segmented nucleus contained an eosinophilic mass or a typical shrunken inclusion.

The relationship between the formation of inclusion bodies and amitotic nuclear division was investigated. It was found that definite early signs of the two different morphological changes appeared at about the same time after infection.

The line of PS cells which we obtained more recently (PS_{II}), also showed amitotic nuclear division. However, the incidence of this was lower than in the PS_I line of cells.

None of the other established cell lines kept in this Institute, (*e. g.* FL or L strains) had such a high frequency of amitotic nuclear division as these cell lines.

Viral yields from infected cells seem to have no relation with this phenomenon. While the culture fluid of infected FL cells contained approximately 10^8 $TCID_{50}$ per ml when cytopathic changes were well advanced, that of L cells and of PS cells contained as low as approximately 10^5-10^6 $TCID_{50}$ per ml. Thus the PS cell lines had a comparatively low viral yield.

Reissig *et al.* reported that single rabbit kidney cells derived from 8 to 10 day old monolayer cultures may undergo amitotic nuclear division after infection with pseudorabies virus, while single cells derived from 3 day old cultures did not exhibit nuclear division and they suggested that this division is correlated with an increased synthesis of DNA.[4]

Although it is uncertain whether nuclear division in infected PS cells is related to increased synthesis of DNA, this phenomenon seems to be more cytological. As cytopathic changes in PS monolayer progressed, infected cells became rounded and paranuclear portions became clearly distinguishable. The appearance of elongated nuclei or amitotic nuclei surrounding paranuclear masses suggested that the former were pressed toward the cellular surface by the latter.

Further cytological and virological studies on this problem are now in progress and will be published later.

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Received on April 12, 1963

EXPLANATION OF FIGURES

Fig. 1. The cell on the left of the plate has a horseshoe-like nucleus. × 3600

Fig. 2. Of the four cells in the plate, the upper two have nuclei like cauliflowers, and the lower two have horsehoe-nuclei. × 3600

Fig. 3. The nucleus of the central cell in the plate is divided into three lobulated nuclei, which are connected together by a fine chromatin strand. The lobulated nucleus, which is shown in the low part of the cell, has one indentation. × 3600

Fig. 4. The cell, the cytoplasm of which occupies the lower and middle part of the plate, has two lobulated nuclei. × 3600

Fig. 5. The lower left cell in the plate has a ringshaped nucleus. × 3600

Fig. 6. The cell in the central right of the plate has a cauliflower-like nucleus. × 3600

Fig. 7. A cell with a cauliflower-like nucleus. × 3600

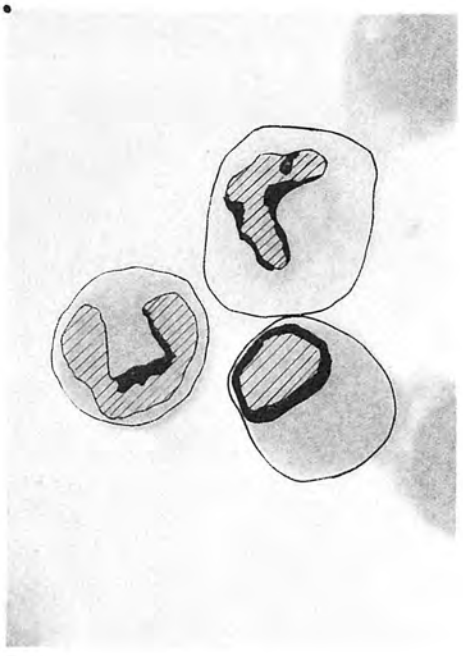

Fig. 1

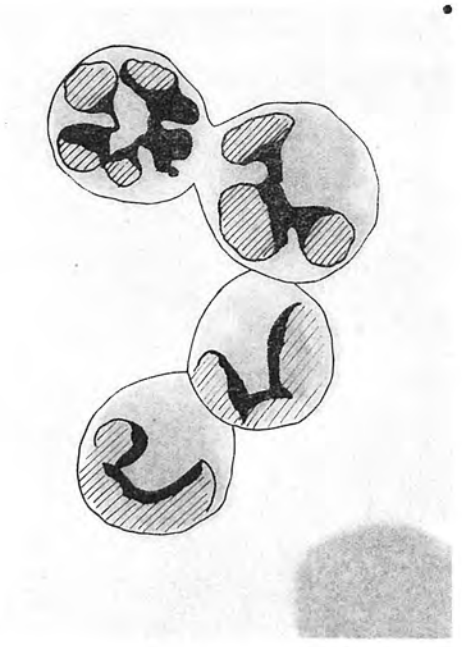

Fig. 2

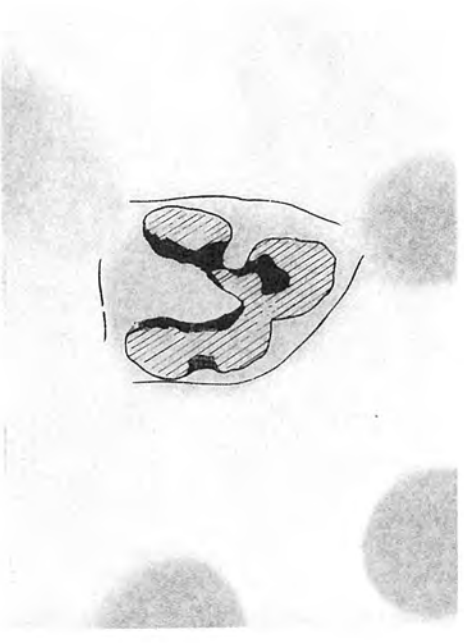

Fig. 3

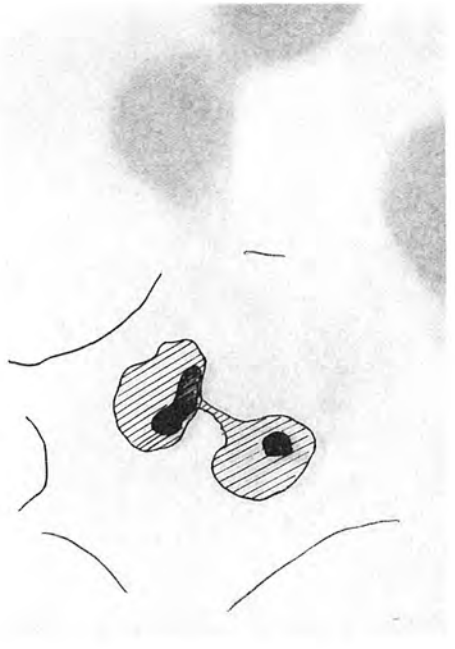

Fig. 4

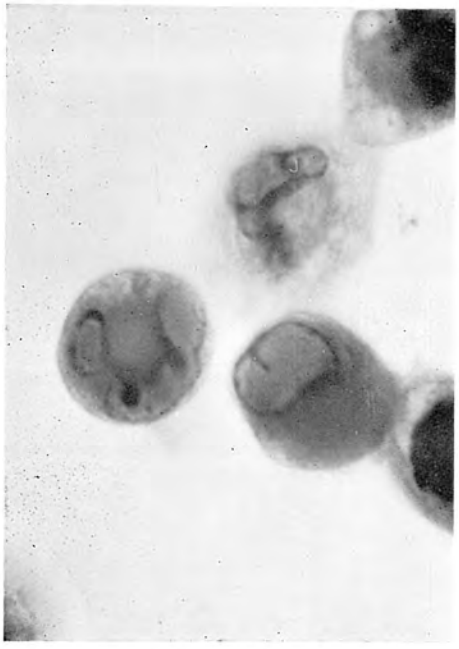

Fig. 1

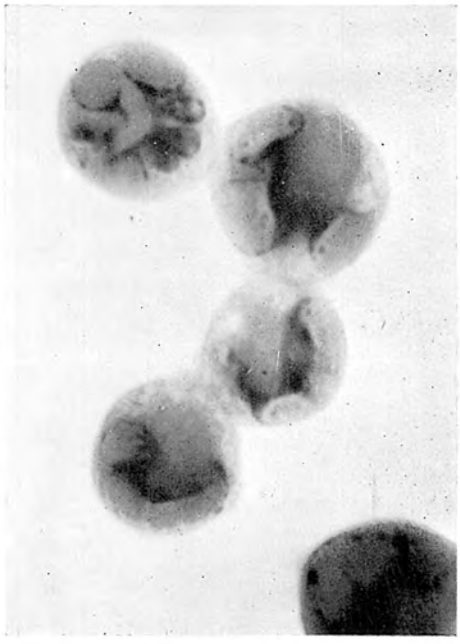

Fig. 2

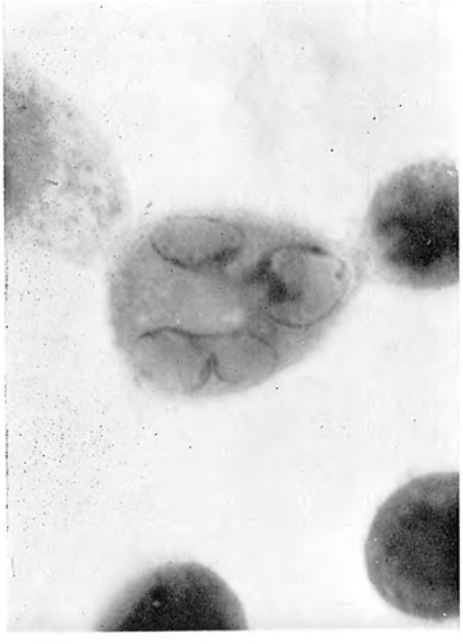

Fig. 3

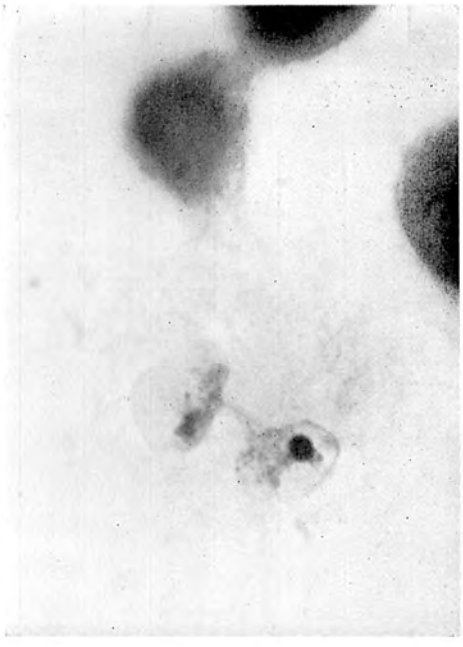

Fig. 4

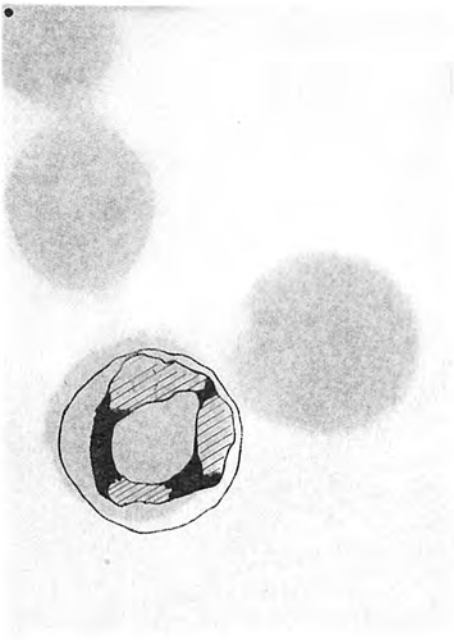

Fig. 5

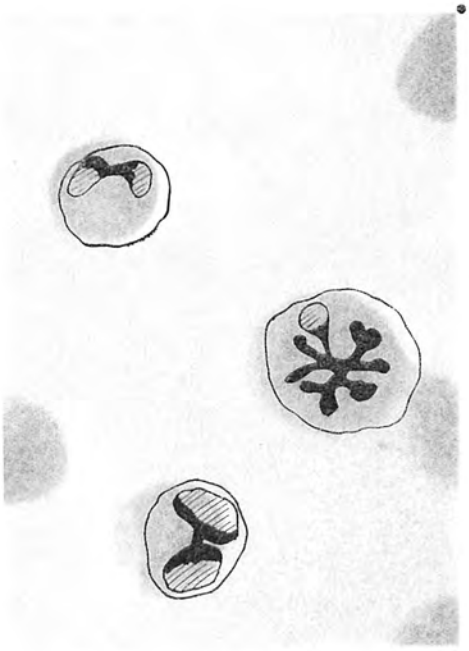

Fig. 6

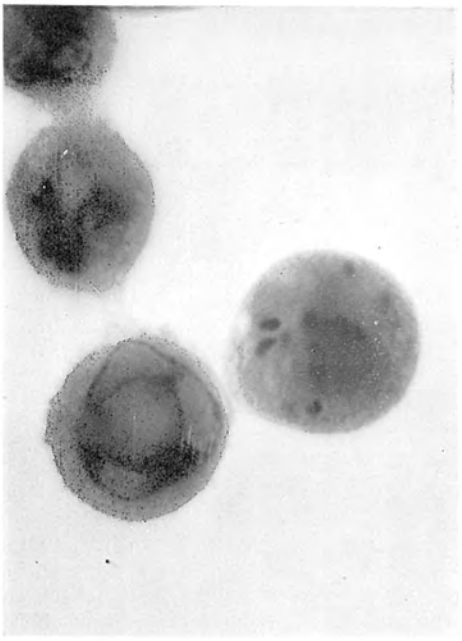

Fig. 5

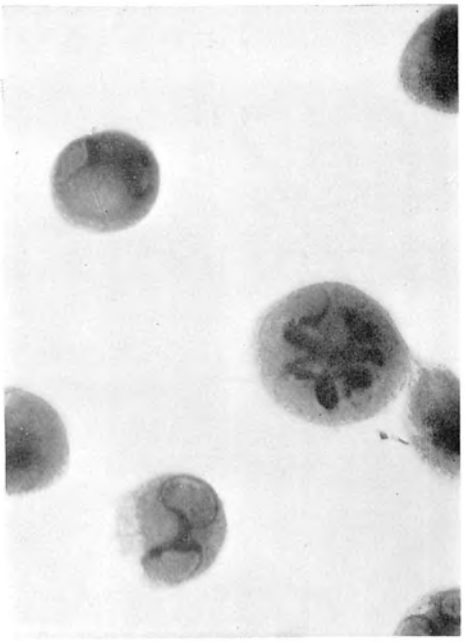

Fig. 6

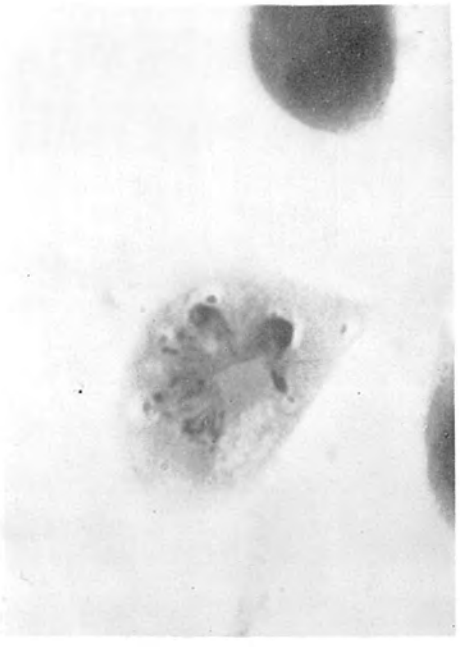

Fig. 7